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Diversified agri-food production systems for nutritional security

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Diversified agri-food production systems for nutritional security

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Table of contents

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Assessing the genetic diversity of guava germplasm characterized by morpho-biochemical traits

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Amid environmental crises, a galloping population, and changing food habits, increasing fruit production with nutritional quality is a global challenge. To address this, there is a necessity to exploit the germplasm accessions in order to develop high-yielding varieties/hybrids with good adaptability and high quality fruit under changing environmental and biological conditions. In the study, a total of 33 morpho-biochemical traits enabled an assessment of the genetic variability, diversity, and structure in a collection of 28 diverse germplasm lines of guava. Results showed that highly significant genetic variability existed in the studied traits in the guava germplasm. The coefficient of variation values for the qualitative and quantitative traits varied from 23.5–72.36 to 1.39–58.62%, respectively. Germplasm Thai, Lucknow-49, Punjab Pink, *Psidium friedrichsthalianum*, and Shweta had the highest fruit weight (359.32 g), ascorbic acid content (197.27 mg/100 g fruit), total phenolic content (186.93 mg GAE/100 g), titratable acidity (0.69 percent), and antioxidant capacity (44.49 μmolTrolox/g), respectively. Fruit weight was positively correlated with ascorbic acid content; however, titratable acidity was negatively correlated with fruit weight. The principal component analysis (PCA) was 84.2% and 93.3% for qualitative and quantitative traits, respectively. Furthermore, K-mean clustering was executed; the population was grouped into three clusters for both traits. Additionally, the dendrogram using agglomerative hierarchical clustering (AHC), where all the germplasm were grouped into four clusters, revealed that among the clusters, clusters III and IV were highly divergent. The high variability, diversity, and structure could be utilized for the breeding programme of guava and also explored for molecular analysis using next-generation technology to enhance the guava yield and nutrition properties and also develop the climate resilient technology to fulfill the existing demand gap and nutrition availability, which could not only mitigate the nutrition requirement but also enhance the easy availability of fruits year-round.

KEYWORDS

qualitative, quantitative, germplasm, guava, variability, diversity

Introduction

The lack of diversity in our current eating habits can lead to long-term health issues like allergies, asthma, heart issues, diabetes, cancer, obesity, and also underdevelopment in children and pregnant women in underdeveloped and developing countries of the world. More than 2 billion people are threatened by a global nutritional crisis (1). A healthy diet is crucial for the good health of human beings, but diets deficient in macro and micronutrients are detrimental to those who cannot afford a variety of cuisines (2). The recommended amount of fruit to be consumed per day is 200 g (3). The availability of fruits worldwide reached 248 g per person per day, but different demographic groups consume different amounts of fruits (https://www.fao.org/3/cb9574en/cb9574en.pdf). Contemporary field-crop-centric agricultural practices challenge the nutritional security of the growing population and cause severe health issues. The health-related problems are further aggravated due to changing climatic conditions. Human beings with nutrient deficits are supposed to be more vulnerable to changing climates; the situation will be more alarming if the same trends continue. The impacts of anthropogenic climate change on the climate at local, regional, and global scales are now beyond dispute. It is noted that there have been variations in the frequency and length of extreme climatic occurrences (4). As a result of extreme hot and cold conditions in major cities and metropolitan areas, urban heat islands (UHI) pose a serious threat to human health, agriculture, and forest fires. Climate change and global warming have an impact on biological, physiological, and biochemical processes in fruit crops (5). Specifically, the effect on vegetative development, blooming, fruit set, fruit quality, the occurrence of physiological problems, the prevalence of pests and diseases, shifting cultivars, shifting crops, and other factors, that are eventually exhibiting indicators of decline in fruit production and productivity. Hence, there is a need to exploit the nutrient-rich, climate-resilient fruit crop germplasm that can withstand changing climes.

Guava belongs to the Myrtaceae family, which encompasses 3,300 species and 150 genera (6). By virtue of its hardness and adaptability, guava is extensively dispersed in the tropics. Guava is also known as the "Apple of the Tropics" (7) and it is also well known as "Poor Man's Apple" because of its availability irrespective of the season and a layman can afford it. Guava can be grown in both tropical and subtropical climate conditions (8). Guava has been grown in many countries in the world, but the top guava-producing nations are India, China, Pakistan, Mexico, Brazil, Egypt, Thailand, Columbia, and Indonesia. According to (9), guava was grown in an area of 0.308 million ha in India, with a production of 4.582 million metric tons and a productivity of 14.87 tons/ha. Guava is wellknown for its flavor, delectable taste, and high nutritional value; it contains a lot of vitamins and minerals, *i.e.,* vitamin C or ascorbic acid (100 g of fresh fruit provides 228 mg), vitamin A (140+ g retinol equivalents/100 g), carbohydrates (13%), and other minerals like calcium, phosphorus, and iron, which are also present in the fruit. Guava fruits have strong antioxidant and free radical scavenging properties (10). Many phytochemical compounds are found in the guava fruits, *viz.*, saponins, lyxopyranoside, oleanolic acid, arabopyranoside, quercetin, guaijavarin, phenolic compounds, and flavonoids. Guava is a delicate, nutritive, and remunerative fruit crop across the tropical and subtropical regions of the world (11). Guava fruits are easy to process into a variety of nutrient-rich products. Vitamin C is indispensable for growth, development, and tissue repair in the human body (12). Guava is a natural source of vitamin C content that is ∼2–5 times higher than that of citrus (13). Furthermore, fruit also possesses an array of medicinal properties (14). Hence there is a need to harness the potential of these wonder fruits by selecting suitable genotypes to cater to the fruit and nutrient requirements of the galloping population. Guava is a self-pollinated crop, but cross-pollination does also occur. The incidence of 35% outcrossing, on the other hand, can provide a heterozygous population with sufficient broader genetic variability to develop attractive commercial varieties (15). Genetic diversity describes the variety of unique inherited traits found in a species (16). Plant breeders have the opportunity to create new and improved cultivars with desirable characteristics that include both farmer-preferred traits (quantity and quality, etc.) and breeder-preferred traits (photosensitivity, pest and disease resistance, etc.). A qualitative technique has been used by (17) for evaluating the fruit diameter of the calyx cavity relative to fruit diameter, skin color, pulp color, and surface texture traits. Similarly, researchers worked on different qualitative traits (18, 19). Based on physical and biochemical characteristics, genetic diversity in the guava and allied *Psidium* species was screened and characterized (20–22). In guava breeding programmes, we must discover underlying genetic diversity across various germplasm/gene pools to find the best parents, which not only enhance yield and fruit quality but also resistance to biotic and abiotic stress environments (23–25).

The studied diverse guava germplasm was maintained at the institute farm, which encompasses cultivars, varieties, hybrids, collections, and related species belonging to the genus *Psidium* (see Figures 1A,B). With a wide range of genetic diversity that includes recombination and transgressive segregates, it is possible to obtain allelic variability from individuals in a population that will help to crop for wider adaptability in vagaries environmental conditions. Studied qualitative factors such as fruit size, shape, pulp texture, peel color, pulp color, fruit dots, pulp flavor, *etc*., are the most important for gaining the acceptance of consumers and farmers. Similarly, the quantitative trait which includes fruit weight, length, pulp thickness, pulp weight, ascorbic acid, total soluble solids, antioxidant capacity, *etc*., are crucial for export and processing firms as well as for farmer and consumer acceptance. Quantitative classification offers a divergence value among individuals and thus enables breeders to understand the racial affinities and evolutionary patterns in various species of cultivated plants. It aids in decision-making on the optimal parental combinations to use in a hybridization programme as well. It provides a solid foundation for combining any two or more genotypes depending on their degree of divergence or similarity.

Hence, the present study was objectively conducted to assess the genetic variability and genetic diversity in the selected guava germplasm for the development of elite genotypes. The outcome of the present investigation will help plant breeders to design and develop nutrient-rich high-yielding guava genotypes that can potentially minimize nutritional deficiency and abridge the guava demand and supply gap.

Materials and methods

Plant materials

The present investigation was carried out on 28 diverse germplasm of guava (pedigree details are mentioned in Table 1 and Figures 1A,B), and these germplasm were evaluated at the orchard of the Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi during the winter seasons of 2018–19, 2019–20, and 2020–21. The trees were 8 years of age, planted at a spacing of 6 × 3 meters apart, and maintained under ideal horticultural operations.

Location

The ICAR-IARI, New Delhi is located in the northern region of India (28.4^{0}N, 77.1^{0}E), with an average rainfall of 797.3 mm per year, 39 mean rainy days, and an average temperature of 34^0 C.

Trait evaluation

A total of 33 qualitative and quantitative traits were recorded in a set of 28 germplasm. The observations are as follows:

Assessing qualitative traits

In the investigation, a total of 17 fruit attributes (qualitative) such as fruit size (FS), shape (FSH), base shape (FBS), shape at the stalk end (FSS), cavity (FC), pulp texture (FPT), puffiness (FP), calyx persistence (FCP), a diameter of calyx concerning that of fruit (DC), fruit ridged collar around calyx cavity (FRC), surface (FSU), peel color (FPC), pulp color (FPU), absence/presence of fruit dots (FD), fruit surface covered in crimson or cherry red color (FSC), taste (FT), and pulp flavor (FPF) were recorded as per (26).

Assessing quantitative traits

A total of 16 morpho-biochemical parameters (quantitative) such as fruit weight (FW), fruit length (FL), fruit breadth (FB), fruit index *i.e.,* fruit length/breadth ratio (FI), pulp thickness (PT), seed core cavity (SC), pulp thickness: seed core cavity ratio (PS), pulp weight (PW), pulp percent (PP), pulp: seed weight ratio (PSW), vitamin C or Ascorbic acid (AA), total soluble solids (TSS), titratable acidity (TA), total soluble solids: titratable acidity ratio (TT), antioxidant capacity (AC), and total phenolic content (TP) were recorded in the guava germplasm. For each trait, samples were analyzed in five replicates.

Methodology for trait measurement

The fruit weight and the pulp weight were recorded using an electronic weighing balance (Aczet CY 223C, India) and expressed in grams. The fruit's length, breadth, pulp thickness, and seed core cavity were measured using Vernier calipers (Mitutoyo 500-754-10, Japan) and expressed in centimeters. A drop of guava juice was placed on the prism of the digital refractometer (MA871 Milwaukee, Romania) to determine TSS and expressed in Brix. Titratable acidity was determined by titrating the aliquot of a known quantity of sample against 0.1N NaOH solution to a pink endpoint using 1% phenolphthalein indicator (27). The recorded titratable acidity was presented as a percent of citric acid. By using a redox titration with potassium iodate and potassium iodide, ascorbic acid was determined (28) and presented in mg/100g of fruit.

The antioxidant capacity in guava fruits was determined by (29). A test tube containing ethanolic fruit extract (0.1 mL) was filled with 1.0 mL each of ammonium acetate buffer, 7.5 × 10^{-3} M neocuproine solution, 10^{-2} M $CuCl_2$ solution,

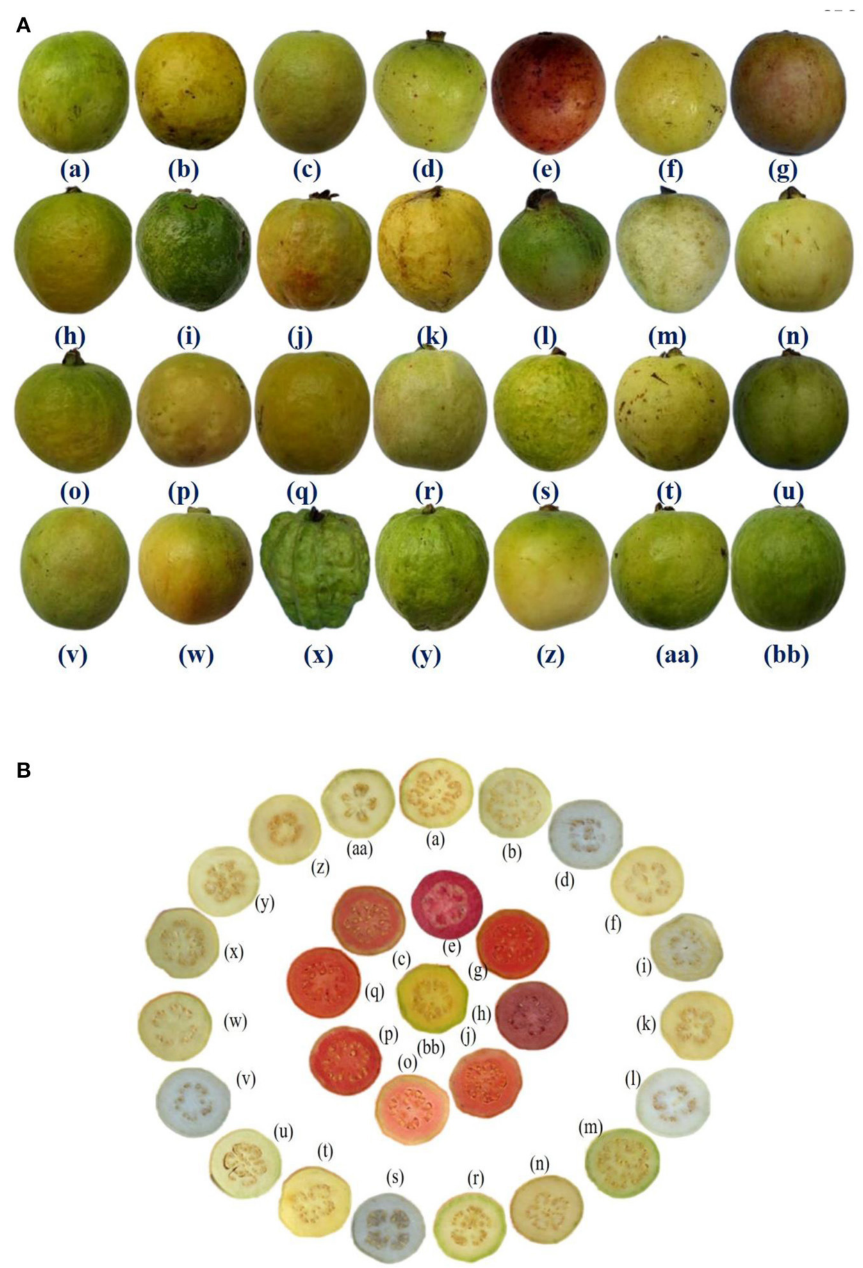

FIGURE 1
(A) Fruit morphology of 28 guava germplasm selected for study. **(B)** Transverse section of fruits of 28 guava germplasm selected for study. (a) Allahabad Safeda, (b) Allahabad Safeda variant, (c) Arka Kiran, (d) Arka Mridula, (e) Black guava (f) Hisar Safeda, (g) Hisar Surkha, (h) Hisar Surkha variant, (i) Kasipur collection, (j) Lalit, (k) Lucknow-49, (l) *P. friedrichsthalianum,* (m) *P. pumilum,* (n) Pant Prabhat, (o) Punjab Pink, (p) Red type I, (q) Red type II, (r) Sasni, (s) Sasri, (t) Shweta, (u) Snow White, (v) Soft Seeded variant, (w) Sour type, (x) Thai, (y) Thai variant, (z) Trichy, (aa) VNR & (bb) Yellow type.

and distilled water. After 30 mins, the absorbance of the sample was recorded at 450 nm with a double beam UV-VIS spectrophotometer (UV5704SS, India) against the reagent blank, and the findings were expressed as Trolox equivalent (μmol Trolox/g FW).

Total phenolic content was determined according to the Folin-Ciocalteu reagent (30) using a UVD-3200 spectrophotometer (Labomed, Inc., Culver city, USA). The 80% ethanol was used to smash 2 g of fruit, which was then centrifuged at 10,000 rpm for 20 mins at 4°C. The supernatant

TABLE 1 Details of 28 guava (*Psidium guajava* L.) germplasm.

Germplasm name	Origin	Species name	Characteristics
Allahabad Safeda	Selection	*P. guajava*	White pulp, round fruits
Allahabad Safeda variant	Selection	*P. guajava*	White pulp
Arka Kiran	Hybrid (Kamsari × Purple Local)	*P. guajava*	Pink pulp
Arka Mridula	Selection (Open pollinated seedlings of Allahaba Safeda)	*P. guajava*	White pulp
Black guava	Selection	*P. guajava*	Pink pulp, purple peel
Hisar Safeda	Hybrid (Allahabad Safeda × Seedless)	*P. guajava*	White pulp
Hisar Surkha	Hybrid (Apple color × Banarasi Surkha)	*P. guajava*	Pink pulp
Hisar Surkha variant	Hybrid (Apple color × Banarasi Surkha)	*P. guajava*	Pink pulp, pyriform fruit
Kasipur collection	Selection	*P. guajava*	White pulp
Lalit	Selection (Half sib population of Apple color)	*P. guajava*	Pink pulp, Transversely elliptic fruits
Lucknow-49	Selection (Open pollinated seedlings of Allahabad Safeda)	*P. guajava*	White pulp
P. friedrichsthalianum	Related species	*P. friedrichsthalianum*	White pulp, small fruits
P. pumilum	Related species	*P. pumilum*	White pulp, small fruits
Pant Prabhat	Selection	*P. guajava*	White pulp
Punjab Pink	Hybrid (Portugal × L-49) × Apple color	*P. guajava*	Pink pulp
Red type I	Selection	*P. guajava*	Pink pulp
Red type II	Selection	*P. guajava*	Pink pulp
Sasni	Selection	*P. guajava*	White pulp
Sasri	Selection	*P. guajava*	White pulp
Shweta	Selection (Half sib population of Apple color)	*P. guajava*	White pulp
Snow White	Selection	*P. guajava*	White pulp
Soft Seeded variant	Selection	*P. guajava*	White pulp, small fruits
Sour type	Selection	*P. guajava*	White pulp, small fruits
Thai	Selection	*P. guajava*	White pulp, large fruits
Thai variant	Selection	*P. guajava*	White pulp, large fruits
Trichy	Selection	*P. guajava*	White pulp, small fruits
VNR	Selection	*P. guajava*	White pulp
Yellow type	Selection	*P. guajava*	Yellow pulp

was added with 2.5 ml of 0.2 N Folin-Ciocalteu reagents (FCR) and kept for 5 mins, later 2 ml of a 20% sodium carbonate solution was added, and the volume was increased to 25 ml by adding 80% ethanol. The mixture was allowed to set for one and a half hours without disturbance. The absorbance of the mixture was measured at 750 nm, where the intensity of the blue color was relative to the concentration of total phenolic content. The standard calibration curve was developed by using gallic acid. The total phenolic content was expressed as gallic acid equivalent per 100 g extract (mg GAE/100 g extract).

Statistical analysis

Descriptive statistics including the minimum, maximum, mean, standard deviation, and coefficient of variation were analyzed using Web Agri Stat Package-2 (WASP-2) developed by ICAR Complex Goa, India. Qualitative data was exposed to non-parametric Spearman correlations, whereas quantitative data was submitted to parametric Pearson correlations. Principal component Analysis (PCA) and K-mean cluster plots were used to analyze data. Data sets may be divided into K clusters, which are represented by their centroids, using the K-means clustering technique (31).

A dendrogram was created by using both qualitative and quantitative traits. Based on Ward's approach and Euclidean distance, respectively, aggregative hierarchical clustering (AHC) and genetic dissimilarity component analysis were performed.

The R statistical software (version 4.2.0; The R Foundation) was used to analyze the reported data for correlation, PCA, K-mean cluster plots, and dendrogram of 33 attributes from the 28 guava germplasm.

Results

Genetic variability and genetic diversity are of prime importance for the selection of the desirable lines, which serve as the basis for designing the breeding programme in the guava crop for improved nutritional status.

Descriptive statistical analysis and correlations that describe the qualitative traits

Table 2 displays the descriptive statistics of the minimum, maximum, mean, standard deviations, and coefficient of variation (CV) for 17 qualitative traits, which include both morphological and fruit quality traits. The study showed that there was wider and more significant diversity and also had high CV values in the studied traits in the guava germplasm.

The variation of CV values for the traits ranged from 23.5 to 72.36 % in the studied qualitative traits. The higher CV values were observed for the FP (72.36%), FSH (58.12%), and FPC (53.91%). FSS (42.36%), FSU (35.71%), FBS (34.68%), FPT and FD (33.95%), FCP (33.09%), FRC (32.07%), FPC (31.81%), and FT (30.15%) all had medium CV values. Similarly, low CV values included FS (28.85%), FSC (28.33%), DC (27.56%), FPF (27.33%), and FC (23.5%). The variation of CV values showed a higher variation prevalence in the studied germplasm.

According to (26), all germplasm were grouped based on traits such as FS (small- 9, medium- 15, large- 4), FSH (subglobose- 14, ovate- 6, pyriform- 6, oblong- 6, transversely elliptic- 1), FBS (flattened- 15, broadly rounded- 13), FSS (broadly rounded- 6, rounded- 10, truncate- 10, pointed- 1, necked- 1), FC (shallow- 4, medium- 18, deep- 6), FPT (gritty- 14, firm-14), FP (absent- 12, present- 16), FCP (persistent- 23, dropping- 5), DC (small- 9, medium-16, large-3), FRC (inconspicuous- 12, conspicuous- 16), FSU (smooth- 17, warty- 11), FPC (Green-yellowish- 8, yellowish- 17, reddish- 2), FPU (white- 17, creamy white- 3, pinkish- 8), FD (absent- 14, present- 14), FSC (low- 24, intermediate- 3, high- 1), FT (low- 24, intermediate- 3, high- 1) and FPF (low- 6, intermediate- 15, high- 7).

Furthermore, large fruit size was observed in Thai, Thai variant, Allahabad Safeda, and Allahabad Safeda variant. Similarly, the reddish peel color was observed in Black guava and Lalit. Pinkish pulp color was observed in Arka Kiran, Black guava, Hisar Surkha, Hisar Surkha variant, Lalit, Punjab Pink, Red type I, and Red type II, whereas yellow pulp color was seen in the Yellow type germplasm. These descriptions will help to identify and easily trace the germplasm during the breeding programme.

Significant correlations (P 0.05) were found in the studied qualitative traits (see Figure 2). The FS and FC (0.488), FS and FP (0.536), FSH and FSS (0.58), FSU and FD (0.658), FSU and FSC (0.506), and FT and FPF (0.602) were shown to have significant positive correlations, whereas FCP and FRC (-0.538) showed a significant negative correlation.

Principal component analysis (PCA) and K-mean clustering for the qualitative traits

PCA was carried out using 17 qualitative traits; these traits were divided into eight components, which account for 84.2% of the total variation observed in the studied germplasm (Table 3).

The first component accounts for 18.2% of the total variation, which encompasses 11 qualitative traits such as FS, FSH, FSS, FC, FP, FRC, FSU, FPC, FSC, FT, and FPF. The second component explained 13.04% of the total variation for the traits (07), *viz.,* FSS, FCP, DC, FRC, FSU, FPC, and FD. The third component explains 11.9% of the total variation, which includes FS, FSH, FPT, FP, DC, FPU, FD, and FSC. The fourth component, the total variation, accounts for 9.9% of the traits like FSH, FPC, FT, and FPF. The fifth component covers 9.6% of the total variation for the traits (FBS, FP, and FSC). The sixth component, explaining 7.7% of the total variation, is comprised of FBS, FSS, FPT, DC and FSU. The seventh component accounts for 5.6% of the total variation for FBS and FT. The eight components explained 5.4% of the total variation for the traits (FCP, DC, and FRC).

The k-means clustering was carried out for 17 qualitative traits of germplasm (a set of 28 germplasm), and this germplasm was grouped into three clusters (see Figure 3). Cluster I encompasses Allahabad Safeda, Lucknow-49, Shweta, Lalit, Punjab Pink, Arka Kiran, Trichy, Black guava, Allahabad Safeda variant, Hisar Surkha variant, and Thai variant. Cluster II comprises Sasri, Pant Prabhat, Hisar Safeda, Sour type, *P. pumilum*, *P. friedrichsthalianum*, Thai, Red type I, Red type II, and Sasni. Cluster III consists of Arka Mridula, Snow White, Yellow type, Kasipur collection, Soft Seeded variant, and VNR. These results suggested that the K-means clustering approach was efficient to explain the genetic material was widely scattered throughout the plot based on the phenotypic composition and attributes.

Descriptive statistical analysis and correlation that describe the quantitative traits

In the study, a total of 16 quantitative traits were examined in a set of 28 germplasm lines of guava. The descriptive statistics revealed an enormous amount of variation present among the studied germplasm. The coefficient of variation (CV) ranged

TABLE 2 Descriptive statistics for 17 qualitative traits in 28 guava germplasm.

Trait	Maximum	Minimum	Mean	Std. deviation	CV
Fruit size	7.00	3.00	4.64	1.34	28.85
Fruit shape	5.00	1.00	1.89	1.10	58.12
Fruit base shape	2.00	1.00	1.46	0.51	34.68
Fruit shape at the stalk end	5.00	1.00	2.32	0.98	42.36
Fruit cavity	2.00	1.00	1.18	0.39	33.09
Fruit pulp texture	2.00	1.00	1.50	0.51	33.95
Fruit puffiness	9.00	1.00	5.57	4.03	72.36
Fruit calyx persistence	7.00	3.00	4.57	1.26	27.56
Diameter of calyx concerning that of fruit	2.00	1.00	1.57	0.50	32.07
Fruit ridged collar around calyx cavity	7.00	3.00	5.14	1.21	23.50
Fruit surface	2.00	1.00	1.39	0.50	35.71
Fruit peel color	3.00	1.00	1.79	0.57	31.81
Fruit pulp color	3.00	1.00	1.68	0.91	53.91
Fruit dots	2.00	1.00	1.50	0.51	33.95
Fruit surface covered by crimson or cherry red color	7.00	3.00	3.36	0.95	28.33
Fruit taste	7.00	3.00	5.21	1.57	30.15
Fruit pulp flavor	7.00	3.00	5.07	1.39	27.33

Trait	FS	FSH	FBS	FSS	FC	FPT	FP	FCP	DC	FRC	FSU	FPC	FPU	FD	FSC	FT	FPF
FS	1																
FSH	0.099	1															
FBS	0.128	0.278	1														
FSS	0.161	0.580*	0.276	1													
FC	0.488*	0.210	0.010	0.410	1												
FPT	0.059	-0.038	0.358	0.130	0.114	1											
FP	0.536*	0.077	0.083	0.113	0.336	0.433	1										
FCP	-0.147	0.087	0.127	0.304	0.095	0.093	0.027	1									
DC	-0.036	0.023	-0.045	-0.443	-0.151	-0.015	-0.177	-0.294	1								
FRC	0.298	0.154	0.083	-0.221	0.221	0.000	0.125	-0.538*	0.046	1							
FSU	0.131	0.210	0.131	0.157	0.138	0.219	0.106	0.389	-0.108	-0.042	1						
FPC	0.411	0.389	-0.168	0.066	0.282	-0.136	0.339	-0.328	0.271	0.307	0.011	1					
FPU	-0.072	0.080	0.107	0.149	0.351	0.153	-0.381	-0.160	0.193	0.201	-0.099	0.028	1				
FD	-0.010	0.005	0.072	0.224	0.114	0.143	-0.144	0.280	-0.265	-0.144	0.658*	-0.168	0.265	1			
FSC	0.140	0.138	-0.182	0.097	0.112	-0.015	0.125	0.323	-0.023	-0.044	0.506*	0.157	-0.070	0.407	1		
FT	0.335	0.135	-0.043	0.012	0.361	-0.240	0.029	-0.074	0.017	0.229	0.174	0.227	0.183	0.127	-0.022	1	
FPF	0.258	-0.117	-0.054	-0.163	0.248	-0.161	0.256	-0.159	0.020	0.153	0.070	0.202	0.111	0.161	-0.021	0.602*	1

-1 0 1

* Significance @ 0.05

FIGURE 2
Spearman correlation coefficient among 17 qualitative traits in 28 guava germplasm. FS, fruit size; FSH, fruit shape; FBS, fruit base shape; FSS, fruit shape at stalk end; FC, fruit cavity; FPT, fruit pulp texture; FP, fruit puffiness; FCP, fruit calyx persistence; DC, diameter of calyx concerning that of fruit; FRC, fruit ridged collar around calyx cavity; FSU, fruit surface; FPC, fruit peel color; FPU, fruit pulp color; FD, fruit dots; FSC, fruit surface covered by crimson or cherry red color; FT, fruit taste and FPF, pulp flavor.

from 1.39 to 58.62%. The traits such as PW (58.62%) and FW (58.17%) had high CV values, the trait PSW (33.05%) had a medium CV value, and the rest of the traits showed lower CV values (Table 4).

Similarly, the morpho-biochemical variability was observed among the studied traits in the germplasm lines *i.e.*, the maximum FW (359.32 g), FL (9.32 cm), and FB (8.82 cm) in the Thai variety; FI (1.29) and TP (186.93 mg GAE/100 g) in

TABLE 3 First 8 components from the PCA analysis of 17 qualitative traits in 28 guava germplasm.

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Fruit size	0.389	−0.126	0.254	0.013	−0.125	−0.004	0.068	−0.003
Fruit shape	0.242	0.067	−0.246	−0.496	−0.042	−0.230	0.290	−0.029
Fruit base shape	0.075	0.170	0.232	−0.221	0.318	0.262	0.615	−0.061
Fruit shape at the stalk end	0.215	0.313	0.123	−0.227	0.263	−0.426	−0.018	0.016
Fruit cavity	0.408	0.036	0.123	0.089	0.181	−0.193	−0.288	0.200
Fruit pulp texture	0.069	0.212	0.338	−0.192	0.137	0.513	−0.269	0.170
Fruit puffiness	0.277	−0.010	0.484	−0.003	−0.311	0.086	−0.108	0.124
Fruit calyx persistence	−0.030	0.466	0	0.114	−0.151	−0.138	0.154	0.318
Diameter of calyx concerning that of fruit	−0.015	−0.269	−0.277	−0.245	−0.048	0.398	0.139	0.518
Fruit ridged collar around calyx cavity	0.233	−0.310	0.022	−0.134	0.129	0.169	−0.058	−0.611
Fruit surface	0.236	0.362	−0.171	0.080	−0.238	0.320	0.117	−0.188
Fruit peel color	0.338	−0.249	−0.113	−0.250	−0.210	−0.092	−0.091	0.124
Fruit pulp color	0.128	−0.018	−0.283	−0.053	0.605	0.088	−0.297	0.191
Absence/presence of fruit dots	0.164	0.388	−0.265	0.219	0.101	0.215	−0.146	−0.228
Fruit surface covered by crimson or cherry red color	0.213	0.166	−0.382	−0.128	−0.357	0.079	−0.206	−0.040
Fruit taste	0.323	−0.132	−0.162	0.379	0.123	−0.063	0.337	0.094
Fruit pulp flavor	0.268	−0.175	−0.031	0.488	0.056	0.087	0.178	0.132
Variability (%)	18.205	15.860	11.892	9.912	9.556	7.681	5.556	5.366

Punjab Pink; PT (1.85 cm) in Allahabad Safeda variant; SC (5.4 cm) in Thai variant; PS (91.58) and TSS (15.02 ^{0}B) in Trichy; AA (197.27 mg/100 g fruit) in Lucknow-49; TA (0.69%) in *P. friedrichsthalianum*; TT (49.35) in Allahabad Safeda; and AC (44.49 µmol Trolox/g) in Shweta.

In contrast, the minimum FW (17.04 g), FB (2.43 cm), PW (15.37 g), PT (0.58 cm), SC (1.58 cm), TSS (9.49 ^{0}B) and TT (13.85) were recorded in *P. friedrichsthalianum*; FL (2.43 cm), FI (0.82), PP (88.34) and PSW (20.16) in *P. pumilum*; AA (110.44 mg/100g fruit) in the Sour type; AC (17.53 µmol Trolox/g) in Kasipur collection; and TP (105.63 mg GAE/ 100 g) in Yellow type.

Highly significant positive as well as negative correlations (p 0.05) were observed among studied quantitative traits in the germplasm (see Figure 4).

The following traits showed highly significant positive correlations: FW and PW (1), FB and SC (0.944), FL and FB (0.923), FW and FB (0.917), FB and PW (0.917), FW and FL (0.893), FL and PW (0.893), FW and SC (0.88), PW and SC (0.879), FB and PT (0.859), FL and SC (0.853), FL and PT (0.823), PP and PT (0.785), PP and PSW (0.775), PW and PT (0.767), FW and PT (0.766), TSS and TT (0.735), FL and PP (0.726), FB and PP (0.717), PT and TT (0.711), AA and TT (0.703).

Similarly, significantly positive correlations were reported among the following traits: FW and PP (0.591), FW and AA (0.49), FW and TT (0.538), FL and AA (0.543), FL and TT (0.653), FB and AA (0.593), FB and TT (0.641), PW and PP (0.595), PW and AA (0.488), PW and TT (0.538), PP and SC (0.567), PP and TT (0.578), PT and SC (0.642), PT and PSW (0.520), PT and AA (0.599), SC and AA (0.503), SC and TT (0.503), PSW and TT (0.49), AA and TSS (0.598), TSS and AC (0.612), and TT and AC (0.517).

Significant negative correlations were observed between the following traits: TA and FW (-0.693), TA and FL (-0.815), TA and FB (-0.766), TA and PW (-0.694), TA and PP (-0.7), TA and PT (-0.806), TA and SC (-0.628), TA and PSW (-0.565), TA and AA (-0.627), TA and TSS (-0.559), TA and TT (-0.934), and SC and PS (-0.497).

Principal component analysis and K-mean clustering for the quantitative traits

In the present investigation, the PCA was performed using 16 quantitative traits. The results revealed that 93.3% of the total variation was observed in the six components of PCA. This shows a large

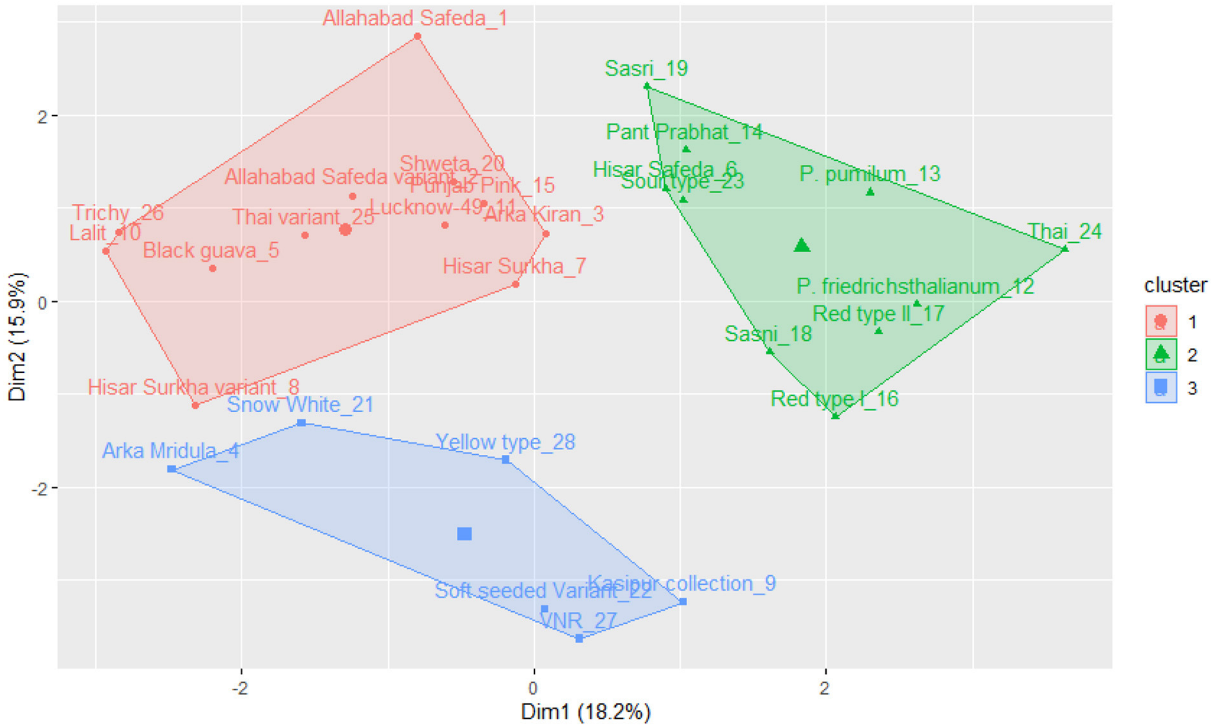

FIGURE 3
K-means cluster solutions plotted against the PCA scatter plot (component 1 on the X axis and component 2 on the Y axis, k-3) based on the 17 qualitative traits.

TABLE 4 Descriptive statistics for 16 quantitative traits in 28 guava germplasm.

Trait	Maximum	Minimum	Mean	Std. deviation	CV
Fruit weight (g)	359.32	17.04	136.39	79.34	58.17
Fruit length (cm)	9.32	2.43	6.08	1.45	23.92
Fruit breadth (cm)	8.82	2.74	6.06	1.41	23.24
Fruit index	1.29	0.82	1.03	0.10	10.19
Pulp weight (g)	336.27	15.37	127.17	74.55	58.62
Pulp percent (%)	93.98	88.34	92.86	1.29	1.39
Pulp thickness (cm)	1.85	0.58	1.27	0.30	23.93
Seed core cavity (cm)	5.40	1.58	3.53	0.94	26.66
Pulp thickness: seed core cavity ratio	1.50	0.50	0.75	0.20	27.05
Pulp: seed weight ratio	91.58	20.16	46.45	15.35	33.05
Ascorbic acid (mg/100 g fruit)	197.27	110.44	147.59	23.29	15.78
Titratable acidity (%)	0.69	0.30	0.44	0.11	25.29
Total soluble solids (^{0}B)	15.02	9.49	12.23	1.51	12.34
TSS: titratable acidity ratio	49.35	13.85	30.57	9.17	29.99
Antioxidant capacity (µmol Trolox/g)	44.49	17.53	27.74	7.71	27.79
Total phenolic content (mg GAE/100 g)	186.93	105.63	142.26	19.88	13.97

Trait	FW	FL	FB	FI	PW	PP	PT	SC	PS	PSW	AA	TSS	TA	TT	AC	TP
FW	1															
FL	0.893*	1														
FB	0.917*	0.923*	1													
FI	-0.010	0.289	-0.090	1												
PW	1.000*	0.893*	0.917*	-0.010	1											
PP	0.591*	0.726*	0.717*	0.192	0.595*	1										
PT	0.766*	0.823*	0.859*	0.038	0.767*	0.785*	1									
SC	0.880*	0.853*	0.944*	-0.159	0.879*	0.567*	0.642*	1								
PS	-0.179	-0.105	-0.209	0.309	-0.176	0.187	0.287	-0.497*	1							
W	0.374	0.436	0.441	0.062	0.379	0.775*	0.520*	0.325	0.153	1						
AA	0.490*	0.543*	0.593*	-0.036	0.488*	0.382	0.599*	0.503*	0.100	0.169	1					
TSS	0.119	0.269	0.329	-0.041	0.118	0.356	0.475	0.188	0.360	0.201	0.598*	1				
TA	-0.693*	-0.815*	-0.766*	-0.232	-0.694*	-0.700*	-0.806*	-0.628*	-0.148	-0.565*	-0.627*	-0.559*	1			
TT	0.538*	0.653*	0.641*	0.131	0.538*	0.578*	0.711*	0.503*	0.208	0.490*	0.703*	0.735*	-0.934*	1		
AC	-0.068	0.129	0.101	0.155	-0.068	0.227	0.283	-0.030	0.292	0.209	0.256	0.612*	-0.395	0.517*	1	
TP	0.024	0.223	0.101	0.296	0.024	0.012	0.061	0.112	-0.151	0.172	0.205	0.261	-0.307	0.377	0.412	1

-1 0 1

* Significance @ 0.05

FIGURE 4
Pearson correlation coefficient among 16 quantitative traits in 28 guava germplasm. FW, fruit weight; FL, fruit length; FB, fruit breadth; FI, fruit index; PT, pulp thickness; SC, seed core cavity; PS, pulp thickness: seed core cavity ratio; PW, pulp weight; PP, pulp percent; PSW, pulp: seed weight ratio; AA, ascorbic acid; TSS, total soluble solids; TA, titratable acidity; TT, TSS, TA ratio; AC, Antioxidant capacity and TP, total phenolic content.

amount of variation exists in the studied germplasm (Table 5).

The first component, explaining 50.6% of the total variation, was observed in traits such as FW, FL, FB, PW, PP, PT, SC, PSW, AA, TSS, TA, and TT. The second component accounts for 16.9% of the total variation and includes FW, PW, SC, PST, TSS, TT, AC, and TP. The third component, explaining 8.9% of the total variation, is the traits, *viz.,* FI, PP, PST, PSW, AA, TSS, and TP. The fourth component showed an 8.1% total variation (FI and TP). The fifth component covers 5.8% of the total variation, which includes FI and PSW. The sixth component showed 3% of the total variation, and the traits included AA and AC.

The k-means cluster was constructed using the same quantitative traits, and it was grouped into three clusters (see Figure 5). Cluster I includes Thai, Thai variant, Allahabad Safeda, and Allahabad Safeda variant, and this germplasm has shown higher FW, FL, FB, and PW. In contrast, Cluster II contains the germplasm of Yellow type, Red type I, Sour type, Soft seeded variant, Red type II, *P. friedrichsthalianum*, and *P. pumilum*, but these germplasm have lower FW, FL, FB, and PW. The germplasm such as Sasni, Kasipur collection, Hisar Safeda, Sasri, Pant Prabhat, VNR, Lucknow-49, Arka Mridula, Hisar Surkha, Lalit, Hisar Surkha variant, Arka Kiran, Black guava, Shweta, Punjab Pink, and Trichy fall under the cluster III.

Dendrogram using agglomerative hierarchical clustering (AHC)

Based on Euclidian distance and agglomeration using the Wards technique, the genetic dissimilarity of the 28 germplasm samples was performed (see Figure 6). Based on the 17 qualitative and 16 quantitative traits, the dendrogram was divided into four clusters. Cluster-I had thirteen germplasm (Hisar Surkha, Arka Kiran, Punjab Pink, Hisar Surkha variant, Trichy, Shweta, Allahabad Safeda variant, Allahabad Safeda, Lucknow-49, Black guava, Snow White, Arka Mridula, and Lalit).

Cluster II consists of eleven germplasm (Yellow type, Soft seeded variant, Pant Prabhat, Hisar Safeda, Sasri, Sour type, VNR, Kasipur collection, Sasni, Red type II, and Red type I). Cluster III included two germplasm, *i.e.,* Thai and Thai variant, which were the most divergent. On the other hand, related species of guava, *i.e.*, *P. pumilum* and *P. friedrichsthalianum* were present in cluster IV. Interestingly, all pink pulp color germplasm falls into clusters I and II.

Discussion

Guava is an important and emerging fruit crop because of its nutritional properties. Its area is expanding in India and it is

TABLE 5 First 6 components from the PCA analysis of 16 quantitative traits in 28 guava germplasm.

Trait	PC1	PC2	PC3	PC4	PC5	PC6
Fruit weight	0.304	−0.258	−0.045	0.016	−0.140	0.060
Fruit length	0.327	−0.108	−0.052	0.207	−0.161	0.124
Fruit breadth	0.331	−0.178	0.054	−0.055	0.013	0.130
Fruit index	0.032	0.215	−0.304	0.634	−0.425	0.045
Pulp weight	0.304	−0.257	−0.049	0.016	−0.135	0.058
Pulp percent	0.280	0.050	−0.355	0.003	0.278	0.040
Pulp thickness	0.319	0.048	−0.170	−0.164	−0.066	0.127
Seed core cavity	0.290	−0.296	0.191	0.022	0.060	0.114
Pulp thickness: seed core cavity ratio	0.006	0.419	−0.465	−0.238	−0.278	−0.082
Pulp weight: seed weight ratio	0.202	0.095	−0.331	0.088	0.659	−0.331
Ascorbic acid	0.240	0.115	0.270	−0.231	−0.314	−0.471
Titratable acidity	0.174	0.391	0.249	−0.308	−0.024	0.014
Total soluble solids	−0.322	−0.144	0.001	−0.071	0.027	0.081
TSS: titratable acidity ratio	0.292	0.254	0.145	−0.038	−0.010	−0.172
Antioxidant capacity	0.100	0.447	0.209	0.027	0.186	0.696
Total phenolic content	0.080	0.224	0.424	0.552	0.158	−0.263
Variability (%)	50.551	16.89	8.939	8.098	5.792	2.979

an excellent source of raw materials for the processing industry. Ascorbic acid, pectin, phenolic compounds, and antioxidants are abundant in guava, which provides health advantages. Researchers have paid a lot of attention to improving the nutritional quality of most fruit crops such as apples, mangos, bananas, and citrus, but guava has high nutritional values even though they have paid less attention to improvement. Hence, there is immense scope for researchers to improve not only the yield potential but also enhance the nutritional value of guava. To produce novel varieties with farmer and market-preferred product profiles, the genetic diversity found in the examined guava germplasm may be investigated for quantity and quality attributes with modern tools. Therefore, this study was initiated to provide a thorough genetic diversity analysis to reveal the performance and nutritional quality attributes among germplasm collections.

Morphological traits

In the study, a total of 33 qualitative as well as quantitative traits were studied in a set of 28 guava germplasm to determine the morphological traits that could be useful for genotype identification. It is necessary to characterize and assess germplasm before selecting the required genetic resources for genetic improvement projects. The majority of the traits under investigation had potential economic value, particularly those contributing to fruit yield and fruit quality. These traits can be further targeted for the improvement of guava varieties and hybrids.

The results from the present investigation support the view that morphology and chemical composition in fruits can be utilized efficiently for cultivar discrimination and estimating the genetic relationships in the diverse groups of guava germplasm. These results, which follow those of earlier research, show that both quantitative and qualitative traits are essential for cultivar identification and evaluation in guava germplasm for traits like fruit dots, puffiness, texture, surface, cavity, pulp taste, etc., which are of interest to taxonomists or breeders (17, 32). Similarly, quality traits are the most important factor in attracting consumers and farmers. In the current research, a significant variance was observed in traits such as fruit length/breadth ratio, fruit breadth, fruit length, fruit size, fruit diameter, fruit ridges on skin, fruit length of the stalk, fruit juiciness, total soluble solid, fruit relief of the surface, fruit ridges, the thickness of outer flesh, fruit skin color, fruit shape at the stalk, longitudinal ridges, flesh color, and fruit acidity (33, 34). Similarly, variation was observed for fruit weight, surface, shape, rind color, pulp color, pulp texture, pulp flavor, taste, and TSS (18, 35, 36).

From the perspective of farmers, consumers, export purposes, and processing industries, quantitative traits such as fruit weight, length, breadth, pulp weight, pulp thickness, pulp percent, pulp: seed core cavity ratio, pulp: seed weight ratio, TSS, antioxidants, and so on are some of the most important.

(22) observed higher vitamin C in red and white guavas than in strawberry guava. Red guavas are suited for processing due to their pulp color, higher beta-carotene, phytochemicals, and minerals. Similarly, (21) reported that the genotypes of cluster IV (Allahabad Safeda, Nagpur Seedless, Parkers Dessert, Kohir Safeda, Lalit, Lucknow-49, and Nagpur Seedless) and cluster V (Lucknow-49, VNR-Bihi selection line) are highly heterozygous, and these lines are valuable for breeding programmes.

Morphological correlations

The degree of correlation between the traits is a crucial consideration, particularly for complicated and economically important traits like yield (37). The ranking values for each variable are used as the basis for the Spearman correlation coefficient rather than the raw data. In the current research, highly positive correlations were observed among qualitative traits (fruit size with cavity and puffiness; fruit size with dots; and fruit taste with flavor). Similarly, some of the qualitative traits showed a significantly negative correlation, *i.e.*, calyx persistence and fruit ridged collar around the calyx cavity. Positive

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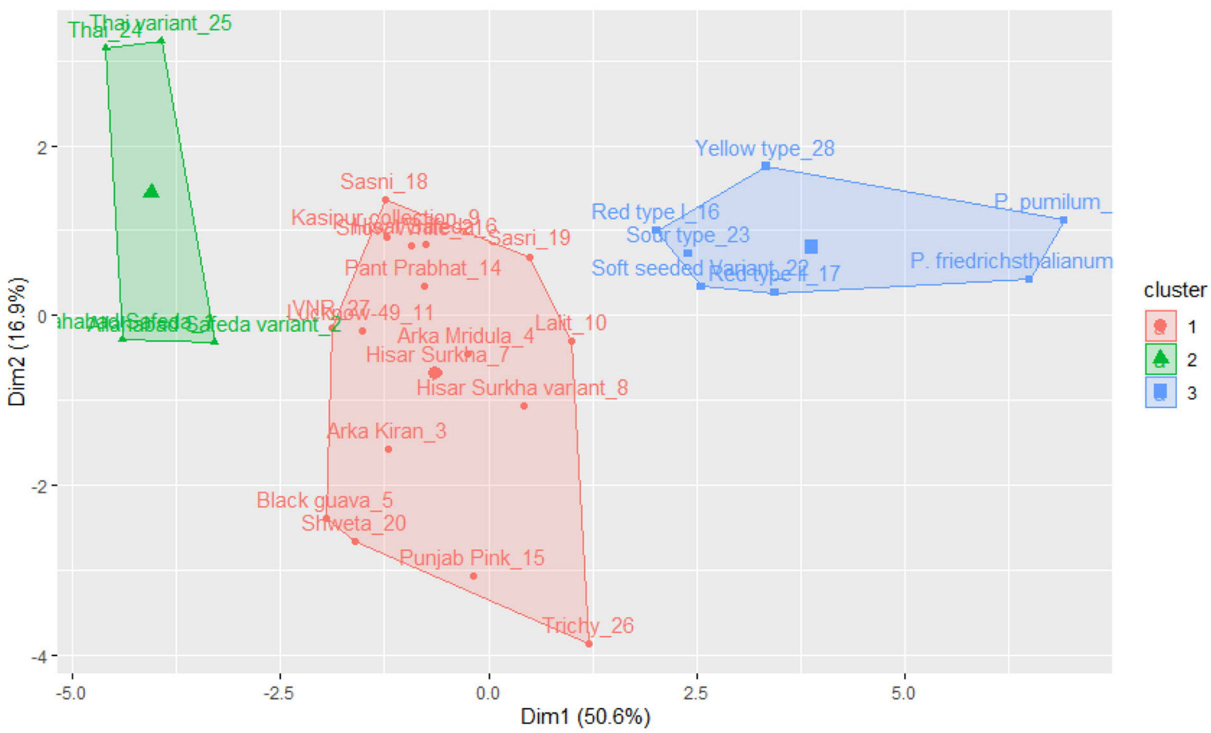

FIGURE 5
K-means cluster solutions plotted against the PCA scatter plot (component 1 on the X axis and component 2 on the Y axis, k-3) based on the 16 quantitative traits.

correlation ensures simultaneous improvement in two or more variables, and negative correlation brings out the need to obtain a compromise between the desirable characteristics (34).

The linear relationship between two continuous variables is assessed using the Pearson correlation (38). A strong and significant correlation was found between quantitative traits, particularly fruit weight, length, breadth, pulp weight, pulp thickness, and vitamin C. Based on the significant positive correlations between the fruit yield and quality traits such as fruit weight, fruit breadth, fruit length, cavity diameter, longitudinal ridge, and longitudinal ridge prominence, it can be understood that these characters positively influence the assessment of cultivar potential. The results are in agreement with previous works on guava fruit characters (34, 39, 40), and biochemical parameters (8, 41).

Principal component analysis (PCA)

PCA is used to uncover patterns and remove duplication in data sets since differences in crop species for yield and quality occur often (42). The main advantage of PCA is that it quantifies the importance of each dimension for correlating a dataset's variability. In the study, a wide range of total variations was observed, *i.e.*, 84.2 and 93.3% for qualitative and quantitative traits, respectively. The primary two components from the PCA demonstrate the highest loading for some traits, including fruit size, shape, cavity, puffiness, peel color, taste, pulp flavor, fruit weight, fruit length, fruit breadth, pulp weight, pulp percent, pulp thickness, seed core cavity, pulp: seed cavity weight, vitamin C, total soluble solids, etc., (43, 44). This outcome helps with the diversity assessment and also with the characterization of guava germplasm.

K-mean clustering for the qualitative and quantitative traits

K-mean clustering was used in this study to group 28 guava germplasm lines into three clusters for qualitative and quantitative traits.

One cluster of varieties exhibited similar traits and had less diversity variation. Cluster analysis makes it possible to identify groups of germplasm that share a variety of traits, which may be helpful for picking the best parent for a crossing. Crossing between varieties from the same group or closely related groups may result in less variation, whereas crossing between groups that are far apart will result in more variation. These lines could

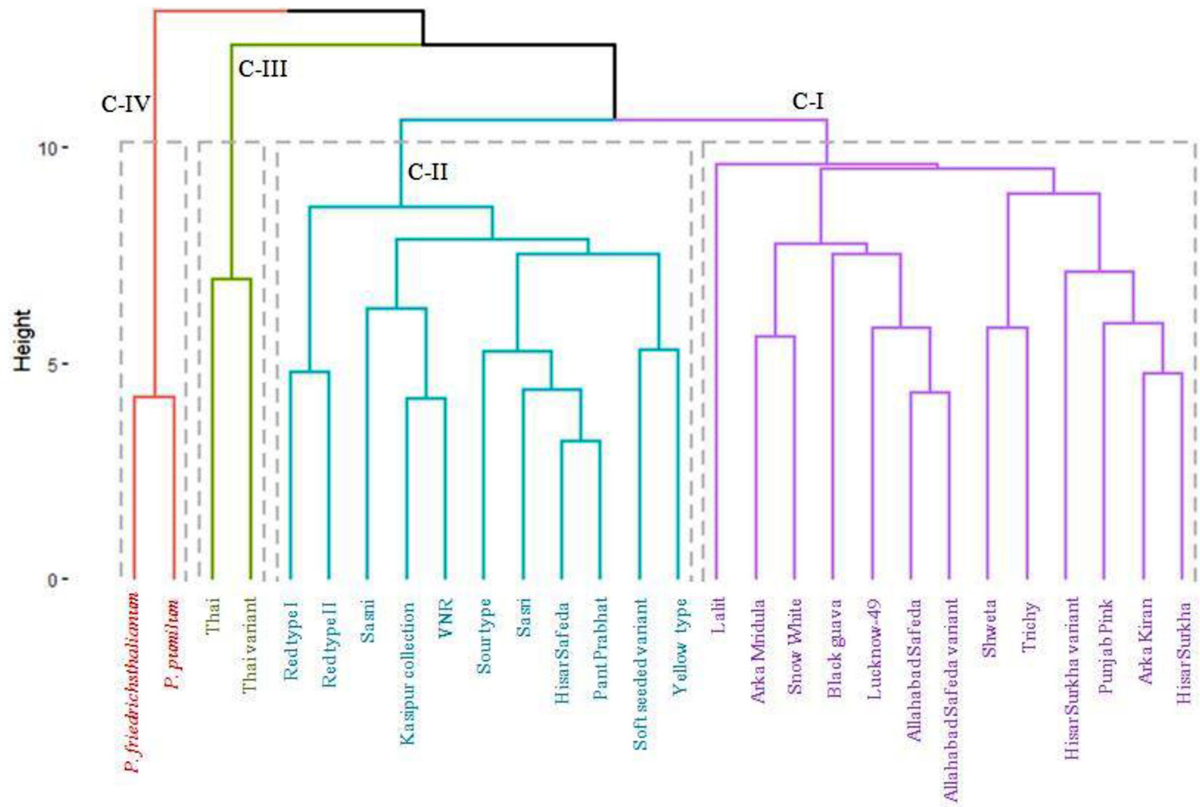

FIGURE 6
Dendrogram for 28 guava germplasm based on 17 qualitative and 16 quantitative traits.

be attempted in genetic improvement programmes, either for obtaining transgressive segregants or hybrids (45).

Dendrogram using agglomerative hierarchical clustering (AHC)

In the present investigation, four clusters were formed among 28 guava germplasm based on the dissimilarity concerning 17 qualitative and 16 quantitative traits. For further advancements, plant breeding is mostly dependent on the genetic variability of cultivated and wild relatives. The pink color of guava pulp is due to the naturally occurring organic pigment carotenoid. All of the pink-colored germplasm is present in clusters I and II, which may be explained by the qualitative and quantitative characteristic values falling within the acceptable range. Among the clusters, cluster III (Thai and Thai variant) and cluster IV (*P. pumilum* and *P. friedrichsthalianum*) were the most divergent for studied traits, hence the selection of such germplasm is important for effective utilize in crop improvement programmes in guava. Similar results were observed by (8), where three distinct clusters based on the dissimilarities of eight indigenous guava cultivars were seen.

Conclusions

Overall, the results revealed the presence of significant genetic variation among the 28 guava germplasm for both qualitative and quantitative traits, which provides an opportunity for the selection of superior genotypes for breeding programmes as parents in hybridization for nutritionally rich variety development. Among the studied germplasm, Thai, Thai variant, Trichy, Lucknow-49, Allahabad Safeda, and Shweta showed promising for some traits. These germplasm can be explored in a breeding programme to enhance the yield as well as nutritional quality.

Even though morphological traits are typically used to infer genetic links, such assessments are better with constraints. Unfortunately, the environment and a plant's stage of development have a big impact on these morphometric properties, which may lead to an unanticipated variety of agronomically useful characteristics. Future marker-assisted breeding and next generation sequence analysis could be used for guava crop improvement may benefit from a combinational approach to identifying and associating DNA-based molecular markers targeting promising fruit characteristic loci. Species that have great genetic diversity are especially capable of overcoming difficulties when new pests, illnesses, and climatic changes develop.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

NG, CA, MV, AN, and MT contributed to the organization of the work plan for the manuscript. NG, AN, and CS drafted the manuscript and composed the outline. VY, MV, KR, and MT contributed to the data analysis. AS, RS, and MS wrote the sections of the manuscript. All the authors had full access to the data and revised and approved the manuscript for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Comparative assessment of nutritional and functional properties of different sorghum genotypes for ensuring nutritional security in dryland agro-ecosystem

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The cultivation of unique sorghum (resistant to abiotic stresses and re-recognized as healthy food) has attracted interest as an environmentally friendly minor cereal and may be a solution to food and nutritional security. However, information about how the use of selected sorghum grains affects nutritive values and its functional properties from sorghum flours is still lacking. To address this question, we selected six sorghum varieties (i.e., JinZa 34, LiaoZa 19, JinNuo 3, JiZa 127, JiNiang 2, and JiaXian) for the comprehensive analysis of the relationship among nutritional compositions, energy value contributions, and functional properties of sorghum grains. Results showed that Carr's index (CI) and angle of repose (AR) of all sorghum flours indicated good flow and compressibility properties in terms of micrometric parameters. All sorghums were considered free of tannin. Based on the scatterplot analysis, the proportions of energy contributions due to protein, fat, and carbohydrate (CHO), were highly positively correlated with protein, fat, and CHO, respectively. The significantly different flours of six sorghum varieties resulted in different functional properties. The amylose content showed a highly negative association with light transmittance and water and oil absorption capacities. In addition, amylose had a highly positive relationship with water solubility (WS) and swelling power (SP). JinNuo 3 had the highest nutritional compositions [proximate, mineral, anti-nutritional values, and amino acid (AA) profiles] and functional properties indicating that it could be used as a brewing liquor. Our findings will provide a new opportunity to cultivate sorghum as an environment friendly minor cereal crop in dryland agro-ecosystems of arid and semi-arid regions of northern China for nutritional security, agriculture processing, and non-food industry in the future.

KEYWORDS

sorghum varieties, nutritive values, quality traits, micrometric properties, functional properties

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], an environmentally friendly crop, is resistance to water and fertilizer efficient than other major cereal crops, such as maize, wheat, and rice (1). Sorghum is a multipurpose minor cereal crop, primarily used as food, feed, and forage, and as important raw materials for brewing liquor (2) and value-added food products (3). According to the public health point of view, sorghum provides many health benefits due to its antioxidant, anti-inflammatory, anti-proliferative, anti-diabetic, and anti-atherogenic properties (4). Its phenolic compounds can prevent many diseases including cancer, diabetes, digestive tract disease, and cardiovascular disease (5, 6).

Sorghum is widely grown in dryland agricultural systems in arid and semiarid specific zones of northern and northeastern parts of China. In those areas, the excessive consumption of water and over-application of inorganic fertilizers have led to serious action on environmental problems for sustainable agriculture (7). These characteristics limit the productivity and make dryland agro-ecosystems both inherently dynamic and vulnerable. It is clear that small-scale farming will continue to play an important role in providing livelihood security for people in those areas. To reduce the poverty and malnutrition it is therefore necessary to improve the productivity of current farming systems, and at the same time safeguard the generation of other ecosystem services, on which local people also depend (7, 8). Therefore, sorghum crop, which is an environmentally friendly minor cereal crop and recognized as healthy food, may be a solution. Nowadays, sorghum research is crucial for environmentally friendly crop and most promising candidate crop as a sorghum-based intercropping for agricultural sustainability (8, 9). Intercropping and sorghum ratooning are popular cultivation techniques among small-scale farmers around the globe (3, 10, 11). Ratooning is a system that grows shortly after cutting the main crop, and the second crop is harvested in the same cropping season. This method is a feasible harvesting practice (11, 12). Sorghum intercropping with some legumes has many economic returns (13–15), and the sorghum ratooning practice also has several advantages, such as no need for new seed and land preparation, covering of time of sowing, and high grain yield and quality (12). Zhou et al. (16) reported that sorghum ratoon crop has high contents of starch, protein, and tannin and low fat content after application of nitrogen fertilizer. This chemical composition indicates good quality for liquor production.

The cultivation of sorghum has decreased many years ago, but has attracted interest recently because of its highly nutritive grains, bioactive compounds, and starch versality (4, 17, 18). Many cereal scientists and food processors extensively studied the characteristics and physicochemical properties of sorghum starches (amylose and amylopectin), which can be adapted for agricultural processing and non-food industry (19–21). The literature reported that the contents of amylose in sorghum flour and starch affect the functional and physicochemical properties (22, 23). Yang et al. (24) reported that flours and starches of non-waxy proso and foxtail millets had higher amylose contents than those of waxy proso and foxtail millets. Potato flours and starches, which have higher amylose content that affects pasting properties than sweet potato flours and starches, have wider applications as functional flours with high nutritive values (25). Some studies also reported that in Tartary buckwheat, the four has the higher amylose content and other nutritional compositions than the starch, thus indicating its suitability for application in the food processing industry (26). However, limited information is available on nutritional compositions, quality traits, physical and functional properties, and anti-nutritional traits in industrial usage of sorghum flours.

The consumption and production of sorghum crops first need vigorous assessment of the ability and feasibility of particular cultivar to provide suitable yield, quality and nutritional value. Moreover, investigation of perceived functional properties is essential to assess the consequences of industrial processing on the crops like mechanical or thermal treatment. This could be due to insolubilization of denatured protein isolates by industrial treatment which may have either beneficial or deleterious impact on the nutritive values of the crops (17, 20, 21). Hence in the present study, sorghum seed flours, obtained from six Chinese sorghum cultivars i.e., JinZa 34, LiaoZa 19, JinNuo 3, JiZa 127, JiNiang 2, and JiaXian have been assayed with a view to evaluating their nutritional and functional properties. After purifying and milling the sorghum seeds, the flours obtained were evaluated for micrometric properties, proximate composition, energy values and contribution, mineral and amino acid (AA) composition, and anti-nutritional traits. Among different functional properties, SP, WS, WAC, OAC, light transmittance, and LGC of the sorghum flours have been assayed. In short, the present study was designed in with the hope of establishing the significance of sorghum as a major cereal grain crop in the human diet and finding the most beneficial cultivar in terms of nutritional value and industrial applicability. Moreover, our findings will provide a new opportunity to cultivate sorghum in dryland agro-ecosystems in arid and semi-arid regions of northern China and the application of sorghum flours in meeting the demand of consumer preference in the food industry.

Materials and methods

Materials

Six sorghum varieties JinZa 34 (JZ 34), LiaoZa 19 (LZ 19), JinNuo 3 (JN 3), JiZa 127 (JZ 127), JiNiang 2 (JN 2), and JiaXian (JX) were used in this study. These varieties were grown under similar planting conditions in an experimental field in Yulin, Shaanxi Province, China in 2021. Purified mature seeds (200 g) were rinsed and turned into flour via a high-speed grinder (FW-100D, XinBaoDe Instruments Ltd., Tianjin, China). Starches were extracted using alkaline steeping methods according to standard procedures of Zhang et al. (27) and Gao et al. (28).

Physical characteristics of sorghum varieties

Purified seeds were used for the determination of physical characteristics. Random grains were selected from a well-mixed sample and weighed by a digital weighing balance/SC-G Automatic Seed Test Instrument (Wan Shen Testing Company, Hangzhou, China) in three replications to measure the thousand-grain weight, and the mean value was recorded. Sorghum grain color and shape were recorded by visual classification. The grain size distribution, average diameter, and lognormal fitting function of sample grains were measured using the Fiji ImageJ software (ImageJ, US, NIH, Bethesda, MD, USA) and Origin 2018 (OriginLab Corporation, Northampton, MA 01060, USA). Bulk density (BD) was determined using a 10 ml graduated cylinder. A known weight of the sample was poured into the cylinder, and the volume was recorded. BD was calculated using the equation: BD (g/ml) = [mass of the sample (g)/volume of the sample (ml)] (29). The tapped density (TPD) of a sample was calculated using the equation: TPD (g/ml) = [mass of the sample (g)/volume of the sample (ml)] (29). The true density (TD) of the sample was calculated using the formula: TD (g/ml) = mass of the sample (g)/volume of sample displaced by the sand (ml) (29). The percentage of porosity (P) in sample was calculated using the formula: P (%) = (TD–BD)/TD $\times$ 100 (30).

Determination of micrometric properties

The Carr's index (CI) of the sample was measured as reported by Menaka et al. (29). CI was calculated using the equation: CI = [(TPD–BD)/TPD] $\times$ 100. The Hausner's ratio (HR) was calculated using the formula: HR = (TPD/BD) $\times$ 100 (29). To measure the angle of repose (AR), a mounted funnel was placed on a laboratory stand at a height of 10 cm from the bench to measure the AR. About 50 g sample was poured into the mounted funnel with the closed tip. The tip-plug was opened, and the sample flour was allowed to pass through the orifice. The height and diameter of the sample heap were measured. AR was calculated using the equation: AR (θ) = $\tan^{-1}$ (h/r), where h represents the height of conical powder heap, and r is the radius of the circular base (29).

Proximate composition and amylose content analyses of sorghum flours

The moisture, crude fiber, and ash contents were analyzed by standard methods of AOAC (31). Fat (F), protein (PT), and starch contents were determined by Soxhlet extractor, Kjeldahl method, and anthrone spectrophotometry method, respectively (23, 24). The content of carbohydrate (CHO) was calculated by difference: CHO = 100 – (%PT + %F + %moisture + %crude fiber + %ash) (32). The percentage of starch yield (SY) was calculated on dry matter basis of 100 g sample grains. SY was calculated using the following formula: SY (%) = (weight of starch/weight of whole grain) $\times$ 100 (30). The starch recovery percent (SR%) of sorghum flour was calculated using the equation: SR (%) = (weight of starch/weight of grain starch) $\times$ 100 (32). The amylose (AM) content was analyzed using the method of Yang et al. (33) with some modifications.

Determination of energy values and percentage of energy contribution

Energy values were determined by multiplying the results of PT, CHO, and F by 17, 17, and 37, respectively (20, 34). Each analysis of the sample was carried out in duplicate. The proportions of energy contributions from F, PT, and CHO to total energy (TE KJ/100 g) were calculated for each proximate composition type. In samples, the utilization of energy due to PT

TABLE 1 Physical properties of sorghum grains.

Varieties	Grain color	Grain shape	1,000 Grain weight (g)	Bulk density (g/ml)	Tapped density (g/ml)	True density (g/ml)	Porosity (%)
JZ 34	White	Round	22.41 ± 0.71^{d}	0.72 ± 0.02^{d}	0.88 ± 0.00^{b}	1.30 ± 0.01^{c}	44.61 ± 1.22^{a}
LZ 19	White	Round	31.85 ± 2.25^{b}	0.79 ± 0.07^{a}	0.96 ± 0.21^{a}	1.42 ± 0.11^{a}	44.37 ± 0.07^{c}
JN 3	White	Round	16.23 ± 0.28^{e}	0.75 ± 0.31^{b}	0.91 ± 0.12^{b}	1.35 ± 0.03^{b}	44.44 ± 1.00^{b}
JZ 127	White	Round	35.80 ± 0.61^{a}	0.74 ± 0.01^{c}	0.90 ± 0.10^{b}	1.33 ± 0.00^{b}	44.36 ± 0.07^{c}
JN 2	White	Round	21.56 ± 0.09^{d}	0.76 ± 0.02^{b}	0.92 ± 0.03^{b}	1.36 ± 0.04^{b}	44.12 ± 0.00^{d}
JX	White	Round	30.14 ± 0.16^{c}	0.75 ± 0.12^{b}	0.91 ± 0.10^{b}	1.35 ± 0.03^{b}	44.44 ± 1.00^{b}

Data are represented as the mean ± SD of triplicate determinations. For each column, values not displaying the same letter are significantly different ($p < 0.05$). JZ 34, jinza 34; LZ 19, liaoza 19; JN 3, jinnuo 3; JZ 127, jiza 127; JN 2, jiniang 2; JX, jiaxian.

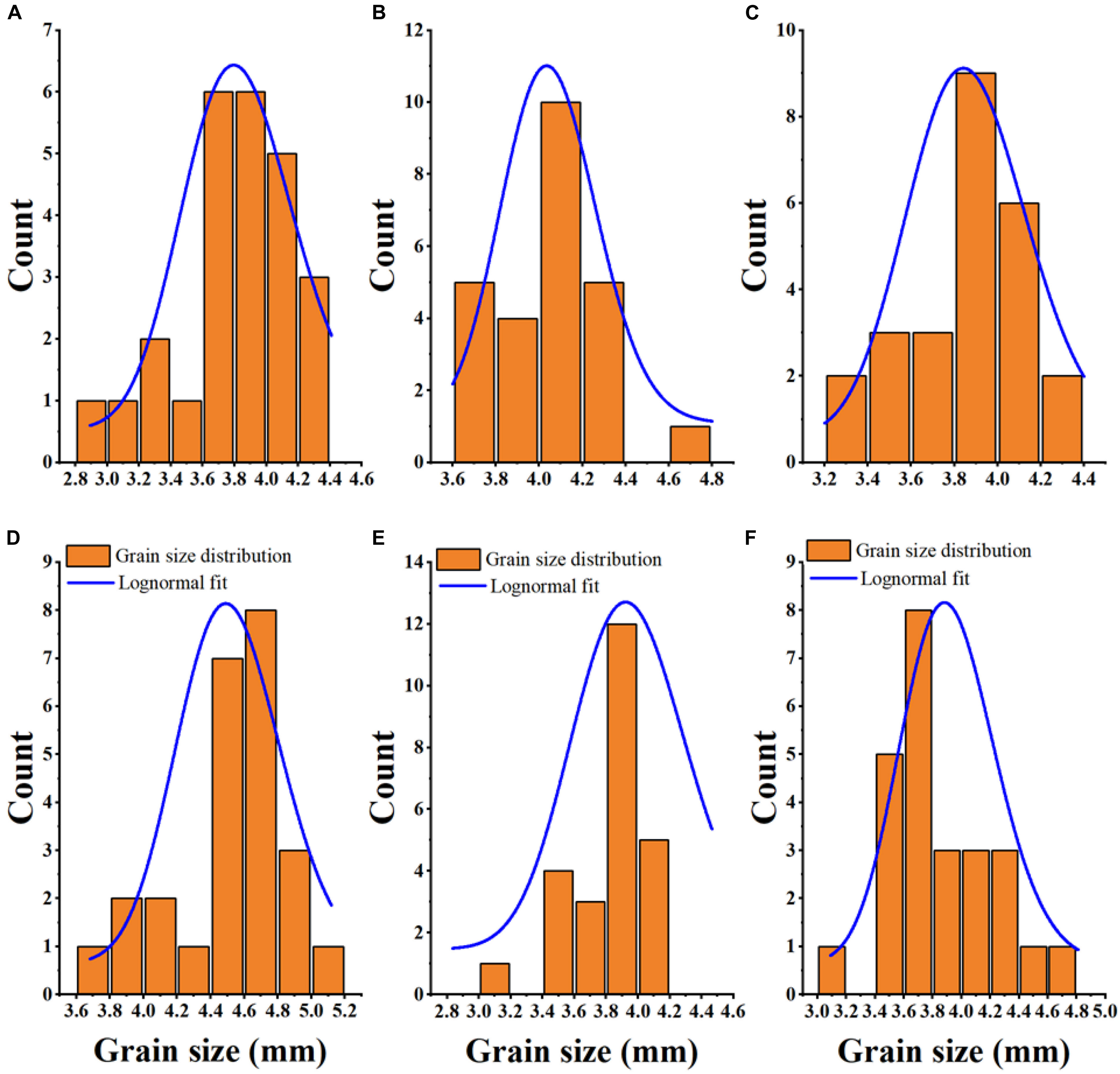

FIGURE 1
Grain size distribution and Lognormal fitting of six sorghum grains. JZ 34, jinza 34 **(A)**; LZ 19, liaoza 19 **(B)**; JN 3, jinnuo 3 **(C)**; JZ 127, jiza 127 **(D)**; JN 2, jiniang 2 **(E)**; JX, jiaxian **(F)**.

(UEDP%) was also calculated in accordance with the formula of Niyi et al. (35).

Determination of mineral and amino acid compositions

The analysis of minerals was carried out from the sample solution obtained by dry-ashing the samples at 550°C to constant weight. Sodium and potassium contents were measured using a flame photometer and the phosphorus content was determined by Vanadomolybdate method (20, 36). Other metals (calcium, magnesium, iron, manganese, and zinc) were measured using an atomic absorption spectrophotometer according to the method described by Adeyeye et al. (20). All determinations were measured in triplicate. The AA compositions of flour samples were determined by the pre-column derivatized AccQ.Tag Ultra method and using the reverse-phase HPLC system (37).

Determination of anti-nutritional factors

The condensed tannin contents of flour samples were determined following the modified vanillin/HCl assay described by Khoddami et al. (38). The absorbance was recorded at 500 nm, and the catechin standard solution was used as template for the condensed tannin assay. Total flavonoids were determined using the assay modified by Khoddami et al. (38) and Afify et al. (39). The reaction mixture was kept for 6 min and then mixed with 2 ml of 1 M NaOH, and the total volume was made up to 10 ml with distilled water. The absorbance and expressed results were 510 nm and μg catechin equivalent/g dry sample (μg/g), respectively (39). Total phenol contents were determined using the Folin-Ciocalteau assay (40, 41) with some modifications. Absorbance was measured at 760 nm. Total phenols were calculated on the basis of standard curves and expressed as mg gallic acid equivalents (GAE)/100 g grain (dry weight, DW).

Functional properties of sorghum flours

Swelling power (SP) and water solubility (WS) were determined at 90°C by using the methods described by Sindhu and Khatkar (26) and Uarrota et al. (42), respectively. The method of Sindhu and Khatkar (26) was used for the determination of water absorption capacity (WAC) and oil absorption capacity (OAC) of different sorghum flours. Exactly 1.5 g sample suspension was added with 10 ml distilled water, and the mixture was agitated with four times for 10 min. After resting periods of 10 min, sediment samples were centrifuged at 3,250 rpm for 25 min. The supernatant was decanted, and tubes were air dried and then weighed to determine WAC. Approximately 3 ml refined groundnut oil was poured into a known weight of 0.5 g sample, and the mixture was stirred for 1 min and kept for 30 min at room temperature. After 30 min, sample tubes were centrifuged at 3,200 rpm for 25 min. The volume of unabsorbed oil was determined for OAC. Light transmittance (%LT) of flour paste was determined by following the methods of Ghada et al. (19) and Yang et al. (23) with some modifications. The percentage of LT was measured using spectrophotometry (Blue Star B, Lab Tech Ltd., Beijing, China). The methods reported by Sindhu and Khatkar (26) and Thilagavathi et al. (43) were followed with slight modifications for the determination of the least gelation concentration (LGC). Solutions 5 ml of concentration of flour [8–30% (w/v)] in test tubes were placed in a water bath maintained at 90°C and kept overnight at 4°C for cooling. Gelation was recorded by inverting the test tubes for determination of LGC.

Data analysis

Data were analyzed using the IBM SPSS version 20.0 software (SPSS Inc. Chicago, IL, USA). All data were presented as mean ± standard deviation. Each test was carried out in triplicate. Data were subjected to one-way ANOVA and the Tukey's multiple range test was performed to compare treatment means. A p-value of 0.05 was considered significant. The Pearson correlation was determined using one-way ANOVA on the SPSS version 22.00 and correlation heatmap created on Microsoft Excel 2016. Column bar and Scatterplot matrix graphs were produced using the Origin 2018 software.

Results and discussion

Physical characteristics and micrometric properties of sorghum grains

The physical characteristics of sorghum grains were assessed. Results are presented in **Table 1**. The physical properties of food are important as they are used in product design and development, process optimization, food quality control, and food process modeling (29). Deepa (30) reported that physical properties have unique characteristics a food material responds to physical treatment, including thermal, mechanical, electrical, optical, electromagnetic, and sonic process. The color and shape of all sorghum grains were white and round, respectively. The thousand grain weight had significantly differ ($p < 0.05$). The thousand-grain weights

TABLE 2 Micrometric properties of sorghum grains.

Varieties	Carr's index	Hausner's ratio	Angle of repose (θ)
JZ 34	18.18 ± 2.10[a]	1.22 ± 0.04[a]	40.25 ± 0.22[a]
LZ 19	17.71 ± 1.20[c]	1.21 ± 0.00[a]	39.92 ± 0.20[b]
JN 3	17.58 ± 1.11[d]	1.21 ± 0.00[a]	39.92 ± 0.21[b]
JZ 127	17.78 ± 1.22[b]	1.22 ± 0.04[a]	40.25 ± 0.22[a]
JN 2	17.39 ± 1.00[e]	1.21 ± 0.01[a]	39.92 ± 0.20[b]
JX	17.58 ± 1.11[d]	1.21 ± 0.03[a]	39.92 ± 0.20[b]

Data are represented as the mean ± SD of triplicate determinations. For each column, values not displaying the same letter are significantly different ($p < 0.05$). JZ 34, jinza 34; LZ 19, liaoza 19; JN 3, jinnuo 3; JZ 127, jiza 127; JN 2, jiniang 2; JX, jiaxian.

of JZ 34, LZ 19, JN 3, JZ 127, JN 2, and JX significantly differed ($p < 0.05$) and were 22.41, 31.85, 16.23, 35.80, 21.56, and 30.14 g, respectively. JZ 127 had the highest thousand grain weight. The highest values of BD, TPD, and TD were recorded in the LZ 19. In the present study, the TD of LZ 19 was significantly higher ($p < 0.05$) than BD and TPD, which might be due to the filling of void space and pores with sand while determining TD in the sand displacement method (30). Porosity, percentage of air between the particles that compared to a unit volume of particles (44). JZ 34 showed the highest P (44.61%). Deepa (30) stated that the difference in P among various samples is attributed to differences in their density values. Deepa (30) and Devi and Sharma (44) reported that high P resulted in high contact with atmospheric oxygen and high rate of auto-oxidation.

The Fiji ImageJ program is useful as magnetophoretic measurements in observing the physics of particle structure, scientific images (digital and scanning electron microscopy), and scattering-intensity data (9, 45, 46). Figure 1 shows the granule size distribution histogram and lognormal fitting of grain size. According to the Fiji ImageJ and Origin 2018 software results, the average grain sizes of JZ 34, LZ 19, JN 3, JZ 127, JN 2, and JX, were 3.80, 4.05, 3.87, 4.54, 3.83, and 3.87, respectively. The size of sorghum grains ranged from 2.8 to 5.2 mm. The average granule size of JZ 127 (4.54 mm) was larger than those of other varieties.The term micrometric represent to a study of well-derived properties of fine particle in science and technology, it is not only used in the development of pharmaceutical and material science (47), but in widely used in food science and technology for functional, pasting, and formulation (48). The micrometric properties including CI, HR, and AR, of sorghum flours were assessed. Results are shown in Table 2. The above three parameters determined the flow characteristics and compressibility of a powder. In the current study, the CI and AR of all sorghum flours indicated good flow and compressibility properties. Deepa (30) reported that CI (23%) and AR (50°) had good flow and compressibility properties. Our results were consistent into their findings. Among the six sorghum flours, JZ 34 had higher CI and AR values ($p < 0.05$). HR did not significantly differ ($p > 0.05$) in our findings. The good flow properties of granules and powders are important in products designed in compressed form like tablets, and capsules (47), and in the creation of new products, like films and coatings (48).

Proximate compositions and energy values of sorghum varieties

The proximate composition was significantly influenced by six sorghum varieties (Table 3). The highest values of crude fiber (2.49%), ash (1.91%), PT (9.34%), and F (5.14%) were recorded in JN 3. In this study, LZ 19 had the lowest crude fiber (1.58%) and F (2.98%) contents, whereas JX showed the lowest PT content (6.24%). Table 3 shows that JX contained considerably CHO content (78.46%), starch content (77.42%), SY (69.43%), and starch recovery (89.68%) than other sorghum varieties. In summary, our results showed that among sorghum varieties, JN 3 and JX had nutritional potential in terms of proximate composition.

Table 4 depicts the percentage of energy values contributed by PT (PEP%), F (PEF%), CHO (PEC%), and utilizable energy due to PT (UEDP%). PEP, PEF, PEC, and UEDP in sorghum flours were 9.07–10.13%, 7.13–12.14%, 77.73–84.57%, and 4.04–6.08%, respectively. The JN 3 with the highest concentrations of F and PT also had the highest proportions of energy contributions due to F (PEF, 12.14%), PT (PEP, 10.13%), and UEDP (6.08%). The energy contribution by CHO was highest in the JX sorghum sample (84.57%). The total energy (TE) values of JZ 34, LZ 19, JN 3, JZ 127, JN 2, and JX were 1545.57, 1546.25, 1577.80, 1566.67, 1569.94, and 1572.17 KJ, respectively. These results indicated that JN 3 and JX had the highest TE values than others. Adeyeye et al. (20) reported that proximate compositions, and energy values and contributions in raw sorghum were higher than those in steeped and germinated sorghum samples.

Scatterplot matrix analysis of some proximate parameters and energy values

The scatterplot matrices of PT, F, CHO, and energy values and its contributions (TE, PEP, PEF, PEC, and UEDP) are shown in Figure 2. PEP, PEF, and PEC were highly positively correlated with PT (r = 0.998), F (r = 0.999), and CHO ($r = 0.952$, $p < 0.05$), respectively. Based on scatterplot analysis, TE had a positive correlation with PEF ($r = 0.428$) and negative correlation with PEP (r = -0.456) and PEC (r = -0.073), respectively. Furthermore, UEDP had a strongly significant positive correlation with PEP ($r = 1.000$, $p < 0.05$).

TABLE 3 Proximate compositions of sorghum grains.

Proximate compositions (%)	JZ 34	LZ 19	JN 3	JZ 127	JN 2	JX
Moisture	9.53 ± 0.18[b]	9.66 ± 0.14[a]	9.49 ± 0.24[b]	8.30 ± 0.19[d]	8.89 ± 0.21[c]	8.04 ± 0.24[e]
Crude fiber	2.13 ± 0.05[b]	1.58 ± 0.05[d]	2.49 ± 0.05[a]	2.20 ± 0.19[b]	2.04 ± 0.11[b]	1.80 ± 0.09[c]
Ash	1.53 ± 0.07[c]	1.31 ± 0.02[e]	1.91 ± 0.12[a]	1.30 ± 0.03[e]	1.45 ± 0.06[d]	1.75 ± 0.08[b]
Protein	8.31 ± 0.03[c]	8.91 ± 0.11[b]	9.34 ± 0.25[a]	8.84 ± 0.30[b]	8.38 ± 0.20[c]	6.24 ± 0.03[d]
Fat	3.49 ± 0.32[b]	2.98 ± 0.19[c]	5.14 ± 0.26[a]	3.67 ± 0.40[b]	4.02 ± 0.46[b]	3.71 ± 0.26[b]
Carbohydrate	75.01 ± 0.10[c]	75.56 ± 0.11[b]	71.63 ± 0.09[d]	75.69 ± 0.14[b]	75.22 ± 0.21[b]	78.46 ± 0.35[a]
Starch	73.82 ± 0.29[b]	75.24 ± 0.42[b]	70.26 ± 0.23[c]	74.98 ± 0.37[b]	74.54 ± 0.19[b]	77.42 ± 0.50[a]
Starch yield	65.83 ± 0.13[c]	67.25 ± 0.21[b]	62.27 ± 0.05[d]	66.99 ± 0.41[b]	66.55 ± 0.11[c]	69.43 ± 0.71[a]
Starch recovery	89.18 ± 0.04[c]	89.38 ± 1.30[b]	88.63 ± 0.02[c]	89.34 ± 1.10[b]	89.28 ± 0.50[b]	89.68 ± 0.08[a]

Data are represented as the mean ± SD of triplicate determinations. Along the same row, values having different letter vary significantly different ($p < 0.05$). JZ 34, jinza 34; LZ 19, liaoza 19; JN 3, jinnuo 3; JZ 127, jiza 127; JN 2, jiniang 2; JX, jiaxian.

TABLE 4 Energy values of different sorghum grains.

Varieties	Total energy (KJ)	PEP (%)	PEF (%)	PEC (%)	UEDP (%)
JZ 34	1545.57 ± 2.20[d]	9.14 ± 0.02[c]	8.35 ± 0.02[c]	82.50 ± 0.04[c]	5.48 ± 0.12[c]
LZ 19	1546.25 ± 1.01[d]	9.80 ± 0.09[b]	7.13 ± 0.03[d]	83.07 ± 0.02[b]	5.88 ± 0.10[b]
JN 3	1577.80 ± 0.03[a]	10.13 ± 1.00[a]	12.14 ± 0.14[a]	77.73 ± 0.01[d]	6.10 ± 0.06[a]
JZ 127	1566.67 ± 0.09[c]	9.55 ± 0.03[b]	8.63 ± 0.11[c]	81.81 ± 0.06[c]	5.73 ± 0.08[b]
JN 2	1569.94 ± 0.0.11[c]	9.07 ± 0.06[c]	9.47 ± 0.09[b]	81.45 ± 0.04[c]	5.44 ± 0.06[c]
JX	1572.17 ± 9.02[b]	6.73 ± 0.00[d]	8.70 ± 0.04[c]	84.57 ± 0.11[a]	4.04 ± 0.03[d]

Data are represented as the mean ± SD of triplicate determinations. For each column, values not displaying the same letter are significantly different ($p < 0.05$). JZ 34, jinza 34; LZ 19, liaoza 19; JN 3, jinnuo 3; JZ 127, jiza 127; JN 2, jiniang 2; JX, jiaxian. PEP, proportion of total energy due to protein; PEF, proportion of total energy due to fat; PEC, proportion of total energy due to carbohydrate; UEDP, utilizable energy due to protein.

Amylose analysis

The amylose content affects the physicochemical and functional properties of flour and starch (22, 26). The amylose contents of JZ 34, LZ 19, JN 3, JZ 127, JN 2, and JX were 15.61, 17.32, 8.00, 19.30, 8.00, and 15.68%, respectively (**Figure 3**). In our studies, JZ 127 had the highest amylose content (19.30%), whereas JN 3 (8.00%) and JN 2 (8.00%) had the lowest amylose content.

Minerals and anti-nutritional quality traits of sorghum

In the present study, the different sorghum flours significantly affected the mineral contents (**Table 5**). However, the highest values of calcium (0.04%), phosphorus (0.35%), potassium (0.38%), sodium (0.05%), magnesium (0.19%), iron (50.00%), manganese (16.30%), and zinc (15.40%) were found in JN 3. Sorghum grains have higher mineral contents than other cereals, including rice (49), wheat (50), millet (51), and maize (52).

Anti-nutritional contents, such as tannin, flavonoids, and total phenols, are shown in **Figure 4**. Sorghum consists of two main anti-nutritional factors, namely, tannin and total phenols, which are located in the grain (53). The major anti-nutritional effects of tannins are reduction in feed intake, thus diminishing the digestibility and utilization of nutrients and adversely affecting the metabolism and toxicity in the livestock industry (53). Khoddami et al. (38) and Shen et al. (41) reported that the level of tannins present in sorghum can be the dominant factor that influences its nutritional value for food and non-food industries. In our studies, JN 3 flour had the highest tannin (1.46%), flavonoid (23.19 mg/g), and total phenol (5.57 mg/g) contents than other sorghum flours (**Figures 4A–C**). Tannin contents ranged from 0.01 to 2.12% (54) who studied 110 Chinese sorghum grains for determination of tannin contents by near-infrared reflectance spectroscopy (NIRS) and designated as tannin-free. The grains of all sorghums were observed free of tannin. Awika (55) stated that sorghums lacking a pigment testa are considered "free of tannin." Different grain colors in the same species and growing environment (41, 56), result in differences in the total phenol contents of varieties.

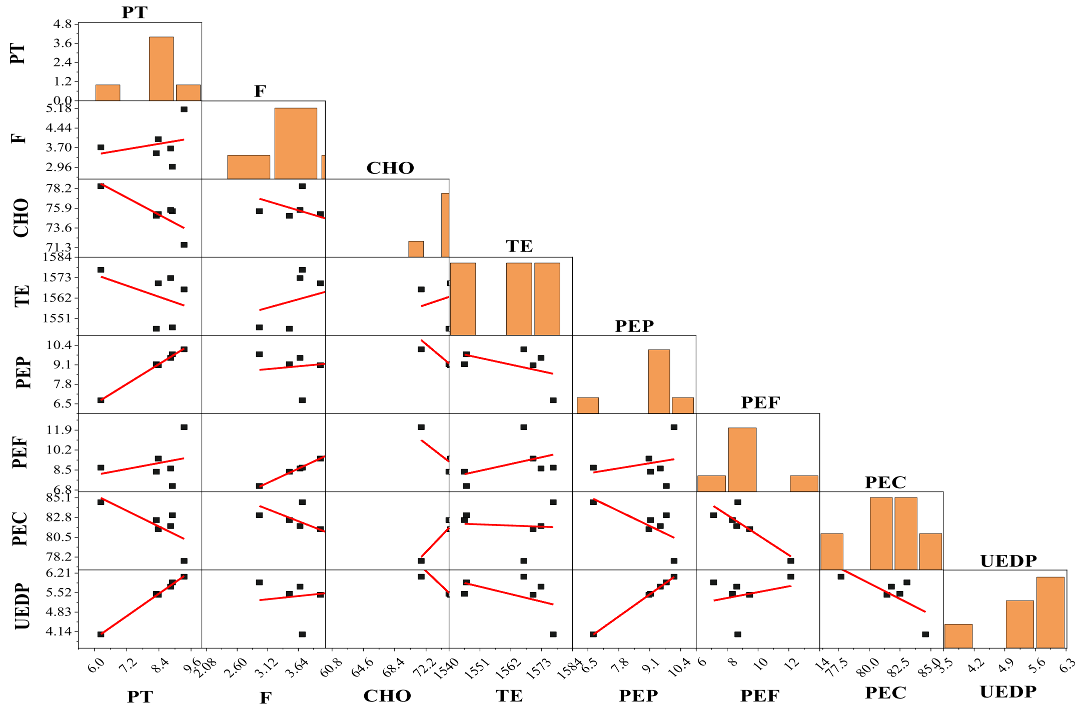

FIGURE 2
Scatterplot matrix analysis of protein (PT), fat (F), carbohydrate (CHO), and energy values contributions. TE, total energy; PEP, proportion of total energy due to protein; PEF, proportion of total energy due to fat; PEC, proportion of total energy due to carbohydrate; UEDP, utilizable energy due to protein.

Amino acid composition analysis

The AA contents of different sorghum varieties are summarized in **Table 6**. JN 3 had the highest essential contents of essential (Thr, Val, Met, Ile, Leu, and Phe), basic (Arg, His, and Lys), acidic (Asp), hydrophobic (Ala), and polar uncharged (Gly and Tyr) AAs. A high Arg content can be used for the treatment of cardiovascular diseases (57, 58). Lys is the first limiting AA in cereal grains, which are staple food (57). Li et al. (21) reported that Glu and Asp are the primary AAs of seed storage PT, and are acidic AA. A high concentration of hydrophobic AA (Ala) affected the functional properties of PTs and provided the dense internal structure of PT form, thus improving its thermal stability (59, 60). In summary, our result showed that JN 3 sorghum grain had remarkable potential for nutritional supplement in industrial food applications.

Functional properties

Among other sorghum grains, JN 2 had the highest LT (34.01%, **Figure 5A**). Sindhu and Khatkar (26) reported that the AM content affects the transmittance value of paste, which can be responsible for the difference in the turbidity of sorghum flour in the current study. JN 2 had the highest LT (34.01%) and decreased AM content, and these findings were consistent with some studies pointing out that an increase in AM content will decreases the transparency of flour paste (61). The WAC

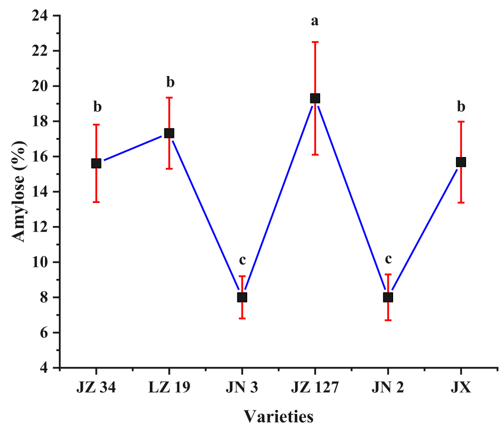

FIGURE 3
Amylose content of sorghum flours. JZ 34, jinza 34; LZ 19, liaoza 19; JN 3, jinnuo 3; JZ 127, jiza 127; JN 2, jiniang 2; JX, jiaxian. The data showed the mean of three replications, and error bars are standard deviations. Different letters **(a–c)** indicate there were significant differences ($p < 0.05$) in the LSD mean comparisons between the treatments mean.

TABLE 5 Mineral compositions of different sorghum grains.

Mineral compositions (%)	JZ 34	LZ 19	JN 3	JZ 127	JN 2	JX
Calcium	0.02 ± 0.01^{b}	0.01 ± 0.00^{b}	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	0.01 ± 0.00^{b}	0.02 ± 0.01^{b}
Phosphorus	0.20 ± 0.01^{c}	0.21 ± 0.01^{c}	0.35 ± 0.03^{a}	0.23 ± 0.02^{b}	0.17 ± 0.03^{d}	0.16 ± 0.00^{d}
Potassium	0.15 ± 0.01^{d}	0.22 ± 0.02^{b}	0.38 ± 0.02^{a}	0.14 ± 0.00^{d}	0.23 ± 0.02^{b}	0.17 ± 0.03^{c}
Sodium	0.01 ± 0.00^{c}	0.03 ± 0.00^{b}	0.05 ± 0.00^{a}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.01 ± 0.00^{c}
Magnesium	0.12 ± 0.01^{c}	0.09 ± 0.00^{d}	0.19 ± 0.02^{a}	0.11 ± 0.01^{c}	0.13 ± 0.02^{b}	0.11 ± 0.01^{c}
Iron	37.00 ± 0.21^{b}	38.00 ± 0.20^{b}	50.00 ± 0.33^{a}	36.00 ± 0.20^{b}	32.00 ± 0.11^{c}	33.00 ± 0.12^{c}
Manganese	13.20 ± 0.01^{e}	14.00 ± 0.05^{d}	16.30 ± 0.06^{a}	14.21 ± 0.06^{c}	14.00 ± 0.00^{d}	14.50 ± 0.03^{b}
Zinc	14.21 ± 0.06^{d}	14.90 ± 0.04^{b}	15.40 ± 0.07^{a}	14.30 ± 0.02^{c}	14.11 ± 0.02^{e}	14.20 ± 0.06^{d}

Data are represented as the mean ± SD of triplicate determinations. Along the same row, values having different letter vary significantly different ($p < 0.05$). JZ 34, jinza 34; LZ 19, liaoza 19; JN 3, jinnuo 3; JZ 127, jiza 127; JN 2, jiniang 2; JX, jiaxian.

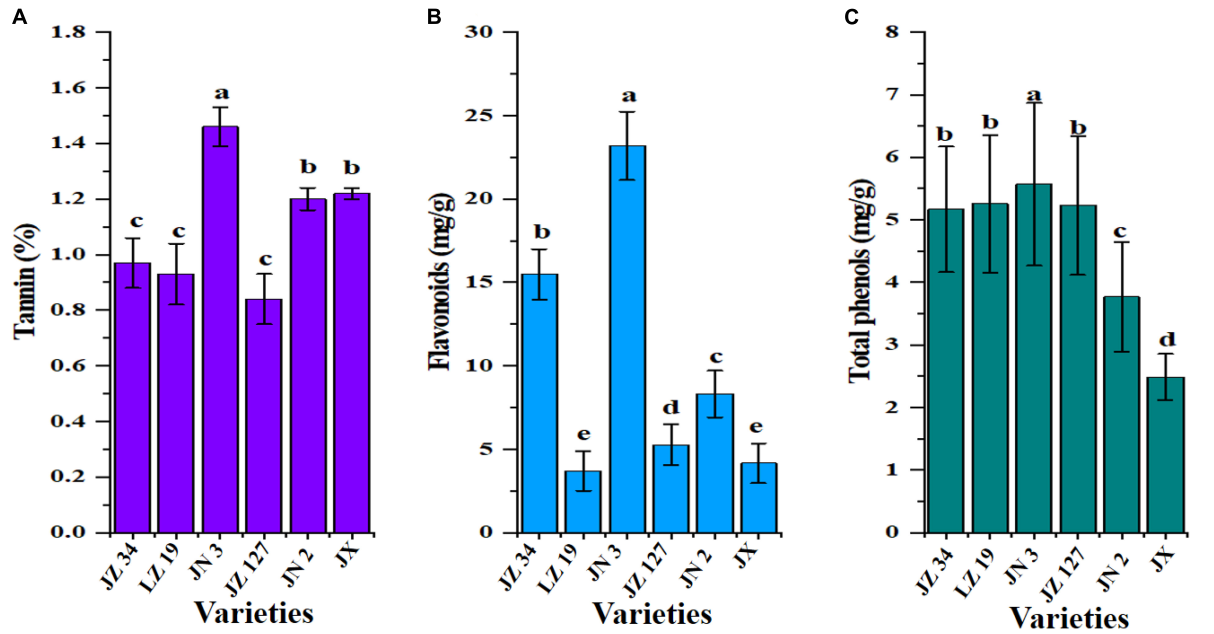

FIGURE 4
Anti-nutritional factors of six sorghum grains, tannin **(A)**, flavonoids **(B)**, and total phenols **(C)**. JZ 34, jinza 34; LZ 19, liaoza 19; JN 3, jinnuo 3; JZ 127, jiza 127; JN 2, jiniang 2; JX, jiaxian. The data showed the mean of three replications, and error bars are standard deviations. Different letters **(a–e)** indicate there were significant differences ($p < 0.05$) in the LSD mean comparisons between the treatments mean.

is the ability of the flour to maintain water against gravity and is improved by PT and CHO by supporting hydrophilic parameters, like polar and charged side chains (26). The WAC of different sorghum varieties ranged from 103.43 to 132.86%, and among the samples, JN 2 had the highest WAC (132.86%, **Figure 5B**). In the food industry, the role of OAC is the interaction between the non-polar AA side chains and hydrocarbon chains of lipid to determine mouthfeel and flavor retention of products (62). As shown in **Figure 5C**, JN 2 had high OAC (140.52%), whereas JZ 34 had the lowest OAC (111.09%).

The functions of SP and WS are the disruption and breakage of hydrogen bonds between water molecules and AM and amylopectin (63). JX had the highest WS (11.11%), and JN 2 had the lowest WS (7.22%, **Figure 5D**). **Figure 5E** shows that JZ 127 had the highest SP (8.25 g/g) compared with other treatments. In our study, among sorghum varieties, SP and WS were different, which might be due to the AM content (9, 26). Uarrota et al. (42) and Yang et al. (23) reported that AM inhibits the SP and WS in the physicochemical properties of cereal starch. The LGC is the amount of starch and gelation of pasting properties (26). The LGC of JZ 34, LZ 19, JN 3, JZ 127, JN 2, and JX were 19.00, 22.42, 24.55, 22.40, 26.77, and 22.39%, respectively (**Figure 5F**). In our studies, JN 2 had the highest LGC (26.77%), whereas JZ 34 had the lowest LGC (19.00%). LGC could be added as composite food for curd formation and could be used as additives of food materials for forming gel in food products (64). The low

TABLE 6 Amino acids compositions of different sorghum grains.

Parameters (%)	JZ 34	LZ 19	JN 3	JZ 127	JN 2	JX
Thr	0.03 ± 0.00^{c}	0.06 ± 0.00^{b}	0.08 ± 0.00^{a}	0.04 ± 0.00^{c}	0.06 ± 0.00^{b}	0.06 ± 0.00^{b}
Val	0.37 ± 0.01^{d}	0.69 ± 0.02^{c}	0.90 ± 0.03^{a}	0.34 ± 0.01^{e}	0.80 ± 0.03^{b}	0.71 ± 0.02^{c}
Met	0.05 ± 0.00^{b}	0.05 ± 0.00^{b}	0.07 ± 0.00^{a}	0.04 ± 0.00^{b}	0.05 ± 0.00^{b}	0.05 ± 0.00^{b}
Ile	0.27 ± 0.01^{d}	0.52 ± 0.02^{c}	0.67 ± 0.02^{a}	0.24 ± 0.01^{e}	0.60 ± 0.02^{b}	0.53 ± 0.01^{c}
Leu	0.76 ± 0.02^{e}	1.86 ± 0.08^{c}	2.32 ± 0.07^{a}	0.71 ± 0.02^{f}	2.21 ± 0.20^{b}	1.71 ± 0.02^{d}
Phe	0.17 ± 0.01^{d}	0.37 ± 0.01^{b}	0.41 ± 0.01^{a}	0.15 ± 0.01^{e}	0.36 ± 0.01^{b}	0.33 ± 0.01^{c}
His	0.03 ± 0.00^{c}	0.05 ± 0.00^{b}	0.07 ± 0.00^{a}	0.02 ± 0.00^{d}	0.04 ± 0.00^{c}	0.05 ± 0.00^{b}
Lys	0.03 ± 0.01^{c}	0.02 ± 0.00^{c}	0.31 ± 0.00^{a}	0.03 ± 0.00^{c}	0.02 ± 0.00^{c}	0.18 ± 0.01^{b}
Asp	0.11 ± 0.01^{e}	0.41 ± 0.01^{d}	0.61 ± 0.02^{a}	0.54 ± 0.01^{b}	0.51 ± 0.01^{c}	0.52 ± 0.01^{c}
Gly	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.03 ± 0.00^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}
Ala	0.63 ± 0.02^{c}	1.65 ± 0.06^{b}	2.21 ± 0.20^{a}	0.60 ± 0.02^{d}	2.00 ± 0.03^{b}	1.67 ± 0.02^{b}
Tyr	0.03 ± 0.00^{c}	0.06 ± 0.00^{b}	0.08 ± 0.00^{a}	0.01 ± 0.00^{d}	0.04 ± 0.00^{c}	0.06 ± 0.00^{b}
Arg	0.09 ± 0.00^{d}	0.13 ± 0.01^{c}	0.20 ± 0.01^{a}	0.04 ± 0.00^{e}	0.17 ± 0.01^{b}	0.10 ± 0.01^{d}

Data are represented as the mean ± SD of triplicate determinations. Along the same row, values having different letter vary significantly different ($p < 0.05$). JZ 34, jinza 34; LZ 19, liaoza 19; JN 3, jinnuo 3; JZ 127, jiza 127; JN 2, Jiniang 2; JX, jiaxian. Thr, thereonine; Val, valine; Met, methionine; Ile, isoleucine; Leu, leucine; Phe, phenaylalanine; His, histidine; Lys, lysine; Asp, aspartic acid; Gly, glycine; Ala, alanine; Tyr, tyrosine; Arg, arginine.

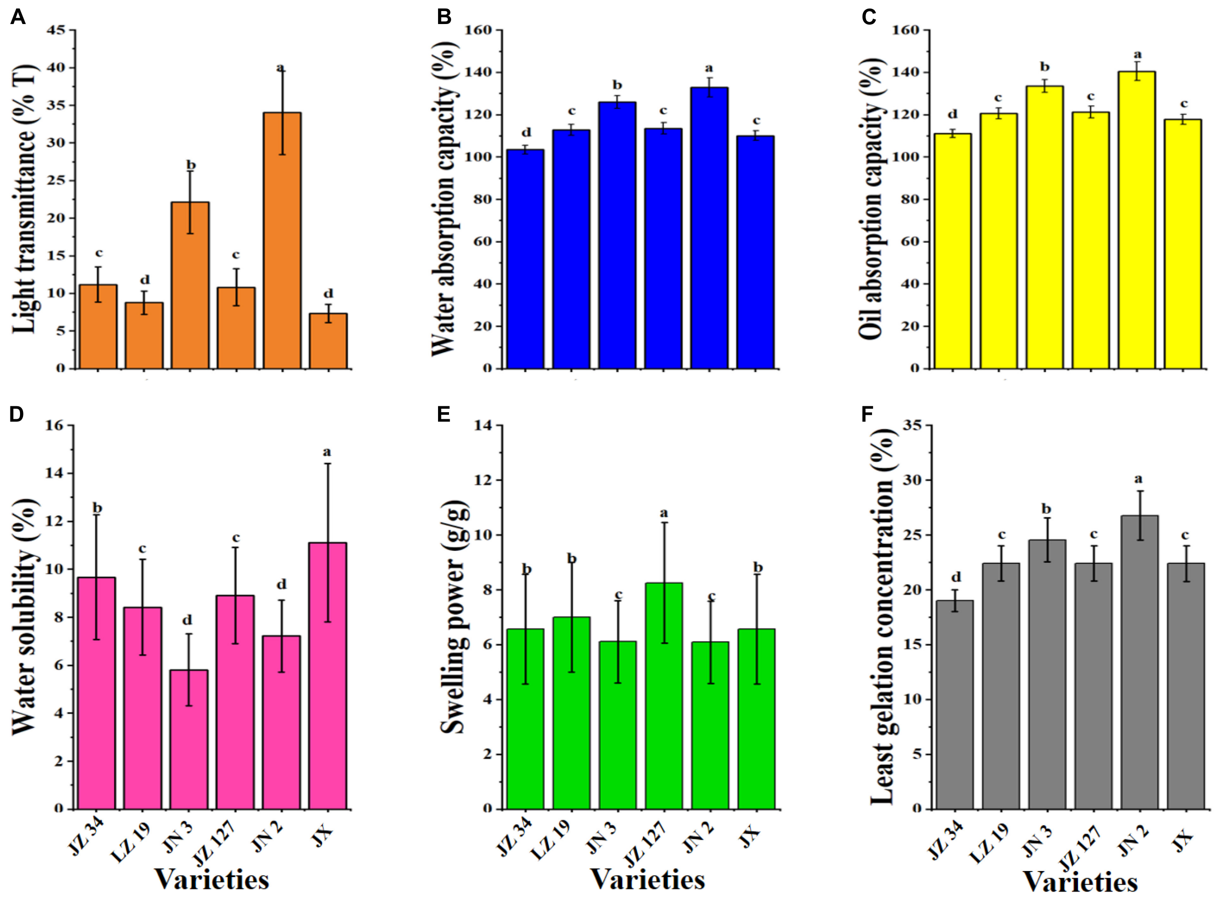

FIGURE 5
Functional properties of sorghum grains. Light transmittance **(A)**, water absorption capacity **(B)**, oil absorption capacity **(C)**, water solubility **(D)**, swelling power **(E)**, and least gelation concentration **(F)**. JZ 34, jinza 34; LZ 19, liaoza 19; JN 3, jinnuo 3; JZ 127, jiza 127; JN 2, jiniang 2; JX, jiaxian. The data showed the mean of three replications, and error bars are standard deviations. Different letters **(a–d)** indicate there were significant differences ($p < 0.05$) in the LSD mean comparisons between the treatments mean.

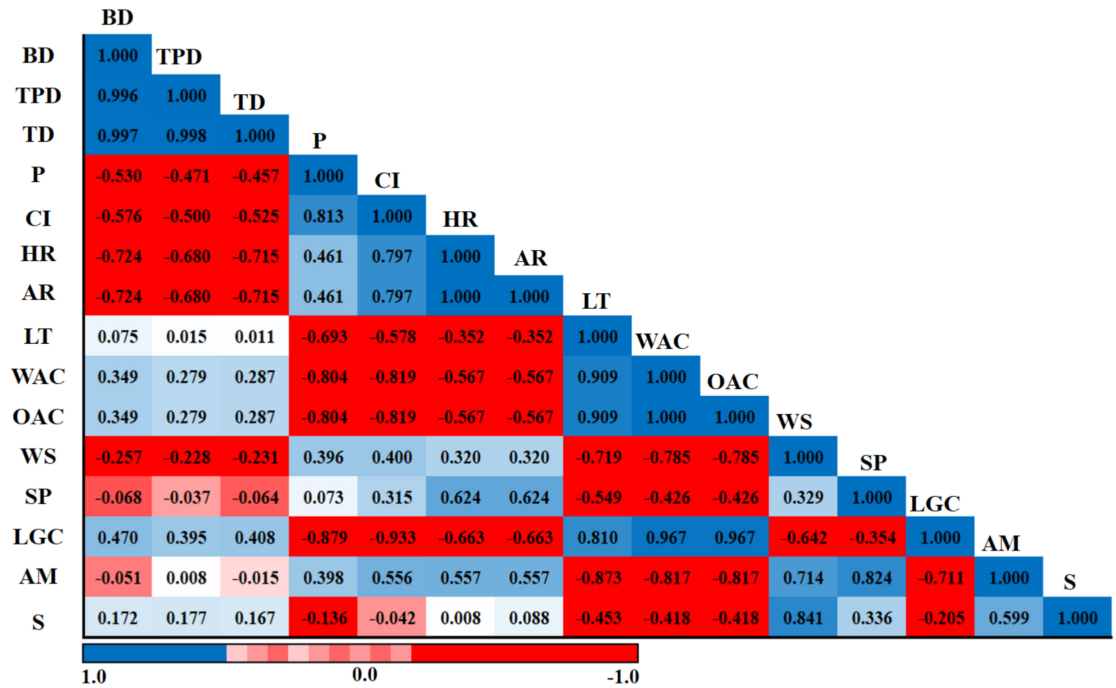

FIGURE 6
Pearson's correlation coefficients of amylose, starch, physical properties, micrometric properties, and functional properties of six sorghum grains. BD, bulk density; TPD, tapped density; TD, true density; P, porosity; CI, Carr's index; HR, Hausner's ratio; AR, angle of repose; LT, light transmittance; WAC, water absorption capacity; OAC, oil absorption capacity; WS, water solubility; SP, swelling power; LGC, least gelation concentration; AM, amylose; S, starch. The numbers in each field represent the correlation extent; the color represents significant correlation ($p < 0.05$); the deeper the color of the field, the more significant the correlation ($p < 0.01$). The blue color means a positive correlation, and the red color means a negative correlation.

LGC of flour is required for improved gelling formation of PT ingredients, and resulting in increased SP of the flour (65, 66).

Correlation heatmap analysis

The Pearson correlation results of amylose, starch, physical properties, micrometric properties, and functional properties of six sorghum grains are presented in **Figure 6**. BD was positively correlated with TPD (r = 0.996) and TD (r = 0.997). TD had a strong positive correlation with TPD (r = 0.998), whereas CI had highly positive association with P (r = 0.813). AR had a strongly significant positive correlation with HR (r = 1.000). WAC had a highly positive correlation with LT (r = 0.909). By contrast, WAC was negatively correlated with CI (r = -0.819). OAC had a highly negative correlation with CI (r = -0.819) and a highly positive association with LT (r = 0.909). Furthermore, OAC had a strongly significant positive correlation with WAC (r = 1.000). LGC was strongly and negatively correlated with CI (r = -0.933). Moreover, LGC was strongly and positively correlated with WAC (r = 0.967) and OAC (r = 0.967). AM showed a highly negative association with LT (r = -0.873), WAC (r = -0.817), and OAC (r = -0.817). In addition, AM had a highly positive relationship with WS (r = 0.714) and SP (r = 0.824).

Conclusion

We studied the physical properties, nutritive quality, and functional properties of six different sorghum varieties. The flour of JN 127 had the highest amylose content. The physical properties of LZ 19 were better than those of other sorghum grains, and JZ 34 had the best micrometric properties. JX produced higher SY and was suitable as a frozen food thickener or food additive and raw material for porridge, couscous, and mayonnaise. All sorghums were free of tannin. Among the six sorghum grains, JN 3 had the highest proximate, mineral, and AA compositions and energy contributions. The six sorghum grains had differences in functional properties, including LT, WAC, OAC, WS, SP, and LGC of flours as composite flours products in sorghum for healthy food. Our results indicated that JN 3 could be utilized for brewing sorghum grain and liquor flavor and could be effective materials for functional foods to improve health. Our assessments in future will stimulate the utilization of sorghum as a potential crop in dryland sustainable agro-ecosystems and rural livelihood nutritional security in arid and semiarid regions of northern China.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MH: conceptualization, investigation, methodology, data, software, writing—original draft preparation, and writing—review and editing. BF: writing—review and editing, supervision, funding acquisition, project administration, and resources. HW and LT: investigation and methodology. VY: writing—review and editing. All authors have read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Biochemical and antioxidant activity of wild edible fruits of the eastern Himalaya, India

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The eastern Himalayas, one of the important hotspots of global biodiversity, have a rich diversity of wild edible fruit trees. The fruits of these tree species have been consumed by the tribal people since time immemorial. However, there is limited information available on the biochemical and antioxidant properties of the fruits. Therefore, the present investigation was undertaken to study the physico-chemical and antioxidant properties of the nine most important wild fruit trees. Among the species, *Pyrus pashia* had the maximum fruit weight (37.83 g), while the highest juice (43.72%) and pulp content (84.67%) were noted in *Haematocarpus validus* and *Myrica esculenta,* respectively. Maximum total soluble solids (18.27%), total sugar (11.27%), moisture content (88.39%), ascorbic acid content (63.82 mg/100 g), total carotenoids (18.47 mg/100 g), and total monomeric anthocyanin (354.04 mg/100 g) were recorded in *H. validus*. *Docynia indica* had the highest total phenolic content (19.37 mg GAE/g), while *H. validus* recorded the highest total flavonoids and flavanol content. The antioxidant activities of the different fruits ranged from 0.17 to 0.67 IC_{50} for DPPH activity and 3.59–13.82 mg AAE/g for FRAP. These fruits had attractive pigmentation of both pulp and juice and were a good potential source for the extraction of natural edible color in the food industry. The fruits also possess high market prices; *Prunus nepalensis* fetched $ 34.10–$ 141.5 per tree. Therefore, these fruits are rich sources of antioxidants, pigments and have a high market value for livelihood and nutritional security.

KEYWORDS

wild edible fruits, bioactive compounds, pigmentation, processing, value addition, livelihood, diversity, conservation

1. Introduction

The eastern Himalayan region of India has a diverse agro-climate, ranging from tropical to alpine, and receives very high rainfall. The region is an important part of the Indo-Myanmar biodiversity hotspot of the world (1). The diverse agro-climatic conditions of this region offer immense scope for the evolution and development of different wild edible species. Of the 800 wild edible tree species found in India, about 300 are consumed by the hill populace of this region alone. Therefore, the region is considered a reservoir of several crop species, including wild relatives, grown naturally in the forests and also in the backyards of the local tribes. The economy of this region is basically rural based; agriculture and allied sectors play a predominant role. The fruits collected from the forest as well as from their own land are consumed locally and also sold in local markets at a premium price. Recently, several trainings and demonstrations have been conducted by various government agencies to impart knowledge and skills to the local people, resulting in improved resource utilization and entrepreneurial skills. Several value-added products, such as wine, vinegar, jam, jelly squash, RTS, pickles, etc., of these wild fruits are prepared and marketed by self-help groups (SHGs) and entrepreneurs. However, the availability of value-added products on the market is still lacking due to poor commercial production.

These fruit plants are found in tropical, subtropical, and temperate regions of the Indian subcontinent, East Asian nations, South East Asian nations, and European nations, which suggests that they are adaptable to a wider range of environments (**Table 1**). These fruits have been important constituents of diet and health care and have contributed significantly to the livelihood security of the local people over centuries (2). Locally, these crops are also used to extract natural pigments (3); and ethnobotanical uses of fruits in the treatment of cancer (4); fever, cough, and jaundice, gastrointestinal, respiratory, and cardiovascular (5); anti-obesity (6); cognitive boosting properties, liver health, and reducing fatty liver buildup (7); leaves of *Pyrus pashia* were used as fodder (8) and the Monpa community of Tawang, Arunachal Pradesh, India, used extracts of *P. pashia* as butter tea beverages (9). This could be due to the nutraceutical properties of these fruits. It has been proven that ingestion of natural antioxidants from fruit sources, such as polyphenols, have significant anticarcinogenic, antipyretic, anticoagulant, anti-inflammatory, and hypoglycemic properties (10). This might be attributed to the powerful antioxidant capacity of polyphenols and their additives, and their synergistic effects with associated bioactive constituents. Such constituents provide protection to the cellular system against oxidative impairment, which consequently reduces the oxidative stress in the human body (11). In addition, fruit-based natural antioxidants are also fascinating because of their safety and wide applications in the cosmetic, pharmaceutical, and food industries as alternative sources to synthetic antioxidants (12). In spite of these potential applications, research on these crops is at an infant stage, although morphological characterization of some fruit crops, such as *Prunus nepalensis*, *Elaeagnus latifolia*, *Pyrus pashia,* and other wild edible fruits has been described (13–15). However, their nutraceutical properties have not yet been scientifically assessed. Hence, the present study was carried out to determine the biochemical and antioxidant properties of popular wild edible fruit trees grown in the eastern Himalayas of India. The information generated could lead to a better understanding of the potential functional food sources and an increased consumption of these fruits. This, in turn, could have a significant impact on the most vulnerable tribal population's long-term economic, nutritional, and health system in the near future.

2. Materials and methods

2.1. Materials and experimental site

The fruits of wild edible plant species such as *Baccaurea sapida* (Roxb.) Müll. Arg., *Docynia indica* (Wall.) Decne., *Elaeagnus latifolia* L., *E. pyriformis* Hook. f., *Haematocarpus validus* (Miers) Bakh. f. ex Forman., *Myrica esculenta* Buch. -Ham. ex D. Don., *Myrica nagi* Thunb., *Prunus nepalensis* Ser., and *Pyrus pashia* Buch.-Ham. ex D. Don. grown in the forests and/or backyards, were collected for the study (**Figure 1**). The collection was made from various locations in the region, particularly the Khasi Hills, Jaintia Hills, Ri Bhoi, and Garo Hills, distributed between 20.1–26.5°N latitude and 85.49–92.52°E longitude with altitude ranging from 100 to 2,000 m amsl (**Figure 2**). The collected fruits were analyzed for different biochemical and functional attributes at the ICAR Research Complex for the North Eastern Hill Region, Umiam, Meghalaya, India, during 2019–2020.

2.2. Quantitative analysis

Twenty-five ripe fruits of each species were used for carrying out all the physical and biochemical analyses. Fruit samples were harvested at an appropriate maturity. The harvested fruits were washed with distilled water, wiped with tissue paper, and kept at room temperature for 10 min to remove the adhering water before analysis. The parameters, *viz.*, fruit, and seed weights, were determined using an electronic balance (Adair Dutt-1620C). Fruit length and diameter were measured using a digital caliper (Code 1108-150). The pulp recovery percentage was estimated using the following formula:

$$\text{Pulp Recovery (\%)} = \frac{\text{Pulp weight (g)}}{\text{Fruit weight (g)}} \times 100$$

2.3. Determination of biochemical attributes

Biochemical parameters such as total soluble solids (TSS) were determined using a hand-held refractometer (HI 96801) and titratable acidity, ascorbic acid, reducing sugars, and total sugars were analyzed according to Rangana (52). The moisture content of the fruits was determined gravimetrically as per the method of Akter et al. (53) and Raaf et al. (54). The fresh fruit samples were weight before and after drying in a hot air oven (thermostatically controlled, Model–IC7). About 20 g finely shredded fresh sample was placed in a clean and dried crucible with a cover, and accurately weighed on an electronic weighing balance (Model–AUX220). The samples were dried in the oven at 105°C for 24 h or until a constant weight was achieved for two consecutive weights. Following drying, the crucible was cooled in a desiccator. The moisture content (MC) was calculated as below and expressed as a percentage.

$$\text{MC (\%)} = \frac{\text{W1} - \text{W2}}{\text{W1}} \times 100$$

Where W_1–fresh weight
W_2–dried weight

TABLE 1 Habitat, distribution, and utilization of wild edible fruit crops of the eastern Himalayas, India.

Crops	Habitat and distribution	Uses
Prunus nepalensis Ser. (Family: Rosaceae and Vernacular name: Sohiong)	• Habitat: Subtropical and temperate Himalayan regions at an altitude of 800–3,000 m amsl. • Native: Eastern Himalayas including the Khasi and Jaintia Hills of Meghalaya, India. • Domestication status: Growing wild and semi cultivated. • Distribution: It is found in Meghalaya, and other parts of Himalayas India (2). The species also found in Bhutan, Nepal, Myanmar, and China (16).	• Edible portion: Epicarp and mesocarp. • Dessert purpose: Fresh fruits. • Processing: The processed products prepared from this fruit are ready to serve drink (RTS), squash, candy, powder, wine, tooty fruity (Sohiong + chow chow). The products developed retained the natural purple color of the fruits for longer period (up to 1 year). • Others: Fruits are used as astringent, leaf as diuretic agent against edema (17).
Elaeagnus latifolia L. (Family: Elaeagnaceae and Vernacular name: Sohshang)	• Habitat: Thrives well in open forest and swamps of the foothills track of Eastern Himalayas up to elevations of 2,600 m amsl. • Native: The lower hill tracks of Himalayas considering its wider genetic variability. • Domestication: The shrub mostly found in back yard as semi-wild and semi cultivated (13). The genus is also reported to be cultivated in warmer parts of southern Europe, North America and Vietnam (18). • Distribution: Subtropical and temperate Himalaya including Myanmar and China.	• Edible portion: Epicarp and mesocarp of fleshy drupe fruits. • Dessert purpose: Fresh fruits. • Processing: pulp used for preparation of pickle, jam, jelly and leather. Refreshing drink prepared from fruit juice possess attractive reddish or pinkish color. • Others: Fruits are astringent (19) and found to reverse the growth of cancers (4). Pulp also used for dye extraction and seed as source of oil. The plant also possesses ornamental values.
Elaeagnus pyriformis Hook. f. (Family: Elaeagnaceae and Vernacular name: Sohkhlur)	• Habitat: Thrives well in open forest up to elevations of 2,600 m amsl. • Native: Foot hill tracks of the Eastern Himalayas India. • Domestication: The shrub mostly found in forest areas. • Distribution: Subtropical and temperate Himalaya including Myanmar and China.	• Edible portion: Epicarp and mesocarp of fleshy drupe fruits. • Dessert purpose: Fresh fruits. • Processing: Processed products such as pickle, jam and jelly were prepared from pulp. Fruit beverage develop an attractive attractive reddish or pinkish color. • Others: Fruits are capable of reducing cancer and reversing the growth of cancers (4).
Myrica esculenta Buch. -Ham. ex D. Don. (Family: Myricaceae and Vernacular name: Sohphie bah)	• Habitat: It flourish well in mixed forests of *Pinus* sp., *Quercus leucotrichophora* and marginal lands of nitrogen depleted soils up to an altitude of 2,000 m amsl in the sub-tropical Himalayas (20). • Native: North east and northern India, southern Bhutan and Nepal. • Domestication: Semi-cultivated. • Distribution: In the Indian subcontinents, it is confined in the sub-tropical Himalayas ranging from Punjab eastward to Assam. It was also found in the temperate and sub-tropical regions of China of both hemispheres except Australia (21).	• Edible portion: Epicarp and mesocarp of drupaceous fruits. • Dessert purpose: Fruits are edible as fresh at all stages of its growth. • Processing: Fruits are used for making refreshing drink and pickle. The extracted juice emits a very attractive sparkling red color. • Others: Fruit juice are used for treatments of jaundice in Khasi Hills (22), fever in Khasi Hills, Vietnam, South China (23), Ulcer, and Anthelmintic in Himachal (24, 25), Bronchitis, dysentery in Nepal (26). The bark is used as aromatic, tonic for rheumatism, astringent, carminative, asthma, odontalgia, diarrhea, lung infection, fever, cough, bronchitis, dysentery, antiseptic indigenous medicine (27, 28). Tannin extract from the barks are used as a yellow dyeing agent (29).
Myrica nagi Thunb. (Family: Elaeagnaceae and Vernacular name: Sohphienam)	• Habitat: The tree is evergreen in the sub-temperate of mid-hill and hill tracks of the Himalayas up to 2,100 m amsl. • Native: Eastern Himalayas, India. • Domestication: Found wild in the forest. • Distribution: It is found in the mid-Himalayas of India including the Khasi Hills. It is also found in Bangladesh, Singapore, Malayan islands, China and Japan (30).	• Edible portion: Epicarp and mesocarp of drupaceous fruits. • Dessert purpose: Fruits are edible as fresh at all stages of its growth. • Processing: Pulp are used for preparation of refreshing drink and pickle. The juice possesses a very attractive sparkling pink color. • Others: Bark powdered is used against dysentery (31).
Baccaurea sapida (Roxb.) Müll.Arg. (Family: Phyllanthaceae and Vernacular name: Sohramdieng/ Sohmyndong)	• Habitat: Grow favorably in moist tropical up to an altitude of 900 m asml. • Native: South East Asian region. • Domestication: Growing wild and semi-cultivated in the sub-Himalayan tract of eastern India. It is cultivated in China, Myanmar, Thailand, Vietnam, and Malaysia (18). • Distribution: It is found from Bihar to Arunachal Pradesh and in the lower hills and valleys (of Meghalaya, Assam, Nagaland, Manipur, Mizoram, and Tripura), Orissa and Andaman and Nicobar Islands. Globally, its distribution is from Indo-Malaysia to the West Pacific.	• Edible portion: Arils is very delicious at ripening stage. • Dessert purpose: Fresh fruits at ripened stage. • Processing: Products prepared are squash, RTS, wine, jam and jelly due to its rich sources of pectin (14.1%). Fruit rinds are also used for making pickle. • Others: Fruits and leaf produced dye of chocolate color which can be used as natural colorants in processed products. Seed was used to extract annatto dye (4.8–6.0%) for coloring of silk, cotton and other textile materials for orange-red color (3). Fruit juice are used for treatment against arthritis, abscesses, injuries, and constipation (32).

(Continued)

TABLE 1 (Continued)

Crops	Habitat and distribution	Uses
Pyrus pashia Buch.-Ham. ex D. Don. (Family: Rosaceae and Vernacular name: Sohjhur)	• Habitat: It thrives well in moist soil up to an elevation 2,700 m amsl. It is tolerant to drought and atmospheric pollutants. • Native: Southern Asia. • Domestication: It is cultivated in Khasi and Jaintia Hills as back yard and border tree crops. • Distribution: It is distributed from East Afghanistan, North Pakistan through Himalaya to Vietnam.	• Edible portion: Thalamus or receptacle of pome fruits. • Dessert purpose: Fruit are eaten fresh, and preferred for its sweetness and grittiness. • Processing: • Others: Fruit juice is astringent and diuretic (33) used against constipation (34), dysentery (35), leishmaniasis (36), eye problems (37), digestive disorder, sore throat, irritability, abdominal pain, anemia, curing eye disorder (38) and curing of gastrointestinal, respiratory and cardiovascular related problems (5). Decoctions of dried fruits with other plant parts improves in spleen and stomach functions (39). Leaf extract as a tonic for hair loss, treatment of digestion related ailments and possess antimicrobial activity (40). Staining of crushed leaves in palm, feet and nails improve cosmetic appearance (41). The barks are used to treat digestive disorders (42), sore throat, fever, peptic ulcer, gastric ulcer and typhoid fever (43). Leaves are used as fodder for goats and sheep (8) and it extracts as butter tea beverages by the Monpa community of Tawang, Arunachal Pradesh, India (9). The fruit is added to cattle fodder to enhance milk production (44). Seedlings can be used as rootstock for pear and quince grafts (28).
Docynia indica (Wall.) Decne. (Family: Rosaceae and Vernacular name: Sohphoh / shoptet)	• Habitat: Grows well in moist soil, open places, and upland temperate to subtropical at an elevations of 700–3,000 m amsl. • Native: Eastern Himalayas of India through Nepal to South Central China. • Domestication status: Growing in the back yard and also naturally in forest areas of Khasi and Jaintia Hills of Meghalaya, India. In Northern Vietnam, the species is domesticated in large area (3,000 ha) with a production of 6,500 tons of fruit (45). • Distribution: Southern foothill tracks of the Himalayan range from Pakistan through India, Nepal, Bhutan, and Bangladesh to the mountains of northern Myanmar, Thailand, Laos, Vietnam, and southern China.	• Edible portion: Mesocarp of matured fruits. • Dessert purpose: The fully ripe fruit is eating as fresh, while, the half ripe fruits are consumed as fresh with salt. • Processing: The fruits soaked in brined solution or boiled with syrup and sundried. Pulp is used as raw materials for production of juice, tea, vinegar, wine, pickles and jelly (13, 45). • Others: Fruits are used as natural remedy in treatment of infectious diseases, obesity (6), digestive problems and possess hypoglycemic and hypolipidemic properties (46). Fruit extract possesses antioxidant and antibacterial properties which have industrial potential as food preservative (47). The species as rootstock imparting semi-dwarf in apple.
Haematocarpus validus (Miers) Bakh. f. ex Forman (Family: Menispermaceae and Vernacular name: Sohsnam)	• Habitat: An evergreen vine and prefers the tropical to subtropical conditions with a large number of herbaceous undergrowth on a hilly landscape up to altitude 1,250 m amsl. • Native: Eastern Himalayas India to West Java. • Domestication status: Growing in the forest naturally. • Distribution: The geographical distribution of this species is limited and it has been categorized as critically endangered in Meghalaya (48). In India, it is found in Meghalaya, Tripura, Assam, Arunachal Pradesh, Sikkim, West Bengal, and Andaman and Nicobar Islands. It is also found in Bangladesh, Indonesia, Pakistan, and South east Asian.	• Edible portion: Mesocarp of ripened fruits. • Dessert purpose: Ripened fruits. • Processing: Immature fruits are used for value-added products such as squash, pickles, and chutney. • Others: Fruits and seeds were consumed for treatments against anemia, roots for curing itching and tender shoots for treatment of jaundice (49), while leaf decoction was used against body ache (50). The fruit is a rich source of choline for cognitive boosting properties, liver health and reduce fatty liver build up (51). Fruits are rich source of purple to bright reddish pigmentation used for dyeing local handicrafts and may have potential application as natural colorant in food industries.

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FIGURE 1
Fruits of wild edible plants grown in the eastern Himalayas, India.

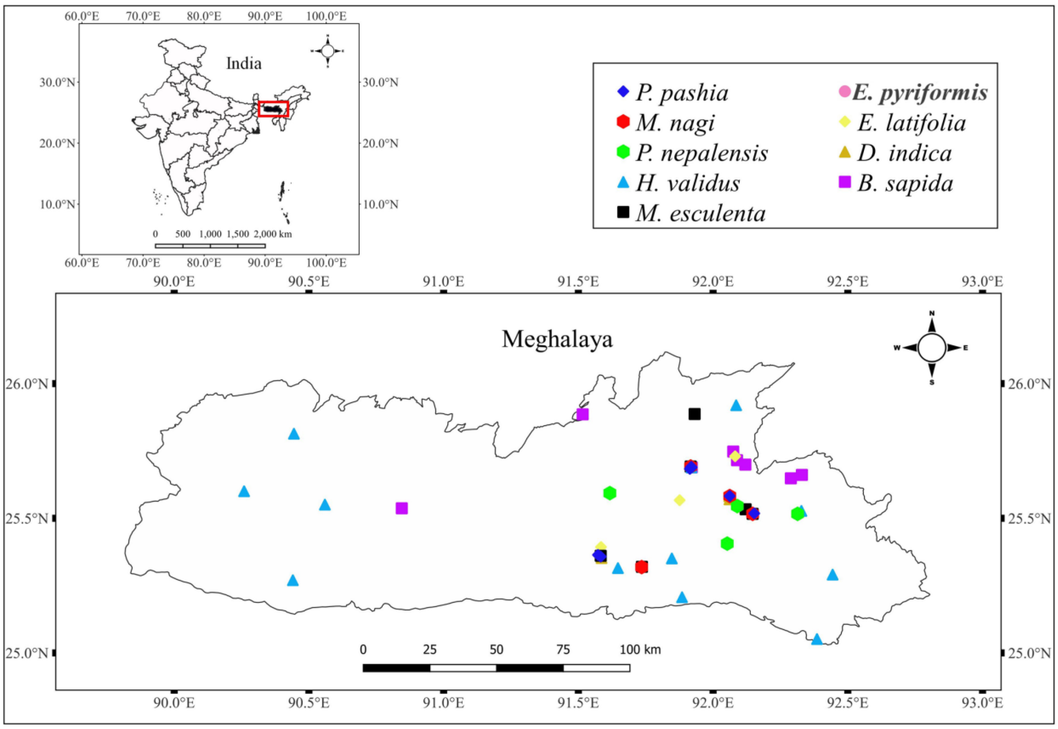

FIGURE 2
Collection sites of wild edible fruits grown in the eastern Himalayas, India (Generated by subjected the global positioning system (GPS) data to quantum geographic information system (QGIS) version 3.20.1).

2.4. Measurement of total carotenoids

Total carotenoids were determined as per the method of Chen et al. (55). The extraction of carotenoids was carried out according to the method developed by Chen et al. (55). Pulp (10 g) was placed in a vessel, protected from light, and mixed with 50 mL of extraction solvent (hexane/acetone/ethanol: 70:15:15, v/v/v). The mixture was stirred for 1 h using an orbital shaker. About 5 mL of a 40% KOH in methanolic solution were added, and the solution was saponified at 25°C in the dark for 2 h. Subsequently, 30 mL of hexane were added, the mixture was shaken vigorously, and the upper layer was collected. The lower layer was extracted twice, and the supernatant was also collected and filtered through sodium sulfate powder to remove traces of water. The supernatant obtained was pooled and stored at −80°C under a nitrogen atmosphere (99.9% purity) in the dark until analysis. The total carotenoid content of the extracts was measured using a UV-Visible spectrophotometer at 450 nm. A calibration curve (0–50 ppm) was prepared using β-carotene as the standard and the results were expressed as mg β-carotene/100 g sample.

2.5. Determination of functional attributes and antioxidant activity

2.5.1. Preparation of fruit extract

The pulp (5 g) of each fruit was grinded, and 50 mL of aqueous methanol was added at ambient temperature. The mixture was incubated for 1 h at room temperature with continuous magnetic stirring at 200 rpm and centrifuged at 1,000 *g* for 20 min. The supernatant was collected and stored at −20°C until analysis. The aliquot was used for assessments of total phenolic content, total monomeric anthocyanins, total flavonoids, total flavonol, DPPH free radical scavenging capacity, and FRAP reducing power.

2.5.2. Determination of total phenolic content

The crude extracts were estimated for total phenolic content using the Folin–Ciocalteu procedure as per the method of Singleton and Rossi (56). About 1 mL of the extract was transferred to 2 mL of Folin–Ciocalteu reagent (1:10 v/v distilled water). After 10 min, 1.6 mL (7.5%) of sodium carbonate was added. The mixture was vortexed for 15 s before being left to stand for 30 min at room temperature to develop its color. The absorption was measured at 743 nm in a UV-visible spectrophotometer (Model: UV 3200). The concentration of polyphenols in samples was derived from a standard curve of Gallic acid, and the total phenolic content was expressed as Gallic acid equivalents (GAE) in mg/g of pulp.

$$\text{TPC (mg GAE/g fw)} = \frac{\text{Conc. of GA from Std curve} \times \text{vol. of extract} \times 100}{\text{Weight of the sample}}$$

2.5.3. Determination of total monomeric anthocyanin content

Total monomeric anthocyanin was determined as per the procedure of Giusti and Wrolstad (57); Lako et al. (58). About 0.4 mL of the extract solution was taken, and 3.6 mL of the corresponding buffer; pH 1.0 buffer (potassium chloride 0.025 M) and pH 4.5 buffer (sodium acetate, 0.4 M) was added. The absorbance of each solution was taken against a blank in a cuvette with a 1 cm path length at 510 nm and 700 nm using a UV-Visible spectrophotometer. Total monomeric anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents (mg cyd-3-gluE / 100 g) as follows:

$$\text{Anthocyanin Pigment (mg cyd-3-gluE/100 g fw)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l}$$

Where A = $(A_{510nm} - A_{700nm})$ pH 1.0 − $(A_{510nm} - A_{700nm})$ pH 4.5;

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu);

DF = dilution factor established in D;

l = pathlength in cm;

ε = 26,900 molar extinction coefficients for cyd-3-glu; and 1,000 = factor for conversion from g to mg.

2.5.4. Measurement of total flavonoids

The total flavonoid content of extracts was estimated using Aluminum chloride ($AlCl_3$) colourimetric assay as previously described by Zhishen et al. (56). About 0.3 mL of 5% $NaNO_2$ was added to 1 mL extract. After 5 min, 0.3 mL of 10% $AlCl_3.6H_2O$ was added, and incubated for 5 min. About 2 mL NaOH (1M) was added, and the final volume of the solution was adjusted to 5 mL with distilled water. After 15 min of incubation, the mixture turned to pink and the absorbance was measured at 510 nm (UV-visible spectrophotometer, Model: UV 3200). Total flavonoid content was presented as mg quercetin equivalent per gram (mg QE/g).

$$\text{Flavonoid content (mg QE/g fw)} = \frac{\text{Conc. of Q from std curve} \times \text{volume of extract}}{\text{Wegiht of the sample}}$$

2.5.5. Determination of total flavonols

Total flavonols in the fruit sample extracts were determined according to the method of Miliauskas et al. (59). 2 mL of 2% $AlCl_3$ and 6 mL (5.0%) sodium acetate solutions were added to 2.0 mL of extract. The mixture was incubated at 25°C for 2.5 h and absorption at 440 nm (UV-visible spectrophotometer, Model-UV 3200) was read. Total flavonol content was expressed as quercetin equivalent (mg QE/g).

$$\text{Flavonol content (mg QE/g fw)} = \frac{\text{Conc. of Q from std curve} \times \text{volume of extract}}{\text{Weight of the sample}}$$

2.5.6. Measurement of DPPH free radical scavenging activity

The free radical scavenging activity of the fruit extracts was estimated with the DPPH (1, 1-diphenyl-2- picrylhydrazyl) method (60). Ascorbic acid was used as a reference standard. 100 μL of aliquot was transferred to test tubes, to which 3.9 mL of freshly prepared DPPH solution (25 mg/L in methanol) were added. The mixtures were then thoroughly mixed and allowed to stand for 30 min. The absorbance was measured at 517 nm (UV-visible spectrophotometer, Model: UV 3200). The percent scavenging activity of DPPH was calculated using the following formula:

$$\text{DPPH Scavenging activity (\%)} = \frac{\text{Ac} - \text{At}}{\text{Ac}} \times 100$$

Where, Ac is the absorbance of the control reaction and At is the absorbance of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50} (the concentration of fruit sample required to decrease the absorption at 517 nm by 50%). The IC_{50} value was expressed as the concentration in milligram of extract per mL that inhibited the formation of DPPH radicals by 50%.

2.5.7. Measurement of FRAP reducing power

The reducing power of the extracts was assessed as per the method of Oyaizu (61). About 100 μL of fruit extracts were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). This mixture was incubated at 50°C for 20 min, to which 2.5 mL aliquots of trichloroacetic acid (10%) was added. The content was centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 mL) was extracted and mixed with 2.5 mL of distilled water and 0.5 mL of freshly prepared ferric chloride solution (0.1%). Then the measurement of absorbance was recorded at 700 nm (UV-visible spectrophotometer, Model: UV 3200) and the reducing power was expressed in terms of ascorbic acid equivalent (AAE) in milligram per gram of extract (mg AAE/g).

2.6. Color, season of availability and market price of fruits

Color measurements of ripened fruits of different fruit tree species were carried out using a Color Hunter meter (HunterLab Color Quest XE). The instrument was calibrated using the black and white tiles. The value was expressed as L* values indicated lightness (black, $L^* = 0$ and white, $L^* = 100$), a* values indicated redness-greenness (red, $a^* = 100$ and green, $a^* = -100$), b* values indicated yellowness-blueness (yellow, $b^* = 100$ and blue, $b^* = -100$). The observation was replicated thrice for each sample. Observations were taken at the base, middle, and apex of fruits at an equidistant space under the aperture of the color meter. Through image analysis, an Android application (Color Grab version 3.9.2) was used to determine the color of fruit juice. A local market survey in Shillong city and 10 weekly markets in Khasi and Jaintia Hills were conducted. Informants (60 no.) were randomly selected among the local vendors and farmers for data collection on period of fruit availability in the markets and the market price of fruits. The selection of key informants was done with the help of village workers and elders as per the ethnoecological methods of Martin (62). The yield of fruits per tree was determined by counting the number of fruits per tree at harvest and multiplying it by its fruit weight, expressed in kg per tree.

2.7. Statistical analysis

The replicated (three of each parameter) data were analyzed using statistical package for the social sciences (SPSS) (Version 14.0) software, and the data were presented as mean ± SE using one-way ANOVA ($p < 0.05$) of Tukey's HSD (honestly significant difference) test. The possible relationship between antioxidant compounds and antioxidant activity was analyzed through Pearson's correlation coefficient. Using quantum geographic information system (QGIS) version 3.20.1, a map of the collection sites was created subjecting the global positioning system (GPS) data.

3. Results and discussion

3.1. Physico-chemical characteristics

The biochemical traits of fruits contribute to the consumer's perception of quality traits, including those associated with taste, mouth feel, and appearance. The results revealed a significant variation among the fruit morphological and biochemical characteristics of different wild edible fruit species ($p < 0.05$, Table 2). The maximum fruit length ranged from 4.38 cm in *H. validus* to 1.52 cm in *M. nagi*; fruit diameter (1.23 cm in *M. nagi* to 4.39 cm in *P. pashia*); fruit circumference (12.38 cm in *P. pashia* to 3.64 cm in *M. nagi*); fruit weight (7.32 g in *E. pyriformis* to 37.83 g in *P. pashia*); fruit volume (39.89 cm^3 in *P. pashia* to 7.32 cm^3 in *E. pyriformis*); juice content (21.22% in *M. nagi* to 43.72% in *H. validus*) and pulp content (56.69% in *B. sapida* to 84.67% in *M. esculenta*). The significant differences in fruit physical characteristics indicated greater variability among fruit crops. The maximum fruit weight was observed in *P. pashia,* followed by *D. indica, H. validus,* and *E. latifolia*; juice content was recorded in *H. validus*, followed by *M. esculenta, B. sapida,* and *E. pyriformis*; and pulp content (>70%) in *M. esculenta,* followed by *M. nagi, P. pashia, P. nepalensis, D. indica, and H. validus*. Similarly, there was a significant difference ($p < 0.05$) among fruits for biochemical attributes as given in Table 3. The moisture content was the highest in *H. validus* (88.39 ± 1.85%) and the lowest in *P. pashia* (73.75 ± 1.88%). The determination of moisture content in food is considered to be one of the most important assays since moisture greatly influences the physical properties and stability of the food (63). The total soluble solids (TSS) was the maximum in *H. validus* (18.27 ± 1.49%) and the minimum in *M. esculenta* (5.83 ± 0.30%). The titratable acidity was the highest in *M. esculenta* (3.32 ± 0.06%), followed by *E. latifolia* (2.68 ± 0.04%) and the lowest in *P. pashia* (0.31 ± 0.03%). Total sugar ranged from 3.26 ± 0.05% in *E. latifolia* to 11.27 ± 1.26% in *H. validus*. Reducing sugar content ranged from 1.32 ± 0.03% to 7.38 ± 0.54%, the minimum was recorded in *E. latifolia* and the maximum in *H. validus*. Our results indicated that the fruits of *H. validus*, *P. nepalensis, B. sapida, E. latifolia, M. esculenta,* and *D. indica* contained higher levels of TSS and acidity. TSS and acidity are the two important factors for determining the quality traits in a fruit, which also influence the taste, sweetness, and also act as an indicator of the maturity of the fruit and its suitability for processing. This was indicated by a strong relationship between TSS and total sugar (0.711**), ascorbic acid (0.838**), total monomeric anthocyanin (0.732**), total carotenoids (0.407*), total flavonoids (0.479**), and total flavonol (0.532**). Similar observations have been reported by Canan et al. (64). Hence, the fruits rich in TSS and acidity were found suitable for fresh

TABLE 2 Fruits and seed physical characteristics of promising wild edible fruits grown in the eastern Himalayas, India.

Characters	Fruit length (cm)	Fruit diameter (cm)	Fruit circumference (cm)	Fruit weight (g)	Fruit volume (cm^3)	Juice content (%)	Pulp (%)	Seed weight (g/seed)	Seed length (mm)	Seed breadth (mm)	Seeds number/ Fruits
Baccaurea sapida	2.82 ± 0.03^{cd}	3.13 ± 0.28^{bc}	9.06 ± 0.89^{c}	11.86 ± 0.77^{cd}	12.39 ± 0.81^{ef}	36.3 ± 1.92^{b}	56.69 ± 2.5^{e}	0.49 ± 0.02^{h}	11.35 ± 0.12^{e}	9.87 ± 0.07^{f}	3.00^{c}
Docynia indica	4.27 ± 0.14^{a}	3.81 ± 0.47^{ab}	10.61 ± 1.33^{b}	33.17 ± 2.68^{a}	33.3 ± 0.12^{b}	29.54 ± 2.10^{c}	78.54 ± 1.60^{b}	0.07 ± 0.01^{i}	10.45 ± 0.04^{f}	3.16 ± 0.03^{h}	4.00^{b}
Elaeagnus latifolia	3.84 ± 0.39^{ab}	2.82 ± 0.35^{cd}	8.52 ± 1.06^{d}	17.82 ± 0.70^{c}	17.92 ± 1.84^{d}	28.26 ± 3.86^{c}	68.10 ± 1.9^{d}	3.37 ± 0.04^{a}	32.25 ± 0.37^{a}	14.11 ± 0.06^{a}	1.00^{d}
Elaeagnus pyriformis	2.34 ± 0.14^{de}	2.04 ± 0.12^{de}	6.48 ± 0.38^{f}	7.32 ± 0.82^{e}	7.32 ± 0.78^{g}	29.72 ± 1.53^{c}	58.27 ± 0.7^{e}	1.06 ± 0.05^{f}	20.86 ± 0.06^{b}	12.63 ± 0.03^{d}	1.00^{d}
Haematocarpus validus	4.38 ± 0.19^{a}	3.08 ± 0.37^{bc}	8.58 ± 1.03^{d}	23.62 ± 1.76^{b}	24.28 ± 0.56^{c}	43.72 ± 1.46^{a}	76.25 ± 1.86^{bc}	2.62 ± 0.02^{b}	20.13 ± 0.48^{c}	10.58 ± 0.13^{e}	1.00^{d}
Myrica esculenta	3.14 ± 0.15^{c}	2.73 ± 0.11^{cd}	$8.13 \pm 0.31d^{e}$	13.26 ± 1.83^{cd}	14.64 ± 1.76^{de}	40.36 ± 1.47^{ab}	84.67 ± 0.53^{a}	2.03 ± 0.06^{d}	19.62 ± 0.18^{cd}	13.74 ± 0.03^{b}	1.00^{d}
Myrica nagi	1.52 ± 0.05^{f}	1.23 ± 0.08^{e}	3.64 ± 0.24^{g}	8.30 ± 0.56^{de}	9.07 ± 0.7^{fg}	21.22 ± 0.92^{d}	84.12 ± 1.97^{a}	1.32 ± 0.06^{e}	10.03 ± 0.05^{f}	0.82 ± 0.04^{i}	1.00^{d}
Prunus nepalensis	2.14 ± 0.08^{e}	2.17 ± 0.02^{d}	6.72 ± 0.05^{ef}	7.82 ± 0.10^{de}	8.23 ± 2.68^{g}	26.16 ± 0.98^{cd}	71.23 ± 1.47^{d}	2.25 ± 0.04^{c}	14.36 ± 0.17^{d}	13.52 ± 0.07^{c}	1.00^{d}
Pyrus pashia	3.71 ± 0.63^{b}	4.39 ± 0.43^{a}	12.38 ± 1.13^{a}	37.83 ± 4.75^{a}	39.89 ± 4.75^{a}	29.72 ± 2.95^{c}	71.83 ± 1.71^{cd}	0.78 ± 0.03^{g}	8.72 ± 0.03^{g}	4.65 ± 0.04^{g}	7.33 ± 24^{a}

Values given are mean ($n = 30$) with ± SE followed by different letters on each column indicate significant difference from each other according to Tukey's test ($p < 0.05$).

consumption as well as processing and value addition (65), and can be promoted for different value-added products such as ready to serve (RTS), wine, etc. as a cottage industry.

3.2. Functional attributes and antioxidant activity

3.2.1. Ascorbic acid content

Ascorbic acid is regarded as the most important antioxidant vitamin. However, it cannot be synthesized by humans due to the lack of gulonolactone oxidase enzyme, and a deficiency of dietary ascorbate results in clinical syndrome and scurvy (66). Hence, supplementing the diet with ascorbic acid-rich foods is very vital. In this study, the ascorbic acid content of wild edible fruits had significant variations (**Figure 3A**). The highest ascorbic acid content was recorded in *H. validus* (63.82 mg/100 g pulp) and the lowest in *P. pashia* (9.62 mg/100 g pulp). These results agree with the reports of Contreras-Calderón et al. (67) on the variability of vitamin C content in several wild edible fruits. Furthermore, the finding demonstrated that these wild edible fruits have a higher vitamin-C content than commercially available major fruits: *Citrus sinensis* (10.13 ± 0.10 mg/100 g), *Ananas comosus* (6.40 ± 0.18 mg/100 g), *Malus domestica* (7.94 ± 0.13 mg/100 g), and *Prunus persica* (5.92 ± 0.12 mg/100 g). However, they had a lesser content than the richest known sources of vitamin C, such as *Psidium guajava* (198.05–221.47 mg/100 g), *Phyllanthus emblica* (375.68 mg/100 g), and *Emblica officinalis* (756.32 mg/100 g) (68–70). Our results showed that the ascorbic acid content of *E. latifolia* was lower than that reported from Sikkim by Dasila and Singh (71). This variation may be attributed to different analytical methods, as reported by Dias et al. (72). The *E. pyriformis* reported in this study had a lower ascorbic acid content than that reported from Manipur (20.10 mg/100 g) by Khomdram et al. (70). The reason for variations may be due to the unique genetic make-up among genotypes and environmental factors (73), and differences in soil physico-chemical attributes such as *p*H, nutrients, and agro-ecology (74). A variation in the pH of the soil is known to determine the availability of nutrients to the roots and their uptake, which could be influenced by soil geology and climatic factors (75). A significant positive correlation of ascorbic acid with total soluble solids (0.838**) and total sugar (0.784**) was observed (**Table 4**). A high positive correlation of ascorbic acids with sugars was due to the recurring and elaborate interactions between organic acids and sugars (76), which may be associated with the synthesis of ascorbic acid from glucose (77). Ascorbic acid also showed a significant negative correlation with total antioxidant activity (−0.397*) which was represented by the IC_{50} of DPPH and analyzed by Pearson's correlation coefficient (r). It is well-established that lower IC_{50} values indicate high antioxidant activity (78). Therefore, an increase in the ascorbic acid content will enhance the antioxidant activity of these fruits, as shown by the lower IC_{50} of DPPH value. Our findings suggested that ascorbic acid may be one of the factors contributing to antioxidant properties, as evidenced by their positive relationship in a variety of other food sources (79). Hence, the daily consumption of these fruit crops will enrich the diet and act as an additional or alternative source of ascorbic acid.

TABLE 3 Biochemical characteristics of wild edible fruits grown in the eastern Himalayas, India.

Species	Moisture (%)	Total soluble solids (%)	Titratable acidity (%)	Total sugar (%)	Reducing sugar (%)
Baccaurea sapida	81.65 ± 2.83^{c}	11.97 ± 1.72^{c}	1.29 ± 0.06^{f}	5.84 ± 0.48^{c}	3.72 ± 0.46^{d}
Docynia indica	76.28 ± 0.5^{de}	8.23 ± 0.45^{g}	1.32 ± 0.03^{f}	5.42 ± 0.11^{cd}	3.81 ± 0.18^{cd}
Elaeagnus latifolia	78.35 ± 2.0^{cd}	9.4 ± 0.80^{e}	2.68 ± 0.04^{b}	3.26 ± 0.05^{e}	1.32 ± 0.03^{fg}
E. Pyriformis	80.49 ± 1.28^{bc}	11.33 ± 1.21^{cd}	2.23 ± 0.05^{d}	3.65 ± 0.05^{e}	1.87 ± 0.02^{f}
Haematocarpus validus	88.39 ± 1.85^{a}	18.27 ± 1.49^{a}	1.83 ± 0.03^{e}	11.27 ± 1.26^{a}	7.38 ± 0.54^{a}
Myrica esculenta	86.75 ± 1.33^{ab}	5.83 ± 0.30^{hi}	3.32 ± 0.06^{a}	4.27 ± 0.08^{de}	2.93 ± 0.03^{e}
Myrica nagi	83.62 ± 1.52^{bc}	6.76 ± 0.43^{h}	2.45 ± 0.04^{c}	6.83 ± 0.05^{c}	3.08 ± 0.10^{de}
Prunus nepalensis	75.26 ± 0.56^{e}	16.73 ± 0.93^{b}	1.21 ± 0.04^{f}	8.74 ± 0.60^{b}	4.46 ± 0.20^{bc}
Pyrus pashia	73.75 ± 1.88^{e}	9.38 ± 0.70^{ef}	0.31 ± 0.03^{g}	6.27 ± 0.49^{c}	4.84 ± 0.14^{b}

Values given are mean ($n = 3$) with ± SE. One-way analysis of variance (ANOVA) plus *post hoc* Tukey test was done to compare means. Superscript lowercase letters on each column designated statistical significance ($p < 0.05$).

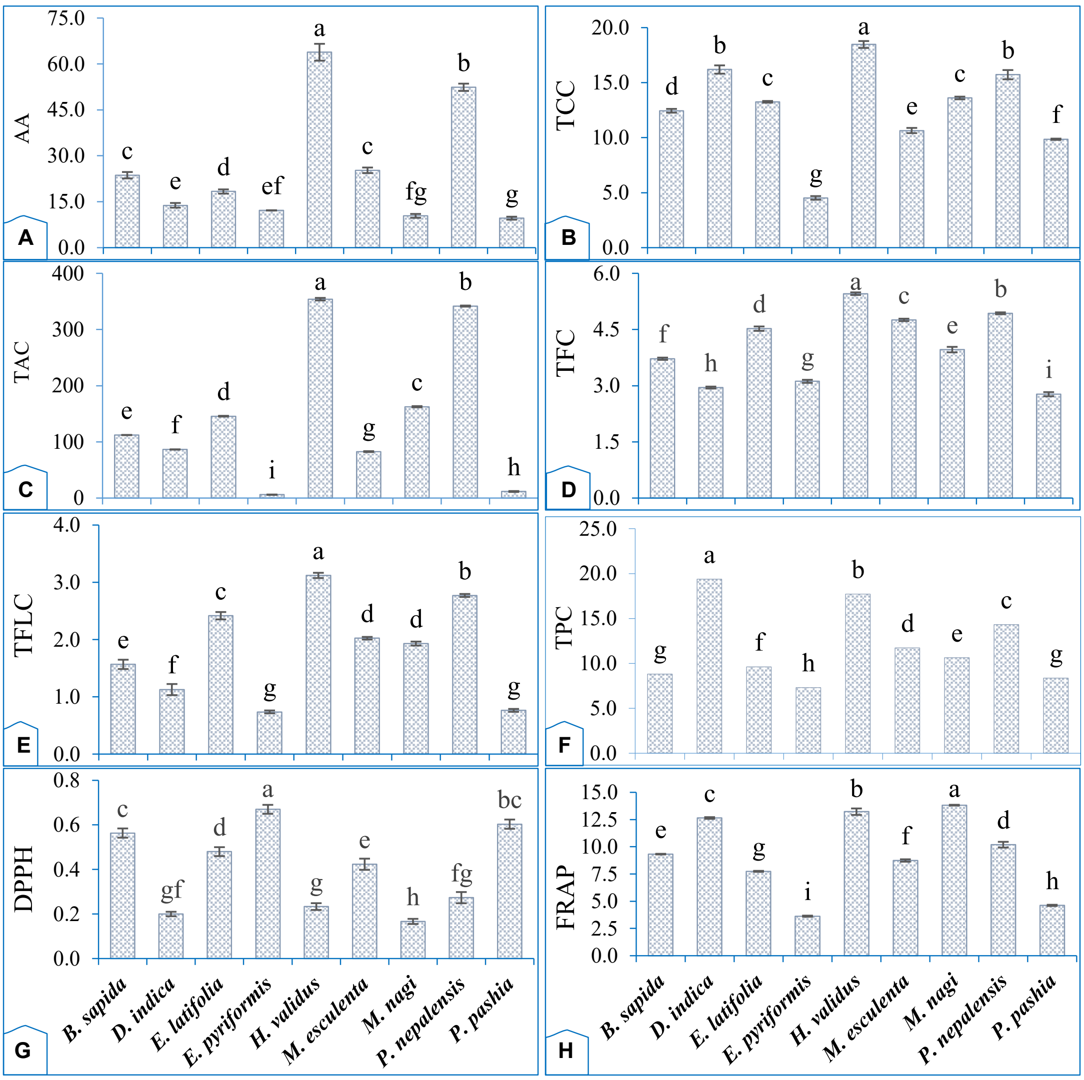

FIGURE 3
Functional attributes of wild edible fruits grown in the eastern Himalayas, India. **(A)** Ascorbic acid content (AA, mg/100 g fw); **(B)** total carotenoids content (TCC, mg/100 g fw); **(C)** total monomeric anthocyanins content (TAC, mg/100 g fw); **(D)** total flavonoids content (TFC, mg QE /g); **(E)** total flavonol content (TFLC, mg QE /g); **(F)** total phenol content (TPC, mg GAE /g); **(G)** DPPH antioxidants capacity (DDPH, IC_{50} value mg /mL); **(H)** FRAP antioxidants capacity (FRAP, mg AAE/g) content in wild edible fruits. IC_{50} ascorbic acid (0.012 ± 0.002). Mean value of three replications (each replication consisted 10 fruits) with ± S.E followed by different letters on each bar indicate significant difference from each other according to Tukey's test ($p < 0.05$).

TABLE 4 Pearson's correlation coefficient (r) among biochemical and antioxidant activities of wild edible fruits grown in the eastern Himalayas, India.

Characteristics	TSS	TSG	AA	TAC	TCC	TPC	TFC	TFLC	DPPH	FRAP
TSS	1	0.711**	0.838**	0.732**	0.407*	0.32	0.479**	0.532**	−0.117	0.134
TSG		1	0.784**	0.799**	0.678**	0.551**	0.498**	0.582**	−0.552**	0.563**
AA			1	0.899**	0.622**	0.540**	0.816**	0.821**	−0.397*	0.405*
TAC				1	0.784**	0.567**	0.824**	0.912**	−0.645**	0.635**
TCC					1	0.809**	0.541**	0.709**	−0.818**	0.851**
TPC						1	0.332*	0.431**	−0.794**	0.732**
TFC							1	0.962**	−0.427**	0.447**
TFLC								1	−0.555**	0.569**
DPPH									1	−0.932**
FRAP										1

*Significant at 0.05 level (2-tailed), **significant at 0.01 level (2-tailed). TSS, total soluble solids (%); TSG, total sugar (%); AA, ascorbic acid content (mg/100 g fw); TCC, total carotenoids content (mg/100 g fw); TAC, total anthocyanins content (mg/100 g fw); TFC, total flavonoids content (mg QE/g); TFLC, total flavonol content (mg QE/g); TPC, total phenol content (mg GAE/g); DPPH, DPPH antioxidants capacity (IC_{50} value mg/mL); FRAP, FRAP antioxidants capacity (mg AAE/g).

TABLE 5 Color, season of availability and market price of wild edible fruits grown in the eastern Himalayas, India.

Fruits	Peel pigmentation			Juice pigmentation			Season of availability	Yield per tree (kg/tree)	Market price per kg ($)
	L* value	a* value	b* value	L* value	a* value	b* value			
Baccaurea sapida	71.8 ± 10.42^{a}	$-6.500.46^{d}$	30.9 ± 4.78^{a}	35.7 ± 4.75^{d}	3.7 ± 0.32^{d}	2.5 ± 1.36^{f}	June to July	45–138	0.85
Docynia indica	54.5 ± 6.80^{b}	-6.7 ± 3.77^{d}	30.0 ± 2.55^{a}	50.3 ± 5.15^{bc}	2.4 ± 1.29^{d}	15.1 ± 0.71^{cd}	November to December	27–125	0.57
Elaeagnus latifolia	22.5 ± 2.93^{cd}	31.5 ± 3.90^{a}	$12.9 \pm 1.66b^{c}$	39.8 ± 2.08^{cd}	23.4 ± 1.9^{a}	17.6 ± 0.71^{bc}	March to May	15–117	0.50
Elaegnus pyriformis	54.5 ± 2.92^{b}	$1.8.0 \pm 2.03^{cd}$	17.7 ± 3.27^{b}	70.9 ± 8.15^{a}	11.1 ± 2.8^{c}	20.4 ± 0.40^{b}	April to May	7–26	0.21
Haematocarpus validus	16.2 ± 4.33^{de}	6.3 ± 2.12^{c}	-0.1 ± 1.22^{d}	14.5 ± 1.56^{e}	19.3 ± 3.04^{b}	6.5 ± 1.25^{e}	June to August	35–83	1.14
Myrica esculenta	54.2 ± 6.36^{b}	15.7 ± 4.29^{b}	29.6 ± 2.05^{a}	67.5 ± 2.61^{a}	1.7 ± 1.47^{e}	17.6 ± 1.29^{bc}	June to July	17–116	0.78
Myrica nagi	25.6 ± 1.57^{cd}	28.5 ± 1.51^{a}	9.3 ± 0.67^{c}	41.5 ± 3.75^{cd}	24.6 ± 2.95^{a}	13.4 ± 1.21^{d}	June to July	12–53	0.99
Prunus nepalensis	15.8 ± 1.30^{e}	2.6 ± 0.44^{cd}	-0.7 ± 0.71^{d}	8.2 ± 1.21^{e}	14.1 ± 2.15^{bc}	4.3 ± 1.90^{ef}	August to October	20–125	1.70
Pyrus pashia	33.2 ± 1.33^{c}	20.1 ± 1.91^{b}	18.1 ± 0.95^{b}	61.1 ± 2.21^{bc}	0.7 ± 0.29^{d}	31.2 ± 0.55^{a}	August to October	40–132	0.64

Mean value of three replications (each replication consisted 10 fruits) with ± S.E followed by different letters on each bar indicate significant difference from each other according to Tukey's test ($p < 0.05$). Based price of 2019, 1 USD = 70.39 INR.

3.2.2. Total carotenoid content

Carotenoids as antioxidant compounds are known to be present in several fruit crops, and the dietary intake of carotenoid-rich foods has been reported to retard cancer, cardiovascular disease, and several other ailments in humans (80). Results showed that the total carotenoids content of different wild edible fruit species varied significantly ($p < 0.05$; **Figure 3B**). *H. validus* recorded the highest total carotenoids (18.47 mg/100 g pulp), followed by *D. indica,* and the lowest total carotenoids (4.52 mg/100 g pulp) were noted in *E. pyriformis*. The fruits of *H. validus*, *D. indica,* and *P. nepalensis* contain higher total carotenoids than mangoes [4,926.76–14,942.46 μg/100 g fw, (81)] and cashews [0.4 mg/100 g fw, (82)]. The variations in genetic make-up among the species may be the cause of the variations in total carotenoids. Dias et al. (72) have also reported the great influences of varieties, maturity, cultural management, environment, postharvest care, storage conditions, and analytical methods on the formation of secondary metabolites, including the total carotenoid content in fruit crops. The high carotenoid content of these fruits is an important indicator of their quality and high nutritional value (83). In addition, our study also found a strong negative correlation between total carotenoid content and DPPH (−0.818**). The presence of high level of total carotenoids in the fruits of *H. validus, P. nepalensis, D. indica, B. sapida, E. latifolia*, and *M. esculenta* indicates their powerful ability to scavenge oxygen free radicals and active oxygen. The previous study (84) revealed that carotenoid scavenging ability would increase due to an increase in the lipophilicity of carotenoid. Lycopene was effective in reducing Fe (III) to Fe (II), given the fact that lycopene contains 11 conjugated double bonds (84). Although lycopene content was not analyzed in our study, Dasila and Singh (71) found that it was 2.5 times higher in *E. latifolia* (2.06 ± 0.38 mg/100 g) than β-carotene (0.83 ± 0.02 mg/100 g). Our results also indicated that the a* value (redness) of the peel (31.5 ± 3.90^{a}) and juice (23.4 ± 1.9^{a}) of *E. latifolia* were the highest among these wild edible fruits (**Table 5**). It is well established that the red color of certain fruits and vegetables, such as tomato, pink grapefruit, red grapes, watermelon, and red guava, is due to the presence of lycopene (85). Therefore, lycopene may be one of the major pigments responsible for the red color in the fruits of *E. latifolia*.

3.2.3. Total monomeric anthocyanin content

Anthocyanins are water-soluble and vacuolar pigments found in most species in the plant kingdom. Its accumulation mostly occurs on flowers and fruits, which impart an attractiveness to the fruit, hence; it is considered a color indicator and a natural colorant (86). It also plays a role in preventing, ameliorating, and scrubbing oxidative stress, thus retarding several diseases and physiological malfunctions (87). A significant variation ($p < 0.05$) in total monomeric anthocyanin content was observed among wild edible fruits (**Figure 3C**). The highest total monomeric anthocyanin content was recorded in *H. Validus* (354.04 mg/100 g), followed by *P. nepalensis* (341.70 mg/100 g) and the lowest was found in *E. pyriformis* (6.02 mg/100 g). These wild fruits contain more total monomeric anthocyanin than commercial fruit cultivars such as sweet cherry *cv.* Black Gold [44.19 ± 1.38 mg/100 g fw, (88)], red currants (12.14 ± 0.87 mg/100 g fw), black currant (287.78 ± 0.08 mg/100 g fw) (89), purple tomato [20.73 ± 2.86 mg/100 g fw, (90)], and guava [0.40–0.69 mg/100 g, (82)]. The varied total anthocyanin levels between species indicate genetic variations in the synthesis of these bioactive substances. Horbowicz et al. (86) have also reported the considerable variation in anthocyanin content of the fruits among different species or cultivars within the same species. This difference in anthocyanin content among these fruits might be due to the effects of genetics, agro-ecological conditions such as pH, light, temperature, and horticultural practices (91). In our study, it was also observed that the fruits with higher anthocyanin content, such as *H. Validus* (−0.7 ± 0.71) and *P. nepalensis* (−0.1 ± 1.22) had the lowest b* value (**Table 5**). Similarly, the lowest L* values were recorded in the darkest colored fruits (*H. Validus*, 16.2 ± 4.33 and *P. nepalensis,* 15.8 ± 1.30). In the previous study by Muzolf-Panek and Waskiewicz (92), it was revealed that the effect of variety was predominant in fruit peel color and that the darkest table grapes had the lowest L* values, indicating blue-black and violet-black peel in varieties of table grapes. According to Ponder et al. (93), anthocyanins are responsible for the specific dark blue color of fruit berries, and the darker the fruit, the more anthocyanins it contains. Therefore, the dark purple and blue fruit color of *H. validus* and *P. nepalensis* might be due to their high anthocyanin content. Furthermore, a significant inverse relationship (−0.645**) between total monomeric anthocyanin content and DPPH demonstrated their high antioxidant properties. Similarly, Katiresh et al. (94) found that anthocyanins in *Sesbania sesban* had high antioxidant activity and were effective at scavenging free radical DPPH. Structurally, monomeric anthocyanin possesses loose structures that are easier to undergo oxidation and thus will exhibit better antioxidant activity compared to non-monomeric anthocyanin (95). This is also in agreement with Castaneda-Ovando et al. (96), who claimed that the molecule that donates a free electron (ionization potential) or hydrogen atoms (bond dissociation energy) to the reactive free radicals is often the best antioxidant, and increasing the stability of the anthocyanin will reduce its antioxidant stability. As a result, consuming fruits with high concentrations of these compounds may provide protection to the body against various illnesses (97).

3.2.4. Total flavonoids and flavonols

Flavonoids and flavonols are naturally occurring phenolic compounds found in fruits, vegetables, and/or medicinal plants. They have significant biological effects and exhibit promising antioxidant activity due to their ability to effectively scavenge reactive oxygen species. Dietary flavonoids are recognized for their antioxidant potential, antiproliferative effects, and protective effects on lipids and vital cells against oxidative damage. These properties also play a significant role in the prevention of cardiovascular disease, inflammation, and antiproliferative or anticancer activities (98). A significant variation was also recorded in total flavonoids and flavonol content among different wild fruit species ($p < 0.05$, **Figures 3D, E**). Total flavonoid content values ranged from 2.77 ± 0.06 mg QE/g (*P. pashia*) to 5.46 ± 0.04 mg QE/g (*H. validus*). Similarly, total flavonol content also varied significantly among the studied fruits, being the maximum in *H. validus* (3.12 ± 0.05 mg QE/g) and the minimum in *E. pyriformis* (0.74 ± 0.03 mg QE/g). These fruits contained a higher level of flavonoids than most of the plants reported by Fouad et al. (99) and also higher concentrations of flavonols than *Prunus mahaleb* [1.24 ± 0.06 g/kg, (100)]. This variation in total flavonoids and flavonol content among different fruit species could be due to various intrinsic and extrinsic factors, such as genetic and environmental factors. Our results revealed a strong negative correlation of total flavonoids content (−0.794**) and total flavonol content (−0.427**) with DPPH content, which indicates that flavonoids and flavonols play an important role in the antioxidant activity of these fruits. Our study is in line with that of Chandra et al. (101), who reported that 32% of the antioxidant activity in crops was contributed by flavonoids, which constitute a major group of antioxidant compounds and act as primary antioxidants (102). The redox properties of total flavonoids were due to the unique positions of OH ortho (C-3′ and C-4′) and oxo functional groups (C-4) in flavonoids (103). Therefore, fruit trees such as *H. validus*, *M. esculenta, B. Sapida, M. nagi, E. latifolia, D. indica,* and *P. nepalensis* are rich in flavonoids and flavonol content, suggesting their consumption can help people meet their nutritional needs and protect them from developing a variety of degenerative diseases.

3.2.5. Total phenolic content

Plant-derived phenolic compounds are a diverse group of secondary metabolites that interact with reactive oxygen species to prevent oxidative damage, thereby aiding plant defense mechanisms and protecting humans from a variety of degenerative diseases (104). Our results indicated the presence of significant variations ($p < 0.05$) in total phenolic content among fruits in the following descending order: *D. indica* (19.37 ± 0.07 mg GAE/g) followed by *H. validus, P. nepalensis, M. esculenta, M. nagi*, *E. latifolia, B. sapida, P. pashia,* and *E. Pyriformis* (7.32 ± 0.11 mg GAE/g) (**Figure 3F**). Interestingly, fruits like *D. indica, H. validus, P. nepalensis, M. esculenta, M. nagi*, and *E. latifolia* had higher total phenolic content than the commercial crops of the region, such as pineapple (47.9 mg GAE/100 g), banana (7.2 ± 0.5–18.9 ± 1.4 mg GAE /g dw), and papaya (57.6 mg GAE/100 g) (105, 106). These fruits are comparable with the known richest sources of total phenolic content, such as Aonla (944.85–4,969.50 mg/100 g pulp), which are grown locally (107). According to Robards et al. (108), phenolic compounds exhibit heterogeneity in distribution and concentration across and within plant species. Furthermore, the higher phenol accumulation in the fruits under our study might depend on several factors, *viz*., agroclimatic conditions, organ, plant developmental stage, and their interaction with the genotype (10). The presence of different concentrations of sugars, carotenoids, or ascorbic acid, as well as extraction methods, may influence the amount of phenolics (109). Czyczyło-Mysza et al. (110) also suggested the importance of both additive and epistatic gene effects on total phenolic content in species, which affect other adaptation traits of the species. Total phenolic content had a positive correlation with total sugar (0.551**), ascorbic acid, DPPH (−0.794**), total monomeric anthocyanin, total carotenoids, total flavonoids, and total flavonol. According to Fitriansyah et al. (111), if the r value is $-0.61 \leq r \leq -0.9723$, it showed a high negative correlation, which indicates that TPC had a strong negative correlation with DPPH. It is well established that phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. The higher the TPC, the greater is the total antioxidant activity of these fruits as demonstrated by low IC_{50} of DPPH. Our study exhibits that TPC was one of the major contributory compounds for antioxidant activity, which was also confirmed by Nariya et al. (112) for their scavenging ability due to their unique hydroxyl groups. This indicates that these wild food resources are highly nutritious and rich sources of bioactive compounds, and their consumption will further help improve nutrition.

3.2.6. DPPH free radical scavenging activity

The free radical chain reaction is widely accepted as the most important mechanism of lipid peroxidation. Radical scavengers terminate the peroxidation chain reaction by directly counteracting and quenching peroxide radicals. The capacity of polyphenols to transport labile H atoms to radicals is a probable mechanism of antioxidant protection, which can be assessed universally and rapidly using DPPH. Furthermore, DPPH is the most common and cost-effective way to determine the free radical scavenging capacity of natural products, which are major factors in biological damage caused by oxidative stress (113). Our results revealed a significant variation ($p < 0.05$) in DPPH free radical scavenging activity among the studied fruits (**Figure 3G**), and it ranges from (0.17 ± 0.01 IC_{50} mg/mL) in *M. nagi* to 0.67 ± 0.02 IC_{50} mg/mL in *E. pyriformis*. The present results showed lesser values than those recorded in commercial fruits such as grapes (0.79 ± 0.34 IC_{50} mg/mL), pineapple (0.83 ± 0.24 IC_{50} mg/mL), and guava (1.71 ± 0.61 IC_{50} mg/mL) (79). The antioxidant capacity of fruits and vegetables was influenced by factors such as genetic makeup, maturity, and other environmental factors such as sunlight exposure, soil, and the gene-environment interaction (10). According to Matuszewska et al. (78), the lower IC_{50} values of DPPH indicate a high level of antioxidant activity, which means that these fruits, *viz*., *M. nagi, D. indica, H. validus,* and *P. nepalensis* with a low IC_{50} value can scavenge the DPPH radicals to form a stable reduced DPPH molecule. The high accumulation of total sugar, ascorbic acid, total monomeric anthocyanin, total carotenoids, total phenolics, total flavonoids, and total flavonol content increases the antioxidant activity, as demonstrated by the lower IC_{50} of DPPH value in our result, which agreed with the finding of Sundaramoorthy and Packiam (114). Therefore, the high antioxidant activity of these fruits might be due to a strong negative correlation of different compounds with IC_{50} DPPH. Previous studies have also found that the antioxidant activity in plant tissue was mainly due to the unusual redox properties of not just one particular compound but also of different bioactive compounds including TPC, tannin, anthocyanin, TFC, phenols, alkaloids, and pro-anthocyanins (115, 116). The antioxidant effect is due to the ability of compounds in the plant extract to transfer electrons or hydrogen atoms to neutralize radicals of DPPH and form neutral DPPH molecules (117). Hence, it is clear from our results that these fruit crops had a greater potential for radical scavenging compounds with proton-donating abilities.

3.2.7. FRAP reducing power

FRAP antioxidants capacity is a simple and inexpensive assay that offers a putative index of the potential antioxidant activity of plant materials. Principally, the FRAP assay treats the antioxidants in the sample as reductants in a redox-linked colourimetric reaction. The reducing power assay, i.e., the transformation of Fe^{3+} to Fe^{2+} in the presence of either the extract or the standard (ascorbic acid), is a measure of reducing capability (79). A significant variation ($p < 0.05$) of FRAP reducing power was observed among the underutilized fruits studied, and it ranged from 3.63 ± 0.05 mg AAE/g in *E. pyriformis* to 13.82 ± 0.04 mg AAE/g in *M. nagi* (**Figure 3H**). These results indicated higher FRAP values in these fruits than in the other wild fruits reported (0.0518 ± 0.49 to 0.111 ± 0.00 mg AAE/g) by Mahadkar et al. (118) from central India. The differences in antioxidant content between species may be due to genetics and environmental factors, as well as their interactions. Our result showed a strong positive correlation of FRAP value with total sugar (0.563**), ascorbic acid (0.405*), total monomeric anthocyanin (0.635**), total carotenoids (0.851**), total phenolics (0.732**), total flavonoids (0.447**), total flavonol (0.569**), and inversely related to DPPH (IC_{50}, −0.932**) (**Table 4**). The finding that the DPPH and FRAP assays of fruit extracts were highly correlated agrees with the work of Szydłowska-Czerniak et al. (119) and is consistent with the view that the two assays share a similar mechanistic basis, *viz*., transfer of electrons from the antioxidant to reduce an oxidant, as proposed by Huang et al. (120). The total carotenoids and total phenolic content were the major compounds contributing to the antioxidant activity in our study. Previous report in amla fruits (*Emblica officinalis* Gaertn) showed that carotenoids had reduction potential lower than 0.44 V, allowing them to reduce Fe (III) to Fe (II) while also being oxidized and acting as antioxidants (84). Similarly, the phenolic compounds largely contribute to the antioxidant activities of these species and therefore could play an important role in the beneficial effects of these fruits. Several studies have found that phenolic compounds are major antioxidant constituents in selected plants and that there are direct relationships between their antioxidant activity and total phenolic content (103). The antioxidant properties of phenolic compounds are directly linked to their unique structure, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (121). This demonstrated that most of these underutilized fruits have strong reducing capabilities as compared to other fruit crops, which might be due to the presence of high total carotenoids, total phenolic content, and other functional compounds that are responsible for their antioxidant activity (122). In general, among the fruits, *M. nagi* showed relatively stronger FRAP activity than other fruits.

FIGURE 4
Different products developed by farmers from wild edible fruits grown in the eastern Himalayas, India. **(A)** Jam of Sohiong (*P. nepalensis* Ser.); **(B)** Squash of *P. nepalensis*; **(C)** Juice of *P. nepalensis*; **(D)** Juice of Sohphie (*M. esculenta*); **(E)** Wine of *P. nepalensis*; **(F)** pickle of Sohphoh (*D. indica*); **(G)** Mixed pickles of *P. nepalensis* and *E. latifolia*; **(H)** pickle of Sohshang (*E. latifolia*).

3.3. Color, season of availability and market price of fruits

These fruits have appealing pigments, both in the peel and juice, as evidenced by a significant variation in value of L*, a* and b* in both peel and juice color ($p < 0.05$, Table 5). *B. sapida* had the highest peel L* value (71.80 ± 10.42a) and b* value (30.90 ± 4.78a), while *E. latifolia* had the highest peel a* value (31.50 ± 3.90a). Similarly, the yellowness and redness of these fruits were found to be higher than many of the Indian commercial mango varieties (123). The color variation (L*, a, b) among the wild edible fruit trees might be due to a genetic effect. It is well established that the L* value is a suitable indicator of darkening that arises either from increasing pigment concentrations or from oxidative browning reactions (124). Furthermore, the higher a* and b* values added a decorative effect toward the consumer's preference. These fruits can be a good potential source for the extraction of natural edible color that is required in the food industry. As per Deka et al. (125), the products prepared from *P. nepalensis*, such as squash and jam, develop an attractive color and remain stable for 1 year (**Figure 4**). They have also prepared ready-to-serve (RTS) products and cherry wine from *P. nepalensis* fruits, which impart a unique natural purple color (13). The suitability of products for processing and extracting natural color helps stabilize the market price. The results also showed that the season of availability of fruits varies from plant to plant. The fruits of these wild edible fruit plants were found to be available throughout the year, with the exception of January and February. The different harvesting periods of these wild edible fruits ensure the year-round availability of these fruits, and particularly during the lean season when other fruits are not available, they provide supplementary food and nutritional security in the region. The yield of wild edible fruit trees varies between 7–26 kg per tree in *E. pyriformis* and 45–138 kg per tree in *B. sapida*. The variation in season of fruit availability and yield among wild edible fruit trees might be due to the contribution of genetic makeup and the growing environment (126). Similarly, the market price of fruits ranged from $ 0.21 per kg in *E. pyriformis* to $ 1.7 per kg in *P. nepalensis*. The variation in market price among wild edible fruits might be due to the contribution of fruit quality factors such as taste, TSS-acidity blend, peel appearance, etc., which determine the appealability and preferences amongst consumers. Tarancon et al. (127) also reported that the consumer's perception of fruit quality is exclusively based on appearance. The market price of the fruits of *P. nepalensis* was about $34.10–$141.5 per tree, which highlights the high potential for income generation from these wild fruit trees. Therefore, expansion of the commercial area under these crops and their utilization may offer an additional source of income, employment generation, and livelihood improvement.

4. Conclusion

About 12% of the world's population lives in mountainous regions. Wild edible fruits have been consumed by the mountainous populace since time immemorial. Many of these genetic resources, however, have become rare and endangered as a result of overexploitation in their natural habitat and a lack of consumer understanding of their antioxidant and biochemical values. Our results would aid in a proper understanding of the potential uses and antioxidant activities of wild edible fruit trees. Therefore, it is concluded that:

Wild fruits such as *H. validus, P. nepalensis, B. sapida, E. latifolia, M. esculenta, and D. indica* are high in total soluble solids, total sugar, and acidity. These fruits have the potential to be used as supplementary bases in the fruit processing industry.

The high antioxidant activities such as ascorbic acid, total phenolic content, total flavonoid, total flavonol, DPPH free scavenging capacity, and FRAP reducing power in *H. validus, P. nepalensis, M. esculenta,* and *M. nagi* suggest their potential as sources of bioactive compounds.

These fruits can be used to extract attractive natural colors and to make high-value processed products such as jams, squash, pickles, and wine.

A proper understanding of the biochemical and antioxidant properties of these fruits will help in their sustainable utilization and conservation.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HR: laboratory studies, design of the figures, and writing the manuscript. VV: data analysis and interpretation of finding. HT, V, RS, and KB: editing of the manuscript. SA, MD, LC, and BM: correction of manuscript. JM, ARS: data collections, laboratory studies, and design of the figures. SH and VM: discussion of the results, critical feedback, and providing help in shaping the content, and evaluation of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Integrating conservation agriculture with intensive crop diversification in the maize-based organic system: Impact on sustaining food and nutritional security

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Introduction: Developing an intensive sustainable model and feeding a rising population are worldwide challenges. The task is much more daunting in the North Eastern Himalayas, where, low productive maize (*Zea mays*)- fallow is the main production system in the upland. To increase farm productivity, nutritional security, and energy dietary returns while maintaining environmental sustainability and economic viability, short-duration crops must be included in the maize–fallow system.

Methods: A field study was conducted in sandy clay loam soil with a randomized complete block design with three replications for three continuous years (2018–2021) under organic management with two crop management practices, *viz.*, (i) conservation agriculture and (ii) conventional agriculture, and six crop diversification options, *viz.*, (i) maize–sweet corn (*Zea mays saccharata*)–vegetable pea (*Pisum sativa*) (M-SC-VP), (ii) maize–sweet corn-mustard (*Brassica juncea*) (M-SC-M), (iii) maize–sweet corn–lentil (*Lens culinaris*) (M-SC-L), (iv) maize–sweet corn–vegetable broad bean (*Vicia faba*) (M-SC-VB), (v) maize (local)–vegetable pea (M-VP), and (vi) maize (local)–fallow (M-F).

Results: The results showed that, the average system productivity was 5.3% lower for conventional agriculture than conservation agriculture. System carbohydrate, protein, fat, dietary fiber, and dietary energy were ~6.9, 6.8, 7.8, 6.7, and 7%, higher in conservation agriculture than in conventional agriculture, respectively. Similarly, system macronutrients (Ca, Mg, P, and K) and system micronutrients yield (Fe, Mn, Zn, and Cu) were, 5.2–8% and 6.9–7.4% higher in conservation agriculture than in conventional agriculture, respectively. On average, over the years, crop diversification with M-SC-VP/M-SC-VB intensive crop rotation had higher system productivity (158%), production efficiency (157%), net returns (benefit–cost ratio) (44%), and dietary net energy returns (16.6%) than the local maize–vegetable pea system. Similarly, the M-SC-VP/M-SC-VB system improved the nutritional security by improving Ca, Mg, P, K, Fe, Mn, Zn, and Cu yield by 35.5–135.7% than the local M-VP system.

Discussion: Conservation agriculture with M-SC-VP/M-SC-VB rotation showed significantly (p<0.05) higher productivity, carbohydrate yield, protein yield, fat yield, and dietary fiber production. It is concluded that conservation agriculture improved soil health and performed better than conventional agriculture in maize-based intensive cropping systems. Overall results indicate that crop diversification with M-SC-VP/M-SC-VB can potentially increase calorie and protein consumption and farm profitability.

KEYWORDS

conservation agriculture, crop intensification, dietary energy returns, food security, production economics, nutritional security

1. Introduction

Increasing environmental crises and resource degradation had a deleterious effect on food and nutritional security throughout the world. The most significant barrier to attaining sustainable development goals (SDGs), like environmental sustainability and societal wellbeing, is the detrimental impact of poor agricultural production management on ecosystem integrity (1). As a direct consequence of this, both researchers and policymakers are confronted with significant obstacles in their efforts to simultaneously achieve their goals (SDGs) in the areas of food, nutrition, and socioeconomic development. The North Eastern Himalayan (NEH) Region of India is a habitat of ~50 million people, is suffering from low-agriculture production as a result of massive soil degradation and inadequate agronomic management (2–4). In this region, large-scale adoption of mono-cropping generally results in yield stagnation (5), low farm income, and poor resource utilization (6, 7). Maize (*Zea mays* L.)-based cropping system is the second most significant food crop after rice, contributing greatly to household food and nutrition security and livelihoods for low-to middle-income rural and urban populations. These practices at farmers' fields have less productivity due to poor crop and land management. However, as more land for expanding agriculture is not readily available in these regions, it is becoming immensely important to make use of existing fallow land through diversification and intensification with the adjustment of more crops in cropping sequence. There is an opportunity to improve cropping systems and implement triple cropping rotations in a year, notably by incorporating short-duration sweet corn in the double cropping of maize–legumes/oilseeds. Although most research on cropping systems and conservation agriculture practices in the NEH region focuses on productivity enhancements, nutritional security improvement through efficient intensive sustainable cropping systems for smallholder farming households and rural communities still has scope for research (6, 8).

In the NEH region, rice/maize-based cropping systems are used with extensive tillage (puddling for rice and recurrent tillage in winter crops) and the burning or total removal of crop residues from the field. Maize is grown with moderate to minimal tillage on slopes/terraces. Intensive tillage practices and improper crop management can negatively influence soil quality and potentially cause soil degradation (6, 9, 10). More resource-efficient practices, such as conservation agriculture with full retention of crop residue and diversified crop rotations, are receiving widespread support as potential solutions for lowering the consumption of non-renewable resources, reversing soil deterioration, and restoring soil quality, all while cutting emissions (11–14). Organic intensive cropping system with conservation practices enhanced crop establishment and provision for timely sowing, maintaining, or increasing per unit productivity, lowering production costs, and increasing net returns along with ensuring nutritional security, potential energy availability, and system resilience (15–17), which ensures to achieve little toward the SDGs-3 (good health and wellbeing) and-12 (zero hunger; responsible consumption and production).

The NEH region is best suited for organic farming since the synthetic fertilizers load on the soil is minimal. Agricultural crop residues and organic manure have enormous potential to restore soil health through organic conservation practices (18–20). NEH region produced 2.55 million tonnes of agricultural biomass and has 2.98 million bovines, which stimulates organic crop production. Over the last several decades, there has been a growing emphasis on using conservation agriculture approaches in organic production systems to reduce soil erosion, enhance soil quality, preserve, boost crop yield and nutritional security, and maintain environmental quality (21–24).

In addition, the government of India is placing a strong emphasis on the promotion of organic and natural farming practices. As part of this initiative, national programs such as the Paramparaghat Krishi Vikas Yojana (PKVY; Traditional Agricultural Development Plan), the Rashtriya Krishi Vikas Yojana (RKVY; National Agricultural Development Plan), and the Mission Organic Value Chain Development for North Eastern Regions (MOVCD-NER) are currently being carried out in these regions. In 2023, the Government of India launched the program, i.e., PM PRANAM Yojana (Prime Minister Program for Restoration, Awareness, Nourishment, and Amelioration of Mother earth) to incentivize alternative fertilizers for the promotion of organic farming. Furthermore, the Government of India aims to set up 500 "waste to wealth plants" under GOBAR-DHAN Scheme to convert organic waste into valuable organic nutrient inputs. It is the goal of this initiative to increase the amount of land that is farmed organically by making use of the organic

Abbreviations: NEH, North Eastern Himalaya; M-SC-VP, Maize–sweet corn–vegetable pea; M-SC-M, Maize–sweet corn–mustard; M-SC-L, Maize–sweet corn–lentil; M-SC-VB, Maize–sweet corn–vegetable broad bean; M-VP, Maize–vegetable pea; M-F, Maize–fallow; PM PRANAM, Prime Minister Program for Restoration, Awareness, Nourishment, and Amelioration of Mother earth; GOBAR-DHAN, Galvanizing Organic Bio-Agro Resources-DHAN; PKVY, Paramparaghat Krishi Vikas Yojana (Traditional Agricultural Development Plan); RKVY, Rashtriya Krishi Vikas Yojana (National Agricultural Development Plan); MOVCD-NER, Mission Organic Value Chain Development for North Eastern Regions; SDGs, Sustainable development goals; MEPE, Maize equivalent production efficiency; MEY, Maize equivalent yield; PEA, Potential energy availability; Gj, Giga joule; kcal, Kilo calorie.

resources that are already in existence, such as manures from livestock, cropping system diversification that includes green manuring, crop residue utilization for soil health restoration, maintaining crop-livestock interactions and crop productivity, and lowering the levels of pollution in the water and air. Because of this, the findings of the current study on the impact of the organic conservation agriculture approach will make it possible for policymakers in the NEH region to put into practice agricultural methods that are efficient.

Few studies have been conducted to analyze the effects of utilizing various types of tillage practices while using double cropping systems, comparing the various conservation agriculture techniques (6, 8). However, robust studies especially on potential nutrition and energy availability, dietary energy returns, or profitability comparing conservation agriculture to conventional agriculture under intensive organic crop diversification systems across the region are lacking. These comparisons would be useful for determining whether conservation agriculture or conventional agriculture is more profitable. A better knowledge of the impacts that conservation agriculture has on crop production, profitability, and nutritional security will assist and explain the performance of these systems and identify the ones that are the most productive and efficient in the region. In light of this, the purpose of this study was to evaluate the organic intensive crop diversification that is the most resilient and sustainable in order to assure the maximum levels of production, profitability, and nutritional security while using the organic conservation agriculture approach in comparison to conventional agriculture.

2. Materials and methods

2.1. Description of the site and soil characteristics

The location of the experimental site was in the NEH region of the Indian state of Manipur. Most of these regions' soils are composed of sedimentary rocks, with parent materials originating from the Disang (Eocene) and Barail (Oligocene) groups of sandstone and shale. Intermontane valleys have 2.23 million hectares (~12% of Manipur's entire geographical area) of cultivable land. During the entire rainy season (April to October), rainfed cereals are mainly grown in the hill and foothill ecosystem (primarily rice and maize) as mono-cropping, with only minor periodic replenishment of plant nutrients from external organic or inorganic sources (5).

The experiment was carried out for three continuous years (2018–2021) at Lapmhel Research Farm (24°49' N latitude, 93°55' E longitude, and 786 m above MSL altitude) of the ICAR NEH Region, Manipur Centre, Imphal, India. The research site's climate is subtropical humid, with a mean (3 years) minimum and maximum temperature variations of 6.2–22.2 and 22.2–30.0°C, respectively. The minimum and maximum relative humidity average varied from 40.0 to 69.9% and 84.6 to 90.9% during experimentation (Supplementary Figure S1). The experimental period of 2018, 2019, and 2020 had a total yearly rainfall of 1326.3, 1147.0, and 1328.9 mm, respectively. The soil at the location of the experiment had the consistency of sandy clay loam. Supplementary Table S1 provides information on the various soil properties that were present at the beginning of the experimentation (0–0.15 m).

2.2. Treatment detail and agronomic crop management

The field experiment was conducted in a randomized completely block design (RCBD) with three replications for three continuous years (2018–2021) under organic management with two crop management practices, *viz.*, (i) conservation agriculture and (ii) conventional agriculture, and six crop diversification, *viz.*, (i) maize (*Zea mays*)–sweet corn (*Zea mays saccharata*)–vegetable pea (*Pisum sativa*) (M-SC-VP), (ii) maize–sweet corn–mustard (*Brassica juncea*) (M-SC-M), (iii) maize–sweet corn–lentil (*Lens culinaris*) (M-SC-L), (iv) maize–sweet corn–vegetable broad bean (*Vicia faba*) (M-SC-VB), (v) maize (local)–vegetable pea (M-VP), and (vi) maize (local)–fallow (M-F). In experimental plots of conventional agriculture, four tilling operations were carried out, i.e., two passes of tillage with harrow and two passes of cultivator followed by planking. The crop residues were completely removed from the conventional agriculture plot. In conservation agriculture, reduced tillage operation was maintained with crop residues. In this plot, only one tilling operation was carried out followed by planking with crop residue retention. Except for sweet corn, the above-ground crop leftovers and retained in the field after the economic parts of the crops were collected. Sweet corn biomass is used for livestock feed. The details of the package and practices are given in Table 1.

2.3. Computation of system productivity

System productivity and maize equivalent production efficiency in terms of maize equivalent yield was computed using Eqs. (1, 2)

$$\text{MEY}(\text{Mg/ha}) = X + \frac{(y^{*}P2)}{P1} \quad (1)$$

where X: maize grain yield (Mg ha^{-1}), Y: grain/cob/pod yield of other crops, *viz.*, sweet corn, vegetable pea, mustard, lentil, and broad bean (Mg ha^{-1}), P1: selling price of other crops (INR Mg^{-1}), and P2: selling price of maize (INR Mg^{-1}).

Maize equivalent production efficiency (MEPE) was computed by the following formula:

$$\text{MEPE} = \text{MEY}\left(\text{Mg ha}^{-1}\right) / \text{Duration cropping system}(\text{days}) \quad (2)$$

2.4. Computation of carbohydrate, protein, fat, dietary fiber, and nutrients yield

Values of carbohydrate yield, protein yield, fat yield, dietary fiber yield, and nutrient yield are provided in Table 2. The corresponding values were multiplied by the grain yield of respective crops and the system yield was obtained after summation in respective cropping

TABLE 1 Crop-wise package of practices under organic management.

S. No.	Particulars	Maize	Sweet corn	Vegetable pea	Mustard	Lentil	Broadbean	Maize (local)
1.	Variety	HQPM-1	Hi Brix-39	Arkel/Makhyatmubi (local)	M-27	HUL-57	Local Hawaimubi	Local Chaochujak
2.	Seed rate	20 kg ha^{-1}	8 kg ha^{-1}	80 kg ha^{-1}	5 kg ha^{-1}	40 kg ha^{-1}	100 kg ha^{-1}	20 kg ha^{-1}
3.	Spacing	60 × 30 cm	60 × 30 cm	40 × 20 cm	40 × 15 cm	30 × 10 cm	30 × 15 cm	60 × 30 cm
4.	Nutrient management	70 kg N from FYM @ 22.58 Mg ha^{-1}, 10 kg N from vermicompost @ 1.69 Mg ha^{-1} and 20 kg N from biofertilizers/neem cake (Azotobacter @ 10 kg ha^{-1} + Phosphate solubilizing bacteria @ 10 kg ha^{-1} + Trichoderma @ 5 kg ha^{-1} + Neem cake @ 200 kg ha^{-1})	70 kg N from FYM @ 22.58 Mg ha^{-1}, 10 kg N from vermicompost @ 1.69 Mg ha^{-1} and 20 kg N from biofertilizers/neem cake (Azotobacter @ 10 kg ha^{-1} + Phosphate solubilizing bacteria @ 10 kg ha^{-1} + Trichoderma @ 5 kg ha^{-1} + Neem cake @ 200 kg ha^{-1})	14 kg N from FYM @ 4.52 Mg ha^{-1}, 2 kg N from vermicompost @ 0.34 Mg ha^{-1} and remaining 4 kg N from biofertilizers/neem cake (Azotobacter @ 10 kg ha^{-1} + Phosphate solubilizing bacteria @ 10 kg ha^{-1} + Trichoderma @ 5 kg ha^{-1} + Neem cake @ 200 kg ha^{-1})	28 kg N from FYM @ 9.03 Mg ha^{-1}, 4 kg N from vermicompost @ 0.68 Mg ha^{-1} and remaining 8 kg N from biofertilizers/neem cake (Azotobacter @ 10 kg ha^{-1} + Phosphate solubilizing bacteria @ 10 kg ha^{-1} + Trichoderma @ 5 kg ha^{-1} + Neem cake @ 200 kg ha^{-1})	14 kg N from FYM @ 4.52 Mg ha^{-1}, 2 kg N from vermicompost @ 0.34 Mg ha^{-1} and remaining 4 kg N from biofertilizers/neem cake (Azotobacter @ 10 kg ha^{-1} + Phosphate solubilizing bacteria @ 10 kg ha^{-1} + Trichoderma @ 5 kg ha^{-1} + Neem cake @ 200 kg ha^{-1})	14 kg N from FYM @ 4.52 Mg ha^{-1}, 2 kg N from vermicompost @ 0.34 Mg ha^{-1} and remaining 4 kg N from biofertilizers/neem cake (Azotobacter @ 10 kg ha^{-1} + Phosphate solubilizing bacteria @ 10 kg ha^{-1} + Trichoderma @ 5 kg ha^{-1} + Neem cake @ 200 kg ha^{-1})	70 kg N from FYM @ 22.58 Mg ha^{-1}, 10 kg N from vermicompost @ 1.69 Mg ha^{-1} and 20 kg N from biofertilizers/neem cake (Azotobacter @ 10 kg ha^{-1} + Phosphate solubilizing bacteria @ 10 kg ha^{-1} + Trichoderma @ 5 kg ha^{-1} + Neem cake @ 200 kg ha^{-1})
5.	Pest management	Seed treatment and soil application of Trichoderma harzianum @ 5 kg/ha, Pheromone traps @ 20 traps/ha, Prophylactic application of organic formulations such as Neem oil/Nimbicidine @ 1 ml litre^{-1}	Seed treatment and soil application of Trichoderma harzianum @ 5 kg/ha, Pheromone traps @ 20 traps/ha, Prophylactic application of organic formulations such as Neem oil/Nimbicidine @ 1 ml litre^{-1}	Seed treatment and soil application of Trichoderma harzianum @ 5 kg/ha, Pheromone traps @ 20 traps/ha, Prophylactic application of organic formulations such as Neem oil/Nimbicidine @ 1 ml litre^{-1}	Seed treatment and soil application of Trichoderma harzianum @ 5 kg/ha, Pheromone traps @ 20 traps/ha, Prophylactic application of organic formulations such as Neem oil/Nimbicidine @ 1 ml litre^{-1}	Seed treatment and soil application of Trichoderma harzianum @ 5 kg/ha, Pheromone traps @ 20 traps/ha, Prophylactic application of organic formulations such as Neem oil/Nimbicidine @ 1 ml litre^{-1}	Seed treatment and soil application of Trichoderma harzianum @ 5 kg/ha, Pheromone traps @ 20 traps/ha, Prophylactic application of organic formulations such as Neem oil/Nimbicidine @ 1 ml litre^{-1}	Seed treatment and soil application of Trichoderma harzianum @ 5 kg/ha, Pheromone traps @ 20 traps/ha, Prophylactic application of organic formulations such as Neem oil/Nimbicidine @ 1 ml litre^{-1}
6.	Weed management	Two manual weeding at 25 and 50 days after sowing	Two manual weeding at 25 and 50 days after sowing	Two manual weeding at 25 and 50 days after sowing	Two manual weeding at 25 and 50 days after sowing	Two manual weeding at 25 and 50 days after sowing	Two manual weeding at 25 and 50 days after sowing	Two manual weeding at 25 and 50 days after sowing
7.	Water management	--	--	Two live saving irrigation at pre flowing and pod development stage	Two live saving irrigation at pre flowing and pod development stage	Two live saving irrigation at pre flowing and pod development stage	Two live saving irrigation at pre flowing and pod development stage	--

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TABLE 2 A total of 100g of seeds contain nutrients from various crops.

Particulars	Maize	Sweet corn	Vegetable pea	Lentil	Mustard	Broadbean
Moisture%	10.4	76.0	78.9	8.3	8.0	81.0
Carbohydrate (%)	74.3	18.7	14.4	63.4	28.1	11.7
Protein (%)	9.42	3.27	5.42	24.60	26.08	5.60
Fat (%)	4.74	1.35	0.40	1.06	36.24	0.60
Dietary Fiber (%)	7.30	2.00	5.60	10.70	12.20	4.20
Ca/mg	7.0	2.0	25.0	35.0	266.0	22.0
Mg, mg	127.0	37.0	33.0	47.0	370.0	38.0
P, mg	210.0	89.0	108.0	281.0	828.0	95.0
K, mg	287.0	270.0	244.0	677.0	738.0	250.0
Fe, mg	2.71	0.52	1.47	6.51	9.21	1.90
Mn, mg	0.49	0.16	0.41	1.39	2.45	0.32
Zn, mg	2.21	0.46	1.24	3.27	6.08	0.58
Cu, mg	0.31	0.05	0.18	0.75	0.65	0.07
Energy, calories	377.5	100.0	82.9	361.5	542.8	74.6
Reference	(25)	(26)	(27)	(28)	(29)	(30)

systems. System energy production was calculated from carbohydrate, protein, and fat by multiplying by 4, 4, and 9, respectively (31).

2.5. Potential energy availability

Potential energy availability (PEA) is computed by the following formula:

$$\text{PEA (Person/ha/year)} = \text{System energy yield (Kcal)} / \text{average requirement of energy per person} \quad (3)$$

where the energy requirement for men is 2,710 kCal, for women is 2,130 kCal, and the average is 2,420 kCal, which is considered for the PEA calculation (32).

2.6. Computation of economics

For economic analysis, the total cost of production, the total return from the system's outputs (main and by-products), gross return, benefit-to-cost ratio, and dietary energy returns were computed using Eqs. (4–7), where the total variable cost of production was considered as the total cost of production. The minimum support price (MSP) was considered as per the prevailing market price in INR (Indian rupees) for maize, sweet corn, vegetable pea, mustard, lentil, and broad bean. The details of prices for accounting of economics are presented in Supplementary Table S2.

$$\text{Gross retrun}\left(\text{GR, INR ha}^{-1}\right) = \text{System output}\left(\text{Mg ha}^{-1}\right) \times \text{output price}\left(\text{INR Mg}^{-1}\right) \quad (4)$$

$$\text{Net return}\left(\text{NR, INR ha}^{-1}\right) = \text{System output}\left(\text{Mg ha}^{-1}\right) - \text{Total cost of production}\left(\text{INR ha}^{-1}\right) \quad (5)$$

$$\text{Benefit to cost ratio (BCR)} = \text{Total return}\left(\text{INR ha}^{-1}\right) / \text{Total cost of production}\left(\text{INR ha}^{-1}\right) \quad (6)$$

$$\text{Dietary energy returns}\left(\text{Kj INR}^{1}\text{investment}\right) = \text{Energy yield}\left(\text{Kj ha}^{-1}\right) / \text{Total cost of production (INR/ha)} \quad (7)$$

2.7. Data analysis

The data from grain yield and carbohydrate yield, protein yield, fat yield, dietary fiber yield, and nutrients yield were processed for analysis of variance (ANOVA) in a factorial RCBD using R version 9.2 to examine the statistical significance of the treatments (crop management and crop diversification). Using SPSS version 16.0, the LSD of the mean was calculated using Duncan's multiple range test (DMRT) ($p < 0.05$).

3. Results

3.1. System productivity and production efficiency

Averaged over the 3 years (2018–2021), conservation agriculture significantly ($p < 0.05$) produced 5.3% higher system productivity

(15.8 Mg ha^{-1}) than conventional agriculture (15.0 Mg ha^{-1}). Among the crop diversification options higher system productivity was recorded in M-SC-VB (22.8 Mg ha^{-1}) ≥ M-SC-VP (22.6 Mg ha^{-1}) than the popularized cropping system on farmers' fields as maize–fallow (M-F: 3.2 Mg ha^{-1}) and maize–vegetable pea (local) (M-VP; 8.8 Mg ha^{-1}) (Table 3). Conservation agriculture enhanced production efficiency by 5.1% than conventional agriculture. On average, the highest production efficiency was recorded in M-SC-VB (62.5 kg ha^{-1} day^{-1}) followed by M-SC-VP (61.9 kg ha^{-1} day^{-1}) than the popularized cropping system of M-VP (8.7 kg ha^{-1} day^{-1}) and M-F (3.7 kg ha^{-1} day^{-1}; Table 3).

3.2. Dietary carbohydrate, protein, fat, and fiber yield

Averaged over the years, conservation agriculture significantly recorded 6.8, 6.9, 7.8, and 6.7% higher dietary carbohydrate, protein, fat, and fiber yield than conventional agriculture (Figure 1). Among the cropping system, the highest dietary carbohydrate (6727.4 kg ha^{-1}), dietary protein (1168.5 kg ha^{-1}), and dietary fiber (956.5 kg ha^{-1}) are observed in M-SC-VP followed by M-SC-VB cropping system, while highest dietary fat was obtained in M-SC-M cropping system and it was comparable with M-SC-VB and M-SC-VP (Figure 1). The minimum dietary carbohydrate, protein, fat and fiber yield were recorded in M-F and M-VP than other cropping systems, where additional sweet corn adjusted in system.

3.3. Dietary essential mineral yield

Cropping system diversification with sustainable agronomic management practices significantly influenced the nutritional yield of Calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu). Conservation agriculture enhanced the essential minerals yield of Ca, Mg, P, K, Fe, Mn, Zn, and Cu by 8.0, 7.0, 5.6, 5.2, 7.4, 6.9, 7.2, and 7.4% than conventional agriculture (Figures 2, 3). Among cropping systems, the highest essential nutrients harvest, *viz.*, P (33.4×10^6 mg ha^{-1}), K (54.8×10^6 mg ha^{-1}), Fe (29.6×10^4 mg ha^{-1}), Mn (6.9×10^4 mg ha^{-1}), Zinc (24.7×10^4 mg ha^{-1}), and Cu (3.42×10^4 mg ha^{-1}) were obtained in M-SC-VP cropping rotation, except for Ca (3.05×10^6 mg ha^{-1}) and Mg (13.5×10^6 mg ha^{-1}), which were obtained in M-SC-M cropping rotation. The minimum essential nutrient production was obtained in M-F and M-VP cropping rotations (Figures 2, 3).

3.4. Energy production and potential energy availability

Conservation agriculture practices (122.5 Gj ha^{-1}) recorded the highest dietary energy production than conventional agriculture (114.5 Gj ha^{-1}) (Figure 4). Consequently, in the same treatment, the higher potential energy availability was recorded for 12,094 persons ha^{-1} $year^{-1}$ than conventional agriculture (11,310 persons ha^{-1} $year^{-1}$). Among the cropping diversification, the highest dietary energy production was recorded in M-SC-VP (153.9 Gj ha^{-1}) followed by M-SC-VB (146.3 Gj ha^{-1}; Figure 4). M-SC-VP cropping rotation could fulfill the dietary energy requirement in terms of PEA of 15,199 persons ha^{-1} $year^{-1}$ followed by M-SC-VB (14,444 persons ha^{-1} $year^{-1}$). The minimum energy production was obtained in M-F (51.4 Gj ha^{-1}) followed by M-VP (72.2 Gj ha^{-1}). Similarly, M-F and M-VP could fulfill the dietary energy requirement for only 5,073 and 7,128 persons ha^{-1} $year^{-1}$, respectively (Figure 4).

3.5. Production economics

Agronomic management practices significantly influence the farm net returns of maize-based cropping diversification. The highest net

TABLE 3 Effect of crop management and cropping system on system productivity, production efficiency, and economics in the maize-based cropping system.

Treatments	System productivity (Mg ha^{-1})	Production efficiency (Kg ha^{-1} day^{-1})	Net returns (INR x 10^3 ha^{-1})	Benefit–cost ratio	Dietary energy returns, Kj INR^{-1} invested
Agronomic management					
Conservation agriculture	15.8	43.2	242.7	2.49	280.3
Conventional agriculture	15.0	41.1	227.4	2.23	247.5
LSD ($p < 0.05$)	0.72	1.91	14.3	0.12	11.48
Crop diversification					
M-SC-VP	22.6	61.9	353.5	2.99	276.4
M-SC-M	16.9	46.3	255.2	2.35	268.9
M-SC-L	17.9	49.1	273.8	2.52	276.1
M-SC-VB	22.8	62.5	356.2	2.97	259.2
M-VP	8.8	24.2	130.7	2.07	229.6
M-F	3.2	8.7	41.2	1.25	273.3
LSD ($p < 0.05$)	1.4	3.7	33.8	0.20	20.7

M-SC-VP, maize–sweet corn–vegetable pea; M-SC-M, maize–sweet corn–mustard; M-SC-L, maize–sweet corn–lentil; M-SC-VB, maize–sweet corn–vegetable broad bean; M-VP, maize (local)–vegetable pea (local); M-F, maize (local)–fallow, LSD, least significant difference.

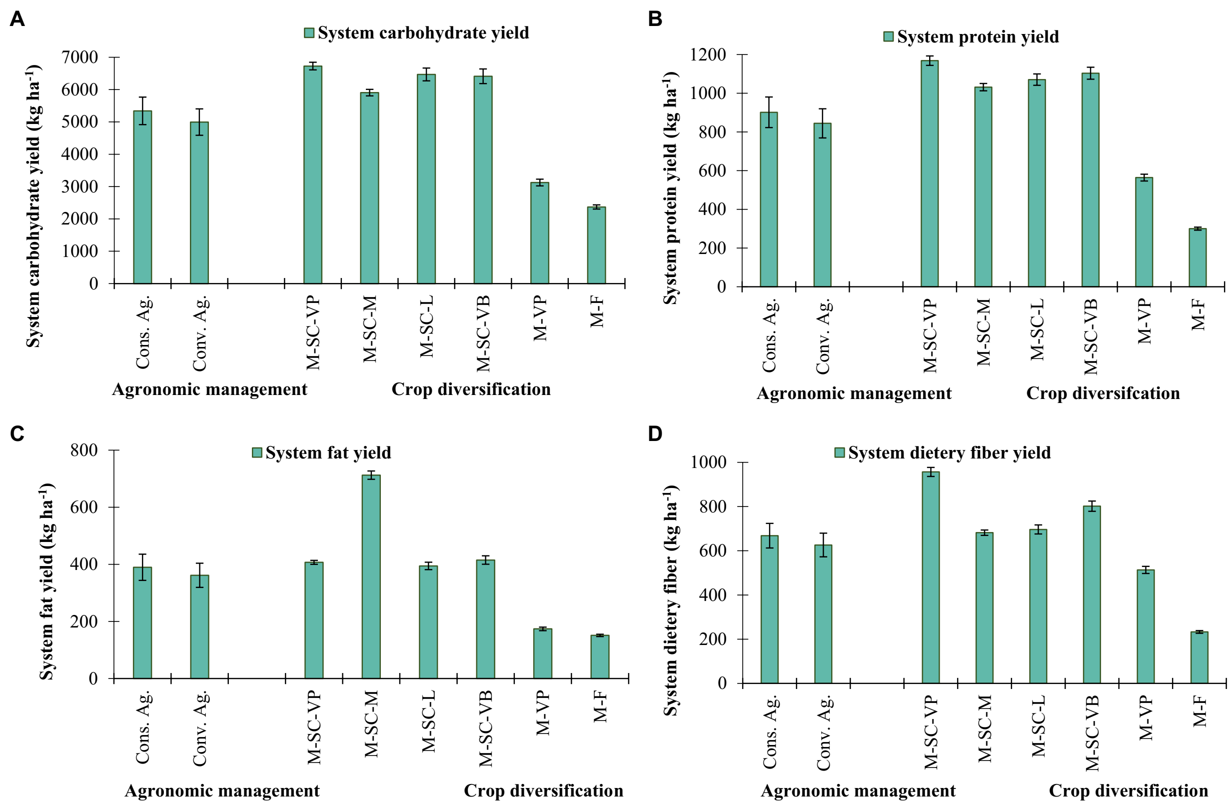

FIGURE 1
Effect of crop management and cropping system on **(A)** system carbohydrate yield, **(B)** system protein yield, **(C)** system fat yield, and **(D)** system dietary fiber yield. The vertical bar represents the standard error of the mean (p<0.05). Cons. Ag., Conservation agriculture; Conv. Ag., conventional agriculture; M-SC-VP, maize–sweet corn–vegetable pea; M-SC-M, maize–sweet corn–mustard; M-SC-L, maize–sweet corn–lentil; M-SC-VB, maize–sweet corn–vegetable broadbean; M-VP, maize–vegetable pea; M-F, maize–fallow.

returns and B:C ratio were recorded in Indian rupees (INR) 242.3×10^3 and 2.49 in conservation agriculture than conventional agriculture INR $227.4 \times 10^3\,ha^{-1}$ and 2.23, respectively. The cropping system significantly ($p < 0.05$) differed the net returns and B:C ratio. The M-S-VB gave the highest net returns of INR $356.2 \times 10^3\,ha^{-1}$ followed by M-SC-VP (INR $353.5 \times 10^3\,ha^{-1}$). However, the M-SC-VB system enhanced the net farm income by 764 and 172% over M-F and M-VP cropping sequences, respectively. Similarly, M-SC-VP performed equally better and recorded significantly higher farm net income by 758 and 170% than M-F and M-VP, respectively. Similarly, the higher benefit–cost ratio was recorded in M-SC-VP (2.99) ≥ (2.97) than the dominant cropping system of M-VP (2.07) and M-*F* (1.25) (Table 3).

3.6. Dietary energy returns

Dietary energy returns in terms of energy produced per INR invested significantly ($p < 0.05$) influenced due to agronomic management and cropping diversification. Averaged over the 3 years, conservation agriculture improved 13.25% dietary energy returns than conventional agriculture. Among cropping diversification, the highest dietary energy returns were obtained in M-SC-VP (280.3 Kj INR^{-1} invested). The least dietary energy return was obtained in M-VP (229.6 Kj INR^{-1} invested; Table 3).

4. Discussion

4.1. Current and future importance of organic conservation agriculture in the north eastern Himalayas

There are seven states that constitute the NEH region of India, these states are Assam, Arunachal Pradesh, Manipur, Mizoram, Nagaland, Meghalaya, and Sikkim. The NEH has a total geographical area of ~18.37 million hectares (M ha) (33), and its net cultivated area is 1.77 M ha (34). The NEH is the most ideal niche location for the development of organic crop production. Because of this, fertilizer usage in the NEH states is almost negligible, with the exception of Manipur (68.3 kg ha^{-1}), in contrast to the overall fertilizer use in India, which is 133 kg ha^{-1} (35). As a result, agricultural crop residues have a tremendous amount of potential to enhance the quality of the soil in the NEH region. Crop residues are a by-product of crop production in NEH of India (2.55 million tonnes of crop residues include 0.40 M t in Arunachal Pradesh, 0.90 M t in Manipur, 0.51 M t in Meghalaya, 0.06 M t in Mizoram, 0.49 M t in Nagaland, 0.04 M t in Tripura, and 0.15 M t in Sikkim). The residues of rice and maize are not being utilized as livestock feed, rather 11% (0.28 M t) of total crop residues are burnt causing pollution, especially air (36). These residues may be used for crop production under organic conservation agriculture.

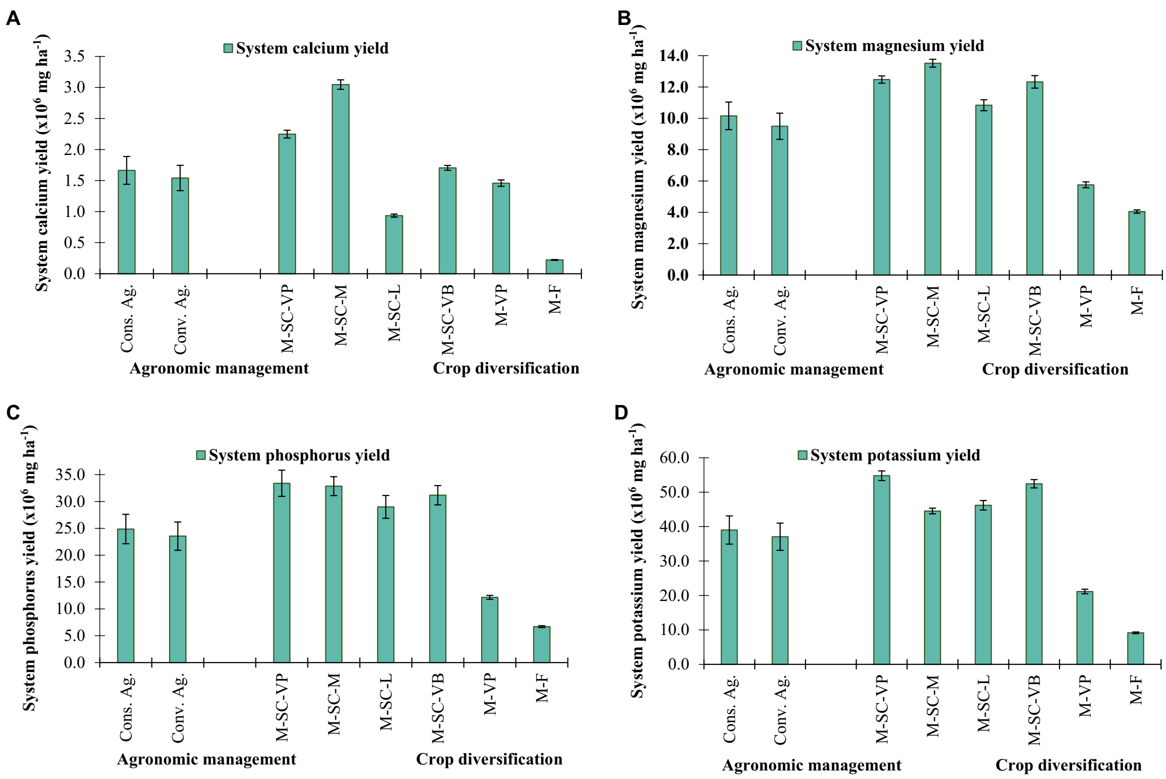

FIGURE 2
Effect of crop management and cropping system on mineral yield of **(A)** calcium, **(B)** magnesium, **(C)** phosphorus, and **(D)** potassium. The vertical bar represents the standard error of the mean (p<0.05). Cons. Ag., Conservation agriculture; Conv. Ag., conventional agriculture; M-SC-VP, maize–sweet corn–vegetable pea; M-SC-M, maize–sweet corn–mustard; M-SC-L, maize–sweet corn–lentil; M-SC-VB, maize–sweet corn–vegetable broadbean; M-VP, maize–vegetable pea; M-F, maize–fallow.

The total bovine population in NEH is estimated to be ~2.98 million, with bovine (mostly cattle and buffalo) providing most of the manure used in agricultural production. To cash these opportunities to convert animal waste into wealth, the Government of India launched the Galvanizing Organic Bio-Agro Resources (GOBAR)-DHAN scheme under Swachh Bharat Mission (Gramin). To encourage organic farming, Indian Government launched the PM PRANAM Yojana to incentivize the alternative options of chemical fertilizers. The traditional symbiotic interactions between crops and livestock in smallholder, mixed farming systems have been disrupted as a result of several factors. These factors include the gradual transition away from the use of draft animals in favor of electrical and mechanical sources of power, the decreasing reliance on crop residues as ruminant fodder, the large-scale burning of straw, and the gradual decline in recycling farmyard manure to enrich soils. There are still some locations in the NEH that use crop residues as feed for animals, although these areas are becoming increasingly rare. According to the findings of the latest research, this indicates that there is a significant possibility to make use of the crop residues that are present in the NEH region in order to promote organic conservation agriculture practices. In this way, the soil's potential for long-term productivity could be preserved.

Over the course of the last few decades, there has been a growing awareness of the importance of utilizing conservation agriculture systems in order to reduce the amount of soil erosion, enhance the quality of the soil, maintain, or increase crop productivity and nutritional security, and keep the environmental quality intact in agricultural systems (6). In order to improve carbon sequestration in agricultural land and reduce green house gas (GHG) emissions, one of the most important factors is the quantity and quality of crop residues that are provided *via* cultivation using conservation agriculture (no-tillage) (37). It is commonly believed that the conservation tillage system can improve soil quality by increasing soil health indicators and, therefore, the functioning of the soil microbial community, which is important for the transformation and mineralization of organic compounds and nutrients in soil ecosystems. Conservation agriculture system involves minimal physical disturbance and soil inversion (38). In the NEH region, the area is suffering from a catastrophic loss of plant cover and top fertile soils as a direct result of the extreme erosion caused by steep slopes.

Practicing shifting cultivation in a 0.756 million ha land area resulted in burning phytomass (including forest floors) of more than 8.5 million tonnes annually (39). This has resulted from disturbances in soil carbon dynamics, mostly due to the loss of topsoil from surface runoff in sloping lands. In this context, conservation agriculture offers a significant, multi-dimensional opportunity to transform large-scale agricultural waste streams from financial and environmental liability to valuable assets. If the agriculture crop biomass is utilized through conservation agriculture, millions of tonnes of carbon can be sequestered and fertile soil will be saved from erosion. In shifting cultivation, instead of "slash and burn," the practice should be "slash and recycle biomass." In addition, the government of India is placing

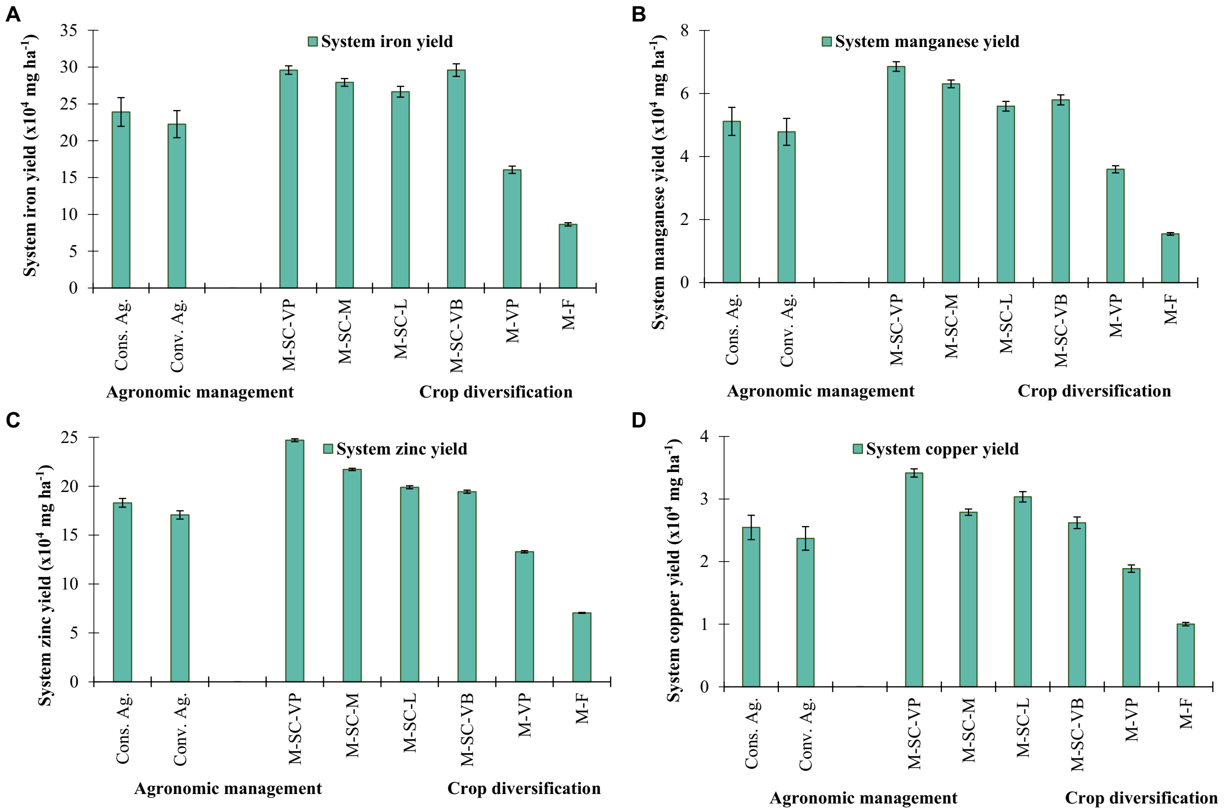

FIGURE 3
Effect of crop management and cropping system on trace mineral yield of **(A)** iron, **(B)** manganese, **(C)** zinc, and **(D)** copper. The vertical bar represents the standard error of the mean (p<0.05). Cons. Ag., Conservation agriculture; Conv. Ag., conventional agriculture; M-SC-VP, maize–sweet corn–vegetable pea; M-SC-M, maize–sweet corn–mustard; M-SC-L, maize–sweet corn–lentil; M-SC-VB, maize–sweet corn–vegetable broadbean; M-VP, maize–vegetable pea; M-F, maize–fallow.

a strong emphasis on the promotion of organic and natural farming practices. As a part of this initiative, national programs such as the Paramparaghat Krishi Vikas Yojana (PKVY; Traditional Agricultural Development Plan), the Rashtriya Krishi Vikas Yojana (RKVY; National Agricultural Development Plan), and the Mission Organic Value Chain Development for North Eastern Regions(MOVCD-NER) are currently being carried out. The goal of this initiative to increase the amount of land that is farmed organically by making use of the organic resources that are already in existence, such as manures from livestock, cropping system diversification that includes green manuring, crop residue utilization for soil health restoration, maintaining crop–livestock interactions and crop productivity, and lowering the levels of pollution in the water and air. Because of this, the findings of the current study on the impact of organic conservation measures will make it possible for policymakers in the NEH area to put into practice agricultural methods that are efficient.

4.2. Production efficiency

Agricultural management strategies those are sustainable from an ecological perspective are essential to the continued provision of ecosystem services (4, 40). The designing of a food and nutrient-efficient cropping system to sustain the livelihood of farmers will achieve little toward SDGs-3 (good health and wellbeing) and-12 (zero hunger; responsible consumption and production). The utilization of leguminous crops within the system and the recycling of biomass led (17.2–18.4 tonnes ha^{-1}; Supplementary Figure S1) to an increase in the production efficiency per unit of land following the implementation of conservation agriculture. The retention of more residues of above-ground biomass helps to improve the quality of the soil's properties (18, 41). Therefore, production efficiency and overall system productivity increased by ~5% due to the retention of residues as compared to the removal of residues in conventional agriculture (6). The possibility to fulfill SDGs, particularly those related to good health and wellbeing, is offered through higher production efficiency and system productivity. In a similar manner, M-SC-VP and M-SC-VB offered a system productivity and production efficiency that was approximately 2.6 and 7.0 times greater than that of the most often used M-F and M-VP cropping systems, respectively.

4.3. Dietary productivity of carbohydrate, protein, fat, fiber, and mineral

The maize–sweet corn–vegetable pea/broad bean intensive cropping system in the NEH region produces a higher dietary carbohydrate (107–115%), dietary protein (96–105%), dietary fat (134–138%), dietary fiber (56–86%), dietary minerals like calcium (17–54%), magnesium (114–116%), phosphorus (156–175%),

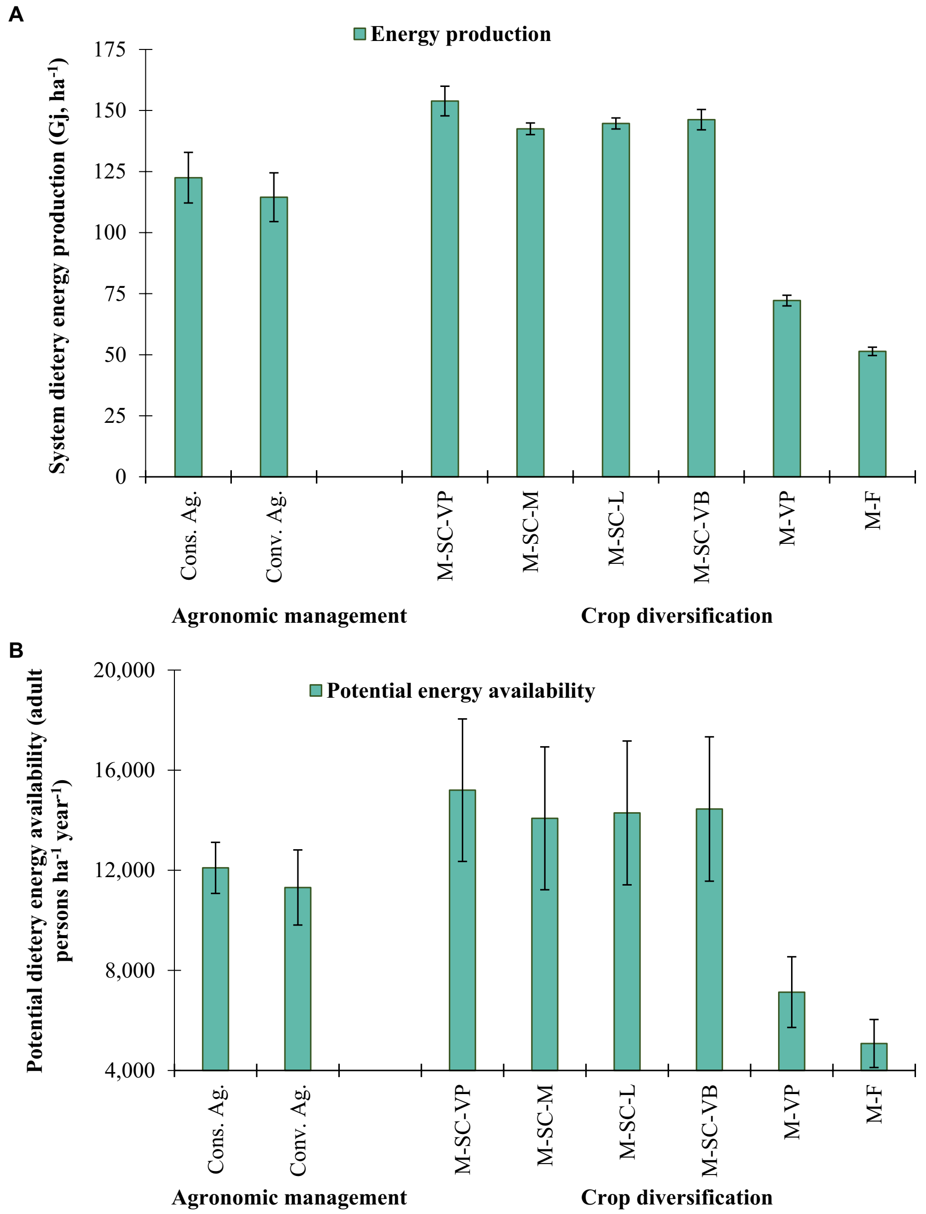

FIGURE 4
Effect of crop management and cropping system on **(A)** system dietary energy production and **(B)** potential dietary energy availability. The vertical bar represents the standard error of the mean (p<0.05). Cons. Ag., Conservation agriculture; Conv. Ag., conventional agriculture; M-SC-VP, maize–sweet corn–vegetable pea; M-SC-M, maize–sweet corn–mustard; M-SC-L, maize–sweet corn–lentil; M-SC-VB, maize–sweet corn–vegetable broadbean; M-VP, maize–vegetable pea; M-F, maize–fallow.

potassium (148–159%), iron (84%), manganese (61–90%), zinc (46–86%), and copper (39–81%) than the most popular local M-VP cropping rotation. In addition, the designing of intensive cropping diversification improved and ensured greater availability of dietary foods and minerals than locally adopted systems, such as local M-VP and M-F cropping diversification. These intensive cropping rotations also reduced the area of land needed for agricultural production. In accordance with the United Nations Sustainable Development Goals, the Northeast Himalayan region faces the challenge of maximizing sustainable agricultural development while simultaneously increasing grain production to a level that satisfies dietary needs for carbohydrates, protein, fat, fiber, and minerals while simultaneously reducing resource use (8, 42, 43). The calorie and protein yields of all crops grown throughout the Kharif, Rabi, and summer seasons, as well as the yields of all cropping systems, were considerably impacted by conservation agriculture. The residue retention of maize and subsequent season crops resulted in an increase of 5–8% in the average output of dietary carbohydrates, proteins, fats, fibers, and minerals. These findings are consistent with those of earlier research conducted in South Asia, where the implementation of conservation agriculture-based management strategies has led to a 3.0–6.0% increase in protein production across a number of cropping systems (44, 45). The greater calorie and protein yields that were achieved *via* the use of conservation agriculture were direct results of the higher grain yield that was achieved through the use of these management strategies. The adoption of appropriate agricultural patterns in conjunction with the utilization of appropriate technology has the potential to enhance the calorie and protein security of smallholder farmers in India and South Asia (44).

4.4. Dietary energy and potential energy availability

The production of food is dependent on ecosystems in which the soil should be in good condition and working properly, which in turn gives services to agriculture by fertilizing the soil with the necessary organic inputs (16, 46). These ecosystem services, which include regulating and providing support, make delivering ecosystem services possible (47). The recycling of agricultural residues and intensification of crop production with a diversity of different crops contribute to an increase in the quality of ecosystem services. The production of food that is required to fulfill nutritionally adequate diets is reliant on the good functioning of the ecosystem, which is based, in turn, on the variety of farming techniques and inputs. India has attained food self-sufficiency or food security in terms of *per capita* calorie availability during the past decade due to its constant and sustainable development in food production. This feat was accomplished over the past 10 years (48). Despite this, the need for sustainable intensification and diversification of sole cropping systems or double to triple cropping systems with manipulation of agronomic management practices under changing climate scenarios and ensuring the food and nutrition security of an increasing population will continue to remain the major challenge in Indian agriculture. This will continue to be the case as long as the population of India continues to rise (49).

Our research has the potential to contribute to the process of formulating policies and establishing strategies for achieving and maintaining food and nutritional security through the sustainable intensification and diversification of crop production. Dietary patterns have been consistently shifting in the NEH region ever since there has been a shift in attitude toward healthy (organic) and complete dietary food. This shift has resulted in a greater emphasis on the consumption of foods that are high in carbohydrates, proteins, dietary fiber, and enriched mineral content. This study investigated the dietary energy and potential energy availability (PEA) for the food intake from the comparative cropping system under conventional agriculture and conservation tillage. The analysis was based on the changes in diet. The results of our research indicated that the utilization of intensive cropping systems (M-SC-VP, M-SC-M, M-SC-L, and M-SC-VB) led to a higher production of dietary energy by 103 and 186%, respectively, when compared to the conventional cropping system, which consisted of growing maize as sole and in conjunction with vegetable peas. Among the systems, M-SC-VP and M-SC-VB had the best overall performance and the highest PEA. This guarantees that the requirements for dietary energy are needed by 15,199–14,444 adult person year^{-1}. The maize–sweet-corn–vegetable pea/broad bean system that was implemented with conventional tillage resulted in the maximum yields of grain, calories, carbohydrates, proteins, and minerals at the system level. In the NEH region, maize does not directly contribute to the composition of human meals. On the contrary, this is a significant source of poultry feed, and chicken is one of the important sources of protein in the diets of people who live in that region. Therefore, there is potential to integrate maize into human diets and to increase its consumption by altering the dietary patterns of human consumers. Our research has shown that implementing these systems into conventional farming to make the most efficient use of available resources is one way to enhance the level of food and nutritional security available to the region's growing population.

4.5. Dietary energy returns and economics

It is crucial to know how smallholder farmers in the NEH area may optimize their dietary energy returns and farm profitability through the efficient and effective use of natural resources in the intensive cropping systems that are used in that region (land, water, energy, and labor). This study examined the impact of six different cropping diversifications and two different alternative options (conservation and conventional) on the productivity of the systems, as well as the nutritional supply and profitability of the systems. Because of the high yields of hybrid maize and the adjustment of one short-duration sweet corn crop that was grown in the system, the maize–sweet corn–vegetable pea/broad bean rotation resulted in a higher net margin (~157%) and dietary energy returns (~20%) than the maize–vegetable pea cropping systems. This was due to the fact that the rotation included maize, sweet corn, vegetable peas, and broad beans. It is consistent with the findings of past studies conducted in the NEH region that the intensive cropping system has a larger gross margin than the conventional double and sole cropping systems (6).

5. Conclusion

Increasing cereal-based cropping systems' productivity, profitability, and long-term sustainability is a challenge for NEH's low-to middle-income rural and urban populace. This study showed that maize–sweet corn–vegetable pea/broad bean systems could increase systems productivity, production efficiency, carbohydrate yield, protein yield, dietary fiber yield, and grain calories by 158, 157, 110, 101, 71.3, and 108% while providing 171.5, 44, and 16.6% higher net margin, benefit–cost ratio, and dietary energy returns per INR invested than the local farmer practice of maize–vegetable pea system. Four cropping systems (excluding the local maize–vegetable pea and maize–fallow system) evaluated here might benefit from conservation tillage. The maize–sweet corn–vegetable pea/broad bean system had higher Ca, Mg, P, K, Fe, Mn, Zn, and Cu nutritional security and PEA than the other system. This study shows that conservation agriculture-based management approaches can benefit maize-based intensive rotations in a subtropical environment of the NEH. Although the study farm is bordered by farmers' fields with comparable climates and soil, crop management procedures in research stations might differ according to natural factors and socio-economic contexts. Depending on farmers' priorities and risk tolerance, our findings propose a basket of technology solutions for smallholders to implement conservation agriculture. The development aims in the NEH as a whole include extending these approaches from research the field of smallholder farmers.

6. Policy implications

Sustainable development goals aim for promoting responsible production and consumption which also include food without any chemical residues. Government of India aim to bring 10% of the net cultivated area under certified organic farming by 2030 which is currently only 3%. The introduction of farmer-friendly certification systems such as the Participatory Guarantee System (PGS) and large area certification also encourages farmers to adopt alternative production systems. Focus is being made on the promotion of chemical-free farming in niche areas (with low consumption of fertilizers and pesticides; for example, north-eastern states, hilly areas in other parts, etc.) and niche crops (crop responding to organic management). Therefore, findings from the study can be integrated with ongoing government-sponsored promotional schemes like the PKVY, Traditional Agricultural Development Plan, the RKVY, National Agricultural Development Plan, and the MOVCD-NER to reap better benefits. Further alternative fertilizing strategies for organic farming can also be integrated through the PM PRANAM Yojana which will encourage organic growers. Furthermore, the Government of India aims to set up 500 "waste to wealth plants" GOBAR-DHAN scheme to convert organic waste into valuable assets (an organic source of nutrients). Soil and human nutritional security can be achieved through the promotion of science-led organic farming including choosing appropriate tillage options along with cropping systems as evident from the study. Prioritized solutions for organic farming will improve productivity leading to better export of safe food to the global market and benefit the larger population looking for chemical residue-free food.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

MA: conceptualization, methodology, investigation, monitoring, data curation, and writing of original and final draft. NR: review and writing, editing, and project administration. MA: writing of original first draft, review and editing, and data analysis. SB: review, writing, and editing. JL: review, writing, and editing. AP: review, editing, and project administration.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1137247/full#supplementary-material

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Assessment of ethnobotanical uses, household, and regional genetic diversity of aroid species grown in northeastern India

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Aroids are an important group of indigenous tuber crops, grown widely for their leaves, petioles, stolons, corms, and cormels. A total of 53 genotypes were evaluated for their genetic diversity in northeastern region of India. At household level, a total of 16 landraces of Aroids were recorded having different ethnobotanical uses. Based on the population study under *Jhum*/Shifting farming, landrace Rengama was dominant in area with 47% of the total population followed by Tamachongkham and Tasakrek. However, Pugarkusu and Chigi occupied 33.0 and 24.0% of the population, respectively under backyard farming, and were considered as major landraces. Tamachongkham, high in acridity and total oxalate content (0.82%), was used for cooking with meat, while Tasakrek was used as a baby food due to high total sugar (>3.0%), low in acridity, and total oxalate content (<0.12%). The Simpson's diversity index of the backyards was higher (0.80) as compared to *Jhum* field (0.63). The genotypes showed wider variability in growth and yield attributes like; plant height (89.4–206.1cm), number of side shoots (1.84–5.92), corm weight (38.0–683.3g), cormel weight (14.0–348.3g), yield (0.24–1.83kg plant^{-1}). Similarly, wide variations were also observed for quality traits like total sugar (1.93–4.94%); starch (15.32–32.49%), total oxalate (0.10–0.82%), and dry matter (16.75–27.08%) content. Except for total oxalate, all the growth and yield attributes have shown high heritability and moderate to high genetic advance. Molecular analysis (33 polymorphic SSR markers) detected a total of 136 alleles, ranged 3 to 8 alleles per marker. The observed heterozygosity (0.24) was less than expected heterozygosity (0.69). The group-wise maximum genetic divergence was observed between *Colocasia fallax* (cv. Chigi) to *C. esculenta* var. *aquatilis* (cv. Tharsing); *C. fallax* (*cv*. Chigi) to *C. gigantea* (*cv*. Ganima) and *C. gigantea* (*cv*. Ganima) to *Xanthosoma* spp., while it was least between eddo and dasheen. The findings indicated, a wider diversity and distinct ethnobotanical uses of Aroid landraces at the house hold levels, which should be conserved and popularized to ensure nutritional security.

KEYWORDS

aroid, taro, genetic diversity, starch, oxalic acid, molecular marker

Introduction

Northeastern states of India are one of the important hotspots of the world's biodiversity. Due to humid subtropical climate, there is a wide range of cultivated and wild plant species available in the region. The majority of the Aroid species are native to the region, and belong to Araceae family; among them *Colocasia esculenta* var. *antiquorum* (Arvi/Eddoe), *Colocasia esculenta* var. *esculenta* (Bunda/Dasheen), *Colocasia esculenta* var. *aquatilis* (edible stolon), *Colocasia fallax* (edible leaves and petioles), *Xanthosoma sagittifolium* (Cocoyam/Tannia for edible petioles), and wild Giant taro (*Alocasia* spp.) are basically used by the farmers for consumption. Taro is considered as one of the world's oldest food crops, dating back over 9,000 years and was domesticated first in South-East Asia and spread throughout the globe (1). Due to wider adaptability of this crop to diverse climatic conditions, in the present scenario, it is being cultivated worldwide ranging from equatorial tropical to southern and northern temperate zones and rank 5th most consumed root crops in the world (2, 3). Aroids have immense potential to play an important role in ensuring nutritional security, as they are rich sources of carbohydrates, proteins, minerals and vitamins. The tubers are rich in starch (21.2%), protein (3.2%), and minerals like calcium (31.0 mg), magnesium (106 mg), and potassium (356 mg) content. Besides, leaves are also rich in protein (4.98 g), and minerals like calcium (107 mg), potassium (648 mg), phosphorous (60 mg), and vitamins like carotene (2,895 ug), folate (126 ug), and vitamin K (108.6 μg) in 100 g FW (4) and could be utilized to ensure the availability of all the nutrients in a balanced manner. It is also one of the finest sources of dietary fiber (4.1%). Proper cooking, removes the acridity causing factor, calcium oxalate. There are different aroid landraces grown in the region for specific plant parts and uses, like tubers for breakfast, baby food, curry preparation and pickles; leaf and petiole for cooking, pig feed, and also as an ornamental foliage.

Aroids have ethnopharmacological importance, as cooked vegetable contains mucilage and is an effective nervine tonic (5). Leaf juice is a stimulant, expectorant, astringent, appetizer, and otalgia. The juice expressed from the leaf stalks with salt is used as an absorbent in cases of inflamed glands and buboes. Decoction of the peel is used as a folk medicine to cure diarrhea. The juice of the corm is used in cases of alopecia. Internally, it acts as a laxative, demulcent, anodyne, galactagogue and is used in cases of piles and congestion of the portal system, as well as an antidote to the stings of wasps and other insects (6). The crops are also possessing several pharmacological properties like, hypoglycemic, hypolipidemic, anti-inflammatory, antifungal, and anticancerous properties (7–12).

The crop is cultivated widely under different ecology by the tribes of the region. In Meghalaya, over 82.5% of the total cropped area is covered by marginal and small farmers, with <2.0 ha land holding. Over 80% of the population is dependent on agriculture for their livelihood and nutritional security (Government of Meghalaya, India). As, rice is the staple food of the population, farmers have to explore other options in order to increase the nutritional quality of their food and thus, aroids, being a native crop of this region, can play an important role in augmenting nutritional quality. Among the tuber crops, aroid species are grown widely and diverse genetic resources are being cultivated under *Jhum,* as well as a backyard farming system for the year round production and uses. *Jhum* farming is a traditional farming practice followed in entire northeastern hill region of the India except Sikkim. The crops are grown under mixed cropping with chilies, brinjal, okra, ginger, taro, sweet potato, yam (near burnt trees/shrubs), tapioca, cucumber, and rice bean on fences after clearing the forest and burning the dried biomass, especially on the hill slopes. This practice is also known as 'slash and burn' or 'fire-fallow cultivation. In spite of the tremendous potential, the productivity of this crop is meager in the state. It might be due to the rainfed farming especially in the hills, poor quality of planting materials, and poor crop management.

Although, the crop is rich in nutritional value, it is considered as an Orphan crop, and there is no systematic study on the uses and extent of diversity within and between the aroid species in the region. Aroid species are commercially propagated vegetatively using clones (corms, cormels etc.), and most of the species, especially *Colocasia* spp. grown in the region are diploid, with some triploid species (13). Due to poor in flowering and fertility, majority of the varieties developed in the country are the products of clonal selection. Hence for crop improvement through clonal selection with desirable traits (high in yield, starch, dry matter content and low in oxalate), estimation of genetic diversity, and genetic parameters are very important.

There are many approaches for the estimation of genetic diversity like quantitative traits, isozyme and DNA markers; and among them DNA markers have been found most reliable as these markers are reproducible and free from effects of environmental factors. DNA markers has been deployed in many crop species, but in aroid, there are very few reports of utilizing biochemical and different generations of molecular markers, namely, isozyme (14, 15); AFLP (16, 17); RAPD (18, 19); ISSR (20); SSR (21–23) and SNP (24, 25) for assessment of genetic diversity, as well as evaluation of stability (26).

Keeping above in view, the present study was aimed to assess the ethnobotanical uses and genetic diversity of Aroids in the region as well as at household level grown by the tribes, and its potential utilization as an important food crop. This study will be useful in the taxonomic studies, identification of the superior genotypes having unique uses, their characteristics, promotion for commercial production to ensure nutritional and livelihood security, conservation, and utilization in future improvement program.

Materials and methods

Survey and collection of germplasm

To study the diversity of aroids at household level, survey was carried out at the project site, i.e., Rombagre village, West Garo Hills District of Meghalaya, India located under humid subtropics and mid-hill (altitude ranges 500–600 m above mean sea level) conditions (Figure 1). A total of 16 landraces belonging to different species/groups with common variants were observed at each household having distinct ethnobotanical uses (Table 1). The population diversity of the landraces was studied at farmers' field under *Jhum* as well as backyard of the villagers at household levels covering the entire area (≈ 0.5 ha *Jhum* land and 0.1 ha backyard) of the individual farmers and 2 households were selected for the studies. The diversity index was measured as Simpson index, Shannon's diversity index, and Evenness Index (27–29).

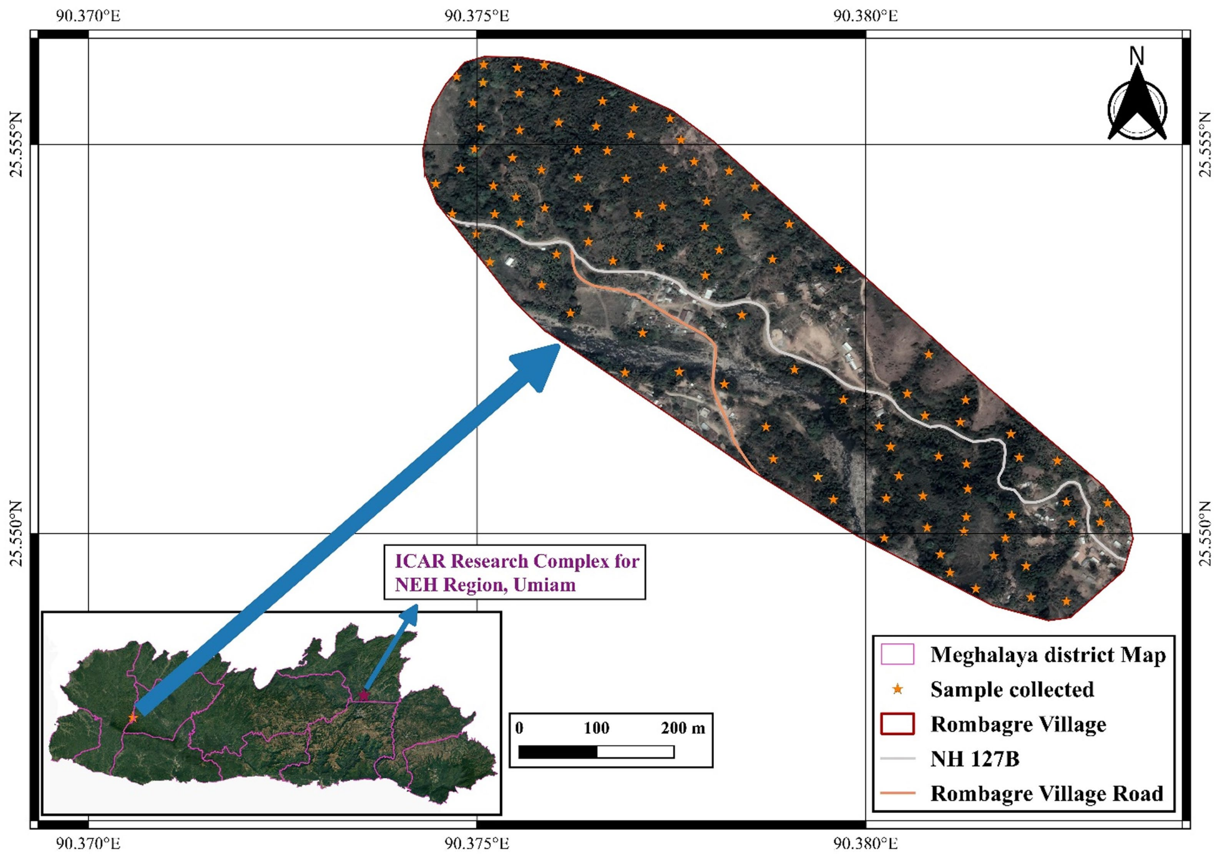

FIGURE 1
Site of Collection of Aroid species in West Garo Hills of Meghalaya (Altitude: 500–600m above mean sea level).

Crop evaluation for yield and quality attributes

Total 53 genotypes comprising of 16 landraces from project site (Rombagre, Meghalaya), 18 popular varieties/lines as well as 19 collections form other northeastern states were evaluated at ICAR Research Complex for NEH Region, Umiam, Meghalaya during March – November, 2016–2018 (3 years). The crops were grown following recommended package of practices for the region (30). The experiment was carried out in a randomized block design with three replications. The observations were recorded for growth and yield-related traits such as plant height (cm), petiole length (cm), number of side shoots, average corm and cormel weight (g), yield per plant (kg) and yield (t ha^{-1}). The mean values of six plants in each replication were used for the statistical analysis.

Proximate analysis

Four important quality parameters namely dry matter, total sugar, starch and oxalate content was estimated for all the 53 cultivated genotypes. The dry-matter content in the samples (corms in dasheen type, and cormels in eddo type) were determined by oven-drying 10 g of the sample at 60°C, till a constant weight was obtained (31). Total sugars were estimated by titration, using Fehling's solution and methylene blue indicator (31). Amount of starch present in the samples was determined using anthrone method (31). After the sugars present in a sample leached out, the starch was hydrolyzed using acid, and estimated as follows:

$$Starch\,(\%) = Reducing\ Sugar\,(\%)\,x\,0.9$$

Moreover, total oxalate content (dry weight basis) was determined as per CTCRI manual (32).

Statistical analysis

Mean values of each replication were used for analysis of variance as per Panse and Sukhatme (33). Phenotypic and genotypic variances of the genotypes were estimated as described by Burton and Devane (34), heritability as described by Hanson et al., 1956 (35) and genetic advance was estimated using the formula suggested by Johnson et al., 1955 (36). The genotypic and phenotypic correlation coefficients and path coefficient were estimated as suggested by Dewey and Lu (37). Clustering was performed using the stats package in R 4.2.1 and visualized using the Factoextra package. Principal component analysis was performed using the Factoshiny package in R 4.2.1.

Molecular characterization

Plant materials

Total 58 genotypes including 16 cultivated local landraces collected from the project site (Rombagre, Meghalaya, India) were used for molecular analysis.

DNA extraction

The total gDNA was extracted from young leaf tissue by using CTAB method (38) with an addition of Polyvinylpyrrolidone (1%). The sample was then ground to a fine powder using liquid nitrogen. DNA sample concentration was determined using a spectrophotometer (Shimadzu, Jiangsu, China) and they were diluted to 20 ng/μL prior to polymerase chain reaction (PCR) amplification.

Molecular analysis

Total 45 SSR markers, reported earlier (22–25, 39) in aroid species were used for the initial screening, 34 shown amplification and except marker Xuqtem-110, remaining 33 were found polymorphic (Table 2). Moreover, markers Ce1B-12, Taro-5, Taro-7, Taro-9, Taro-10, Taro-15, Taro-16, Taro-17, and Taro-18 failed to amplify. Marker Taro-2 and Taro-6 shown amplification in few genotypes only. The PCR analysis was carried out in 20 μL volume containing 40 ng template DNA, 0.5 U TaqDNA polymerase, 0.2 mM each dNTP, 0.2 μM forward and reverse primer each in (1×) reaction buffer that contained 10 mMTris–HCl (pH 8.3), 50 mMKCl, and 2.5 mM $MgCl_2$ (Thermo Scientific, Bangalore, India). Amplification conditions (Applied Biosystems Veriti™, Singapore) were initial denaturation at 94°C for 5 min and 35 cycles at 94°C for 60 s and then 50 – 66°C for 60 s, and extension at 72°C for 2 min, followed by 10 min at 72°C and indefinite soak at 4°C. Amplified products were resolved on 3.5% SFR agarose gel containing ethidium bromide (10 mg/mL) at a constant voltage of 80 V for 3 h using a horizontal gel electrophoresis system (Biorad, Singapore). The gel was run in 1 × TBE buffer. A 50 bp DNA ladder (MBI Fermentas) was run alongside the amplified products to determine their approximate band size. Similarly, the amplified products were visualized under UV by image analyses.

Data analysis

Only consistent, bright, reproducible (i.e., band absence was randomly verified) SSR bands were scored as present (1) or absent (0), where each character state was treated independently. The summary statistics of SSR markers such as the number of alleles per locus, allele frequency, heterozygosity and polymorphic information index (PIC) were determined using Power Marker version 3.25 (40). Genetic diversity was assessed using both model-based approach and distance based approach. For distance based approach, the unrooted phylogenetic tree was constructed based on genetic distance as per Nei distance (41). Clustering using model based approach was performed using Structure 2.3.4 software with 50,000 burning period length followed by 50,000 Markov Chain Monte Carlo (MCMC) replication (42) with a K value ranging from 1–10. The optimum K value was determined based on delta K value using a web program namely Structure Harvester.[1]

1 http://taylor0.biology.ucla.edu/structureHarvester/

Results

Population diversity and ethnobotanical uses

The household population diversity of aroids was assessed at the project site under backyard and *Jhum* farming systems in Rombagre village of West Garo Hills in Meghalaya. A total of sixteen diverse genotypes of aroids were found at household levels including *Xanthosoma* spp., *Colocasia esculenta* var. *aquatilis* (cv. Tharsing), *Colocasia fallax* (*cv*. Chigi), and *Colocasia gigantea* (*cv*. Ganima). These genotypes were of a diverse group with unique ethnobotanical uses (Table 1; Figure 2). Out of 16 genotypes, 6 landraces were grown under *Jhum* land as well as in backyards, while a total of 13 landraces were grown only under the backyards conditions. Based on the population study in *Jhum* land, it was found that landrace Rengama was most dominant in area with a share of 47% of the total population, followed by Tamachonkham (36%), and Tasakrek (12%). However, the maximum area in backyards was under Pugarkusu (33%) followed by *Colocasia fallax* cv. Chigi (24%). The percent populations of the other genotypes are presented in the pie diagram (Figure 3). All the genotypes grown at household level were different from each other for agro-morphological traits and ethnobotanical uses (Table 1). The Simpson's diversity index value was higher for the backyard (0.80) as compared to *Jhum* field (0.63). Similarly, the Shannon diversity index was also higher (42.59) in the backyards as compared to *Jhum* field (17.81). Likewise, the evenness index (EI) was 0.42 for the *Jhum* field, and 1.0 for the backyards. Besides, some of the genotypes differ in agro-morphological traits like, Rengama with cream and pink bud eyes, and Tasakrek with a difference in the size of the corm and cormels but known by a common local name.

Estimation of genetic variation

The analysis of genetic parameters has shown the presence of significant variation ($p < 0.05$) among the genotypes of the aroid species for all the yield and quality attributes (Table 3). Yield attributing traits like, plant height range from 89.4–206.1 cm, petiole length (61.4–145.08 cm), number of side shoots (1.84–5.92), corm weight (38.0–683.3 g), cormel weight (14.87–348.3 g), and yield per plant (0.24–1.83 kg). Among the genotypes, maximum plant height (206.1 cm) and petiole length (145.08 cm) were observed in Rengama-1, a dasheen type genotype collected from the Garo Hills of Meghalaya, while the maximum number of side shoots (>5.0) was recorded from commercial cultivars, namely, BCC-1, BCC-1A, Muktakeshi, Sel C-1 and ML-2. Further, the highest corm weight was recorded in dasheen type genotypes C-3 (683.3 g), followed by Panchmukhi (633.3 g), Arcol-7(600 g) and Selection C-3 (550.0 g). Further, the cormel weight was the highest in dasheen type genotypes Rengama-2 (348.3 g), and Rongrem (270.0 g). There were lateral stolons (5–7 per plant) in place of cormels in genotype Tharsing (*Colocasia esculenta* var. *aquatilis*). The average length, diameter, weight and yield of the stolon were 44.8 cm, 1.8 cm, 86.66 g and 476.66 g, respectively. Tamachongkham (31.4 t ha^{-1}), Tama (30.15 t ha^{-1}), Rengama-2 (28.10 t ha^{-1}), Rongrem (28.10 t ha^{-1}) and Rengama (27.0 t ha^{-1}) were identified as high yielding genotypes under dasheen types. While, White Gauriya (27.53 t ha^{-1}) and SJ-1 (25.19 t ha^{-1}) were identified under eddo types.

TABLE 1 Popular land races of aroid landraces in the Rombagre village of West Garo Hills of Meghalaya and their ethnobotanical uses.

Sl. No.	Landraces	Description
1	Tamachongkam	• Landrace with fused multi corm type • Leaves used for pig feeding • High yield and market value • Cover largest areas under *Jhum* field • Tuber are rich in oxalate content (0.82%) and acridity used for curry preparation with meat • The acridity is removed by boiling in water before curry preparation.
2	Rengama	• A dasheen type landrace, grown widely in *Jhum* land • Corms are medium in oxalate content (0.18–0.23%) and acridity, cooked for snacks and curry preparation. • High in yield, quality and storage life • Rank second in terms of areas under *Jhum* field • High incidence of corm borer. • There are two type of genotype, i.e., yellowish white and pink budded landraces
3	Tamachok	• Bunda type, grown in *Jhum* land for peduncle and corms for cooking • Good in taste, low in oxalate content (0.18–0.22%) content and acridity and used for curry preparation. • Rank third in terms of area under *Jhum* land
4	Tasakrek	• Eddo multicormel type, grown in Jhum field and characterized by number of shoots from single corm. • Low in oxalate (0.12%) and acridity, used for baby food. • The peduncle and petioles are also used for cooking with fish and meat.
5	Tagiting	• *Xanthosoma* spp. • There are two variants, i.e., white and purple type. • Vigorous in growth habit. • Cultivated for the petiole as well as leaves.
6	Ganima	• *Colocasia gigantea* a cultivated for petioles, corms are rich in starch (23.87%) and medium in oxalate content (0.28%) content.
7	Chigi	• *Colocasia fallax*, grown in backyard nearby drain water under partial shade. • Leaves and petioles is used for the cooking during lean period.
8	Other land laces: • Pugarkusu • Rangdubi • Takiltom • Tamitim • Tadikil • Gilasa	• These landraces are grown in backyards for the family consumption • Rangdubi: Not cultivated widely due to low yield and poor marketing
9	Extinct landraces: • Tasupok • Tarengsi • Tachongchang	• Extinct due to smaller size of corm and cormels and poor yield.
10	Giant taro	• *Giant taro* (*Alocasia* spp.) grown wild near streams and ponds. The top of big size corms are consumed in some parts of the Tripura.

Similarly, quality traits including total sugar range from 1.93–4.94%, starch content 15.32–32.49%, total oxalate 0.10–0.82%, and dry matter 16.75–27.08%. Among the genotypes, the highest sugar content was recorded in Arcol-2 (4.94%) followed by Nainital (4.90%). Moreover, the highest starch content was observed in the popular cultivar Kandha-5 (32.49%) followed by White Gauriya (30.32%). The starch content in *Xanthosoma* spp. ranged from 17.67 (Tajiting Purple) to 20.65% (Tagiting White), while in *Colocasia esculenta* var. *aquatilis* (Tharsing) it was 20.23%. Further, among the genotypes, the lowest total oxalate was recorded in SJ-1(0.10%) followed by BK Coll-2 and

TABLE 2 Details of SSR markers used for molecular analysis of aroid species.

Marker	Forward	Reverse	Allele size (bp)	Ann. temp	NA	NE	Obs Hom	Obs Het	Nei	PIC
Ce1A06	GCTTGTCGGATCTATTGT	GGAATCAGTAGCCACATC	95–140	60	6.00	3.41	0.98	0.02	0.71	0.51
Ce1B02	GCACGTTAGACTATTGGA	GTGCTTAGATGGTTGAGA	90–100	60	3.00	2.83	1.00	0.00	0.65	0.36
Ce1B03	TTGCTTGGTGTGAATG	CTAGCTGTGTATGCAGTGT	70–110	50	6.00	3.60	0.83	0.17	0.72	0.61
Ce1B09	AACACTCCCAGAAGAACC	CGTCTTTCAAACTGATCG	50–80	60	5.00	3.72	0.66	0.34	0.73	0.59
Ce1D12	GAAACGTGGGGATTG	CGTTGTGTAAACGGAAG	80–120	56	4.00	3.24	0.79	0.21	0.69	0.51
Ce1F04	AGGGAATACAATGGCTC	ACGAGGGAAGAGTGTAAA	70–90	60	4.00	1.56	0.88	0.12	0.36	0.13
Ce1F12	CTTAGCGTTGTTCCCTAC	GATGCCTGTCCTTATGTTT	50–70	57	6.00	4.88	0.41	0.59	0.79	0.73
Ce1H12	TAGTTAGCGTGCCTTTC	CAACAACTTAATGCTTCAC	50–70	55	4.00	2.63	0.91	0.09	0.62	0.50
AC3	AGTGGCATCAATGGAGGA	CCACTAAACGACGACCCAC	120–200	54	5.00	3.64	0.98	0.02	0.73	0.60
HK5	CCCACCTCTTCCCATTCGCTT	CGATCCTTCCAGCTCCGACAT	160–260	55	7.00	5.10	0.33	0.67	0.80	0.76
HK7	GTTGTCCGCCTGTGCGTTCT	CTCTTGGGAATTCTCCGGGTG	125–165	56	6.00	3.32	0.74	0.26	0.70	0.61
HK22	ACATCAAACCTCTGGTGGGC	AGCAATCCTAGCCGAGGTG	150–300	53	7.00	3.25	0.33	0.67	0.69	0.62
HK25	TGACTAGGCAGGAAGGTAA	CAAGCATTCTCTGAACTATG	120–200	51	5.00	3.85	0.50	0.50	0.74	0.67
HK26	GGGTGTTATCGCCATAGTCAT	GAAACACCACAACGGAGAAAC	110–175	51	7.00	4.04	0.53	0.47	0.75	0.70
HK29	GTCTGTGGAACCCTCAAGC	ATTGTGGGAGCGATAGGG	130–210	54	6.00	3.48	0.88	0.12	0.71	0.62
HK31	TACCGCCGAGTGCTTATC	TACGGCTGGAATCAAAGC	140–220	51	5.00	1.83	0.59	0.41	0.45	0.37
HK34	TTACTCCAAACGAGGCAAAC	CCTTCAAGATGTTACCAAATGC	180–290	56	8.00	5.09	0.16	0.84	0.80	0.74
HK-35	TACTAGAACCCCGTCAGTCT	CGTCGATTTATCAGTGAGC	240–300	53	5.00	3.83	0.52	0.48	0.74	0.66
HK38	AAACGCGGCCAGAAGATC	GAATAGCGGAACAAGGTAGA	120–190	54	5.00	2.82	0.98	0.02	0.65	0.54
Taro01	CTGACTCTTGTAAGGTCGCTC	CAAAAGCAGGTCTGGATG	100–130	56	5.00	4.36	1.00	0.00	0.77	0.65
Taro03	CGTGAGGGCGGTTTTGTCAGG	ACGAGCGAGCAGCTCACCGC	200–210	60	3.00	2.94	1.00	0.00	0.66	0.37
Taro04	ACTTTATGTAATAGTGAACATT	CGAAGCAGCGCCACCGGC	420–450	57	4.00	3.74	0.98	0.02	0.73	0.59
Taro11	CGGCCAAGAAGGAGAGCCA	ACAAGCTTATTTATAGTGGCTA	300–550	58	4.00	2.85	1.00	0.00	0.65	0.45
Taro12	CGCTTTGCCTTTCGGTGTTGAGA	ACTTGGTGTGCAGCAAGACTT	710–810	56	4.00	3.36	0.97	0.03	0.70	0.52
Taro13	GTTAATGGGATATAAACGGCA	CGCCAAAGTCTATTGAGTGTT	620–650	58	4.00	2.29	1.00	0.00	0.56	0.32
Taro14	ACAAAATATGTTCTCTGTGATAT	ACCTAGTCTACTATCGAGCCA	590–660	55	6.00	5.55	0.21	0.79	0.82	0.74
Taro19	TTCGACGTACCGATCGAGACCG	TTACCGAGACTGACGAAGCTAG	380–425	54	4.00	2.59	1.00	0.00	0.61	0.40
Xuqtem-84	AGGACAAAATAGCATCAGCAC	CCCATTGGAGAGATAGAGAGAC	325–500	65	7.00	4.79	0.88	0.12	0.79	0.69
Xuqtem-110	AGCCACGACACTCAACTATC	GCCCAGTATATCTTGCATCTCC	510–600	66	4.00	3.55	1.00	0.00	0.72	0.54
Xuqtem-73	ATGCCAATGGAGGATGGCAG	CGTCTAGCTTAGGACAACATGC	310–330	66	3.00	1.94	0.81	0.19	0.48	0.23
Xuqtem-55	CTTTTGTGACATTTGTGGAGC	CAATAATGGTGGTGGAAGTGG	200–220	60	5.00	4.86	0.31	0.69	0.79	0.69
Xuqtem-88	CACACATACCCACATACACG	CCAGGCTCTAATGATGATGATG	120–220	65	6.00	4.93	1.00	0.00	0.80	0.72
Xuqtem-91	GTCCAGTGTAGAGAAAAACCAG	CACAACCAAACATACGGAAAC	500–520	65	4.00	3.00	1.00	0.00	0.67	0.41
Mean			–		5.06	3.54	0.76	0.24	0.69	0.55

Ann, Annealing temp (°C); NA, Observed number of alleles; NE, Effective number of alleles; Obs Hom, Observed homozygosity; Obs Het, Observed heterozygosity; Nei, Nei's expected heterozygosity; PIC, Polymorphism information content.

ML-2 (0.11% each), while the maximum total oxalate was observed in landrace Tamachongkham (0.82%) followed by Tama (0.42) and Panchmukhi (0.38%). The total oxalate content in *Xanthosoma* spp. was about 0.24%. While, in *Colocasia esculenta* var. *aquatilis* (cv. Tharsing), the average starch and total oxalate content were 0.17 and 19.0%, respectively. Further, dry matter content was highest (27.08%) in KCA-1 and Panchmukhi.

The phenotypic coefficients of variation (PCV) were higher than their corresponding values of genotypic coefficients of variation (GCV) for all the characters. The genotypic coefficient of variation contributed significantly to the phenotypic variation for all the traits. All the traits except plant height, starch, and dry matter content have shown the highest GCV and PCV (>20%) indicating higher variability for these traits in the population. Moreover, except total oxalate content, other traits have shown high heritability (> 60%). Further, all the traits have also shown higher genetic advance (> 40%), except for plant height, dry matter, and starch content.

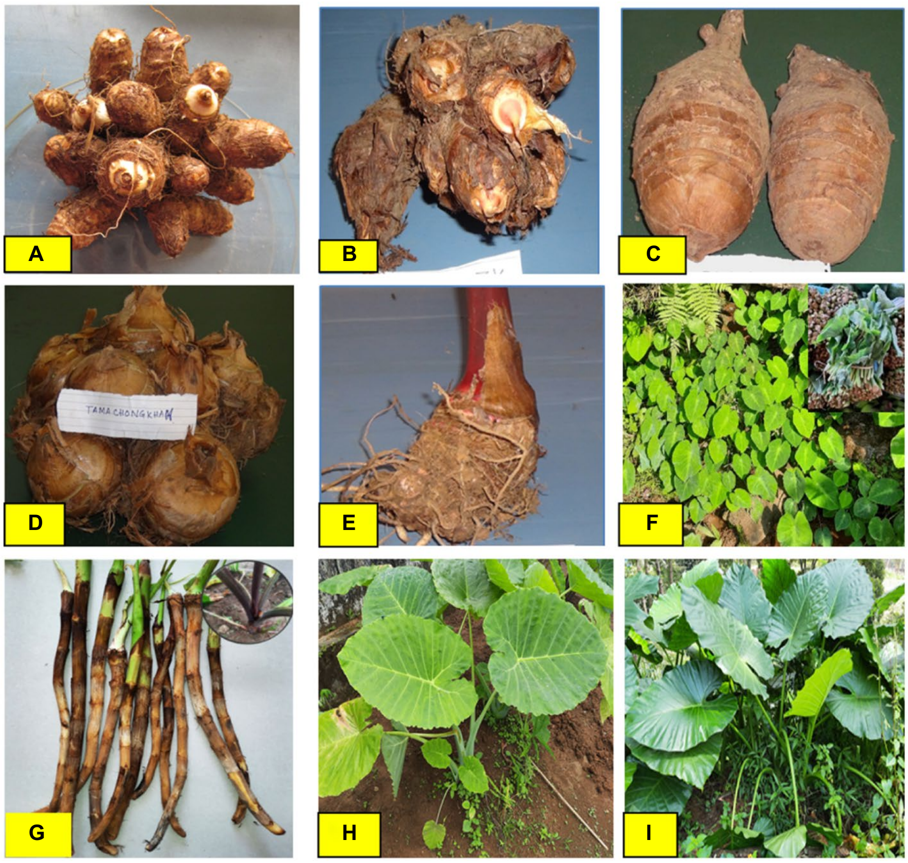

FIGURE 2
Diverse group of Aroid genotypes in Garo Hills of Meghalaya. **(A)** Eddo (cv. Sunajuli). **(B)** Eddo with fused multi cormels (*cv.* Tasakrek-1). **(C)** Dasheen (*cv.* Rengama). **(D)** buda type with fused multi-corm (*cv.* Tamachongkham). **(E)** *Xanthosoma* spp. (*cv.* Tagiting Purple). **(F)** *Colocasia fallax* (leafy type *cv.* Chigi). **(G)** *Colocasia esculenta* var. *aquatilis* (*cv.* Tharsing). **(H)** *Colocasia gigantea* (*cv.* Ganima). **(I)** Wild Giant taro (*Alocasia* spp.)

TABLE 3 Estimation of genetic parameters for growth, yield, and quality attributes in Aroid species.

Traits/ genetic parameters	Plant height (cm)	Petiole length (cm)	No. of side shoots	Average corm wt. (g)	Average cormel wt. (g)	Yield/ plant (kg)	Total sugars (%)	Starch (%)	Oxalate (%)	Dry matter (%)
Mean	118.65	89.35	3.71	249.21	48.14	0.93	3.26	21.65	0.20	21.68
Minimum	89.35	61.47	1.84	38.04	14.87	0.24	1.93	15.34	0.10	16.75
Maximum	206.04	145.08	5.92	683.33	348.33	1.83	4.95	32.49	0.82	27.08
SE(m)	2.33	1.52	0.27	20.15	2.29	0.05	0.09	0.45	0.04	0.23
SE(d)	3.29	2.15	0.39	28.50	3.24	0.01	0.12	0.64	0.06	0.32
CV	3.40	2.94	12.80	14.01	8.24	8.24	4.58	3.62	35.64	1.81
GCV	16.46	22.14	24.54	61.26	116.90	39.26	22.63	16.14	32.72	11.78
PCV	16.80	22.33	27.67	62.84	117.19	40.11	23.09	16.54	48.39	11.92
h2	95.91	98.27	78.61	95.03	99.51	95.78	96.06	95.20	45.73	97.70
GA	39.39	40.39	1.66	306.61	115.63	0.75	1.49	7.02	0.09	5.20
GAM	33.20	45.21	44.81	123.03	240.22	79.01	45.70	32.45	45.59	23.98

Principal component analysis

The results of PCA analysis also revealed the presence of variability for different traits (Figure 4). The first four components with an Eigenvalue of >1, and contributed 65.97% of the total variation. Principal component 1(PC1), contributed 29.0% of the total variability (with loading >3.19), which was positively attributed by growth and yield attributing traits such as plant height, petiole length, yield per plant, yield per ha, corm and cormels weight. PC2 contributed 14.4% to the total variability and was depicted mainly by plant height, petiole

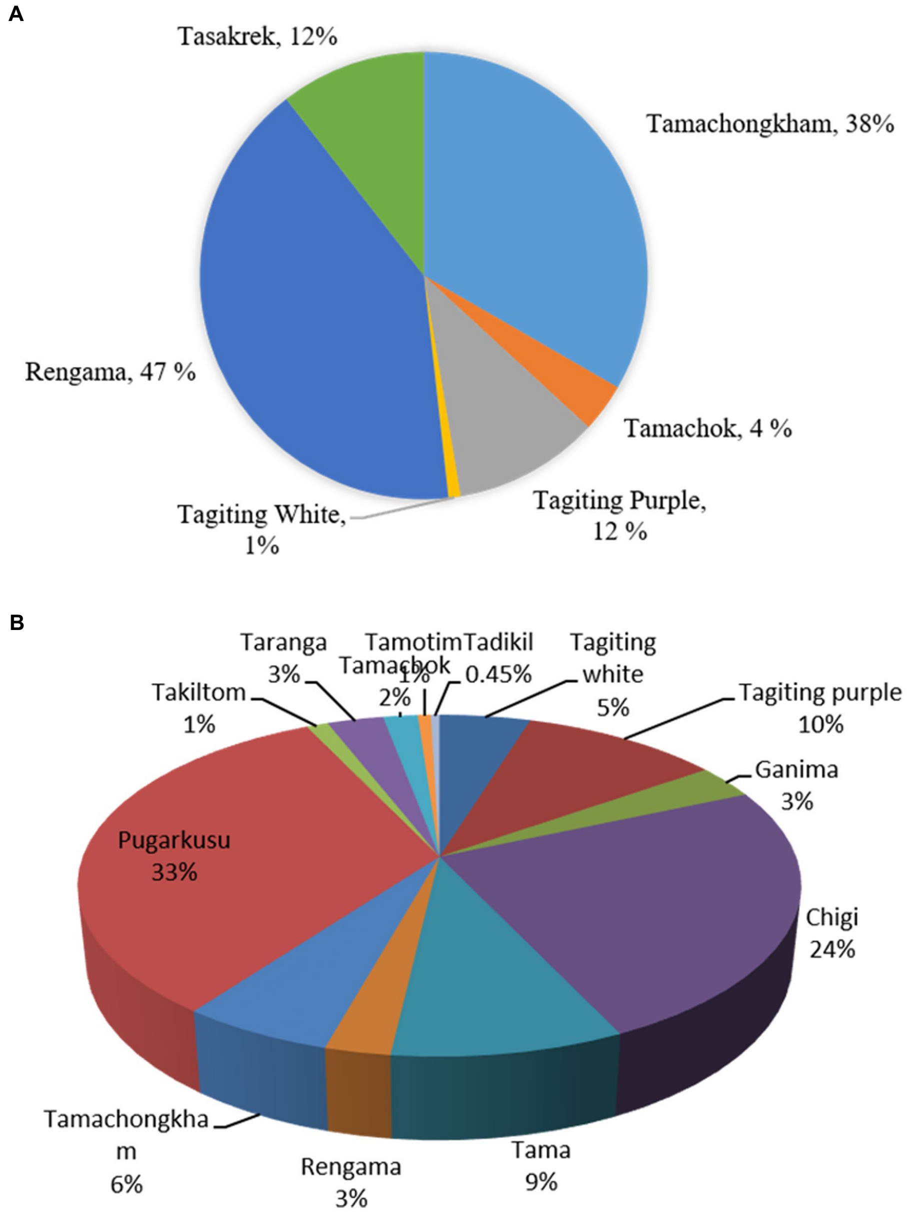

FIGURE 3
Population diversity of the taro landraces under **(A)**. Jhum field, **(B)**. backyards in Rombagre village of West Garo Hills, Meghalaya (India).

length, and the number of side shoots. The PC3 contributed 12.19% of the total variability and was mainly attributed to quality traits such as total sugar, dry matter, and starch content. PC4 contributed 10.36% to the number of side shoots, cormel weight, and starch content. The PCA biplot analysis also differentiated the genotypes for the different traits like Tamachongkham and Tama for higher oxalate content, Rengama for plant height and petiole length, C-3, Panchmukhi, Rengama-2, and Rongrem for corm weight and total yield (Figure 4).

Association between yield and quality attributes

The genotypic correlation coefficients for yield and quality contributing characters were higher than phenotypic correlation coefficients in most cases (Table 4). For traits, like the number of side shoots, total sugar, total oxalate, starch, and dry matter contents, phenotypic correlation coefficients were the same as or higher than the genotypic correlation coefficients. Among the traits at the genotypic and phenotypic level, yield per plant was significant and positively correlated with plant height, petiole length, number of side shoots, corm, and cormel weight (Table 4). However, among the quality parameters the sugar content was negatively correlated with all the traits except for corm weight. Further, starch content was significant and positively correlated with yield, corm weight, petiole length, and plant height. Moreover, total oxalate content was negatively correlated with the number of side shoots, and total sugar content at both genotypic and phenotypic levels. Likewise, dry matter content was significant and positively correlated with quality traits like, total sugar and starch content but negatively correlated with total oxalate, as also indicated by phenotypic correlation.

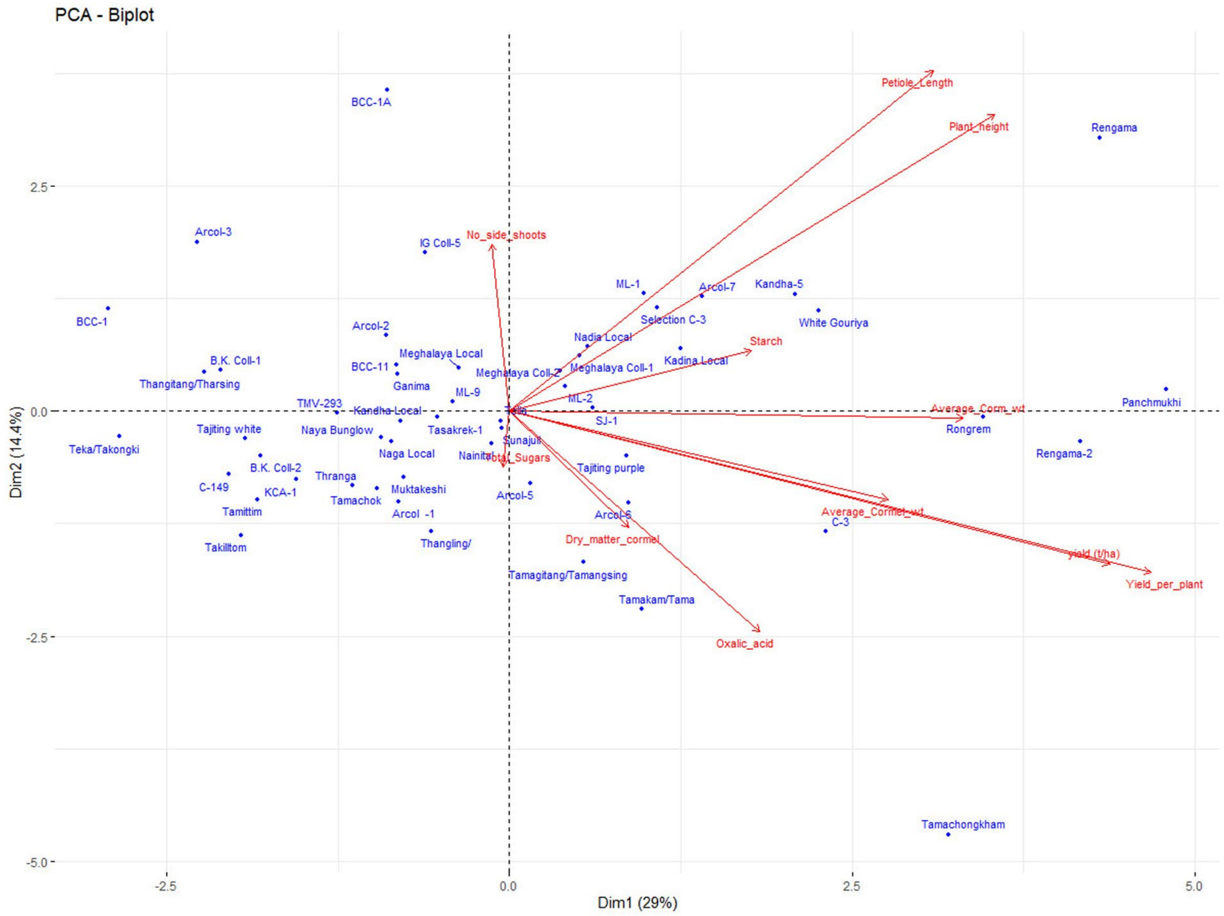

FIGURE 4
Principal components analysis biplot for traits and genotypes.

Genetic diversity

Genetic diversity based on quantitative traits

Based on 10 yield and quality related attributes, all 53 genotypes were grouped into 4 major clusters (Table 5). Cluster-I was comprised of 7 genotypes and characterized by small size corms (<150 g) and cormels (< 25 g). Cluster-II was the largest cluster with 24 genotypes including Ganima (*Colocasia gigantea*) having medium size corms (150–250 g) and cormels (25–50 g) which was further grouped into two sub groups, i.e., Cluster II-A (13 genotypes), and cluster II-B (11 genotypes including Tajiting White a *Xanthosoma* spp.). Cluster III was comprised of 6 high yielding (1.53 kg/plant) dasheen type genotypes like Tamachokgkham, Rengama-2, Rongrem, and Rengama collections from Garo hills and popular varieties/lines like Panchmukhi and C-3 with higher plant height, petiole length, maximum yield per plant, and rich in dry matter content. Furthermore, cluster-IV comprised 16 genotypes, including Tagiting Purple (*Xanthosoma* spp.) having the maximum number of side shoots. It was further grouped into two subgroups, i.e., IV-A having prominent corm (>400 g) with medium size cormels (25–50 g) and IV-B with medium size corm (>150 g) and cormels (25–50 g) with low total oxalate and higher in starch content.

Genetic diversity based on molecular markers

The SSR markers (33) used in the study has shown wider allelic variations among the genotypes (Table 2; Figure 5). A total of 136 alleles were observed with the minimum (3) in markers Ce1B-02, Taro-03, Taro-13, and Xuqtem-73 each and a maximum (8) in HK-34. Likewise, the effective number of alleles varied from 1.56 (Ce1F-04) to 5.10 (HK-5). The population has also shown the presence of heterozygosity, and it ranges varied 0.00–0.84. The observed average heterozygosity (0.24) was less than expected heterozygosity (0.69). Among the groups, the maximum heterozygosity was observed in eddo and dasheen groups (0.66 each) followed by dasheen fused type (0.56), eddo fused type (0.51), *Xanthosoma* spp. (0.27), swamp taro (0.06), and the least in eddo leafy types (0.09). Further, the polymorphic information content (PIC) value ranged from 0.13 to 0.76 across these 56 genotypes with a mean PIC value of 0.55. Out of 33 SSR markers 24 markers showed PIC value ≥0.50.

The results of molecular variance analysis (AMOVA) showed that the presence of diversity within and between the groups of the genotypes (Table 6). The maximum variation was observed within populations (57.11%) followed by between individuals (32.46%) of the same population and among populations (10.43%). Further cluster analysis has also shown wider diversity among the genotypes of aroid species (Figure 6). All the genotypes were grouped into 3 major groups

TABLE 4 Genotypic (G) and phenotypic (P) correlation for growth, yield and quality attributes in Aroid spp.

Traits		Petiole length (cm)	No. of side shoots	Average corm wt. (g)	Average cormel wt. (g)	Yield/plant (kg)	Total sugars (%)	Starch (%)	Oxalate (%)	Dry matter (%)
Plant height (cm)	G	0.873**	0.024	0.405**	0.234*	0.498**	−0.082	0.170*	0.119	0.015
	P	0.837**	0.027	0.384**	0.232*	0.478**	−0.082	0.159*	0.122	0.019
Petiole length (cm)	G		0.114	0.245**	0.209*	0.417**	−0.063	0.167*	0.078	−0.058
	P		0.089	0.240**	0.207*	0.410**	−0.063	0.167*	0.077	−0.059
No. of side shoots	G			−0.175	0.203*	0.049	−0.043	0.065	−0.349**	0.149
	P			−0.145	0.178*	0.055	−0.013	0.052	−0.279**	0.129
Average corm wt. (g)	G				0.238**	0.538**	0.141	0.178*	0.027	0.290**
	P				0.231**	0.523**	0.137	0.178*	0.023	0.273**
Average cormel wt. (g)	G					0.485**	−0.002	0.098	0.106	0.043
	P					0.477**	−0.002	0.094	0.101	0.043
Yield/plant (kg)	G						−0.030	0.287**	−0.104	0.184*
	P						−0.021	0.285**	−0.101	0.178*
Total sugars (%)	G							0.042	−0.183*	0.180*
	P							0.041	−0.173*	0.180*
Starch (%)	G								−0.033	−0.005
	P								−0.035	−0.002
Oxalate (%)	G									−0.172*
	P									−0.168*

*, ** significant at $p < 0.05$ and $p < 0.01$, respectively.

TABLE 5 Cluster mean values for different yield and quality attributes.

Cluster	Plant height (cm)	Petiole length (cm)	No. of side shoots	Average corm wt. (g)	Average cormel wt. (g)	Yield / plant (kg)	Total sugars (%)	Starch (%)	Oxalate (%)	Dry matter (%)
Cluster-I	114.92	89.52	4.11	130.01	17.97	0.341	2.93	21.11	0.20	20.48
Cluster-II-A	113.19	88.11	3.58	182.44	36.74	0.921	3.40	20.24	0.19	21.50
Cluster-II-B	111.50	77.53	3.27	228.16	29.82	0.656	3.45	20.97	0.20	21.95
Cluster-III	146.49	107.37	3.43	464.63	146.06	1.537	3.44	22.43	0.23	22.85
Cluster-IV-A	123.47	97.73	4.61	510.22	49.73	1.110	3.45	23.58	0.27	21.57
Cluster-IV-B	117.26	88.88	3.78	188.89	44.98	1.148	2.92	23.02	0.17	21.79

and Cluster-I (21 genotypes) and II (12 genotypes) were comprised of popular cultivars and landraces collected from the other parts of the country while the landraces of Garo Hills including related species *Colocasia gigantea* (cv. Ganima), *Colocasia esculenta* var. *aquatilis* (*cv*. Tharsing), *Xanthosoma* spp. (Tajiting Purple and Tagiting White) grouped in Cluster-III (25 genotypes). Tharsing a stolon-type taro was found to be most diverse from rest of the species. Moreover, *Colocasia fallax cv*. Chigi a leafy type accession was found closer to the local landrace Takiltom and Tama.

Further, principal coordinates analysis (PCoA) revealed that the three most important principal coordinate axes accounted for 36.84% of the total variance (Figure 7). The first coordinate axes differentiated the local landraces with popular cultivars. The second axes also differentiated the related aroid species (*Xanthosoma* spp.,) and *Colocasia fallax* (*cv*. Chigi) to *Colocasia esculenta* and species *Colocasia gigantea* (*cv*. Ganima), *Colocasia esculenta* var. *aquatilis* (*cv*. Tharsing) and genotypes having multi-corm, multi-cormels with other popular cultivars of *Colocasia* spp.

Genetic structure and interrelationship

Genetic structure analysis was carried out based on the 33 microsatellite markers as per the procedure described by Evanno et al., 2005 (43). The analysis has detected the maximal ΔK (109.33) at $K=2$. The Δ K value decreases with a further increase in K (Figure 8). Out

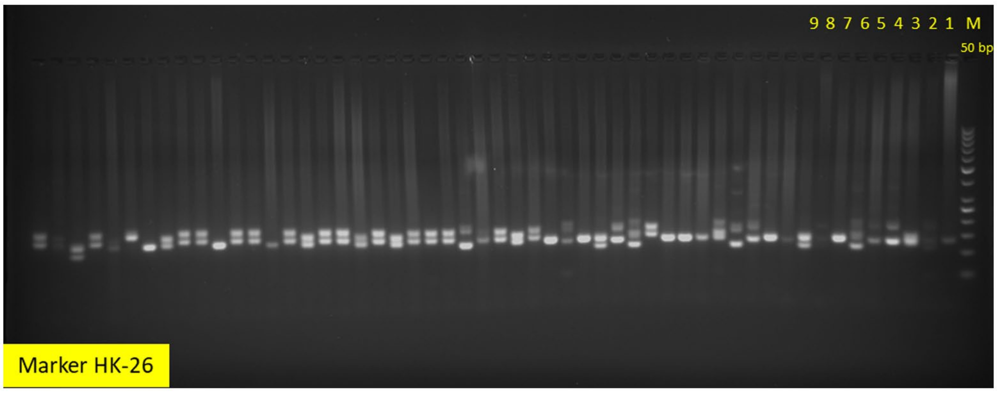

FIGURE 5
Allelic variations among aroid genotypes by SSR marker HK-26.

TABLE 6 AMOVA design and results (average over 33 loci).

Source of variation	Degree of Freedom	Sum of squares	Variance components	Percentage variation
Among populations	6	190.98	1.26	10.43
Among individuals within populations	51	904.07	6.90	57.11
Within individuals	58	227.50	3.92	32.46
Total	115	1322.55	12.08	

of 58 genotypes, 34 were admixture, and it was more common among the popular cultivars and landraces collected from other parts of the country (Figure 8). Moreover, there was the least and no admixture was observed in other aroid species *Xanthosoma* spp. (1 & 8), leafy type *Colocasia fallax* (2), stolon type *Colocasia esculenta* var. *aquatilis* (16) and *Colocasia gigantea* (23). The genetic differentiation between the groups was also from low to very high with fixation index (F_{ST}) values of 0.03–0.80 (mean = 0.28). The maximum differentiation was observed between *Colocasia fallax* (cv. Chigi) to *Colocasia esculenta* var. *aquatilis* (*cv*. Tharsing); *Colocasia fallax* (*cv*. Chigi) to *Colocasia gigantea* (*cv*. Ganima) and *Colocasia gigantea* (*cv*. Ganima) to *Xanthosoma* spp. (Figure 9). Moreover, the least differentiation was observed between Eddo/Arvi (*Colocasia esculenta* var. *antiquorum*) and bunda/dasheen type (*Colocasia esculenta* var. *esculenta*) genotypes.

Discussion

Aroid species are a group of multipurpose tuber crops, grown widely in tropical and subtropical parts of the world for their tubers, petioles and leaves, and playing an important role in the nutritional security of the population. The present study has indicated the presence of diverse genotypes (16 nos.) of aroid species with distinct ethnobotanical uses at household levels in the backyards and *Jhum* land in West Garo Hills of Meghalaya. The maximum diversity was observed in the backyards over the *Jhum* field. The Simpson's and Shannon's diversity index values were 0.80 and 42.59, respectively, for backyards and *Jhum* field, indicating the presence of higher diversity in the backyards as compared to the *Jhum* field. Moreover, the low evenness index value (0.42) for the *Jhum* field as compared to the backyard (1.0) indicated the dominance of some genotypes in the backyards as compared to the *Jhum* field. Further, evaluation trials have also shown wider variability for growth, yield, and quality attributes in aroid genotypes collected from the region. Angami et al., 2015 (44) also observed wider variability in taro landraces of the northeastern India.

Although, the crop is propagated clonally using corms, cormels, and shoots, there is scope for crop improvement through the selection of superior lines, as indicated by both the genotypic and the phenotypic coefficient of variation for all the traits (Table 3). All the traits, except, plant height, starch and dry matter content has shown the highest GCV and PCV (> 20%) indicating higher variability for these traits in the population. Except for total oxalate content, other traits have shown high heritability (> 60%). Further, except for plant height and dry matter content all the traits have shown a higher genetic advance (> 40%). Similar findings were also observed by Singh et al., 2003 (45) in taro. The high heritability and genetic advance of traits, suggest the least effect of environment and possibly the prevalence of additive gene action. Such characters would be responsive to selection (34, 36). The traits like plant height, starch, and dry matter content shown high heritability and low or moderate genetic advance indicating expression of these traits are influenced by non-additive gene action and environment. Further, PCA analysis also revealed the presence of variability for different traits. Economically important traits like yield, corm weight, dry matter, total sugar, and total oxalate content, as explained by principal components, contributing 65.9% of the total variation and selection can be followed for these traits in the population. PCA biplot also differentiated the genotypes for most of the traits, and hence the

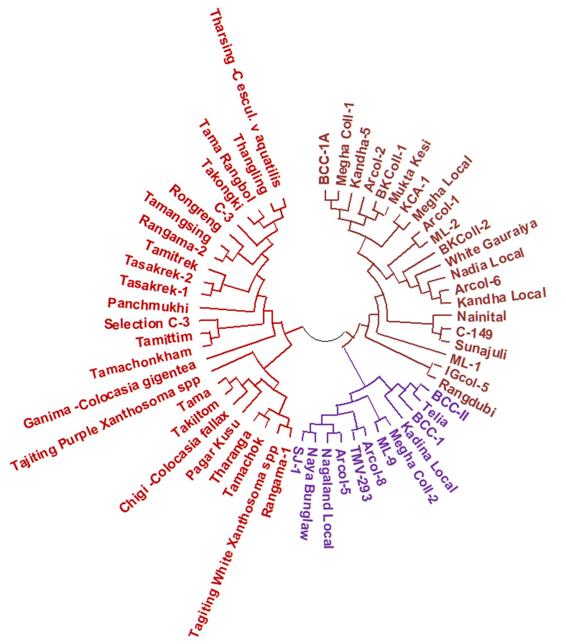

FIGURE 6
Dendogram depicting genetic relationship among the Aroid genotypes based on SSR markers.

superior genotypes from PC-I can be selected for specific traits (Figure 4).

Further, cluster analysis based on quantitative traits has also revealed the presence of wider diversity among the groups (Table 5). The identified high yielding (> 25.0 t ha^{-1}) landraces Tamachongkham, Tama, Rengama-2, Rongrem and Rengama collections from Garo Hills (all dasheen type) and eddo type genotypes White Gauriya and SJ-1, of cluster –III can be promoted for commercial production in the region. These genotypes outperformed the recommended cultivar Muktakeshi for yield by 11.71–29.18%. As yield-attributing traits like, corms and cormels weight was found highly heritable, the genotypes can be selected form the respective groups as per the choice of the consumer and market. For example, a genotype with smaller corm and cormels from Cluster-I, a prominent corm with small cormels from Cluster IV-A and a genotype with medium size corm and cormels from the cluster IV-B. Moreover, the collection of aroids from the project site (Rombagre, Garo Hills) have also shown wider variability for different traits (Table 1).

Under ethnobotanical uses, genotypes like Tamachongkham, Tama with high acridity and total oxalate content was used only for cooking with meat (Supplementary Table S1). For curry preparation, they use genotypes like Rongrem, Tamachok etc. which are low to medium in acridity and total oxalate (< 0.25%) content. Tasakrek, a landrace with low in acridity, total oxalate content (0.10–0.12%) and high in total sugar content (> 3.0%) was utilized as baby food. As

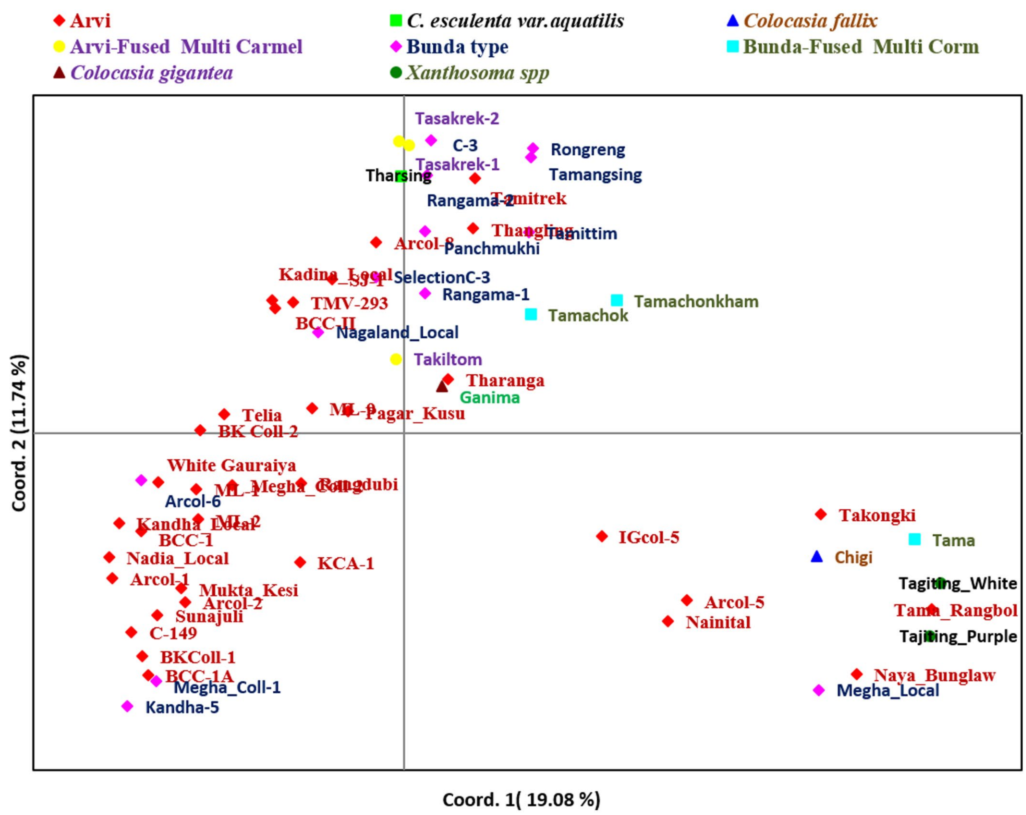

FIGURE 7
Principal coordinate analysis of Aroid genotypes based on SSR markers.

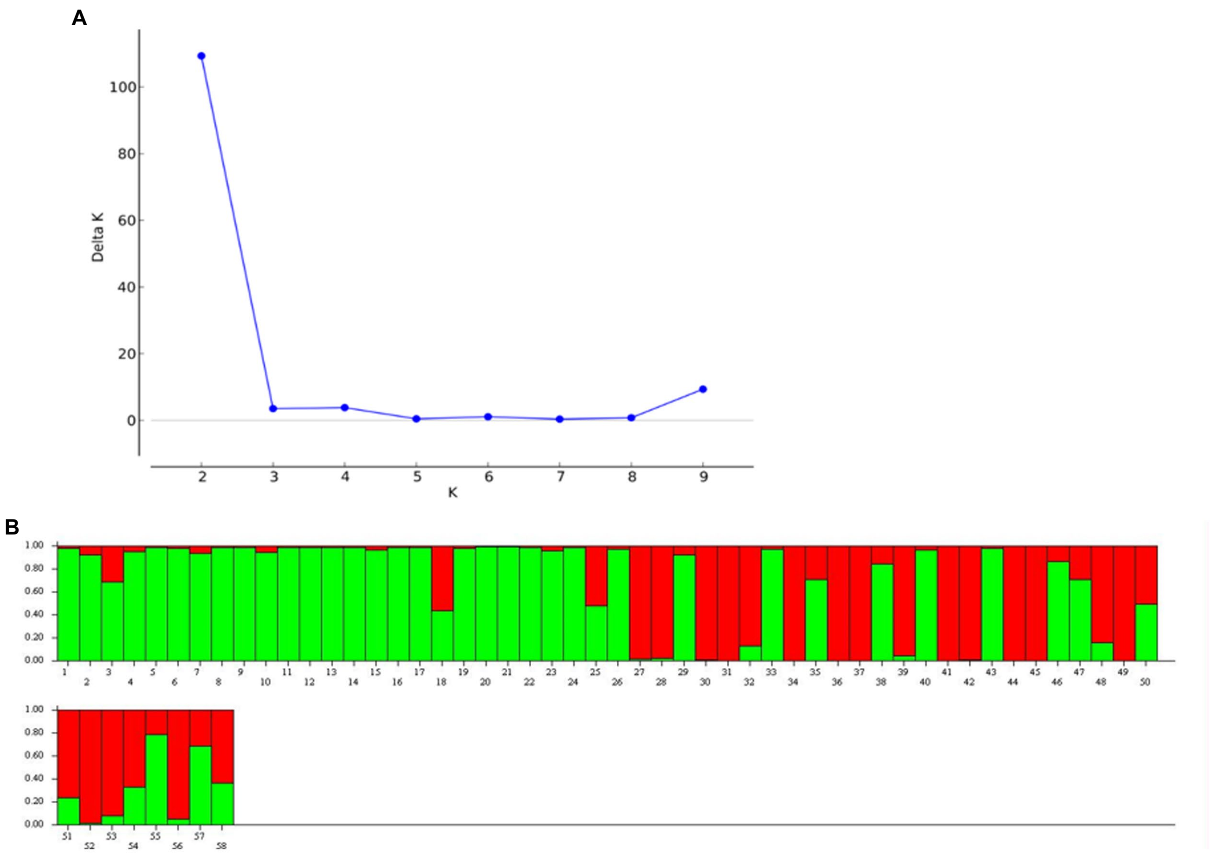

FIGURE 8
Population structure of 58 Aroid genotypes based on 33 SSR markers. **(A)** Δ K graph, **(B)** population structure at ΔK=2.

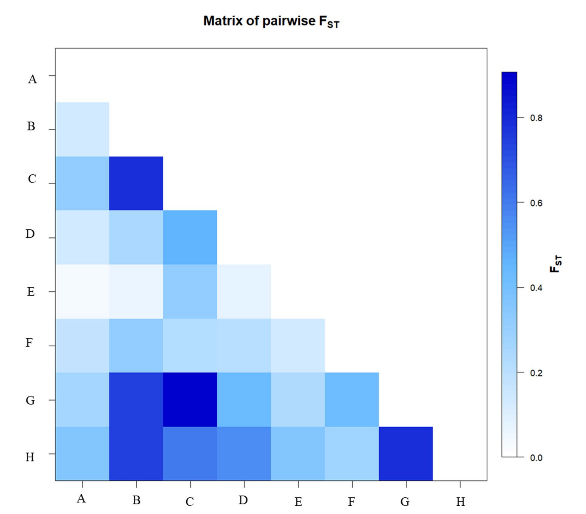

FIGURE 9
Matrix of pairwise F_{ST}. Where: A: Eddo; B: *Colocasia esculenta* var. *aquatilis* (cv. *Tharsing*); C: *Colocasia fallax* (cv. Chigi); D: Eddo with fused multi-cormel; E: Dasheen type; F: Dasheen type with fused multi-corm; G: *Colocasia gigantea* (cv. Ganima); H: *Xanthosoma* spp.

breakfast snacks, landraces like Rengama and Tamittim having higher level of starch (>22%), and lower level of acridity and total oxalate (< 0. 20%) were used. Tania (*Xanthosoma* spp.) landraces such as, Tagiting Purple and Tagiting White grew vigorously and were grown mostly for their petioles for cooking and pig feed especially during dry winter season, when there is scarcity of vegetables. This could be due to prolonged periods of availability, tolerance to leaf blight, and low temperature as compared to other *Colocasia* species, as *Xanthosoma* spp. are rich in cuticular wax content (46). Moreover, tania has been found rich in phosphorous in leaves (388 mg/100 g), petioles (80 mg/100 g), and β-carotene (3,300 ug/100 g) content in leaves (47). Further, the landrace Chigi (*Colocasia fallax*) is grown in the backyards for its leaves and petioles as green leafy vegetables during winter and spring–summer when there is a scarcity of vegetables in the villages. It has been found tolerant to shade and excessive moisture, as it is grown near the drains having multiple fruit plants (banana, orange, jackfruit, guava etc.). Ganima (*Colocasia gigantea*) corms are rich in starch (23.87%) and medium in total oxalate (0.28%) content. Its vigorous growth habit/higher petioles yield with low acridity and poor tuber yield (8.3 t ha^{-1}) may be the possible reasons for petioles production. At household levels, the loss of genetic diversity was also observed, and which was mainly due to factors like poor yield, poor marketing and being susceptible to pests and diseases etc. (Table 1).

The genotypic correlation coefficients for yield and quality contributing characters were higher than the phenotypic correlation coefficients in most cases (Table 4), indicating that the effects of environment has suppressed the phenotypic relationship between these characters. Higher genotypic than phenotypic correlation coefficients among the various traits have been reported by Paul et al., 2014 (48) and Mukherjee et al., 2016 (49) in taro. For traits like the number of side shoots, total sugar, total oxalate, starch, and dry matter contents, phenotypic correlation coefficients were the same as or higher than the genotypic correlation coefficients, indicating that both environmental and genotypic correlations in these cases acted in the same direction and finally maximized their expression at the phenotypic level.

Among the traits at the genotypic and phenotypic levels, yield per plant was significant and positively correlated with plant height, petiole length, number of side shoots, and average corm and cormel weight (Table 4). Similar findings were also reported by Paul et al., 2014 (48). However, among the quality parameters, the total sugar content was negatively correlated with all the traits except for corm weight. Further, starch content was significant and positively correlated with yield, corm weight, petiole length, and plant height. Moreover, total oxalate content was negatively correlated with the number of side shoots and total sugar content at both genotypic and phenotypic level. Likewise, dry matter content was significant and positively correlated with quality traits like, sugar and starch content but negatively correlated with total oxalate. Similar is the case with phenotypic correlation. This could be due to the higher accumulation of photosynthates by the plants and translocation towards corm and cormel production. Moreover, selection based on higher dry matter, starch, and sugar content will be effective to identify the superior accession low in acridity and total oxalate content.

The results of molecular analysis (33 SSR), have generated a total of 136 alleles with wider allelic variations among the genotypes and ranges from 3 (Ce1B-02, Taro-03, Taro-13 and Xuqtem-73) to 8 (HK-34). The presence of heterozygosity (0.0–0.84), indicated the natural hybridization and evolution of the different landraces. Further, group wise maximum heterozygosity in eddo and dasheen also indicated the natural hybridization among the accessions of the same group. Moreover, the least heterozygosity in eddo leafy type and Swamp taro may be due to difference in ecological habitat and a lack of natural crossing. Among the markers (33), the PIC values ranges from 0.13–0.74 and majority of the markers (24) showed high polymorphism (PIC >0.5), and thus indicated the presences of a higher level of genetic diversity among the genotypes which markers can easily differentiate. Similarly, higher polymorphism has also been observed by Khatemenla et al., 2019 (50) in the genotypes of northeastern India. Such greater diversity is expected, as the region is considered the primary center of diversity of the Aroid species. Further, marker HK-34 differentiated Chigi, Tamachok, Tasakrek-1, Taskrek-2, Megha Local, and Rengama having multiple copies (3) of the alleles from other genotypes with unique alleles at an extra locus. This could be due to differences in ploidy levels of the genotypes. The observed average heterozygosity (0.24) was less than the expected heterozygosity (0.69). Similar finding was also observed by Lu et al. (23) in the genotypes from China. This could be due to lack of hybridization as the crop is has erratic flowering, lack of seed set (51), and clonal propagation. The poor natural crossing/fertility may be a result of triploidy as well as its localization to backyard places. Chaïr et al., 2016 (52) observed over 74.2% population of the taro from India were triploid, majority of which (72%) were collected from the Meghalaya state itself.

The results of analysis of molecular variance (AMOVA) have shown the presence of 89.57% of diversity within the population which also supported our findings of less hybridization possibilities (Table 6). Further, cluster analysis based on SSR genotyping (Figure 6) has also shown wider diversity among the genotypes of aroid species. All the genotypes were grouped into 3 major groups, Cluster-I (21 genotypes) and II (12 genotypes) were comprised of popular cultivars and landraces collected from the other parts of the country, while the genotypes of Garo Hills such as *Colocasia fallax* cv. Chigi a leafy type taro, Tajiting Purple and Tagiting White (*Xanthosoma* spp.) grouped in Cluster-III. Genotype, Ganima (*Colocasia gigantia*) cultivated for the petioles and leaves was found to be most diverse form rest of the species. Moreover, *Colocasia fallax* cv. Chigi a leafy, dwarf type and shade-loving, accession found wild and also cultivated in kitchen garden for their year-round leaves and petioles was closer to local landrace Takiltom and Tama. Similarly, under principal coordinate analysis, the landraces were distributed with genotypes from different geographic origins. Similar findings were also observed in the genotypes of taro in Pacific Ocean island, Vanua Lava, Vanuatu (53). The cluster analysis, also indicated the presence of the higher genetic variation within the local genotypes of project site (Rombage, Meghalaya), which are mainly known by their local name like, Rengama and Tasakrek each with two genetically distinct variants, i.e., Rengama-1, Rengama-2 and Tasakrek-1, Tasakrek-2, respectively.

The genotypes were differentiated into 2 genetic groups with maximal ΔK at K = 2 according to the analysis of genetic structure of the 33 microsatellite markers. The 58.62% of the population showed admixture, and it was more common among the popular cultivars and landraces collected from other parts of the country (Figure 8). The least admixture was observed in other aroid species including *Xanthosoma* spp. (1 & 8) and *Colocasia esculenta* var. *aquatilis* (23), which might be attributed to poor natural crossing/fertility. Further, pairwise genetic divergence between the groups were also from low (0.03) to high (0.80) F_{ST} value. Values between 0.00–0.05 indicate little divergence, 0.05–0.15 moderate divergence, 0.15–0.25 high divergence, and over 0.25 a very high degree of divergence (40). The maximum differentiation (Figure 9) was observed between *Colocasia fallax* cv. Chigi (leafy type) to Tharsing (*Colocasia esculenta* var. *aquatilis*.) and *Colocasia fallax cv.* Chigi (leafy type) to Ganima (*Colocasia gigantea*), and Ganima (*Colocasia gigantea*) to tannia (*Xanthosoma* spp). The high degree of genetic differentiation could be due to restricted gene flow between the populations, evolution in different ecology of the region, and somaclonal variations. Moreover, the least differentiation was observed between eddo (*Colocasia esculenta* var. *antiquorum*) and dasheen type (*Colocasia esculenta* var. *esculenta*) genotypes. The stolon farming landrace Tharsing (*Colocasia esculenta* var. *aquatilis*.) was found closer to dasheen/bunda type species (*Colocasia esculenta* var. *esculenta*) with the least differentiation, and this could be due to evolution and selection of the dasheen type genotypes form the stolon farming landraces. Mathews (54), also reported that the cultivated taro originated from the *Colocasia esculenta* var. *aquatilis* through natural differentiation and human selection in tropical areas from northeast India to Australia/New Guinea. However, comparatively least differentiation has been observed in the core set having multi-corm, and cormel genotypes of different geographical origin in China (55).

The above findings of the present investigation have shown the presence of wider genetic diversity in aroids species in the region. The population has low heterozygosity and wider genetic diversity at the molecular level. The superior genotypes can be selected based on consumer demands for the size or weight of the corms/cormels, as they are governed by additive gene action and responsive to selection. The present study has identified landraces at the project site with unique ethnobotanical uses and that have certain nutritional values, like genotypes rich in total oxalate used for cooking with only meat, high starch and low to medium oxalate for breakfast, and genotypes higher in sugar content and low in acridity for baby food need to be conserved and utilized for sustainable *Jhum*/shifting farming, enhancing nutritional security, and strengthening future crop improvement programs. Moreover, in addition to common eddo and dasheen type genotypes, more taxonomic studies on other groups of cultivated aroids, especially fused type eddo and dasheen, as well as leafy type are required. Further, this study also advocate for nutritional profiling, especially genotypes grown for the leaf and petioles purpose in the region as they also have alternative uses in feed ingredients for rearing of pigs, ducks, and poultry.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

VV conceptualize the study and prepared the manuscript with contributions from co-authors. VV, HT, and NS acquired the fund for the research work. VV and PC carried the field trails and data collection, biochemical, and molecular analysis. AK and NS carried the data analysis. All authors approved the final version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1065745/full#supplementary-material

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Impact of organic and integrated production systems on yield and seed quality of rainfed crops and on soil properties

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Mineral and vitamin deficiencies together affect a greater number of human populations in the world than does protein malnutrition. Organic farming is reported to improve nutritional quality of food grains while also improving soil health. However, sufficient scientific information on several aspects of organic farming based on long-term studies is lacking particularly under rainfed conditions of India. The purpose of this study was to assess the long-term impact of organic and integrated production systems on crops yield and quality, economic returns and soil properties. The study was conducted with three crops, sunflower (*Helianthus annuus* L.), pigeonpea (*Cajanus cajan* L.), and greengram [*Vigna radiata* (L.) Wilczek] under three different production systems, control (use of chemical inputs alone), organic and integrated. The results of the 10-year study revealed that, the average production of integrated system was on par with organic management and recorded significantly higher pigeonpea equivalent yield (PEY) (827kgha^{-1}) compared to control (chemical inputs) (748kgha^{-1}). In general, the yield gap between organic and integrated production systems declined from fourth year for greengram and eighth year for sunflower, during the 10-year experimental period whereas the pigeonpea yield was similar under both production systems from first year. Plots under organic management had significantly lower bulk density (1.18mgm^{-3}), higher water holding capacity (38.72%) and porosity (53.79%) compared to integrated production system and control (chemical inputs). The soil organic C (SOC) content in the plots under organic production system was 32.6% more than the initial organic carbon of the soil (0.43%), with higher soil N (205.2kgha^{-1}). Plots under integrated production system, however, had higher soil P (26.5kgha^{-1}) compared with other treatments. The dehydrogenase activity (5.86μg TPF g^{-1} soil h^{-1}) and microbial biomass carbon (317.3μgg^{-1} soil) content was higher in the plots under organic production system than under other systems. Organically produced pigeonpea and greengram seeds had similar protein content with that of integrated system, and higher K and micronutrient (Fe, Zn, Cu, and Mn) contents than other treatments. The results show the potential of organic production system in improving crop yields, soil properties and produce quality in semiarid rainfed areas.

KEYWORDS
organic production system, pigeonpea, greengram, sunflower, soil health, rainfed areas

Introduction

Several countries have started promoting organic farming as an alternative to high-input agriculture (conventional farming). Organic farming is one of the fastest growing sectors of agricultural production. As per the latest FiBL survey, 74.9 million ha were under organic agricultural management worldwide (1). Organic farming is not new to India as this nature friendly farming practice is done in the country from ancient times (2). Recently, promotion of organic farming is one of the priority areas of Government of India to improve agricultural productivity while reducing the use of external inputs; The Government is promoting adoption of organic farming in India through various schemes such as Paramparagat Krishi Vikas Yojana (PKVY) and Mission Organic Value Chain Development for North East Region (MOVCDNER). Last decade witnessed a huge jump under the area of certified organic farming in India. Presently, India has about 2.7 million ha under certified organic farming with the highest number of organic producers (1.6 million) in the world (1).

The productivity of rainfed agriculture which constitutes about 51% of the cultivated area in India is constrained by the aberrant monsoon, low and unstable yield, small farm size, degraded soil and resource poor farmers. Smallholders in rainfed regions may have the chance to increase their output through organic farming without relying on outside resources like capital or inputs, and they may also be able to sell their food for higher prices. Numerous studies have compared the output, impact on the environment, and financial returns of organic and conventional farming. In general, some loss in crop yields is observed after discarding synthetic inputs and converting the operations from the conventional systems to organic production (3, 4), while others have reported that organic systems can be as productive as conventional ones (5, 6). By adding organic manures, the soil's available nutrients usually benefit in the form of increased yields (7, 8). However, literature on performance of rainfed organic production systems is scanty. Though organic farming systems are low-impact and low-yielding than conventional or integrated management systems, they are reported to be more resilient and offer nutrient-dense quality food (9, 10). Further, the reduction in crop yields from organic systems can very well be compensated by the higher economic returns fetching from the price premium (11, 12).

On the other hand, organic amendments like farmyard manure (FYM), vermicompost and green manures lowers bulk density, improves porosity and infiltration rates, reduces surface runoff, increase water-holding capacity thus improving soil physical properties (13–16). Furthermore, numerous studies demonstrate that soil fertility is increased over time by organic farming (14, 17–19). In comparison to conventionally maintained systems, these organic systems also result in superior soil quality and greater soil biological activity (19, 20). Unlike chemical fertilizers, organic amendments are characterized with their slower nutrient release pattern coupled with higher residual effect on the subsequent crops (21, 22). Judicious application of organic amendments improve the crop productivity in addition to maintaining the sustainability of the system (23, 24) because of the organic manure being the basic source of organic matter in soil. In fact, one of the greatest challenges in the present world is to feed the ever-increasing population, still maintaining soil health along with environmental quality (25).

Legume and oilseed crops are the most relevant crop type in global food security, and as such, the move toward resilient and more sustainable cropping systems by reducing the chemical input is a major challenge. Sunflower (*Helianthus annuus* L.), greengram [*Vigna radiata* (L.) Wilczek] and pigeonpea [*Cajanus cajan* (L.) Millsp.] are well suited for the rainfed regions of semi-arid tropics and are widely grown in this region. Further, no attempts were made so far to assess the impact of different production systems on performance of these crops and on different soil properties. Hence, we carried out a study to assess the impact of organic and conventional production systems on performance of sunflower, greengram and pigeonpea, crop quality and soil properties in semiarid rainfed conditions. Here, the first hypothesis we tested was that organic production system would improve crops yield and quality compared to that of conventional production systems due to improvement of soil properties. The second hypothesis tested was that crops respond differently to different production systems.

Materials and methods

Study area

The study location is situated in India's 7.2 agro-ecological subregion, and the growing season lasts between 120 and 150 days. The region has a semi-arid (dry) climate with three distinct seasons: the summer (March to May), the rainy season (*kharif*), which lasts from June to September, and the winter (*rabi*) (October to February). At the Gungal Research farm of the ICAR-Central Research Institute for Dryland Agriculture (17°40′ 40.4″ N latitude and 78°39′, 55.7″ E longitude and at a mean sea level of 626 m), Hyderabad, Telangana, India, the field experiment was carried out for 10 years between 2012 and 2021.The farm represents a semi-arid tropical region with a mean annual temperature of 25.7°C and rainfall of 746 mm. The monthly rainfall during the crop season (July–December) during the study period (2012–2021) and the monthly maximum and minimum temperature prevailed during the period are given in Figures 1, 2. Soil of the experimental site is sandy loam; slightly acidic in reaction (pH 6.51), EC was in normal range (0.05–0.07 dS m^{-1}), low in organic carbon (0.43%), available N (229.1 kg ha^{-1}), high in available P (24.7 kg ha^{-1}), and medium in available K (218.1 kg ha^{-1}) (3).

Treatments

Three production systems *viz.* organic, integrated and control (chemical inputs), and three field crops *viz.* sunflower (*Helianthus annuus* L.), pigeonpea [*Cajanus cajan* (L.) Millsp.], and greengram [*Vigna radiata* (L) Wilczek] were studied in this experiment every year. The experiment was laid out in strip plot design. All the treatments were replicated thrice in a plot size of 12 m × 4 m. The package of practices in each crop are presented in Table 1. The FYM was sourced from the same place every year and the average composition of 0.5% N, 0.25% P, 0.4% K, 27.9 ppm Cu, 228.7 ppm Mn, 452 ppm Fe, and 143.1 ppm Zn (3).

The FYM was treated with *Trichoderma viridae* at 2.5 kg ha^{-1}, as a prophylactic measure against soil borne diseases as explained by

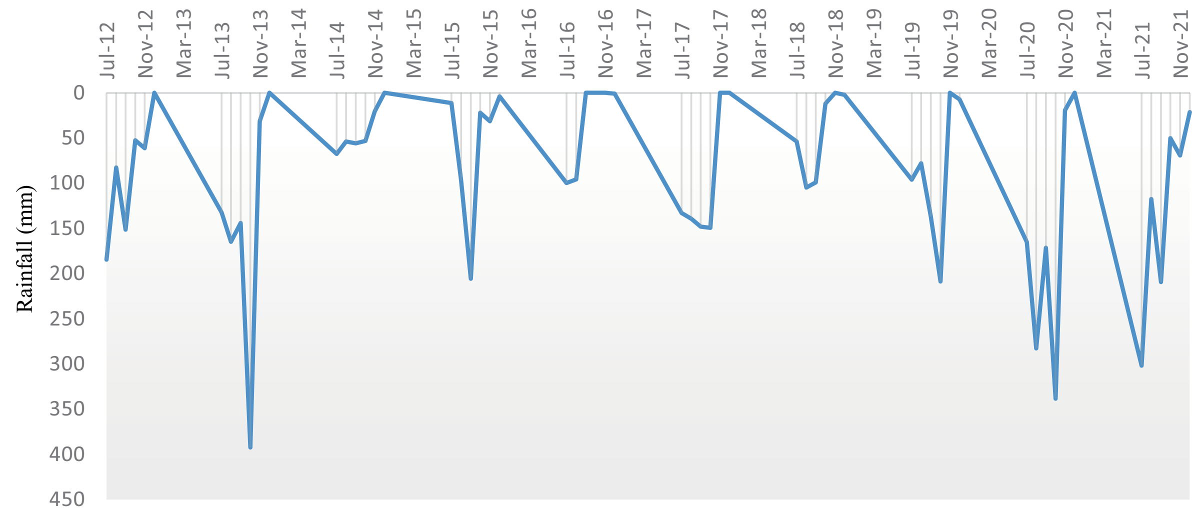

FIGURE 1
Monthly total rainfall (mm) during the cropping period (July–December) during 2012–2021.

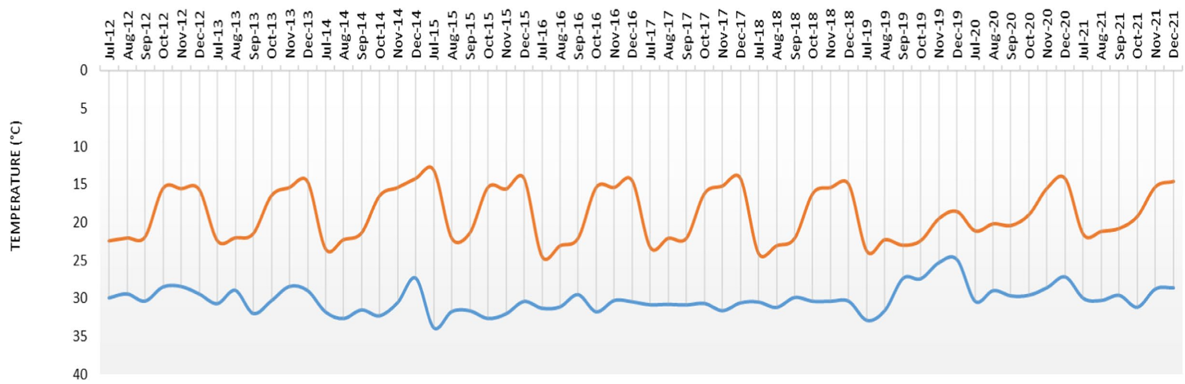

FIGURE 2
Mean monthly maximum and minimum temperature during the crop period (July–December) during 2012–2021.

Gopinath et al. (26). The details of application of nutrients in different treatments are given in Tables 2, 3. Under integrated method, one fourth of the N was applied through FYM. The remaining N and total P and K was applied through mineral fertilizers. In sunflower crop, N was applied in basal and two other splits (30 and 60 DAS). However, in pigeonpea and greengram the nutrients were applied basal.

Every year, the crops were sown after the receipt of monsoon rainfall in the month of June. The organic plots were maintained chemical free throughout the years. Manual weeding and manually operated wheel-hoe were used to keep the plots wee free. The prophylactic measure used are described by the authors in their earlier paper (3). Crops were harvested and the yields were converted to pigeonpea equivalent yield (PEY) as per De Wit (27):

$PEY\ (kg\ ha^{-1})$ = [Yield of sunflower or greengram (kg ha^{-1}) × price of sunflower or greengram seed (Rs kg^{-1})/price of pigeonpea (Rs kg^{-1})].

TABLE 1 Variety, seed rate and planting geometry used for each crop.

Crop	Variety	Seed rate (kgha^{-1})	Planting geometry (cm)
Sunflower	DRSH-1	6	60 × 30
Greengram	WGG-37	15	30 × 15
Pigeonpea	PRG-158	15	90 × 20

Soil sampling and analysis

A core sampler was used to collect the soil samples before the application of various nutrients and after the crop harvest in 2021 from various soil depts (0–20, 20–40, and 40–60 cm). The soil samples for various microbial activity were kept at 4°C and analyzed within

2 weeks. Bulk density of the soil was determined using metallic cores of known volume. The detailed procedures are explained by the author in their earlier paper (3).

Keen Rackzowski box method was used to determine the WHC and total porosity (28). Potentiometric method as described by Jackson (29) was used for determining soil pH. Available N, P, and K were determined using Kjeldahl method, Olsen's method and flame photometer method (29–31). Micronutrients were determined using the atomic absorption spectrometry (32). Soil organic carbon was multiplied with BD to arrive at soil carbon stock (33).

Various microbial activities like dehydrogenase (DHA), soil microbial biomass carbon (MBC), urease and acid phosphatase was analyzed/measured using methods of Casida et al. (34), Vance et al. (35), Tabatabai and Bremner (36), and Tabatabai and Bremner (37).

Crop quality analysis

The dried seeds were stored at room temperature prior to analysis for various parameters. The samples of greengram and pigeonpea were analyzed for chemical parameters after tri-acid digestion. Nitrogen content was determined by Kjeldahl method (38). Protein content was determined by using the formula: N × 5.4 (39). Phosphorus content was analyzed photometrically (40). Potassium content was determined by using flame photometer and micronutrients (Fe, Cu, Mn, and Zn) by using atomic absorption spectroscopy. Sunflower oil was extracted using hexane on Soxhlet apparatus using the methodology of Anjani and Yadav (41).

Statistical analysis

Using the International Rice Research Institute (IRRI) Star and ANOVA, data were statistically evaluated. Tukey's HSD *post hoc* comparisons were used to clarify significant differences in means ($p < 0.05$).

Results

Crop quality

Different production systems significantly influenced the protein content of pigeonpea seed (Table 4). Pigeonpea grown under integrated production system being on par with organic system recorded significantly higher protein content (20.0%) than that of Control (chemical inputs). However, different production systems had no significant influence on protein content of greengram seed. Organic production system being on par with integrated production system recorded significantly greater P content in both pigeonpea and greengram seeds compared to Control (chemical inputs). Significantly greater K content of both pigeonpea and greengram was recorded with organic production system than other treatments. The micronutrient (Fe, Zn, Cu, and Mn) contents of both pigeonpea and greengram seeds varied significantly with different production systems (Table 4). Organic production system being on par with integrated production system registered significantly higher Fe and Zn contents in seeds of both crops compared to Control (chemical inputs). The Zn and Cu

TABLE 2 Application rates of farmyard manure and mineral fertilizers in different treatments.

Treatment	Sunflower					Pigeonpea					Greengram				
	FYM ($Mgha^{-1}$)	Mineral fertilizers ($kgha^{-1}$)			Rock phosphate ($kgha^{-1}$)	FYM ($Mgha^{-1}$)	Mineral fertilizers ($kgha^{-1}$)			Rock phosphate ($kgha^{-1}$)	FYM ($Mgha^{-1}$)	Mineral fertilizers ($kgha^{-1}$)			Rock phosphate ($kgha^{-1}$)
		N	P_2O_5	K_2O			N	P_2O_5	K_2O			N	P_2O_5	K_2O	
Organic	13.3	0	0	0	0	4.4	0	0	0	167	4.4	0	0	0	167
Integrated	3.3	45	60	30	0	1.1	15	50	0	0	1.1	15	50	0	0
Control*	0	60	60	30	0	0	20	50	0	0	0	20	50	0	0

*Use of chemical inputs alone.

contents of organically grown pigeonpea and greengram seed was greater than that of other production systems.

Different production systems had significant effect on sunflower oil content (Figure 3). Integrated production system being on par with Control (chemical inputs) had greater oil content than that of organic production system. Regarding fatty acid composition of oil, organically produced sunflower oil being on par with that of integrated production system had a higher content of oleic acid than Control (chemical inputs). However, no statistical differences were evident among different production systems in terms of palmitic acid, stearic acid and linoleic acid contents.

Crop yield

Yield of all the different crops and cropping system was significantly different in terms of pigeonpea equivalent yield (PEY) in all the years (Figure 4). Rainfall distribution and amount has a greater impact on the yield. Sufficient and well distributed rainfall during 2012 and 2013 resulted in higher yield due to less crop stress. Intermittent dry spells and less rainfall during the others years resulted in PEY less than 1,000 kg ha^{-1} (Figure 1).

In the first year, the integrated production system greatly outperformed the organic and control (chemical inputs) treatments in

TABLE 3 Amount of nutrients applied each year through farmyard manure and rock phosphate in different crops under organic management.

Crop	FYM ($Mgha^{-1}$)	Rock phosphate ($kgha^{-1}$)	Nutrients applied through FYM ($kgha^{-1}$)			P_2O_5 applied through rock phosphate ($kgha^{-1}$)	Total major nutrients applied ($kgha^{-1}$)			Total micronutrients applied ($kgha^{-1}$)			
			N	P_2O_5	K_2O		N	P_2O_5	K_2O	Fe	Cu	Mn	Zn
Sunflower	12.0	0	60	68.7	57.6	0	60	68.7	57.6	5.42	0.33	2.75	1.71
Pigeonpea	4.0	165	20	22.9	19.2	27.2	15	50.1	19.2	1.81	0.11	0.91	0.57
Greengram	4.0	165	20	22.9	19.2	27.2	20	50.1	19.2	1.81	0.11	0.91	0.57

TABLE 4 Seed protein, P, K and micronutrient contents mas influenced by different production systems.

Production system	Protein (%)	P (%)	K (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
Pigeonpea							
Organic	19.6 ± 0.1a	0.19 ± 0.02a	2.39 ± 0.01a	35.6 ± 1.5a	25.0 ± 0.2.1a	10.4 ± 1.8a	11.3 ± 2.3a
Integrated	20.0 ± 0.2a	0.18 ± 0.01ab	2.33 ± 0.03a	31.7 ± 2.1ab	22.7 ± 3.1b	9.7 ± 2.2b	10.8 ± 2.0ab
Control	17.9 ± 0.1b	0.17 ± 0.03b	2.20 ± 0.03b	36.7 ± 1.4b	21.8 ± 2.8b	7.9 ± 1.2c	10.2 ± 2.1b
Greengram							
Organic	21.8 ± 0.2a	0.19 ± 0.02a	2.85 ± 0.01a	37.0 ± 1.2a	29.6 ± 2.1a	10.8 ± 1.2a	12.7 ± 2.1a
Integrated	21.4 ± 0.1a	0.19 ± 0.01a	2.61 ± 0.02b	35.4 ± 1.8ab	29.1 ± 1.7b	8.0 ± 1.8b	12.2 ± 1.8ab
Control*	21.2 ± 0.1a	0.17 ± 0.02b	2.59 ± 0.01b	30.9 ± 1.6b	28.8 ± 1.8b	7.2 ± 0.9c	10.7 ± 1.4b

*Use of chemical inputs alone. Means in the same columns with different letters are significantly ($p < 0.05$) different.

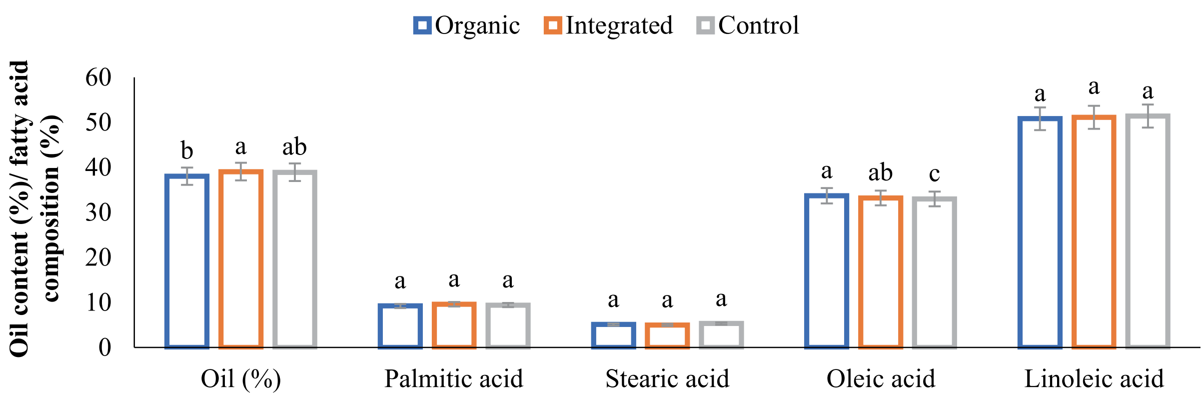

FIGURE 3
Effect of different production systems on oil content and fatty acid composition of sunflower seed. Bars with different letters within each parameter are significantly ($p < 0.05$) different.

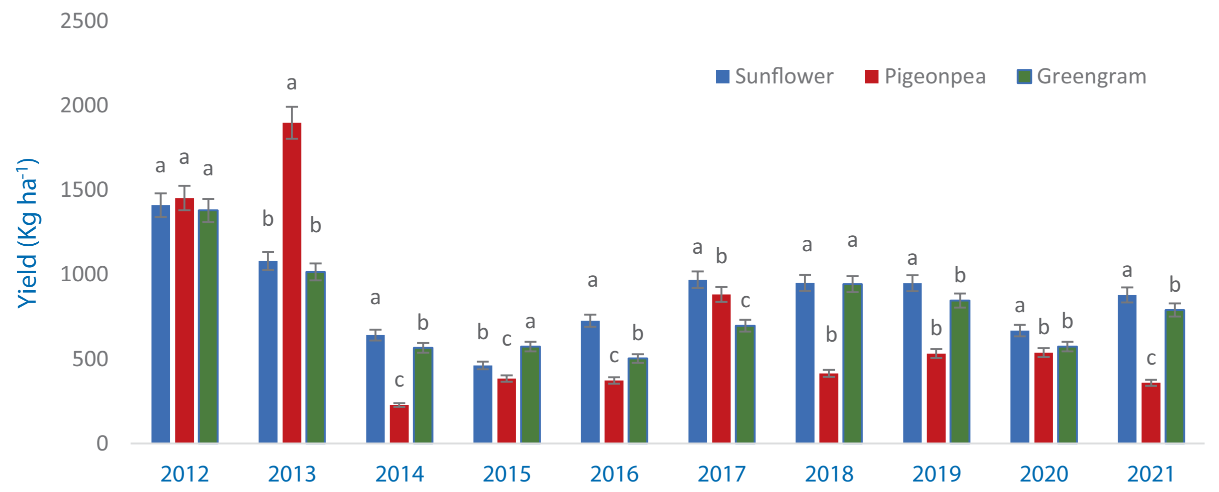

FIGURE 4
Pigeonpea equivalent yield (PEY) of crops during 2012–2021 (averaged across three production systems). Bars with different letters within each year are significantly (p<0.05) different.

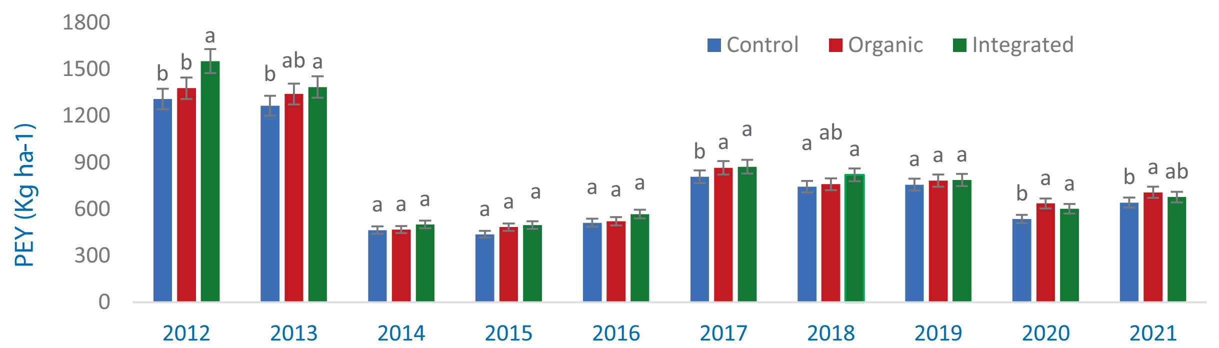

FIGURE 5
Effect of different production systems on pigeonpea equivalent yield (PEY) of crops during 2012–2021 (averaged across three crops). Bars with different letters within each year are significantly (p<0.05) different.

terms of PEY production (2012). In the second year, both integrated and organic production systems were comparable (Figure 5) implying the narrowing of gap. However, no significant changes in PEY was observed during the 2014–2017, presumably as a result of extremely low yields in all treatment groups. When compared to the control (chemical inputs), integrated production system produced more PEY in 2017 and 2018 than an organic production system did (Figure 5). During 2019–2021, both organic and integrated production systems recorded similar but significantly higher PEY than Control (chemical inputs).

Soil parameters

Physical properties

Among the crops, plots under pigeonpea had considerably lower bulk density compared to other crops after 10 years. Plots under organic production system and integrated production system had significantly lower bulk density of soil than that under control (chemical inputs) (Table 5). Cultivation of pigeonpea crop resulted in significantly higher soil porosity (54.40%) compared to other crops.

TABLE 5 Effect of crops and production systems on soil physical parameters.

Treatment	Bulk density (Mgm^{-3})	Porosity (%)	Water holding capacity (%)
Crop			
Sunflower	1.25 ± 0.1a	51.31 ± 0.2b	37.13 ± 0.2b
Pigeonpea	1.20 ± 0.2b	54.40 ± 0.1a	38.94 ± 0.2a
Greengram	1.21 ± 0.1ab	51.09 ± 0.3b	37.45 ± 0.2b
Production system			
Control*	1.26 ± 0.0a	51.00 ± 0.1b	37.21 ± 0.1b
Organic	1.18 ± 0.2b	53.79 ± 0.2a	38.72 ± 0.2a
Integrated	1.21 ± 0.2b	52.41 ± 0.1a	37.84 ± 0.1b

*Use of chemical inputs alone; initial bulk density was 1.21 $Mg m^{-3}$; means in the same columns with different letters are significantly (p < 0.05) different.

Plots managed organically had significantly higher porosity (53.79%) than control (chemical inputs) across the production systems. The soil's ability to retain water was also noticeably higher in the plots grown with pigeonpea crops. When comparing the various production systems, organically managed soils had much more water retention capacity than the control (chemical inputs) (Table 5).

Chemical properties

The 10-year long experiment had no significant effect on soil pH, although pH was marginally higher in the plots under organic management (Table 6). The soil organic C (SOC) content in the plots under organic production system was 32.6% more than the initial organic carbon of the soil, with higher soil N. The SOC was significantly higher in FYM amended plots compared with mineral fertilizer and integrated production treatments. Plots under integrated production system had higher soil P compared with other treatments (Table 6). However, plots under organic production system being on par with integrated system had significantly higher K content than under control (chemical inputs) plots (Table 6). In our study, DTPA-extractable micronutrient (Cu, Mn, Fe, and Zn) contents were significantly higher in the plots under organic production system than under other treatments.

Biological properties

Under this experiment, we observed higher DHA and MBC in plots under organic production system than under other systems for all the three crops (Table 6). Improved DHA, in our study, in plots under organic production system is a result of diversified nutritional amendments which led to the improvement of soil biological health. Similarly, higher activity of acid phosphatase with organic production system (Figure 6) might be attributed to the accelerated microbial activity due to improved organic carbon content of the soil. However, increased level of urease enzyme under organic management (Figure 7) suggested persistent availability of substrates with C-N bonds for the enzyme to work.

Discussion

Crop yield and quality

In our study, the performance of crops varied under different production systems. When the yield data was adjusted for year effect, pigeonpea performed better under organic production system than under other treatments across all the years except 2018 (Figure 8). Averaged across the years, pigeonpea seed yield (721–737 kg ha^{-1}) was similar under both organic and integrated production systems compared to Control (chemical inputs) (672 kg ha^{-1}). In greengram, the seed yields were higher under integrated production system during initial 5 years, whereas organic system recorded marginally higher yields during the latter 5 years compared to other treatments (Figure 9). On average, greengram seed yield (699–706 kg ha^{-1}) was similar under both organic and integrated production systems. Results here are compared with past studies (3, 42–45), where organic crop yields were lower than conventional crop yields during initial years. As the nutrient cycling processes in organic systems change from inorganic N fertilization to organic amendments, lower crop yields in the plots under organic production systems may have been related to the less readily available nutrients in the early years of transition (46–49).

However, integrated production system recorded higher seed yield of sunflower in all the years except during last 2 years where

TABLE 6 Effect of different production systems on soil properties.

Production system	pH	Organic C (%)	Av. macronutrients (kg ha^{-1})			DTPA-extractable micronutrients (ppm)				DHA (μg TPF g^{-1} soil h^{-1})	MBC (μg g^{-1} soil)
			N	P	K	Cu	Mn	Fe	Zn		
Control*	6.45 ± 0.15a	0.42 ± 0.02c	188.1 ± 38c	24.3 ± 22b	217.2 ± 8b	1.64 ± 0.2b	19.0 ± 0.2c	6.8 ± 0.6c	0.43 ± 0.2c	3.61 ± 0.2c	252.8 ± 38b
Organic	6.63 ± 0.18a	0.57 ± 0.03a	205.2 ± 16ab	25.2 ± 11a	244.8 ± 12a	2.08 ± 0.7a	29.9 ± 0.6a	13.8 ± 0.1a	0.68 ± 0.4a	5.86 ± 0.6a	317.3 ± 26a
Integrated	6.55 ± 0.14a	0.49 ± 0.03b	191.5 ± 25bc	26.5 ± 23a	231.4 ± 16a	1.83 ± 0.3a	26.7 ± 0.4b	11.5 ± 0.4b	0.55 ± 0.1b	4.99 ± 0.2b	298.5 ± 31a
Initial values	6.51	0.43	179.0	24.7	218.1	1.69	20.5	6.6	0.47	3.56	262.4

*Use of chemical inputs alone; means in the same columns with different letters are significantly ($p < 0.05$) different.

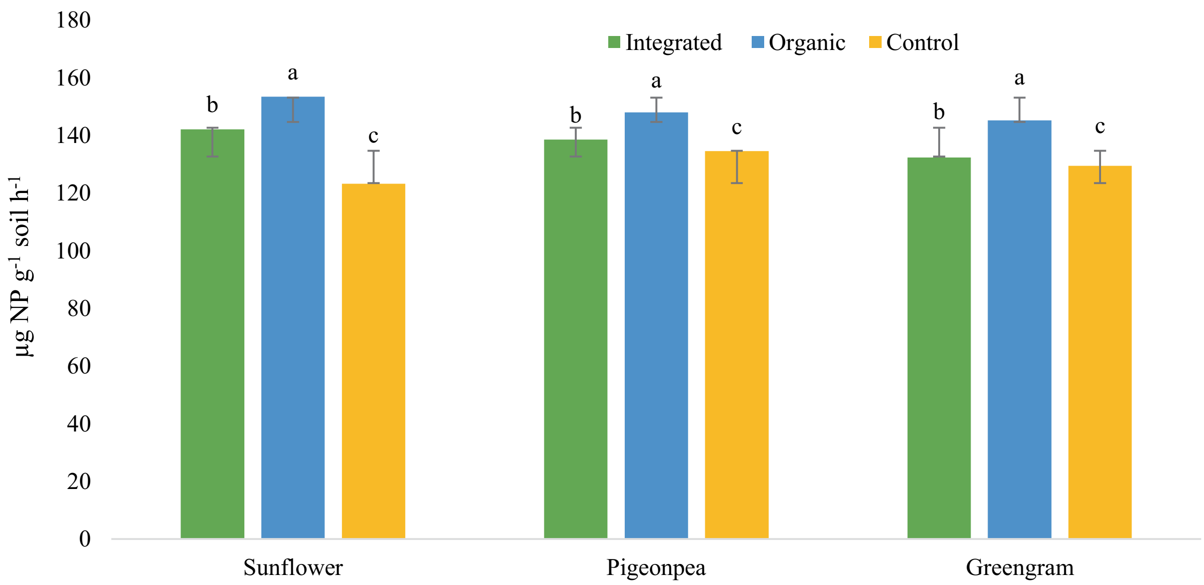

FIGURE 6
Effect of different production systems and crops on soil acid phosphatase activity. Bars with different letters within each crop are significantly ($p < 0.05$) different.

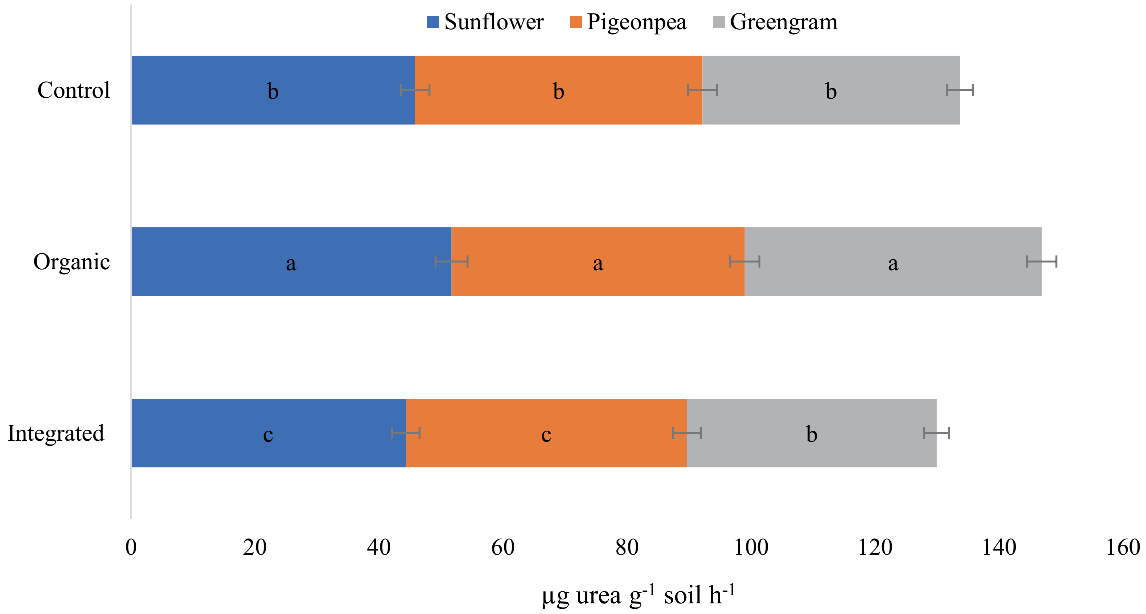

FIGURE 7
Effect of different production systems and crops on soil urease activity. Bars with different letters within each crop are significantly ($p < 0.05$) different.

seed yield was marginally higher under organic production system than other treatments (Figure 10). A gradual improvement in seed yield of sunflower was observed under organic production system whereas, the seed yield showed a declining trend in the plots under Control (chemical inputs), over the years. The yield gap between organic and integrated production systems narrowed down after 8 years of study. Integrated production system, averaged across the years, recorded 9.2–10.0% higher seed yield than that of organic and Control (chemical inputs) treatments. Many comparisons between organic and conventional production systems are mostly from relatively short-term experiments (50, 51). However, there are few well documented long-term (more than 10 years) comparisons between organic and conventional production systems. Similarly to our study, Schrama et al. (45) reported that the yield gap between organic and conventional production systems declined during a 13-year experimental period, suggesting that the yield gap between organic and conventional production systems may decline over time.

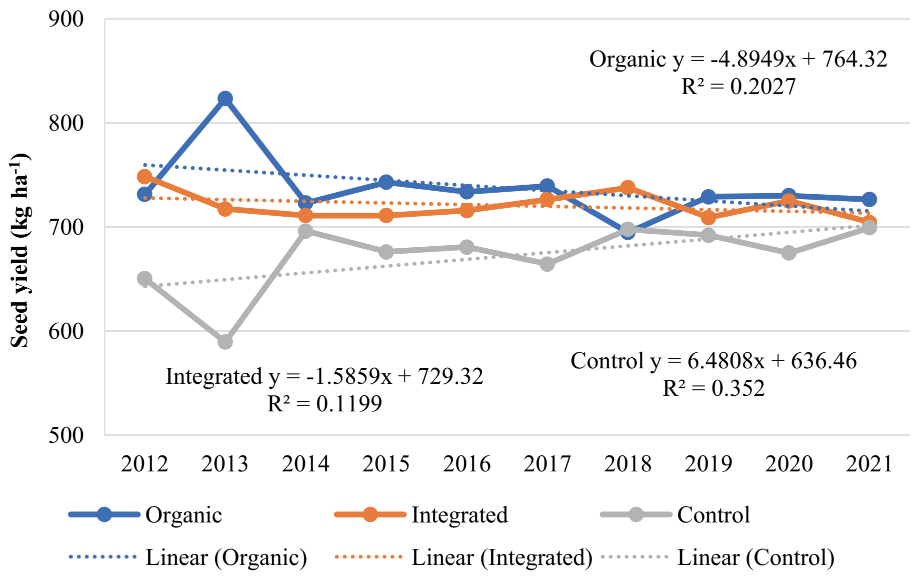

FIGURE 8
Performance of pigeonpea under different production systems during 2012–2021.

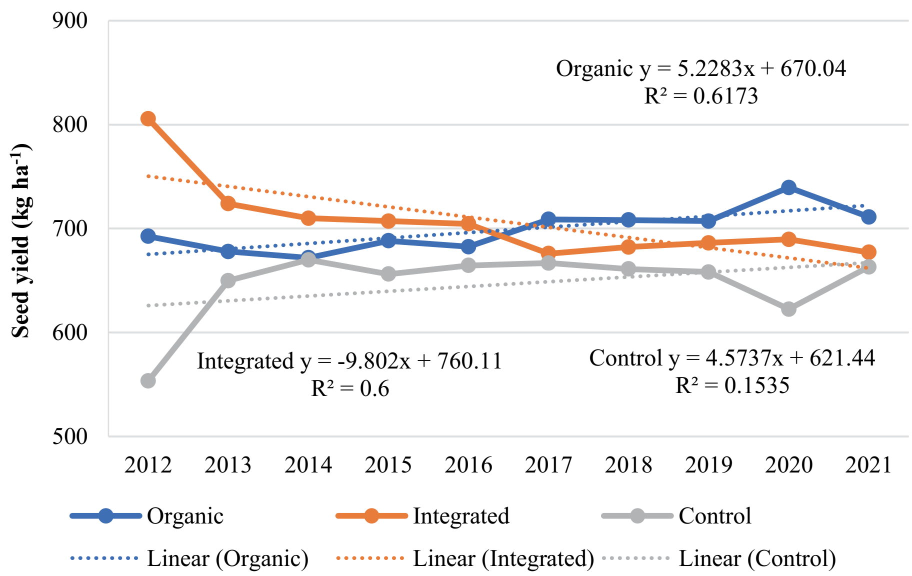

FIGURE 9
Performance of greengram under different production systems during 2012–2021.

A large number of studies have been reported that attempt to investigate if there is a difference in the nutritional value of organically and conventionally grown food (52, 53). In general, our results showed marginally lower protein, and higher K and micronutrient (Fe, Zn, Cu, and Mn) contents of both pigeonpea and greengram seeds under organic management than other treatments. Gopinath et al. (54) and Saha et al. (55) also reported similar results. According to Worthington (56), organic produce from various crops had 21% more iron, 29% more magnesium, and 13.6% more phosphorus. According to a study by Lairon (53), organic food has 21 and 29% more iron and magnesium than non-organic food. In sunflower, integrated production system being on par with Control (chemical inputs) had greater oil content than that of organic production system. However, different production systems had no significant effect on fatty acid composition except that organic sunflower oil had higher content of oleic acid.

Soil properties

Numerous desirable soil characteristics, such as a reduction in bulk density, increased porosity, and increased water-holding capacity,

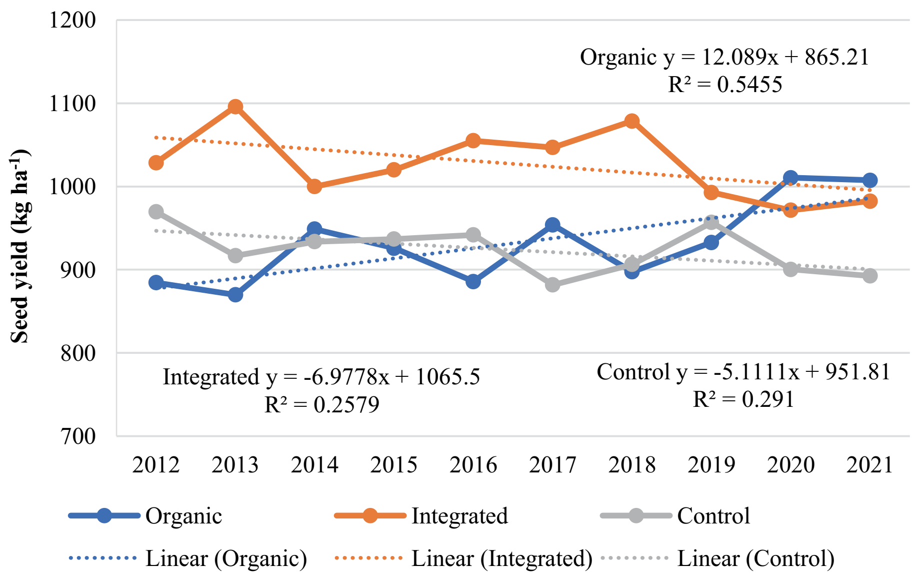

FIGURE 10
Performance of sunflower under different production systems during 2012–2021.

have been linked to the use of organic amendments (14, 16, 26, 45, 57, 58). According to earlier studies (16, 18, 19, 59), organic production systems had higher pH levels in mildly acidic soils than their conventional counterparts. Our findings on the impact of organic systems on various chemical properties of soil are similar to those of those earlier studies.

One of the important environmental benefits due to a shift from conventional to organic production systems is an improvement in soil carbon content (11, 60). The SOC was significantly higher in the plots under organic management compared with mineral fertilizer and integrated production treatments. This increment in SOC might be attributed to the direct addition of organic source of plant nutrients which in turn led to lesser mineralization owing to its wider C: N ratio (61, 62). Previously, some long-term experiments reported notable improvements in SOC content through incorporation of organic manures (63). In a similar line, Aoyama et al. (64) observed increased level of organic matter in soil after 18 years of experimentation with the addition of organic manure. Hati et al. (65), Ramesh et al. (66), and Gopinath et al. (3) also reported higher SOC with organic nutrients application on long term basis.

Higher soil P was found in integrated system as reported by Chen et al. (67). Slower release of organic materials, particularly during initial years under organic production results in lower availability of plant nutrients in organic plots (47, 48). Patel et al. (68) also reported an increase in available P with integrated application of NPK and FYM in a long-term experiment with soybean-wheat cropping system. On the other hand, available K was found higher in organic system. Greater available K with organic nutrition has been documented by Bulluck et al. (57) and Panwar et al. (69). This beneficial effect with organic manure application might be attributed to organic source induced release of organic colloids with more cation exchange sites which adds up more amount of available K by attracting them from the non-exchangeable pool (70). The improved agricultural practices such as soil organic amendments play vital role in soil micronutrient availability (22). Higher DTPA-extractable micronutrient (Cu, Mn, Fe, and Zn) contents under organic production system may be attributed to FYM addition and enhanced soil microbial properties might have improved the micronutrient status of the soil.

Soil organic carbon (SOC) is consisted of a vital fraction termed as microbial biomass carbon (MBC) of soil (71). Microbial biomass carbon (MBC) and dehydrogenase activity (DHA) are crucial indicators for soil quality (72). These indicators also provide clear reflection of soil microbial activity, specifically representing the metabolically active fraction of soil microbial population (73, 74). Improved MBC might be attributed to the property of organic manure to be a more soluble source of substrate for better microbial proliferation in soil (75). Accordingly, implementation of organic management might have accelerated the availability of substrates and stimulated the metabolic activity of soil microbes, which leads to enhanced dehydrogenase activity as confirmed by the outcomes of Basak et al. (76). The reason behind this stimulation of soil dehydrogenase may be due to addition of substrates containing several intra- and extra-cellular enzymes through the incorporation of organic manures. Similar result was also reported by Saviozzi et al. (77) and Smitha et al. (78). Similarly, phosphatase activity in soil is likely to get amplified in response to organic nutrient management compared to chemical inputs (25, 79). In our study, higher activity of acid phosphatase with organic production system might be attributed to the accelerated microbial activity due to improved organic carbon content of the soil. However, increased level of urease enzyme under organic management may be because of persistent availability of substrates with C-N bonds for the enzyme to work. Few other researchers have also reported similar type of improvement in urease activity with the application of organic manures (80–82).

Conclusion

Long-term research based recommendations must be developed for suitable production system that provide higher crop yields, seed quality and improve soil fertility in rainfed areas of India's semiarid tropics. The results of 10-year experiment revealed that the crop yields were lower under organic production system than that of other production systems, particularly during initial years. The yield gap of both the legumes (pigeonpea and greengram) between organic and integrated production systems was less even during initial years, indicating that they may be better suited for organic production under rainfed areas. In general, yield gap of all the three crops between organic and conventional production systems declined over the years. Organic production also improved most of the quality parameters of pigeonpea, greengram, and sunflower relative to integrated production system. Consumption of organic produce, therefore, is one of the approaches to address nutritional security particularly the micronutrient malnutrition of the people. Organic management improved soil properties such as bulk density, porosity, water holding capacity, organic carbon, micronutrient contents and soil microbial activities. In general, the soil fertility parameters were the poorest under Control (use of chemical inputs alone). We conclude that, in the long-run, organic farming has the potential to improve crop yields and soil properties in rainfed semiarid tropics of India.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

KG: conceptualization. KG and GV: methodology. MM, MJ, KG, and BMR: formal analysis. KG, GV, and TP: investigation. MP: resources. KG and VV: data curation, writing, review, and editing. GC: supervision. VS: project administration. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Harnessing sponge gourd: an alternative source of oil and protein for nutritional security

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Luffa cylindrica (L.) Roem. is an important cucurbit crop that assures food security and dietary diversity among the poor communities. In the present study, seeds of 42 genotypes of *Luffa cylindrica* were evaluated for their potential use as oil seed crop. Seed moisture, oil and protein content and fatty acids profile were estimated along with total phenol and sugar content. Significant differences were observed among the various genotypes where oil content ranged from 15.4–29.8% and protein 19.9–30.8%. Total phenol content was high 6.43–12.84 mg/100 g, which bodes well for the sponge gourd seeds' ability to act as antioxidants. Significant correlation were found between important constituents studied like protein and oil, palmitic acid, stearic acid and oleic acid. Total unsaturated fatty acids were in higher amount comparable to saturated fatty acids signifying the good quality of *Luffa* seed oil. Our research revealed that the NDSG-1, Pusa Sneha, DSG-95, DSG-98, DSG-108, and DSG-26 genotypes were very nutritious due to their high levels of protein, oleic acid, and oil output. Additionally, selection of traits having considerable correlation will be beneficial and help in improved varietal development for usage as an alternative oilseed crop. Our research sheds light on the nutritional value of sponge gourd seeds and suggests using them as a potential source for oil and protein, particularly in underdeveloped countries.

KEYWORDS
protein, fatty acids, sponge gourd (*Luffa cylindrica* R.), seed, oil and nutrition

1. Introduction

India is one of the world's 12 centers of crop plant diversity, and its gene pool includes 320 species of wild relatives in addition to 166 species of agro-horticultural crop plants that are dispersed across eight agroecological zones (1, 2). The long history of agriculture and the ethnic diversity on the subcontinent have been significant factors in the diversification of crop resources, leading to the accumulation of rich genetic diversity in a number of crop species and their wild ancestors in this area. The Sanskrit term "Koshataki" denotes the plant's early cultivation history in India.

Vegetable crops known as cucurbits are members of the Cucurbitaceae family, which is mostly made up of species that are eaten as food all over the world. About 118 genera and

825 species make up the family. Despite the fact that the majority of them were Old World originators, numerous species originated in the New World and at least seven genera were found in both hemispheres. Within the family, there is a great deal of genetic variation, and cucurbit species can thrive in temperate, arid deserts, tropical, and subtropical climates. The genetic variety of cucurbits includes both vegetative and reproductive traits, as well as a wide range in the number of monoploid (x) chromosomes, including 7 (*Cucumis sativus*), 11 (*Citrullus* spp., *Momordica* spp., *Lagenaria* spp., *Sechium* spp., and *Trichosanthes* spp.), 12 (*Benincasa hispida*, *Coccinia cordifolia*, *Cucumis* spp. other than *C. sativus*, and *Praecitrullus fistulosus*), 13 (*Luffa* spp.), and 20 (*Cucurbita* spp.) (3). India's various agroecological and phyto-geographical regions are incredibly diverse, and many domesticated and wild cucurbit species are thought to have originated on the subcontinent (4) and the primary center of origin of crops such as smooth or sponge gourd (*L. cylindrica* M. Roem.), ridged gourd [*L. acutangula* (L.) Roxb.] and pointed gourd (*Trichosanthes dioica* Roxb.) (5) particularly in the eastern peninsula tracts, Indo-gangetic plains and north-eastern area (6). In India's north-eastern region, *Luffa* sp. are growing in their natural habitat. Peninsular India is home to *L. acutangula* var. *amara*, while the western Himalaya and upper Gangetic plains are home to *L. echinata*. *L. graveolens*, another crucial species, is found in Tamil Nadu, Sikkim, and Bihar (7).

Through introgression and selection from wild forms that exist in various regions of the country, many species of *Luffa* must have gradually evolved. These species or landraces have useful genes that are adaptable to a wide range of agroecological zones and have resilience to stress, diseases, and pests (6). Recently, sponge gourd genome was sequenced and it was found its genome size sponge was 656.19 Mb which is substantially larger than that of most other sequenced cucurbitaceous species (269–469 Mb) (8). Further phylogenetic analysis allowed the divergence times between sponge gourd genes and their homologs in the other plants to be estimated, indicating that the sponge gourd lineage diverged from the bitter gourd lineage (*M. charantia*) approximately 41.6 million years ago, with subsequent divergence from other cucurbitaceous plants occurring approximately 32.5 million years ago (8).

Sponge gourd [*Luffa cylindrica* (L.) Roem.], is a herbaceous vine of Cucurbitaceae family. The cross-pollinated crop *Luffa* is a diploid species with 26 chromosomes ($2n = 26$) (9). When completely grown, *Luffa* produces tasty green fruits with a cylinder form that can be used as sponges. The young immature fruits and leaves can be prepared as curry or eaten fresh or dry. According to one study, there were significant differences between wild and domesticated species of *Luffa* in terms of their morphological (seed size, color, surface of the seed coat, and 100-seed weight) and biochemical (oil and protein) characteristics (10). They recommended investigating the potential of this valuable crop as a source of edible oil, food, and fodder or as a source of industrial oil/biodiesel. The proximate composition and mineral contents as well as the levels of tannin, oxalate, phytin phosphorus, and phytic acid of *L. cylindrica* were examined. The findings suggested that it may have application as a source of vegetable protein in the diets of both animals and people (11). The oil content and quality features of *Luffa* seeds were evaluated, and this research demonstrated that *Luffa* seed oil is a semi-drying oil that may be employed in surface coating applications such paints, resins, and printing inks. Additionally, seed oil has the potential to be used as a feedstock for the manufacture of soap and biodiesel (12).

In the past, studies have been carried out in India to highlight the genetic diversity of *Luffa* based on morphological data and molecular markers (13–19). *Luffa cylindrica* has chemicals that affect hypersensitive reactions, act as immunostimulants, anti-inflammatories, and participate in glycosidase activity. They also decrease protein synthesis with type I RIPs' structural-function relationships suggesting potential for antitumor and antiviral activities. Finally, they promote uterine contraction to speed up labor (20). Due to abundance of unsaturated fatty acids (80%), as well as the primary fatty acids like linoleic acid (60–65%) and oleic acid (15–20%), in seed oil, tests on mice showed an increase in high density lipoproteins (21).

Additionally, the sponge gourd's leaves, seeds, and fruits display remarkable therapeutic properties, including anti-inflammatory, analgesic, anticancer, hepatoprotective, antibacterial, and wound healing action, and the triterpenoids (sapogenins 1 and 2) that were derived from the sponge gourd exhibit immunomodulatory effects (22–27).

Seeds can be used to cure illnesses like leprosy, sinusitis, and others because they contain a variety of phytochemicals that have the ability to heal wounds and kill bacteria (28, 29). The seeds have alcalase or tryptic protein hydrolysates that are useful for treating diabetes and hypertension (30). Because sponge gourd has 462 NBS-LRR genes, which are involved in nucleic acid metabolic and defense response processes, it has higher stress resistance than other cucurbitaceous species and is frequently used as a rootstock in bitter melon and bitter gourd to increase crop yields, combat soil-borne diseases, and improve flooding tolerance (31, 32).

Due to its antifungal, anti-inflammatory, and anti-tumor qualities, seed oil is utilized in the cosmetics industry (33). The presence of luffin, a ribosome-inactivating protein that prevents the growth of HIV and other diseases, may be the cause of the therapeutic benefits of seeds (34). Despite being poisonous and bitter, seed cake can be utilized as fertilizer due to its high levels of phosphate and nitrogen (35).

Sponge gourd is considered as an emerging income crop due to its several uses as a food, medicine, bath sponge, seed oil, and seed protein (36). The mature fruits of sponge gourds are increasingly prized for their exceptional seeds and high-quality sponge. Standardized solvent extraction techniques have been developed for oil extraction from sponge gourd seeds (37). These findings give a justification for the amazing medical potency of sponge gourd, which has garnered contemporary scientific interest. Nevertheless, there is not much information about this crop's nutritional makeup.

For this reason, it is crucial to examine the nutritional makeup of *Luffa cylindrica* samples gathered from various geographic locations. With the aforementioned information in mind, the objective of the current study was to assess the genetic diversity of 42 genotypes of sponge gourds collected from various Indian regions for quality traits of seeds in order to select the best parent for crop improvement. The quality traits included phenol content, protein, oil, and fatty acid profile.

2. Materials and methods

2.1. Plant material

The study used 42 sponge gourd genotypes collected from various parts of India and maintained as inbreeds at the Division of Vegetable

Science, ICAR-Indian Agricultural Research Institute (IARI), New Delhi (Table 1; Figure 1). The experiment was set up in a randomized block design with three replications during the spring and summer of 2021–2022, with an average temperature of 26°C, an average relative humidity of 46.1%, and average effective precipitation of 5.7 mm and a 14 h light/10 h dark cycle, respectively. The soil has 109.0 Kg ha^{-1} of nitrogen and 9.05 Kg ha^{-1} of phosphorus, respectively. The lines were sown in rows of 2.5 m with 75 cm spacing between the plants, with 15 plants per replication. All the plants were grown at the same time and in same location. All cultural practices recommended for the successful cultivation of the crop were followed. Fruits are harvested when the skin has changed from green to brown or yellowish-brown, which typically takes 150 days. Observations were made on 10 plants at random in each replication. Fruits from each row were composited to make single replicate. Harvested fruits were oven dried at 60°C for 72 h and seeds were collected from dried fruits.

2.2. Seed and oil yield

Fully matured dried fruits were harvested at regular intervals, the seeds were extracted and seed weight was recorded. Seed yield per unit area was recorded in Kg ha-1. Seed yield of each replicate was multiplied by oil percentage to calculate oil yield.

2.3. Sample preparation for biochemical analysis

Fifty gram seeds in each of the 42 genotypes were homogenized by using a stainless-steel mixer grinder. Fine powder was prepared by grinding and then sieved using a test sieve of ASTM 35 to ensure homogeneity and kept at −20°C till further investigation.

2.4. Moisture content

Five gram sample was dried in oven at 110°C overnight and constant dry weight (DW) was attained. The equation used for moisture content determination was $100 \times \left[\left(\mathrm{FW} - \mathrm{DW}\right) / \mathrm{FW}\right]$ and presented in percentage.

2.5. Ash content estimation

Five gram of dried ground sample was taken in silica crucible and initial charring was done at 250°C for 1 h and then the temperature was raised to 450°C and retained for 2 h. For complete ashing ash was moistened with double distilled water and 2–3 drops of concentrated HNO_3 were added. Crucible were again kept in muffle furnace at 450° C for 30 min. Ash content was presented in g/100 g fresh weight.

2.6. Crude fat estimation

Extraction of 10 g of homogenized sample was done with petroleum ether at 40–60°C (38). Extraction was performed for 24 h and overnight drying was done at 60°C till constant weight before and after extraction. Food reference material AS-FRM 6 (fish meal 2) provided by Institute of Nutrition, Mahidol University, Thailand was used and recovery of 95.8 ± 3.6% was obtained.

2.7. Estimation of total protein

Total protein was estimated with some modifications (39). Hundred mg of dried and homogenized sample was digested and estimated for nitrogen content as per (40) using sulfuric acid–selenium–anhydrous sodium sulfate–hydrogen peroxide digestion mixture. To ascertain recovery, food reference material AS-FRM 14 (provided by Institute of Nutrition, Mahidol University, Thailand) was used as a control. The recovery percentage of 98.9 ± 1.9 for AS-FRM 14 was obtained.

2.8. Soluble sugar and total phenol extraction

Hundred mg of homogenized flour was extracted with 5.0 ml of 80% ethanol in an ultrasonic bath at 70°C for 60 min. The contents were centrifuged at 5,000 g for 20 min. The residue was extracted thrice with 5.0 ml of 80% ethanol and supernatants were pooled and volume was made up to 25 ml. This supernatant was kept at −20°C in the dark.

2.9. Total soluble sugar estimation

The total soluble sugar in the sample's 80% ethanolic extract was calculated by anthrone reagent method (41) with minor modifications as mentioned in Padhi et al. (42). Briefly 100 μl of the extract was evaporated in test tubes on a water bath until dry. The residue was dissolved in 1.0 ml of water and 4.0 ml of anthrone reagent was added. The absorbance was read at 660 nm and calibrated against sample blank and dextrose as standard.

2.10. Phenols estimation

The Folin–Ciocalteu Reagent (FCR) method was used to evaluate the phenol content of the ethanolic extract using gallic acid (0–100 g/ml) by method of (43) with minor modifications as described (42). Before extraction, samples were spiked with known concentrations of the standard to determine recovery. The recovery rate was 98.7 ± 1.2.

2.11. Estimation of fatty acids

Luffa seed samples were freshly ground (with a Remi homogenizer) and weighed so that 40 mg of oil could be extracted using a solvent mixture of chloroform, hexane, and methanol (8:5:2 *v*/*v*/*v*) in 10 ml. The resulting extracts were dried for 30 min. at 60°C in nitrogen gas and methyl esters of oil samples were prepared with a few minor modifications (44) as applied to Cucurbitaceous species (45). 1 μl of the derivatized hexane extract was injected onto a highly polar HP Innowax capillary column that was 30 m long (inner diameter: 0.32 m, film thickness: 0.5 μm, split: 1:80). The gas chromatograph in question

TABLE 1 Description of place of origin of sponge gourd genotypes along with their geographical locations and mean annual temperature (MAT).

S. No.	Genotype name	Place of origin	Latitude	Longitude	MAT
1	DSG-6	Hoogly, West Bengal	22°53′60″	88° 23′24″	30.44
2	Pusa Supriya	IARI, New Delhi	28°38′23″	77°10′10″	25.0
3	DSG-7	Moradabad, Uttar Pradesh	28°49′53″	78°46′42″	24.1
4	VRSL-1	Hoogly, West Bengal	22°53′60″	88° 23′24″	30.44
5	VRSL-2	Hoogly, West Bengal	22°53′60″	88° 23′24″	30.44
6	VRSL-4	Hoogly, West Bengal	22°53′60″	88° 23′24″	30.44
7	VRSL-5	Hoogly, West Bengal	22°53′60″	88° 23′24″	30.44
8	VRSL-6	Farrukhabad, Uttar Pradesh	27°22′58″	79°35′30″	30.22
9	VRSL-7	Farrukhabad, Uttar Pradesh	27°22′58″	79°35′30″	30.22
10	VRSL-8	Farrukhabad, Uttar Pradesh	27°22′58″	79°35′30″	30.22
11	VRSL-9	Farrukhabad, Uttar Pradesh	27°22′58″	79°35′30″	30.22
12	VRSL-10	Farrukhabad, Uttar Pradesh	27°22′58″	79°35′30″	30.22
13	VRSL-11	Moradabad, Uttar Pradesh	28°49′53″	78°46′42″	24.1
14	VRSL-12	Moradabad, Uttar Pradesh	28°49′53″	78°46′42″	24.1
15	VRSL-13	Moradabad, Uttar Pradesh	28°49′53″	78°46′42″	24.1
16	VRSL-14	Moradabad, Uttar Pradesh	28°49′53″	78°46′42″	24.1
17	VRSL15	Moradabad, Uttar Pradesh	28°49′53″	78°46′42″	24.1
18	NDSG-1	Faizabad, Uttar Pradesh	26°46′12″	82° 9′0″	24.0
29	PSG-9	Ludhiana, Punjab	30°54′11″	75°51′21″	23.5
19	DSG-31	Barwani, Madhya Pradesh	22°1′48″	74°54′0″	29.93
20	CHSG-1	Ranchi, Jharkhand	23°20′38″	85°18′34″	27.24
21	CHSG-2	Ranchi, Jharkhand	23°20′38″	85°18′34″	27.24
22	DSG-43	Hoogly, West Bengal	22°53′60″	88° 23′24″	30.44
23	DSG-48	Barasat, West Bengal	22°43′22″	88°28′50″	30.4
24	Pusa Sneha	IARI, New Delhi	28°38′23″	77°10′10″	25.0
25	HASG-5	Ranchi, Jharkhand	23°20′38″	85°18′34″	27.24
26	PSG-93	Ludhiana, Punjab	30°54′11″	75°51′21″	23.5
27	PSG-100	Ludhiana, Punjab	30°54′11″	75°51′21″	23.5
28	PSG-110	Ludhiana, Punjab	30°54′11″	75°51′21″	23.5
30	NSG-1-11	Raigarh, Maharashtra	18°30′56″	73°10′55″	24.75
31	NSG-28	Raigarh, Maharashtra	18°30′56″	73°10′55″	24.75
32	JSLG-55	Junagadh, Gujarat	21°31′19″	70 27′28″	25.7
33	DSG-47	Firozabad, Uttar Pradesh	27°9′32″	78 23′44″	24.0
34	Improved Chikni	IARI, New Delhi	28°38′23″	77°10′10″	25.0
35	DSG-95	Una, Himachal Pradesh	31°28′6″	76°16′14″	21.6
36	DSG-98	Dehradun, Uttarakhand	30°18′59″	78°1′55″	20.4
37	DSG-104	Dehradun, Uttarakhand	30°18′59″	78°1′55″	20.4
38	DSG-108	IARI, New Delhi	28°38′23″	77°10′10″	25.0
39	DSG-26	Hoogly, West Bengal	22°53′60″	88° 23′24″	30.4
40	DSG-30	Bokaro, Jharkhand	23°40′9″	86°9′4″	29.77
41	DSG-32	Barwani, Madhya Pradesh	22°1′48″	74°54′0″	29.93
42	DSG-34	Indore, Madhya Pradesh	22°43′10″	75°51′27″	25.3

MAT (mean annual temperature) was computed using information from weather stations located in the areas where samples were taken.

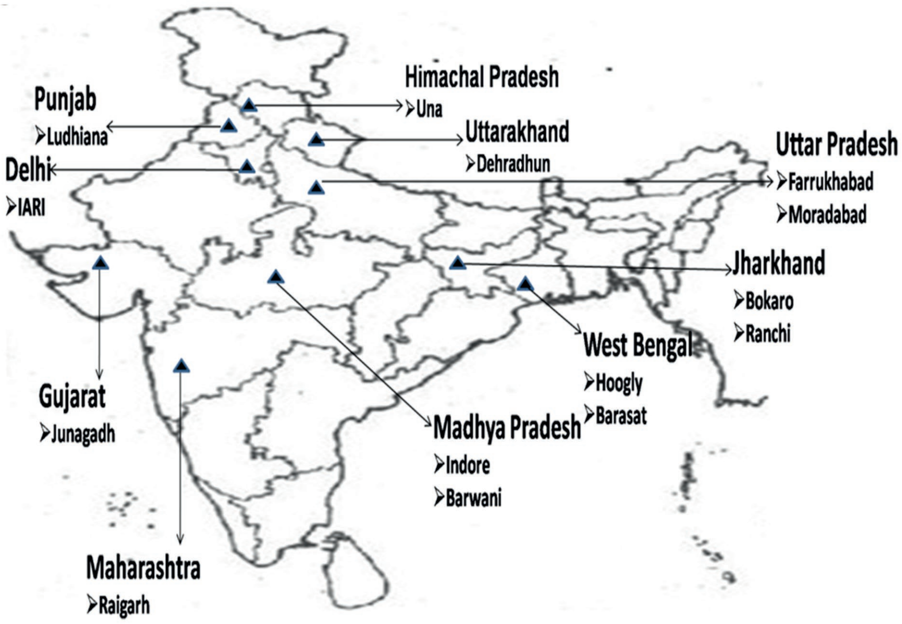

FIGURE 1
Representation of different geographical regions of India from where samples were collected.

was a Hewlett Packard model 6,890 one with a flame ionization detector (FID). Temperatures for the injector and detector were 260°C and 275°C, respectively. The temperature of the oven was designed to rise from 150°C holding for 1 min to 210°C at a rate of 15°C/min, then from 210°C to 250°C at a rate of 5°C/min for 12 min.

Fatty acid methyl esters peaks were identified by comparing the retention times of fatty acid peaks with those of FAMES standard mixture done on 30 m HP Innowax column while running under similar separation circumstances using HP3398A software.

2.12. Data analyses

The data were subjected to statistical analysis program SPSS v. 16 (In., Chicago, IL, United States) to calculate mean, standard deviation (S.D.), one-way ANOVA (analysis of variance) as well as the significance of the difference between the mean by Duncan's multiple range test ($p < 0.05$). After normalizing the data with eigenvalues > 1, principal component analysis (PCA) was carried out to identify the primary components causing the variation in the data. Additionally, the Pearson's correlation between several quality parameters was established. The link between different accessions based on various features was also determined by creating a dendrogram using Ward's approach based on Euclidean distance. Values are presented as the average over three replicates ± the standard deviation (S.D.).

3. Results

3.1. Seed and oil yield

Table 2 shows the seed and oil yield data of *L. cylindrica*. The seed yield ranged from 334 to 600 Kg ha-1 while oil percentage varied from 15.39 to 29.77%. Oil yield was calculated as the product of oil percentage and seed yield, which ranged from 58.1 to 136.9 Kg ha^{-1} with an average of 91 Kg ha^{-1}. However, there was a negative association between seed yield and oil percentage. Hence, the accession with the highest seed yield, i.e., DSG43 was not the highest in oil yield. The highest oil yield potential was observed in DSG108.

3.2. Biochemical analysis

The biochemical parameters with the lowest variability included moisture content (4.35–7.51%) and ash content (1.25–5.17%; Table 3). Two traits, sugar and oil, showed the biggest variability. The sugar concentration varied between 11.91 (PSG-9, Punjab) and 26.43% (CSHG-2, Jharkhand), with 14.52% being the largest difference. Significant variation was seen in the sugar content of the genotypes from Uttar Pradesh, which ranged from 14.57 (VRSL-8, Uttar Pradesh) to 23.63 (VRSL-12, Uttar Pradesh). While the sponge gourd's seed oil content varied from 15.39 (DSG-31, Madhya Pradesh) to 29.77% (VRSL-12, Uttar Pradesh). Moderate variations were found in protein (20.17–27.67%) and phenol content (6.49–12.85 mg 100 g^{-1}) respectively.

The standards mixture gas chromatography (GC) chromatogram along with GC chromatogram for genotypes of *Luffa* as determined by GC–MS is depicted in Figures 2, 3, respectively. Linoleic (C18:2), oleic (C18:1), palmitic (C16:0) and stearic (C18:0) are the four major fatty acids present in sponge gourds. Most prevalent fatty acid with a range of 30.86–68.92% was linoleic acid, followed by oleic acid (13.43–45.03%), palmitic acid (10.76–17.75%) and stearic acid (5.93–11.12%; Table 4). The total polyunsaturated fatty acids (PUFAs) of the seed oil ranged from 72.46–82.76% and formed 10 statistically different groups. Total saturated fatty acids (SFAs) had a very low level (17.24–28.87%)

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TABLE 2 Seed and oil yield of 42 genotypes of sponge gourd.

Genotypes	Seed yield (Kg ha-1)	Oil yield (Kg ha-1)
DSG-6	415 ± 12.6	69.3 ± 2.1
Pusa Supriya	531 ± 16.2	98.1 ± 3.0
DSG-7	392 ± 11.9	80 ± 2.4
VRSL-1	363 ± 11.1	58.1 ± 1.8
VRSL-2	375 ± 8.2	79 ± 1.7
VRSL-4	346 ± 7.5	72.3 ± 1.6
VRSL-5	450 ± 9.8	97.6 ± 2.1
VRSL-6	392 ± 8.6	80.0 ± 1.7
VRSL-7	369 ± 14.4	75.3 ± 2.9
VRSL-8	334 ± 13.1	74.6 ± 2.9
VRSL-9	375 ± 14.7	91.5 ± 3.6
VRSL-10	392 ± 15.3	84.3 ± 3.3
VRSL-11	369 ± 14.4	79.7 ± 3.1
VRSL-12	346 ± 10.5	103.1 ± 3.1
VRSL-13	404 ± 12.3	86.0 ± 2.6
VRSL-14	381 ± 11.6	86.4 ± 2.6
VRSL-15	369 ± 11.2	81.9 ± 2.5
NDSG-1	548 ± 21.4	120.0 ± 4.7
PSG-9	484 ± 18.9	90.1 ± 3.5
DSG-31	577 ± 22.5	88.8 ± 3.5
CHSG-1	450 ± 17.6	96.7 ± 3.8
CHSG-2	484 ± 18.9	95.9 ± 3.7
DSG-43	600 ± 13.1	97.8 ± 2.1
DSG-48	565 ± 12.3	104.3 ± 2.3
Pusa Sneha	554 ± 12.1	122.9 ± 2.7
HASG-5	450 ± 9.8	86.8 ± 1.9
PSG-93	405 ± 7.3	76.5 ± 1.4
PSG-100	428 ± 7.7	101.9 ± 1.8
PSG-110	440 ± 7.9	90.5 ± 1.6
NSG-1-11	497 ± 9.0	101.5 ± 1.8
NSG-28	463 ± 8.3	78.2 ± 1.4
JSLG-55	393 ± 10.4	87.3 ± 2.3
DSG-47	428 ± 11.3	90 ± 2.4
Improved Chikni	486 ± 12.8	76.3 ± 2.0
DSG-95	532 ± 14.1	105.4 ± 2.8
DSG-98	484 ± 14.7	106.6 ± 3.2
DSG-104	507 ± 15.5	111.1 ± 3.4
DSG-108	496 ± 15.1	136.9 ± 4.2
DSG-26	438 ± 13.3	127.7 ± 3.7
DSG-30	416 ± 11.0	98.7 ± 2.6
DSG-32	393 ± 10.4	79.0 ± 2.1
DSG-34	521 ± 13.7	126 ± 3.3

Values are means ± s.d.

with 19 statistically distinct groups. Pusa Supriya (New Delhi) had the highest PUFAs in the seed oil. HASG-5 (Jharkhand) has the highest SFAs concentration.

3.3. Correlation analysis

Table 5 displays the correlation between the moisture, ash, protein, oil, sugar, phenol, linoleic acid, oleic acid, palmitic acid and stearic acid that was determined at two distinct significant levels. There was a significant positive correlation between the moisture and oil (0.302, $p < 0.005$ level), the sugar and palmitic acid (0.360, $p < 0.005$ level), the stearic acid and oleic acid (0.338, $p < 0.005$ level). The oil and protein had a strong positive correlation (0.464, $p < 0.01$ level). At the $p < 0.01$ level, palmitic acid exhibited a strong positive association with stearic acid (0.392) and oleic acid (0.796), whereas at the $p < 0.05$ level, it showed a strong negative correlation with linoleic acid (−0.814). The sugar had a statistically significant negative correlation with protein (−0.344, $p < 0.05$ level) and oil (−0.342, $p < 0.05$ level). Linoleic acid showed a strong negative connection with the oleic acid (−0.979) at $p < 0.05$ level and with the palmitic acid (−0.814) at the $p < 0.01$ level. We examined the correlations between longitude, latitude, mean annual temperature (MAT), and the amount of oil, fatty acids, and protein (Table 6). The mean annual temperature was significantly negatively correlated with oleic acid (−0.326, $p < 0.05$ level) and significantly positively correlated with linoleic acid (0.312, $p < 0.05$ level), despite longitude and latitude having no correlation with these quality traits.

3.4. Principal component analysis

In order to further investigate how the traits, contribute to the observed variability and in order to improve the correlation analysis, the principal component analysis (PCA) was carried out to compute the eigenvalues for 10 traits. The findings showed that the first four principal components with eigen values greater than 1 accounted 74.07% of the overall variation among the genotypes (Table 7). The first principal component which was mostly influenced by sugar, palmitic acid, stearic acid, and oleic acid explained 33.58% of the overall variation. The oil, protein, and phenol were primarily related to the second main component, which accounted for 16.87% of the variation. The protein, phenol, stearic acid and linoleic acid made up the third component and explained 12.69% of the variation. While the moisture, oil, phenol, sugar and linoleic acid comprised the fourth component and contributed 10.95% of the variation. According to PCA data, oleic acid, stearic acid, palmitic acid, total phenol, and protein content contributed the most variability among all the attributes being positive in more than one PC.

3.5. Clustering pattern

The four clusters were produced based on the Euclidean distance (Figure 4). Cluster I comprised of 10 genotypes (VRSL-7, VRSL-15, VRSL-6, VRSL-11, DSG-6, VRSL-1, Improved Chikni, Pusa Supriya, VRSL-2, and JSLG-55) which showed oleic acid less than 20% and linoleic acid more than 60%. DSG-26 and VRSL-9 were present as outgroups. Cluster II encompassed 14 genotypes (VRSL-10, DSG-30, VRSL-13, VRSL-14, VRSL-8, VRSL-12, PSG-100, CHSG-2, DSG-43, NSG-28, DSG-42 VRSL-5, NSG-1-11 and DSG-47) with 20–31% oleic acid and 45–55% of linoleic acid. There were 4 genotypes in Cluster III (Pusa Sneha, DSG98, DSG95 and CHSG1) which displayed 43.5–45% oleic acid and 30.8–31.8%

TABLE 3 Nutritional composition of 42 genotypes of sponge gourd.

Genotypes	Moisture (%)	Ash (%)	Protein (%)	Sugars (%)	Phenol (mg100g-1)	Oil (%)	SFAs (%)	PUFAs (%)
DSG-6	5.21 ± 0.07	4.41 ± 0.04	23.88 ± 0.79	21.42 ± 1.73	7.45 ± 0.10	16.67 ± 1.29	19.12 ± 0.21	80.885 ± 0.21
Pusa Supriya	4.91 ± 0.12	5.17 ± 0.15	22.83 ± 1.02	18.71 ± 0.64	7.29 ± 0.09	18.45 ± 0.73	17.24 ± 0.35	82.76 ± 0.34
DSG-7	7.51 ± 0.24	2.84 ± 0.08	23.10 ± 1.66	14.95 ± 0.77	6.99 ± 0.10	20.49 ± 0.88	21.56 ± 1.29	78.445 ± 1.94
VRSL-1	6.83 ± 0.23	4.62 ± 0.05	21.30 ± 0.89	17.40 ± 0.69	9.76 ± 0.10	16.01 ± 1.26	18.47 ± 0.69	81.53 ± 0.69
VRSL-2	4.53 ± 0.13	4.40 ± 046	26.47 ± 0.90	13.29 ± 0.47	7.33 ± 0.09	20.95 ± 1.42	17.94 ± 0.10	82.055 ± 0.09
VRSL-4	5.14 ± 0.30	3.78 ± 0.04	24.35 ± 1.72	13.73 ± 1.04	6.49 ± 0.09	20.86 ± 1.97	25.24 ± 1.06	74.76 ± 1.05
VRSL-5	4.49 ± 0.18	1.95 ± 0.05	24.44 ± 1.02	17.69 ± 1.33	8.12 ± 0.15	21.69 ± 0.48	22.08 ± 0.59	77.92 ± 0.61
VRSL-6	4.35 ± 0.12	2.88 ± 0.08	24.03 ± 1.54	22.55 ± 1.41	8.95 ± 0.26	20.41 ± 1.20	18.72 ± 1	81.28 ± 1
VRSL-7	5.33 ± 0.13	3.78 ± 0.06	24.45 ± 1.35	19.02 ± 2.73	7.55 ± 0.09	20.35 ± 2.32	19.74 ± 0.26	80.245 ± 0.26
VRSL-8	4.56 ± 0.06	3.42 ± 0.05	24.45 ± 0.93	14.57 ± 0.64	7.35 ± 0.10	22.25 ± 0.80	20.60 ± 0.29	79.405 ± 0.29
VRSL-9	4.62 ± 0.06	3.25 ± 0.07[l]	24.93 ± 1.51	18.92 ± 1.55	8.05 ± 0.01	24.41 ± 1.59	19.79 ± 0.00	80.21 ± 0.01
VRSL-10	5.73 ± 0.12	3.47 ± 0.06	21.84 ± 1.25	19.70 ± 1.43	8.23 ± 0.13	21.53 ± 0.66	21.31 ± 0.04	78.69 ± 0.03
VRSL-11	6.25 ± 0.24	3.16 ± 0.05	23.18 ± 1.60	21.49 ± 1.43	9.45 ± 0.17	21.63 ± 0.69	19.62 ± 0.18	80.395 ± 0.18
VRSL-12	5.81 ± 0.07	4.37 ± 0.06	27.67 ± 0.99	23.32 ± 0.75	7.16 ± 0.11	29.77 ± 0.40	19.76 ± 0.03	75.735 ± 6.36
VRSL-13	6.36 ± 0.13	2.24 ± 0.05	26.11 ± 1.60	18.33 ± 1.35	7.87 ± 0.09	21.33 ± 1.50	21.14 ± 0.21	78.87 ± 0.2
VRSL-14	5.18 ± 0.09	3.10 ± 0.07	24.56 ± 1.86	16.88 ± 2.28	6.82 ± 0.11	22.70 ± 1.46	21.25 ± 0.03	79.365 ± 0.87
VRSL-15	6.28 ± 0.08	3.57 ± 0.06	27.43 ± 0.77	23.63 ± 1.44	7.91 ± 0.13	22.20 ± 1.17	20.0 ± 0.08	81.88 ± 1.54
NDSG-1	5.19 ± 0.06	2.99 ± 0.08	23.78 ± 0.98	17.45 ± 0.85	7.83 ± 0.12	21.92 ± 1.74	22.42 ± 0.30	78.66 ± 1.29
PSG-9	6.49 ± 0.18	3.53 ± 0.06	24.57 ± 1.12[j]	11.91 ± 1.99	9.75 ± 0.10	18.59 ± 1.12	23.17 ± 0.25	76.495 ± 0.7
DSG-31	6.38 ± 0.13	2.36 ± 0.05	20.17 ± 1.23	19.71 ± 1.35	11.54 ± 0.11	15.39 ± 1.40	21.75 ± 0.51	78.24 ± 0.51
CHSG-1	6.22 ± 0.12	2.45 ± 0.05	22.85 ± 1.58	23.61 ± 1.29	11.32 ± 0.20	21.52 ± 0.81	25.31 ± 0.69	74.685 ± 0.7
CHSG-2	6.29 ± 0.06	3.46 ± 0.04	23.75 ± 1.97	26.43 ± 0.80	8.40 ± 0.12	19.78 ± 0.60	25.99 ± 1.83	74 ± 0.82
DSG-43	5.33 ± 0.08	2.87 ± 0.05	22.81 ± 1.87	25.52 ± 0.84	12.18 ± 0.11	16.34 ± 0.89	24.70 ± 0.59	75.285 ± 0.6
DSG-48	6.56 ± 0.14	3.43 ± 0.48	23.22 ± 1.17	15.43 ± 0.81	12.84 ± 0.12	18.45 ± 0.57	24.61 ± 0.47	75.38 ± 0.47
Pusa Sneha	5.75 ± 0.12	2.70 ± 0.05	23.47 ± 0.89	19.43 ± 1.13	8.36 ± 0.07	22.21 ± 0.60	24.40 ± 1.40	75.59 ± 1.4
HASG-5	6.90 ± 0.04	4.28 ± 0.05	24.42 ± 2.44	18.58 ± 0.88	7.13 ± 0.07	19.33 ± 1.92	28.87 ± 5.77	72.46 ± 7.54
PSG-93	6.96 ± 0.06	3.39 ± 0.08	24.99 ± 2.02	17.12 ± 1.58[j]	10.97 ± 0.10	18.88 ± 1.21	22.10 ± 0.53	77.885 ± 0.53
PSG-100	6.50 ± 0.03	2.52 ± 0.05	22.99 ± 1.73	24.29 ± 0.39	11.28 ± 0.12	23.76 ± 1.39	20.96 ± 0.49	78.52 ± 6.67
PSG-110	6.65 ± 0.24	3.43 ± 0.06	24.68 ± 2.73	17.65 ± 1.33	8.46 ± 0.19	20.59 ± 0.78	22.53 ± 0.30	77.455 ± 0.19
NSG-1-11	6.63 ± 0.12	1.25 ± 0.06	24.26 ± 0.78	19.72 ± 1.46	7.54 ± 0.08	20.37 ± 0.68	23.30 ± 0.16	76.695 ± 0.13
NSG-28	6.38 ± 0.06	2.97 ± 0.06	21.11 ± 1.57	16.64 ± 0.75	9.19 ± 0.13	16.87 ± 1.98	22.32 ± 0.14	77.67 ± 0.16
JSLG-55	5.69 ± 0.12	3.71 ± 0.06	21.19 ± 1.43	14.64 ± 1.08	6.43 ± 0.21	22.22 ± 1.59	19.99 ± 0.59	80.005 ± 0.57
DSG-47	5.60 ± 0.06	4.05 ± 0.08	22.52 ± 2.45	16.36 ± 0.91	7.75 ± 0.09	21.13 ± 0.97	21.02 ± 2.33	77.4650.21
Improved Chikni	6.46 ± 0.13	2.34 ± 0.05	21.39 ± 1.16	17.52 ± 1.32	10.25 ± 0.12	15.67 ± 0.95	20 ± 0.18	79.985 ± 0.18
DSG-95	6.57 ± 0.24	3.55 ± 0.64	22.79 ± 0.96	19.57 ± 0.72	10.30 ± 0.07	19.79 ± 0.98	25.50 ± 0.16	74.495 ± 0.16
DSG-98	5.80 ± 0.29	2.16 ± 0.05	24.57 ± 1.47	21.66 ± 2.36	7.44 ± 0.08	21.99 ± 0.75	23.93 ± 0.55	76.05 ± 0.31
DSG-104	6.45 ± 0.24	1.96 ± 0.09	21.67 ± 1.14	14.54 ± 1.35	8.18 ± 0.05	21.87 ± 0.49	22.57 ± 0.25	77.415 ± 0.25[j]
DSG-108	6.64 ± 0.13	2.18 ± 0.06	24.40 ± 1.60	17.8 ± 0.5	7.27 ± 0.06	27.62 ± 1.22	22.97 ± 0.14	77.02 ± 1.12
DSG-26	5.46 ± 0.06	3.24 ± 0.04	25.02 ± 1.33	19.27 ± 0.41	6.87 ± 0.11	27.98 ± 0.63	17.66 ± 0.06	82.345 ± 0.08
DSG-30	6.18 ± 0.07	3.40 ± 0.02	22.35 ± 1.24	21.17 ± 0.13	11.55 ± 0.08	23.72 ± 1.03	21.92 ± 0.06	78.24 ± 0.28
DSG-32	6.02 ± 0.12	2.43 ± 0.07	23.15 ± 1.44	18.91 ± 0.86	12.85 ± 0.07	20.13 ± 1.08	24.08 ± 0.52	75.925 ± 0.53
DSG-34	6.59 ± 0.18	4.11 ± 0.08	24.08 ± 1.68	22.27 ± 0.20	12.22 ± 0.16	24.31 ± 0.51	22.49 ± 0.56	77.52 ± 0.57

Values are means ± S.d.
Total saturated fatty acids—SFAs; Total polyunsaturated fatty acids—PUFAs.

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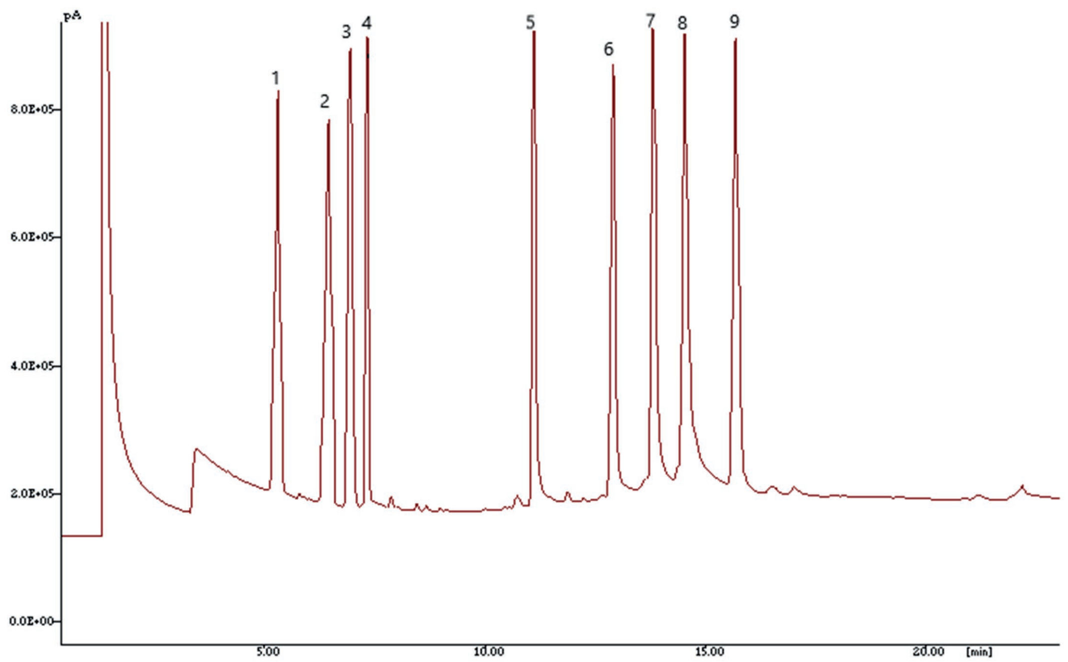

FIGURE 2
Gas chromatography (GC)-flame ionization detector (FID) profile for FAMES standard mixture done on 30m HP Innowax column 1—Myristic acid (C14:0); 2—Myristoleic acid (C14:1); 3—Palmitic acid (C16:0); 4—Palmitoleic acid (C16:1); 5—Margaric acid (C17:0); 6—Stearic acid (C18:0); 7—Oleic acid (C18:1); 8—Linoleic acid (C18:2); 9—Linolenic acid (C18:3).

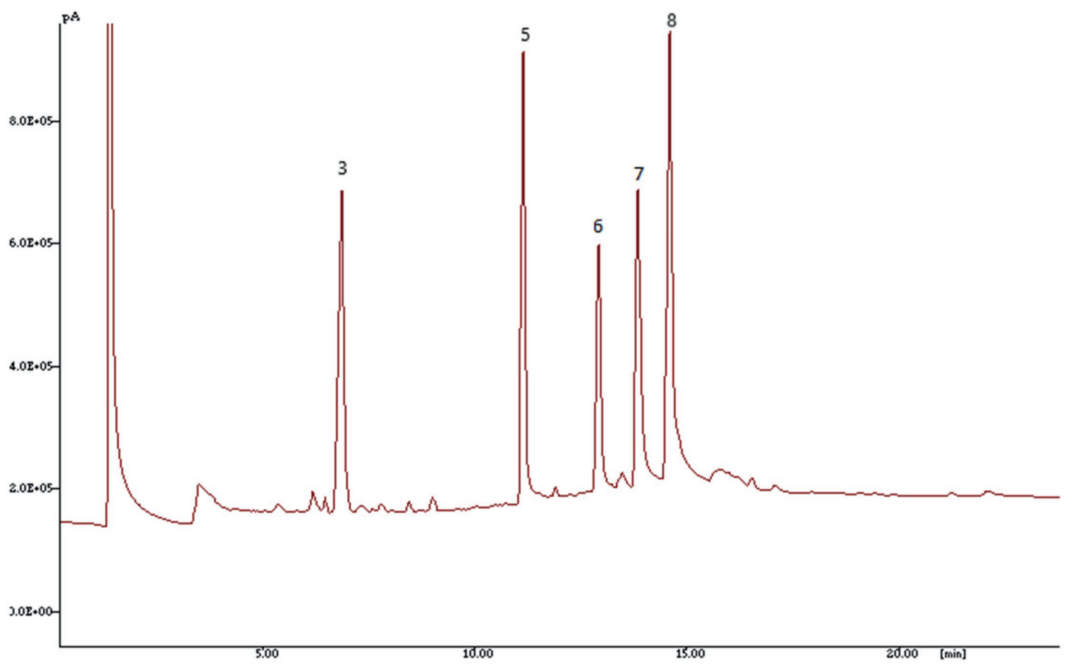

FIGURE 3
Chromatogram of fatty acids in sponge gourd detected by GC-FID done on 30m HP Innowax column 3—Palmitic acid (C16:0); 5—Margaric acid (C17:0); 6—Stearic acid (C18:0); 7—Oleic acid (C18:1); 8—Linoleic acid (C18:2).

linoleic acid. Cluster IV comprised of 14 genotypes (PSG-93, DSG-34, DSG-108, VRSL-4, PSG-9, DSG-38, NDSG-1, PSG-110, DSG-7, DSG-104, DSG-48, DSG-32, HASG-5 and DSG-31) which showed 34.5–42.3% of oleic acid, 35–43% of linoleic acid and moderate oil content.

4. Discussion

This in-depth biochemical investigation of sponge gourd genetic resources reveals that the nutritional traits under study demonstrate high inter-genotype variability and can be applied to both the

TABLE 4 Fatty acid composition of 42 genotypes of sponge gourd.

Genotypes	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)
DSG-6	10.76 ± 0.11	8.36 ± 0.32	19.61 ± 0.88	61.28 ± 0.67
Pusa Supriya	11.31 ± 0.22	5.93 ± 0.13	15.56 ± 0.46	67.20 ± 0.80
DSG-7	12.40 ± 0.10	7.87 ± 0.72	39.92 ± 0.33	38.53 ± 1.62
VRSL-1	10.85 ± 0.06	6.07 ± 0.59	19.17 ± 2.43	62.36 ± 3.13
VRSL-2	12.33 ± 0.30	7.09 ± 0.04	16.18 ± 1.20	65.88 ± 1.29
VRSL-4	15.30 ± 0.08	9.95 ± 1.14	34.78 ± 2.78	39.99 ± 3.83
VRSL-5	13.36 ± 0.13	8.73 ± 0.73	31.54 ± 0.64	46.39 ± 0.04
VRSL-6	11.46 ± 0.72	7.26 ± 0.28	18.93 ± 0.65	62.35 ± 1.65
VRSL-7	11.18 ± 0.12	8.56 ± 0.14	16.98 ± 0.59	63.27 ± 0.86
VRSL-8	12.58 ± 0.02	8.02 ± 0.18	27.39 ± 0.55	52.02 ± 0.84
VRSL-9	11.04 ± 0.06	8.75 ± 0.06	19.18 ± 0.78	61.03 ± 0.76
VRSL-10	13.50 ± 0.01	7.81 ± 0.02	25.73 ± 0.37	52.96 ± 0.40
VRSL-11	12.25 ± 0.19	7.37 ± 0.01	19.37 ± 0.34	61.03 ± 0.52
VRSL-12	12.34 ± 0.06	7.43 ± 0.09	19.06 ± 0.78	59.61 ± 2.98
VRSL-13	13.27 ± 0.06	7.88 ± 0.15	24 ± 0.45	54.87 ± 0.65
VRSL-14	13.74 ± 0.04	7.52 ± 0.06	23.57 ± 2.25	55.79 ± 1.92
VRSL-15	11.93 ± 0.43	8.15 ± 0.35	18.48 ± 0.64	63.4 ± 1.92
NDSG-1	14.13 ± 0.42	8.29 ± 0.72	40.95 ± 0.69	37.72 ± 0.60
PSG-9	15.26 ± 0.42	7.91 ± 0.18	36.83 ± 1.05	39.67 ± 0.35
DSG-31	14.75 ± 0.15	7.01 ± 0.36	40.53 ± 0.09	37.72 ± 0.60
CHSG-1	16.37 ± 0.32	8.95 ± 0.37	43.83 ± 0.25	30.86 ± 0.45
CHSG-2	17.66 ± 0.50	8.33 ± 1.33	30.24 ± 1.77	42.26 ± 4.71
DSG-43	17.15 ± 0.17	7.55 ± 0.42	34.6 ± 1.13	43.07 ± 3.90
DSG-48	16.72 ± 0.78	7.90 ± 0.32	36.99 ± 1.63	38.39 ± 1.16
Pusa Sneha	15.60 ± 0.80	8.81 ± 0.6	43.84 ± 0.52	31.76 ± 0.88
HASG-5	17.75 ± 1.21	11.12 ± 1.21	37.91 ± 2.89	34.55 ± 2.95
PSG-93	15.13 ± 0.31	6.97 ± 0.22	34.52 ± 0.86	43.37 ± 1.39
PSG-100	12.99 ± 0.23	7.97 ± 0.26	21.79 ± 0.31	56.31 ± 3.33
PSG-110	14.65 ± 0.24	7.88 ± 0.06	41.48 ± 0.06	35.98 ± 0.25
NSG-1-11	15.86 ± 0.12	7.45 ± 0.28	29.71 ± 0.35	46.99 ± 0.48
NSG-28	15.14 ± 0.06	7.19 ± 0.08	29.41 ± 1.58	48.27 ± 1.42
JSLG-55	11.26 ± 0.22	8.73 ± 0.37	18.87 ± 1.14	61.14 ± 1.71
DSG-47	13.3 ± 1.22	7.72 ± 0.30	30.97 ± 1.37	46.50 ± 1.58
Improved Chikni	11.53 ± 0.13	8.47 ± 0.31	18.06 ± 1.8	61.93 ± 1.62
DSG-95	17.52 ± 0.18	7.98 ± 0.01	43.54 ± 1.43	30.96 ± 1.27
DSG-98	15.74 ± 0.12	8.20 ± 0.43	45.03 ± 0.43	31.03 ± 0.12
DSG-104	14.83 ± 0.01	7.74 ± 0.24	42.26 ± 0.07	35.16 ± 0.18
DSG-108	14.39 ± 0.04	8.58 ± 0.18	35.69 ± 0.66	41.34 ± 0.46
DSG-26	11.24 ± 0.08	6.42 ± 0.14	13.43 ± 0.56	68.92 ± 0.48
DSG-30	13.42 ± 0.05	8.50 ± 0.11	26.76 ± 0.28	51.48 ± 0.0
DSG-32	15.64 ± 0.21	8.44 ± 0.31	39.04 ± 0.89	36.89 ± 0.36
DSG-34	15.19 ± 0.45	7.30 ± 0.11	34.47 ± 0.83	43.06 ± 0.26

Values are means ± s.d.

industrial and food sectors. The selection and assessment of genetic resources for the intended nutritional qualities are directly related to their use. This is the first in-depth examination of phenolics and fatty acid composition in a broader group of sponge gourds.

According to Chisholm and Hopkins's research, the oil content of nine species of Cucurbitaceae ranged from 20.1 to 38.3% (46); the oil content of wild and cultivated species of *Luffa* was 25–27% (10). In our study, we found seed oil content in range of 15.39–29.77%. which was in agreement to above researchers. A very low moisture content of 4.35–7.51% was recorded in the genotypes and it is ideal for a long shelf life and less susceptibility to microbial attack. Thus, moisture is a key aspect of food stability and preservation (47). The ash level was found to be between 1.25 and 5.17%, which is consistent with the range of 3.00–5.8% for legumes including cowpea, groundnut, and fluted pumpkin seeds (47). The total amount of minerals in a food can be calculated using the ash value.

This study's protein content (19.91–30.77%) was much higher than Prakash's study's which found that both domesticated and wild sponge gourd species had (8–10%) protein in their seeds (10). The differing approaches taken in the two investigations could be the cause of the discrepancy in protein results. As Lowry's method, which assesses soluble proteins and is frequently employed when absolute quantities are not required, was used in Prakash's work (48). Based on the aforementioned findings, the genotypes in our study showed excellent promise as possible sources of protein in the future.

One past investigation reported the protein content of 35.83% in *Luffa cylindrica*, which is slightly greater than what we discovered in our study (47). When the sponge gourd defatted seed's proximate composition was examined, it was discovered that its protein content ranged from 45.06 to 50.06%, which was higher than that of fatted seeds (49). However, > 25% protein content is considered even more for pulses which consumed as a staple food in several areas of the world (50). The variance might be explained by different ecological factors that have an impact on the plant growth. The primary purpose of nutrition is to provide an adequate amount of amino acids, and proteins are a vital part of the food required for both animal and human survival. Thus, *Luffa cylindrica* could be utilized as a substitute source of protein in diet supplements, especially in regions where the bulk of the population consumes starchy foods and cereals, which serve as the basis for nutrition by providing sufficient amounts of needed amino acids or as a feed additive for cattle (51).

Total polyunsaturated fatty acids (PUFAs) content was determined to be 72.46–82.76%, while total saturated fatty acids (SFAs) content was found to be 17.24–28.87% in our study. However, prior study reported 52.02% of PUFAs and 33.07% of SFAs and in seeds of *Luffa cylindrica* (52). The sponge gourd's fatty acid composition was found to be comparable (palmitic 10.76–17.75%, stearic 5.93–11.12%, oleic 13.43–45.03% and linoleic of 30.86–68.92%) to oilseed crop Niger (palmitic 5.8–3.0%, stearic 5.0–7.5%, oleic 13.4–9.3%, and linoleic of 45.4–65.8%) (53). There was a little change in the trend of the fatty acids profile in the study conducted by Lucy and Abidemi (52) in sponge gourd seeds, where palmitic acid and stearic acid content were found to be 12.86 and 15.17% similar to our results, but the percentage of oleic acid (2.57%) and linoleic acid (31.47%) is much lower. Genotypes with oleic acid up to 45% is a novel finding in our research as compared to earlier reports (46, 52).

One of the most important unsaturated fatty acids known to serve a critical role in human nutrition is oleic acid. Oils with a high oleic acid content are highly resistant to heating and oxidation. Therefore, high oleic acid oils can be used in place of saturated fats in food service applications requiring long-life stability because it has been demonstrated that they exhibit heat stability that is equivalent to saturated fats (54). The most

TABLE 5 Pearson's correlation between the traits studied.

	Moisture	Ash	Oil	Protein	Phenol	Sugar	Palmitic acid	Stearic acid	Oleic acid
Ash	−0.048								
Oil	0.302*	−0.045							
Protein	0.310	0.105	0.464**						
Phenol	−0.099	−0.026	0.197	0.067					
Sugar	−0.058	−0.159	−0.342*	−0.344*	0.022				
Palmitic acid	−0.082	−0.245	−0.211	−0.067	−0.180	0.360*			
Stearic acid	−0.208	−0.074	0.020	0.005	0.106	−0.142	0.392*		
Oleic acid	−0.139	−0.374	−0.141	−0.065	−0.204	0.255	0.796**	0.338*	
Linoleic acid	0.143	0.399	0.147	0.111	0.187	−0.321	−0.814**	−0.313	−0.979*

*Correlation is significant at $p < 0.05$ (2-tailed).
**Correlation is significant at $p < 0.01$ (2-tailed).

TABLE 6 Correlation of fatty acids, protein, and oil with climatic and geographic variables.

	Oil	Protein	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
Latitude	0.213	0.233	−0.116	−0.037	0.102	−0.070
Longitude	−0.075	0.151	0.009	0.149	−0.114	0.093
MAT	−0.135	−0.066	−0.216	0.070	−0.326*	0.312*

MAT—Mean annual temperature.
*Correlation is significant at $p < 0.05$ level (2-tailed).

TABLE 7 Eigenvalues and their proportion for 10 biochemical parameters based on 4 principal components.

Traits	1	2	3	4
Moisture	−0.218	0.166	−0.776	0.042
Ash	−0.439	−0.167	0.327	−0.499
Oil	−0.345	0.746	−0.240	0.195
Protein	−0.258	0.703	0.019	−0.179
Phenol	−0.222	0.155	0.398	0.794
Sugar	0.459	−0.529	−0.181	0.359
Palmitic acid	0.881	0.145	−0.017	−0.091
Stearic acid	0.396	0.409	0.548	−0.023
Oleic acid	0.924	0.231	−0.062	−0.073
Linoleic acid	−0.941	−0.187	0.081	0.021
Total of Eigenvalues	3.36	1.69	1.27	1.10
Percent of total variance	33.58	16.87	12.69	10.95
Cumulative percent of total variance	33.58	50.45	63.14	74.10

prevalent fatty acid found in the sponge gourd seeds was linoleic acid. It affects blood lipid levels favorably and is connected to a lower risk of coronary heart disease. Since linoleic acid and any of its derivative fatty acids cannot be produced by humans, they must be obtained through diet. The significant reduction in atherosclerosis caused by the high linoleic acid content and both linoleic and oleic acids lower blood cholesterol levels (55).

The results of this study showed that genotypes having the greatest levels of oleic acid and linoleic acid can be used for genetic testing and breeding initiatives. We discovered that the genotypes NDSG-1, Pusa Sneha, DSG-95, DSG8, and DSG108 had higher oil yields (> 105 Kg ha^{-1}), high oleic acid levels (> 35%), and high protein levels (25% or more). A high oil output, protein, and linoleic acid (68.9%) were found in the DSG-26 genotype, although it had a low oleic acid content (13.4%). Therefore, these genotypes of sponge gourd could be exploited as a good source of oil and protein.

We found no association of the latitude and longitude with the oil and fatty acids. Yunxia Ma also discovered no connection between longitude and the 26 provenances of *Xanthoceras sorbifolium* Bunge (56). Nevertheless, the oil content of *Helianthus annuus* L. seeds showed significant positive correlation with longitude and latitude (57). Temperature is one of the primary factors that affects linoleic

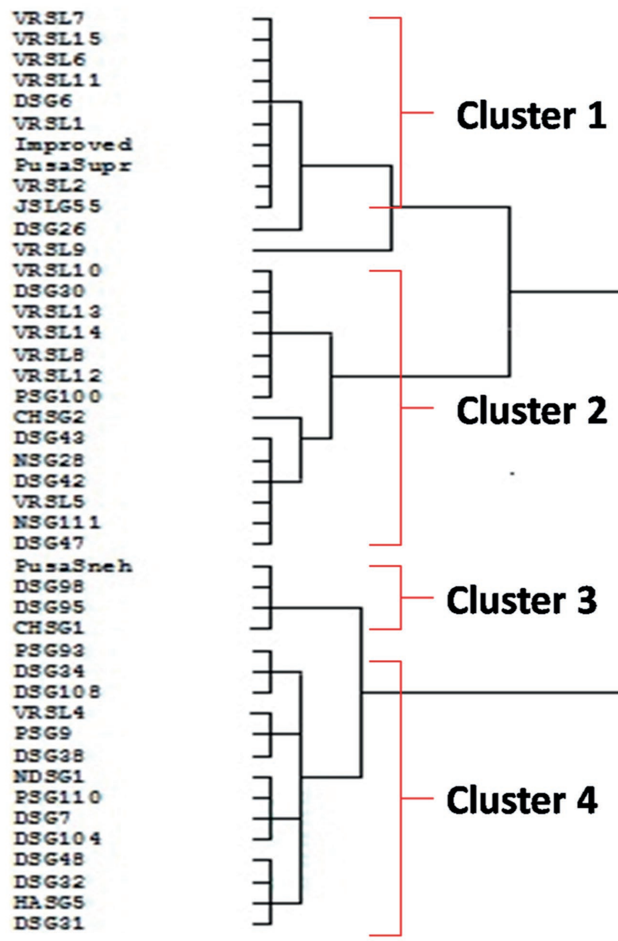

FIGURE 4
Dendrogram showing relationships among 42 genotypes of sponge gourd based on 10 biochemical characters.

acid and oleic acid content in oilseed crops. MAT was observed to be significantly negatively correlated with oleic acid and significantly positively correlated with linoleic acid. Several researchers reported the significant negative association between low temperature and amount of oleic acid present for example, in maize and soybean; sunflower (58, 59). It is stated that seed oils of plants grown in cool climates tend to be more unsaturated than those grown in warm climates. This might be due to increase in accessible oxygen, which was the rate-limiting element for desaturation, and thus increase in the level of unsaturated fatty acids in seeds at low temperatures (60). However, different plant species's fatty acids showed different response to temperature (60). A genetic correlation between traits is produced via pleiotropy and linkage disequilibrium, which prevents traits from varying independently of one another (61). Because choosing one trait indirectly chooses a genetically associated quality, correlation studies are helpful for selecting varieties with enhanced composition (62). Significant positive relationship was observed between oil and protein and both were significantly negatively correlated with sugar, respectively. Therefore, utilizing such traits in breeding programs will be beneficial and contribute to improved varietal development for use as a replacement oilseed crop. Consequently, selecting genotypes with a high oil content and high protein is possible as such features which shows significant relations are controlled by tightly linked genes. It was discovered that linoleic and oleic acids were negatively associated, and other oilseed crops, including crucifer species, soybean, peanut, sesame, and safflower, also had this type of linkage (45, 63, 64).

A crucial tool in the parent-selection process is the measurement and classification of genetic variation between genetic materials. PCA and cluster analysis are practical statistical techniques that complement one another effectively for this objective. While PCA is used to assess the variability's magnitude, cluster analysis is used to categorize the variability. The clustering of genotypes based on their genetic similarity facilitates the discovery and choice of the ideal parents for specific breeding operations (65). The PCA revealed that factors like oleic acid, stearic acid, palmitic acid, total phenol, and protein content were responsible for the majority of the difference between genotypes. The clustering pattern produced four clusters, but they were largely clustered according to the fatty acid profile rather than the place where the seeds were collected.

The Indian subcontinent is rich in genetic variety in cucurbits, with domesticated, semi-domesticated, or wild species occurring in small pockets (66). In addition to fruits, as a vegetable it can also play a significant part in ensuring nutritional security by utilizing the seeds which are a great source of protein and oil. Its commercial use offers great potential for diversifying the types of vegetables that can be farmed. Sponge gourd has got wider adaptability and is found to grow in semi-arid to high rainfall areas. Moreover, there is considerable tolerance in sponge gourd to biotic and abiotic stresses and the crop requires nominal outside efforts leading to the small cost of cultivation and high economic returns (67).

5. Conclusion

Our findings show that there is large variability in sponge gourd genotypes for biochemical parameters, and these variations have a tremendous potential to be used to create genotypes with improved oil quality and nutrient content. This study found the genotypes (NDSG-1, Pusa Sneha, DSG-95, DSG-98, DSG-26 and DSG-108) with the greatest number of good nutritional characteristics like oil yield, high oleic acid and protein; consequently, choosing such genotypes for creating new crosses in breeding would be helpful. Additionally, the nutritional value, fatty acid composition, and phenolic content of sponge gourd seeds enable for both their use as an oil source for industrial purposes as well as a dietary food supplement to meet the nutritional demands of developing nations. As a result, NDSG-1, Pusa Sneha, DSG-95, DSG-98, DSG-26 genotypes of sponge gourd can be used as a nutritious vegetable, while DSG-10 high oil yield can be used to make edible or industrial oil. More research is required before sponge gourd seeds are recommended for human nutrition.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MV and LA conceived and designed the experiment. RT performed the experiment and drafted the manuscript. RB analyzed the data and revised the manuscript. PS assisted or supported in GLC operation. AM and AS provided the seed samples of sponge gourd inbred lines. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Interplay between nano zinc oxide-coated urea and summer green manuring in basmati rice under basmati rice-wheat cropping system: implications on yield response, nutrient acquisition and grain fortification

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Identifying appropriate nutrient management options is crucial for reversing the yield plateau and enhancing the nutritional status of basmati rice under the basmati rice-wheat cropping system of the Indo Gangetic Plain (IGP). Alternative to the conventional chemical fertilizer, ZnO nanoparticles as carrier material for the micronutrient Zn has shown promise in reducing the bulkiness of fertilizer use in the soil–plant environment. But whether its integration and interaction with an organic source such as green manuring could enrich basmati rice grain with micronutrients and promote protein nutrition is not well investigated. Therefore, we conducted a field experiment during the summer and rainy seasons (April–October) of 2020 and 2021 at the research farm of the ICAR-Indian Agricultural Research Institute, New Delhi in a split-plot design with two summer green manuring (SGM) options (*Sesbania* and cowpea, along with fallow) as main plots and six fertilization strategies as subplots: 5 kg Zn ha^{-1} as bulk ZnO, N at 120 kgNha^{-1} as prilled urea (PU), N at 120 kgNha^{-1} as PU+5 kg Zn ha^{-1} as bulk ZnO, 1% bulk ZnO-coated PU, 0.1% nano ZnO coated PU and 0.2% nano ZnO coated PU replicated thrice. On average, SGM increased basmati rice grain yield by 13.2 and 12.3% during 2020 and 2021, respectively compared to fallow. Integrated application of zinc with urea significantly (p<0.05) increased the grain yield of basmati rice by 9.56% and by 10.5% relative to urea without zinc and by 33.7 and 33.8% than the sole application of 5 kg Zn ha^{-1} through ZnO, respectively during 2020 and 2021. On average, SGM boosted Zn, Cu, Mn, and Fe content in milled rice by 25, 22.38, 20.0, and 18.85% during 2020 and 23.75, 21.4, 19.6, and 13.3% during 2021, respectively compared to fallow. Relative to sole urea application, zinc, and urea together improved the Zn and Fe content in milled rice by 2.99mgkg^{-1} and 2.62 mgkg$^{-1,}$ respectively during the first year and by

2.83 $mgkg^{-1}$ and 2.6 $mgkg^{-1}$, respectively during the second year of study. The highest protein content in basmati rice grain was observed when it was grown after *Sesbania aculeata* residue incorporated plot during both the years and it decreased in the order: *Sesbania aculeata*>*Vigna unguiculata*>summer fallow. Our findings revealed that with the application of 1% bulk ZnO coated PU with *Sesbania*; the yield response, micronutrient acquisition, and protein accumulation in milled rice was higher than in other plant fertilization methods. However, in plots treated with *Sesbania*, along with 0.2% nano ZnO-coated PU exhibited statistically equivalent yield and micronutrient loading in edible tissues. Hence, this study unveils the critical role of nano ZnO-coated urea and summer green manuring in elevating micronutrient and protein bioavailability in basmati rice and concurrently reducing Zn dose by 20%, making it a profitable option for farmers.

KEYWORDS

basmati rice, green manuring, micronutrients, nano ZnO, protein

1. Introduction

In Asia, about 24 million hectares (Mha) of land is devoted to the rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) cropping system (RWCS) (Nawaz et al., 2019). This system is most prevalent in South Asia's Indo-Gangetic Plains (IGP), where India alone holds 10.3 Mha land under this particular cropping system (Timsina and Connor, 2001). Rice and wheat together contribute about 45% of digestible calories, 30% of protein, and a considerable percentage of animal feed worldwide (Evans, 1996). In India, RWCS contributes 31% of food grain output (Prasad, 2005) demonstrating its significance in ensuring national food security. India produces 20% of the world's rice, making it the second-largest rice producer. To meet the needs of its expanding population, India plans to increase rice production to the tune of 130 million tonnes by 2030 (Kumar et al., 2021). India relies extensively on RWCS to provide not just food security, but also nutritional security. However, the sustainability of the rice under RWCS has emerged as a major emerging challenge during the last decade. Some of the predominant reasons include stagnating yields, decreasing crop responses to external mineral fertilizer, multi-nutrient deficiencies, dwindling factor productivity (land, water, labor, etc.), and the use of repeated wet tillage, which oxidizes buried organic matter and breaks down macro-aggregates negatively impacting soil health (Bhatt et al., 2016). Furthermore, keeping the land idle during the intervening period of two crops does not provide any financial benefits to producers. All these factors contribute to the poor financial situations of farmers leading to agriculture becoming a non-profitable livelihood. The Global Hunger Index (2022) depicts that India ranks 107th out of 121 nations, with a score of 29.1 which is an indicator of a serious hunger problem in the country. Anthropogenic climate change will further negatively impact the grain quality of rice (Lin et al., 2010). Since soil, plant, and human health are interconnected, India's agri-food system, especially rice in the RWCS, must be transformed to provide food and nutritional security, increasing profits without compromising soil quality.

Increasing the profitability of rice under RWCS may need adopting a vertical crop diversification approach, such as switching from ordinary rice genotypes to high-yielding basmati genotypes that have a high value in international trade. Due to its unique characteristics (such as a pleasant aroma and an extended grain length after cooking), basmati rice has a lot of potential for export. In the fiscal year 2021–22, India earned US $3,540.40 million from the export of basmati rice, making it the world's largest exporter (APEDA, n.d.). Improving the nutritional quality of the basmati rice to combat mineral shortages and malnutrition would be a win-win situation for everyone involved in RWCS, in addition to bringing in much-needed foreign currency.

The manifestation of any trait in a plant depends on genotype, environment and the dynamic interplay between genotype and environment. Since soil is one of the most significant environmental factors affecting yield and nutritional content in grains, it must be carefully attended to break open the abeyant high yield potential and nutrient-dense grains of basmati rice genotypes. In this perspective, sustainable intensification of the fallow period in RWCS by integrating summer green manuring crops (SGM) might be one of the feasible solutions for sustainable land use and most crucially recovering soil health affected by soil harmful intense tillage operations performed under the present system. Numerous studies have demonstrated the efficacy of green manure in boosting agricultural productivity, mitigating soil erosion, lowering runoff and nitrate leaching during the fallow period, decreasing soil bulk density, raising the levels of organic matter, N, P, K, and other nutrients in the soil to lessen the need for synthetic fertilizers, maintenance of soil biodiversity and enzyme activity, etc. (Mandal et al., 2003; Salahin et al., 2013; Ma et al., 2021). Additionally, it is well established that leguminous green manures have high fertilizer value on the succeeding crops due to their ability to fix atmospheric nitrogen. Green manuring coupled with chemical fertilizer can help ensure the long-term viability of RWCS (Yadav et al., 2000). An earlier study reported that *Sesbania* green manure could partially replace chemical fertilizer, increase rice yield, and reverse the declining trend of partial factor productivity (PFP) in wheat. Leguminous green manures mineralize more quickly and release both macro- and micronutrients factored by their lower C/N ratio. Additionally, the incorporation of green manure can improve the bioavailability of micronutrients by chelation through the production of organic acids (Singh and Shivay, 2019).

Among the essential micronutrients, zinc (Zn) is one of the most crucial micronutrients for crop production because it is a key structural component or regulatory co-factor of numerous enzymes and proteins in many crucial biochemical pathways, including protein metabolism, auxin synthesis, pollen formation, the preservation of the integrity of biological membranes, and carbohydrate metabolism (Alloway, 2008). However, its deficiency is more evident in high pH calcareous soils in the North Western Indo-Gangetic Plains (IGP), which is linked to greater Zn sorption on the soil as hydroxides and carbonates (Prasad, 2006). Further, the prolonged submerged condition of the rice is one of the causes of the Zn deficiency in rice in particular. In this context, green manuring with Sesbania (*Sesbania aculeata* Pers.) has shown promising results for improving soil zinc bioavailability and producing Fe, Mn, Cu, and Zn-dense grains in basmati rice (Pooniya and Shivay, 2013). Limited scientific research has also indicated the benefits of SGM in rice under RWCS (Thind et al., 2019; Sharma et al., 2021), but it is important to consider how its integration and adoption in the present system can increase productivity, nutritional security, and profitability. Organic manures, such as green manuring, can improve soil fertility and health, but their slower mineralization rate is out of sync with plant needs, as they cannot be relied on alone to provide the plants' nutritional demands during the crop season. Thus, the present focus on enhancing agricultural productivity and crop quality is preferred to the use of chemical fertilizers, however it is linked to a number of unfavorable environmental outcomes, such as soil hardening, decreased fertility, reduced activity of native soil microorganisms (and thus a decrease in soil biodiversity), alteration in soil pH, and the formation of a crust on the soil's surface (Pahalvi et al., 2021). Rice being nutrient intensive, there is a pressing need to pay crucial attention to make optimal use of fertilizers, which are becoming more expensive with time.

As a result, the co-application of organic and inorganic nutrient sources needs to be appropriately maneuvered in order to capitalize on the synergism that exists between them. Green manure increases nitrogenous fertilizer efficiency for two main reasons: (a) green manure N is as efficient as urea N in rice (Ladha and Kundu, 1997), and (b) higher root density of subsequent wheat due to improved soil physical conditions enhances nutrient acquisition capacity and biological yield at a given fertilizer level (Boparai et al., 1992). In this context, the use of slow-release fertilizer has been an effective method of nutrient administration because it releases nutrients gradually, at the rate preferred by the plant, and it keeps those nutrients in the soil for an extended length of time. A study by Cole et al. (2016) suggested the benefits of slow-release fertilizer and reported it as an efficient way of administering nutrients as they are made available to the target at the desired rate or concentration level, hence sustaining the nutrients in the soil for a longer period. Enhanced nutrient use efficiency, especially for nitrogen (N) has been achieved through the use of conventional coated urea category of fertilizers such as neem-coated urea in tropical lowland rice (Mohanty et al., 2021), sulfur-coated urea in spring wheat (Shivay et al., 2016), and zinc-coated urea in RWCS (Shivay et al., 2008), etc.

The use of innovative nano-fertilizers like nano zinc oxide (ZnO) has recently reshaped the definition of enhanced fertilizer use efficiency in the agriculture sector. In comparison to traditional fertilizers, it is fundamentally different due to its unique physical, chemical, and biological properties. These fertilizers have a nutrient release rate that corresponds with crop needs, which helps in maximizing nutrient recovery and reducing nutrient losses. Also, because plants only need small amounts of these fertilizers, the greenhouse gas emissions during their production and transportation are very low. According to Pavithra et al. (2017), ZnO nanoparticles increased rice grain Zn content and grain production. The use of zinc nano fertilizer (ZnNFs) resulted in increased plant growth in pearl millet (Tarafdar et al., 2014). Applying ZnNFs (<100 nm) in maize crop has reportedly increased the plant height, root length, root volume, and dry weight of the root (Adhikari et al., 2015). In wheat, seed priming with 75 and 100 mg/l of ZnNFs has reported enhanced shoot Zn concentration (12–24%) and root Zn concentration (13–19%), compared to the control (Rizwan et al., 2019). Further, zinc oxide-coated urea has a favorable influence on grain yield and Zn content in a rice-wheat system (Shivay et al., 2008). Another study by Dimkpa et al. (2020) suggested that coating urea with nano-ZnO improved plant performance and Zn accumulation in wheat. However, the majority of the investigations have concentrated on the effect of the sole application of ZnO nanoparticles on plants. There is a dearth of research on the combining effect of summer green manuring (SGM) with the use of nano-ZnO-coated urea (NZCU). In addition, the relative efficacies of NZCU vis-à-vis bulk ZnO-coated urea with respect to grain quality of rice under RWCS have not been tested.

Therefore, the present study was contemplated with two primary objectives: (1) To elucidate the comparative efficacies of different green manure crops and diverse zinc sources on the yield response of basmati rice under RWCS, and (2) To decipher the nutritional composition particularly micronutrient and crude protein content of basmati rice as influenced by the integrated use of summer green manuring and zinc sources.

2. Materials and methods

2.1. Experimental site and soil descriptions

The field experiment was conducted for two consecutive years at the research farm of the ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India during the summer-rainy seasons of 2020 and 2021. The Institute farm lies at latitude 28°38′ 24″ N, longitude 77°10′ 26″ E and 228.6 m above sea level. During the basmati rice crop period of 2020 and 2021, mean rainfall was 802.5 mm and 1534.3 mm, mean evaporation was 772.4 mm and 688.3 mm, maximum daily temperature range was 28.4–43.3°C and 24.2.6–43.8°C, and minimum daily temperature range 10.9–31.7°C and 12.6–32.5°C, respectively. Daily variation in the agrometeorological parameter is presented in Supplementary Figure 1 (SF1).

Table 1 depicts the soil's physical and chemical parameters.

2.2. Experimental design

The experiment was conducted in a split-plot design with one summer fallow (G1) and two green manure crops [*Sesbania aculeata* (G2), *Vigna unguiculata* (G3)] as the main plot and six subplots comprising 5 kg Zn ha^{-1} as bulk ZnO (80% Zn) [(control for N), Z1], nitrogen (N) at 120 kg N ha^{-1} as prilled urea [(control for Zn), Z2], N at 120 kg N ha^{-1} as prilled urea +5 kg Zn ha^{-1} as bulk ZnO (80% Zn) (Z3), 1% bulk ZnO coated prilled urea [N at 120 kg N ha^{-1} + 2.08 kg Zn ha^{-1} as

TABLE 1 Soil properties of the experimental field at the beginning of the experiment.

Particulars	Value
Soil depth	0–15 cm
Sand (%)	10.9
Silt (%)	36.3
Clay (%)	52.8
Bulk density ($Mg\,m^{-3}$)	1.51
pH (1:2.5 soil: water) (Prasad et al., 2006)	7.68
Electrical conductivity (1:2.5 soil-water extracts) ($dS\,m^{-1}$ at 25°C) (Piper, 1966)	0.41
Organic carbon ($g\,kg^{-1}$) (Walkley and Black, 1934)	6.5
Available nitrogen ($kg\,ha^{-1}$) (Subbiah and Asija, 1956)	171
Available phosphorus ($kg\,ha^{-1}$) (Olsen et al., 1954)	15.9
Available potassium ($kg\,ha^{-1}$) (Hanway and Heidel, 1952)	309
DTPA-extractable micronutrients ($mg\,kg^{-1}$) (Lindsay and Norvell, 1978)	Zn (0.63), Cu (1.68), Mn (3.10) and Fe (3.35)

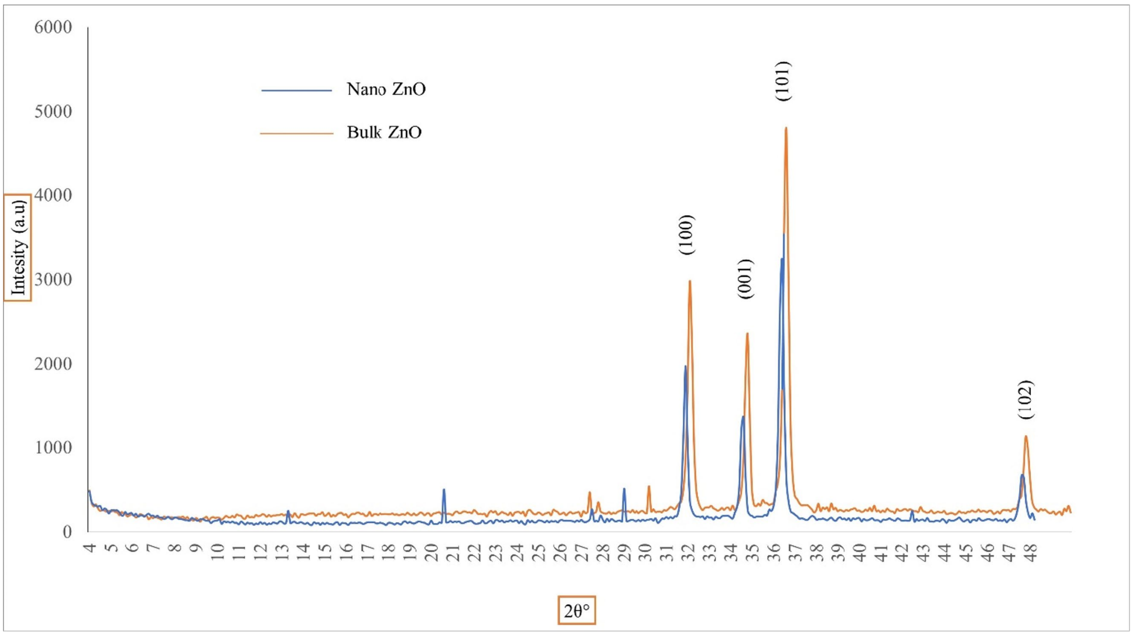

FIGURE 1
XRD spectrum of bulk and nano ZnO.

bulk ZnO, Z4], 0.1% nano ZnO coated prilled urea [N at $120\,kg\,N\,ha^{-1} + 0.208\,kg$ Zn ha^{-1} as nano ZnO, Z5] and 0.2% nano ZnO coated prilled urea [N at $120\,kg\,N\,ha^{-1} + 0.416\,kg$ Zn ha^{-1} as nano ZnO, Z6] replicated three times. The *Sesbania* (variety: Punjab Dhaincha 1) and cowpea (variety: Pusa Komal) seeds were sown at $50\,kg\,ha^{-1}$ and $35\,kg\,ha^{-1}$, respectively, and at 22.5 cm apart, using a seed drill during the third week of May during both years of study and incorporated into the soil at 45 days after sowing (1 week before transplanting of basmati rice).

2.3. Characterization of zinc oxide

The ZnO used in our experiment was purchased from Sigma Aldrich Chemical Co., United States and characterized using the following techniques.

2.3.1. X-ray diffraction

X-ray diffraction (XRD) data of materials used (both bulk and nano) was collected using an X-ray diffractometer (Philips PW 1710), equipped with copper Kα radiation as the X-ray source (2θ range of 4–48°) operated at 40 kV and 20 Ma tube current. It was observed that all the peaks were matching with the standard data of the hexagonal ZnO wurtzite structure (JCPDS card no. 36–1,451; Figure 1).

2.3.2. Dynamic light scattering

Both bulk and nano zinc oxide were characterized for their particle size by Zetatrac™ particle size analyzer based on the Dynamic Light Scattering (DLS). The 95% of the nano ZnO particle size had a diameter of <74 nm (confirming the nano size of nano ZnO, i.e., <100 nm) and it was < 3,430 nm (beyond 100 nm) in bulk ZnO (Figures 2A,B; Table 2).

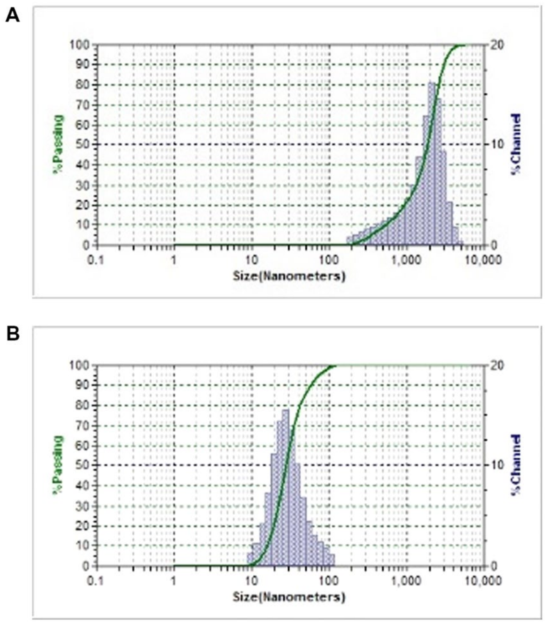

FIGURE 2
(A) DLS of bulk ZnO particles. **(B)** DLS of nano ZnO particles.

TABLE 2 Comparison of the particle size distribution of bulk and nano zinc obtained using DLS.

Particle size distribution (% Passing)	Bulk ZnO [Diameter (nm)]	Nano ZnO [Diameter (nm)]
10	533	16.07
50	1872	28.26
80	2,607	42.8
95	3,430	74

2.3.3. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX)

SEM was used to examine the surface morphology and cross-section. The presence of bulk and nano ZnO on the urea granules was clearly depicted in SEM micrographs, demonstrating efficient surface coating, whereas the absence of ZnO provided evidence of a lack of zinc coating (Figures 3A–F). Furthermore, a Field Emission Scanning Electron Microscope (FESEM) FEI Quanta 200 FESM (FEI, Netherlands) equipped with an Oxford EDX system IE 250 X Max 80 was used to conduct elemental analysis and to determine the presence or absence of Zn. As expected, peaks for carbon, oxygen, and nitrogen were observed in the sole urea granules. The Zn peak was seen in all of the coated urea granules, in addition to the peaks seen in the uncoated granules (Figures 4A–F). The granules that were not coated had no zinc.

2.3.4. High-resolution transmission electron microscopy

The sample for transmission electron microscopy (TEM) was prepared from dispersions of nano zinc oxide sonicated for 40 min at room temperature and followed by placing it on a carbon-coated grid using a micropipette and staining the grid with 2% uranyl acetate. The materials placed were observed for morphology and size using a TEM (Jeol 1,011, Japan) operating at 100 Kv. The ZnO nanoparticles were well in the nano range (1–100 nm; Figure 5). Some larger aggregates in the sample are linked to the high surface energy of nano-particles.

2.4. Total dry matter, N, Zn, Cu, Mn, and Fe added through summer green manuring

The total dry matter, N, and micronutrients added by green manuring are presented in the Supplementary Tables S1, S2, respectively.

2.5. Crop establishment

Two or three seedlings (25 days old) of rice variety 'Pusa Basmati 1401' (seed rate 20 kg ha^{-1} used to raise the nursery) were transplanted at a spacing of 20 cm by 10 cm in the first fortnight of July 2020 and 2021, respectively. Recommended doses of N, P, and K (120.0, 26.2, and 33.3 kg ha^{-1}) were applied in the form of urea, single super phosphate, and muriate of potash. P, K, and 5 kg Zn ha^{-1} as soil application was applied as basal during the transplanting of basmati rice. Nitrogen (in the form of prilled urea and zinc-coated urea) was applied in three equal splits: one-third as basal, one-third at 50% tillering, and one-third at the panicle initiation stage. The rice crop was grown as per the recommended agronomic practices and was harvested in the last week of October during both study years.

2.6. Chemical analysis

Harvested plant samples were sun-dried, oven-dried at 60 ± 2°C in a hot-air oven for 6 h, and crushed. Chemical analysis was performed on 0.5 g of plant samples from different parts of rice grain and straw. Micronutrient content in dry matter was evaluated by the di-acid digestion method using atomic absorption spectrophotometry (Prasad et al., 2006), and uptake was calculated by multiplying corresponding values by dry matter and expressed in g ha^{-1}. Plant samples of 0.5 g were digested in 10 ml of analytical-grade concentrated H_2SO_4 with a digestion mixture and analyzed in a Kjeldahl device to determine N% (Prasad et al., 2006). Husk, white rice kernel, and bran protein were calculated by multiplying N concentration by 5.95 (Juliano, 1985).

2.7. Statistical analysis

Statistical computation was performed using R software (version 4.2.2) and R-Studio (version 2022.12.0 + 353) by using "agricolae" (De Mendiburu and de Mendiburu, 2019) and "psych" packages (Revelle and Revelle, 2015). Duncan's multiple range test (DMRT) was performed for grouping of treatment means. p values were computed for estimating the significance of the treatment mean.

3. Results

3.1. Grain yield

Both summer green manuring (SGM) and zinc fertilization (ZF) significantly influenced the grain yield of basmati rice during both years of study (Figures 6, 7). Results of contrast analysis (Table 3) indicated

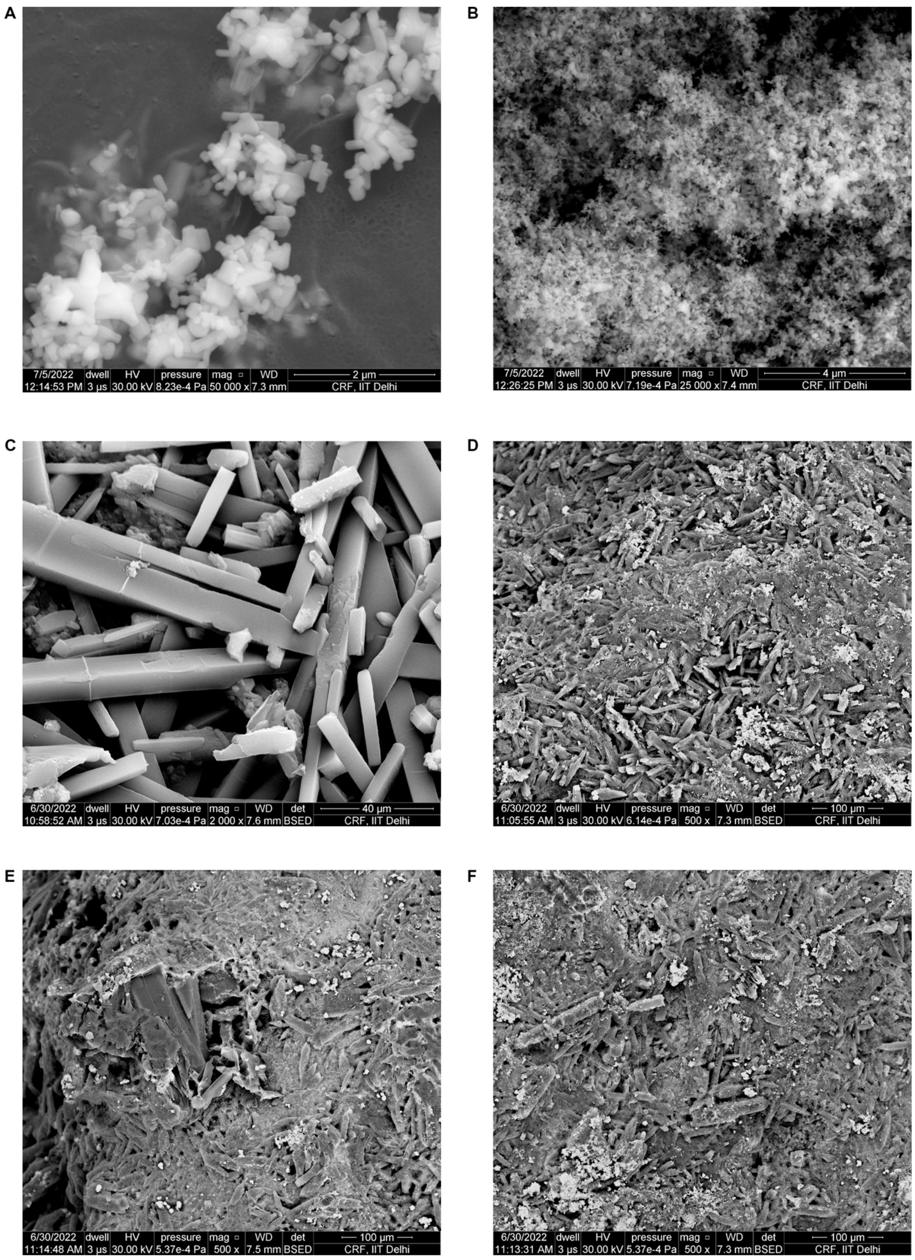

FIGURE 3
(A) SEM of bulk ZnO. **(B)** SEM of nano ZnO. **(C)** SEM of urea granule. **(D)** SEM of 1% bulk zinc-coated urea. **(E)** SEM of 0.1% nano zinc-coated urea. **(F)** SEM of 0.2% nano zinc coated urea.

that; averaged over ZF treatments, SGM significantly produced a higher yield ($p < 0.001$) than fallow (G1). On average, SGM increased basmati rice grain yield by 13.2 and 12.3% during 2020 and 2021, respectively compared to fallow (G1) (Figure 6). Of the SGM, *Sesbania* green manuring (G2) recorded numerically higher grain yields during both years of study which was also significantly different from cowpea green manuring (G3) (Figure 6). Averaged over ZF treatment, *Sesbania* green manuring (G2) increased basmati rice grain yield by 18 and 17% compared to fallow (Figure 6) during 2020 and 2021, respectively. Likewise, cowpea green manuring (G3) increased basmati rice grain yield by 8.42% and by 7.85% compared to fallow (Figure 6) during 2020 and 2021, respectively. Further, conjoint use of zinc with urea (both coating and separate application) significantly improved the grain yield ($p < 0.05$) of basmati rice than sole urea application (Z2) and soil application of 5 kg Zn ha^{-1} through ZnO (Z1) as evident by contrast analysis (C2 and C3; Table 3) during both years of study. Integrated application of zinc with urea significantly ($p < 0.05$) increased the grain yield of basmati rice by 9.56% and by 10.5% relative to urea without zinc and by 33.7 and 33.8% than the sole application of 5 kg Zn ha^{-1} through ZnO (Z1), respectively during 2020 and 2021 (Figure 7). Among ZF, application 1% BZCU (Z4) gave the highest yields, although not significantly different from the application of 0.2% NZCU (Figure 7), however, both outperformed other ZF treatments. There was a significant SGM × ZF effect for grain yield response (Table 4). A

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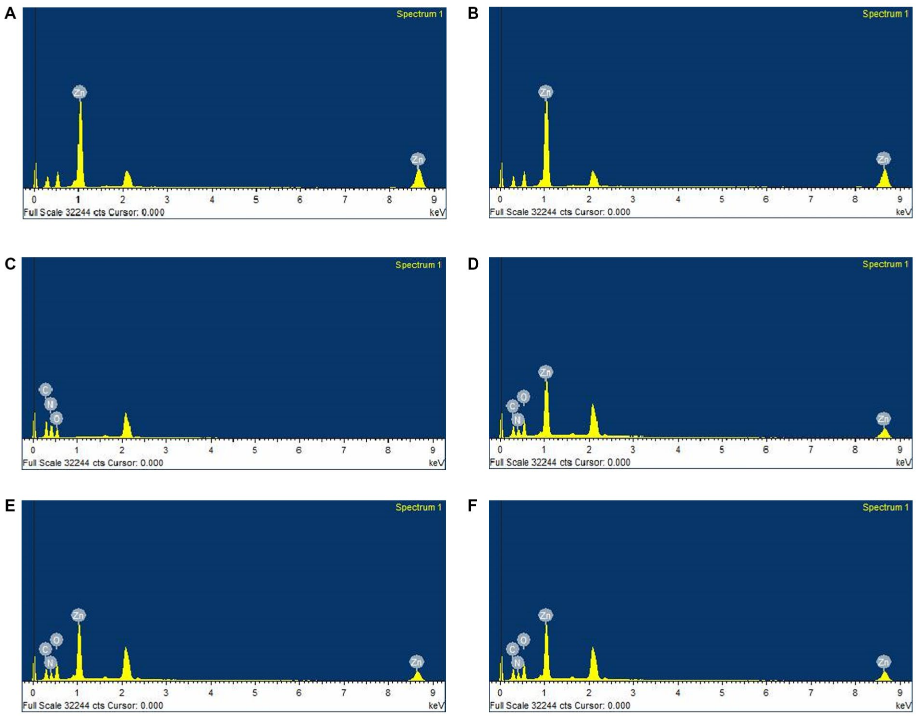

FIGURE 4
(A) EDX of bulk ZnO. **(B)** EDX of nano ZnO. **(C)** EDX of urea granule. **(D)** EDX of 1% bulk zinc-coated urea. **(E)** EDX of 0.1% nano zinc-coated urea. **(F)** EDX of 0.2% nano zinc-coated urea.

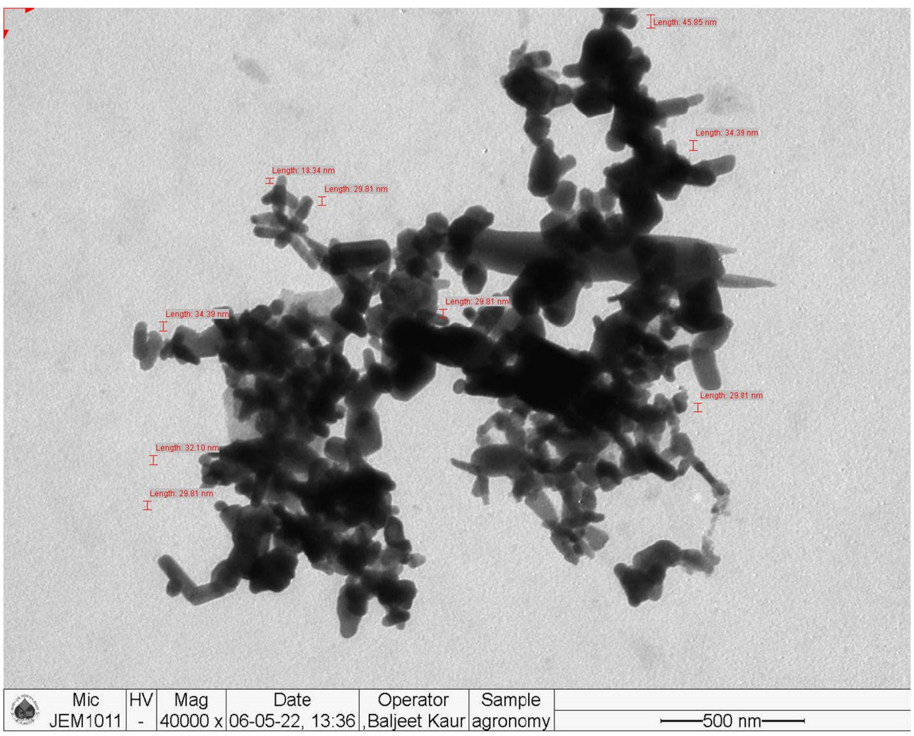

FIGURE 5
TEM of nano ZnO particles.

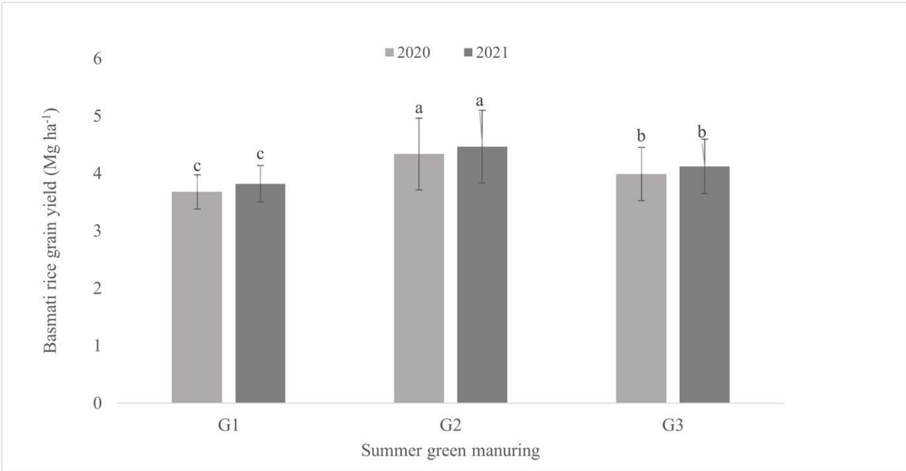

FIGURE 6
Grain yield response of basmati rice influenced by summer green manuring (SGM).

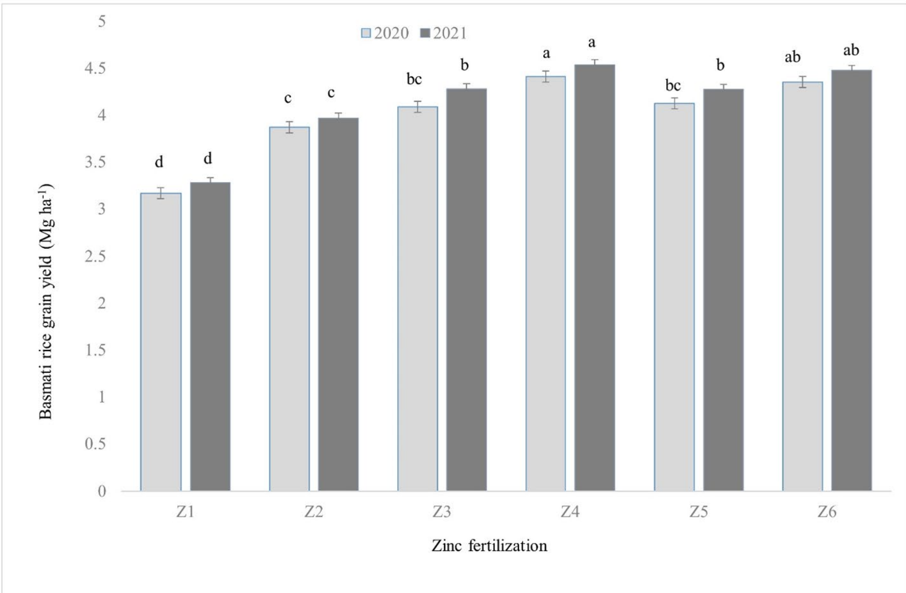

FIGURE 7
Grain yield response of basmati rice influenced by zinc fertilization (ZF).

combination of *Sesbania* green manuring (G2) and the application of 0.2% NZCU demonstrated a higher grain yield response (Table 5 and Figure 8).

3.2. Micronutrient concentrations (Zn, Cu, Mn, and Fe) in grain and straw

The Zn, Cu, Mn, and Fe concentrations in different parts of grain and straw in response to SGM and ZF are presented in Tables 6–10. Across all SGM and ZF levels, straw had higher micronutrient concentrations than grain (Tables 6–10), with the order being Fe > Zn > Mn > Cu. In both years, micronutrient concentration decreased in the order of bran > hull > milled rice. On average, the content of Zn, Cu, Mn, and Fe milled rice was 13.57, 4.61, 42.56, and 144.9 mg kg^{-1} during 2020 and 13.9, 4.81, 42.8, and 145.8 mg kg^{-1} during 2021. Likewise, in the straw, the average concentrations of Zn, Cu, Mn, and Fe were 70.16, 8.03, 5.67, and 9.54 mg kg^{-1} and 69.91, 8.13, 5.77 and 9.77 during 2020 and 2021, respectively, irrespective of SGM and ZF. Both SGM and ZF significantly influenced the micronutrient content during both years of study (Table 4). Contrast analysis (Table 3) revealed that; averaged over ZF treatments, SGM significantly produced micronutrient-dense milled rice ($p < 0.001$) than fallow (G1). Of the SGM, *Sesbania* green manuring (G2) recorded numerically higher Zn, Cu, and Fe content in milled rice than fallow (G1) during both years of study, however, Cu and Fe concentration in milled rice was statistically

TABLE 3 Orthogonal contrasts (depicting value of *p*) for grain yield and micronutrient content in milled grain of basmati rice as influenced by summer green manuring (SGM) and zinc fertilization (ZF).

Source of variation	Grain yield		Zn		Cu		Mn		Fe	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Fallow vs. SGM (C1)	0.0016	0.0014	<0.0001	<0.0001	0.002	0.003	0.001	0.001	<0.0001	<0.0001
Z1 vs. (Z3, Z4, Z5, Z6) (C2)	<0.0001	<0.0001	<0.0001	<0.0001	0.6	0.65	0.64	0.62	<0.0001	<0.0001
Z2 vs. (Z3, Z4, Z5, Z6) (C3)	0.034	0.045	<0.0001	<0.0001	0.4	0.45	0.44	0.4	<0.0001	<0.0001

SGM, Summer green manuring; Z1, 5 kg Zn/ha-ZnO soil application (Control for N); Z2, N through prilled urea + No Zn (Control for Zn); Z3, N through prilled urea + 5 kg Zn/ha-ZnO; Z4, 1% BZCU; Z5, 0.1% NZCU; Z6, 0.2% NZCU.

TABLE 4 Analysis of variance (ANOVA) results (value of *p*) for the effect of summer green manuring and zinc fertilization on Zn, Cu, Mn, and Fe concentration in the different parts of grain, straw, and grain yield of *basmati rice.*

	Summer green manuring (SGM)		Zinc fertilization (ZF)		SGM×ZF	
	2020	2021	2020	2021	2020	2021
Zn concentration						
Husk	<0.0001	<0.0001	<0.0001	<0.0001	0.02	0.02
Bran	<0.0001	<0.0001	<0.0001	<0.0001	0.01	0.02
Milled rice	<0.0001	<0.0001	<0.0001	<0.0001	0.0012	<0.0001
Straw	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.006
Cu concentration						
Husk	0.007	0.006	0.05	0.05	1.00	0.97
Bran	0.004	0.005	0.051	0.051	0.98	1.00
Milled rice	0.009	0.008	0.052	0.069	0.996	0.99
Straw	<0.0001	<0.0001	0.053	<0.0051	1.00	0.97
Mn concentration						
Husk	<0.0001	<0.0001	0.07	0.07	0.99	1.00
Bran	<0.001	<0.001	0.07	0.06	0.97	0.93
Milled rice	0.005	0.004	0.051	0.053	0.96	0.96
Straw	<0.0001	<0.0001	0.07	0.08	0.99	1.00
Fe concentration						
Husk	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Bran	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Milled rice	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Straw	<0.002	0.0016	<0.0001	<0.0001	0.7	0.6
Grain yield	0.0009	0.002	<0.0001	<0.0001	0.0007	0.003

equivalent with G3. In contrast, cowpea green manuring (G3) significantly outperformed the other two (G1 and G2) with respect to Mn content both in milled grain and straw (Table 9). On average, SGM boosted Zn, Cu, Mn, and Fe content in milled rice by 25, 22.38, 20.0, and 18.85% during 2020 and 23.75, 21.4, 19.6, and 13.3% 2021, respectively compared to fallow (G1). Averaged over ZF treatment, *Sesbania* green manuring(G2) increased Zn, Cu, Mn, and Fe content in milled rice by 30, 30.3, 11.8, 20, and 22.5%, 28.57, 11.7, 15.5% compared to fallow during 2020 and 2021, respectively. Likewise, cowpea green manuring (G3) enhanced Zn, Cu, Mn, and Fe content in milled rice 21.42, 14.4, 28.2, 17.64 and 20%, 14.28, 27.4, 11.11% compared to fallow during 2020 and 2021, respectively.

Further, the application of zinc in conjunction with urea (both coating and separate application) significantly improved the Zn and Fe content ($p < 0.01$) in milled rice than sole urea application (Z2) and soil application of 5 kg Zn ha^{-1} through ZnO (Z1) as evident by contrast analysis (C2 and C3, Table 3) during both years of study. In contrast, averaged over SGM, Cu, and Mn content in milled rice were not significantly affected regardless of ZF treatment. Averaged over SGM, integrated application of zinc with urea significantly ($p < 0.01$) enhanced the Zn and Fe content in milled rice by 2.45 mg kg^{-1} and 2.52 mg kg^{-1}, respectively during 2020 and by 2.63 mg kg^{-1} and 2.6 mg kg^{-1}, respectively during 2021 than the sole application of 5 kg Zn ha^{-1} through ZnO (Z1). Relative to sole urea application, zinc, and urea together improved the Zn and Fe content in milled rice by 2.99 mg kg^{-1} and 2.62 mg kg^{-1}, respectively during the first year and by 2.83 mg kg^{-1} and 2.6 mg kg^{-1}, respectively during the second year of study.

TABLE 5 Grain yield response of basmati rice to combine the application of summer green manuring (SGM) and zinc fertilization (SGM×ZF).

	Basmati rice grain yield (Mgha^{-1})					
	2020			2021		
	Summer green manuring (SGM)					
Zinc fertilization (ZF)	G1	G2	G3	G1	G2	G3
Z1	3.10^{g}	3.27^{fg}	3.13^{g}	3.21^{h}	3.38^{gh}	3.25^{h}
Z2	3.76^{ef}	3.96^{cde}	3.89^{de}	3.79^{fg}	4.09^{def}	4.03^{ef}
Z3	3.78^{ef}	4.43^{abc}	4.06^{cde}	4.08^{def}	4.56^{abcd}	4.20^{def}
Z4	3.90^{de}	4.94^{a}	4.39^{bcd}	4.01^{ef}	5.07^{a}	4.53^{bcde}
Z5	3.68^{ef}	4.62^{ab}	4.07^{cde}	3.87^{fg}	4.74^{abc}	4.21^{cdef}
Z6	3.87^{de}	4.80^{ab}	4.39^{bcd}	3.96^{f}	4.94^{ab}	4.53^{bcde}

Means followed by the same letter(s) within a column do not differ significantly at 5% probability level by DMRT.

TABLE 6 Summer green manuring (SGM) and zinc fertilization (ZF) on Zn concentration (mgkg^{-1}) in different parts of grain and straw of basmati rice.

Zinc concentration in different parts of grain and straw of *Basmati* rice								
	Husk		Bran		Milled rice		Straw	
	2020	2021	2020	2021	2020	2021	2020	2021
SGM								
G1	43.0^{c}	45.0^{c}	53.1^{c}	55.1^{c}	11.53^{c}	12.0^{c}	64.0^{c}	65.5^{c}
G2	48.5^{a}	50.5^{a}	61.5^{a}	63.5^{a}	15.0^{a}	15.3^{a}	73.0^{a}	74.5^{a}
G3	46.0^{b}	47.6^{b}	57.0^{b}	59.0^{b}	14.0^{b}	14.4^{b}	68.0^{b}	70.4^{b}
ZF								
Z1	43.4^{c}	45.4^{d}	54.8^{c}	57.0^{d}	12.0^{c}	12.2^{c}	65.5^{d}	67.5^{c}
Z2	40.4^{d}	42.4^{e}	52.0^{d}	54.0^{e}	11.46^{c}	12.0^{c}	63.0^{e}	65.0^{d}
Z3	46.2^{b}	48.2^{c}	57.6^{b}	60.0^{c}	14.0^{b}	14.4^{b}	68.4^{c}	70.4^{b}
Z4	50.0^{a}	52.0^{a}	61.2^{a}	63.0^{a}	15.1^{a}	15.5^{a}	72.4^{a}	74.0^{a}
Z5	45.2^{b}	47.2^{b}	56.6^{b}	59.0^{b}	14.0^{b}	14.25^{b}	69.4^{b}	71.0^{b}
Z6	49.1^{a}	51.1^{a}	61.0^{a}	62.6^{a}	14.8^{a}	15.2^{a}	72.0^{a}	73.4^{a}

Means followed by the same letter(s) within a column do not differ significantly at 5% probability level by DMRT.

TABLE 7 Summer green manuring (SGM) and zinc fertilization (ZF) on Cu concentration (mgkg^{-1}) in different parts of grain and straw of basmati rice.

Cu concentration in different parts of grain and straw of *Basmati* rice								
	Husk		Bran		Milled rice		Straw	
	2020	2021	2020	2021	2020	2021	2020	2021
SGM								
G1	6.15^{b}	5.90^{b}	10.1^{b}	10.0^{b}	4.20^{b}	4.02^{b}	8.00^{c}	7.79^{c}
G2	7.44^{a}	7.20^{a}	11.6^{a}	11.4^{a}	5.4^{a}	5.24^{a}	8.31^{a}	8.21^{a}
G3	6.80^{ab}	6.60^{ab}	11.0^{a}	10.8^{a}	4.80^{ab}	4.60^{ab}	8.18^{b}	8.08^{b}
ZF								
Z1	6.70^{a}	6.50^{a}	11.0^{a}	10.6^{a}	4.70^{a}	4.52^{a}	8.12^{a}	8.02^{a}
Z2	6.60^{a}	6.40^{a}	10.7^{a}	10.5^{a}	4.60^{a}	4.39^{a}	8.10^{a}	8.00^{a}
Z3	6.8^{a}	6.60^{a}	11.0^{a}	10.7^{a}	5.00^{a}	4.66^{a}	8.13^{a}	8.03^{a}
Z4	7.00^{a}	6.80^{a}	11.1^{a}	10.9^{a}	5.02^{a}	4.82^{a}	8.15^{a}	8.05^{a}
Z5	6.70^{a}	6.49^{a}	10.8^{a}	10.6^{a}	4.71^{a}	4.51^{a}	8.12^{a}	8.02^{a}
Z6	6.98^{a}	6.80^{a}	11.1^{a}	11.0^{a}	5.00^{a}	4.80^{a}	8.14^{a}	8.04^{a}

Means followed by the same letter(s) within a column do not differ significantly at 5% probability level by DMRT.

TABLE 8 Summer green manuring (SGM) and zinc fertilization (ZF) on total micronutrient (Zn, Cu, Mn, and Fe) uptake (g ha^{-1}) by *basmati rice.*

Total micronutrient (Zn, Cu, Mn, and Fe) uptake by basmati rice								
	Zn		Cu		Mn		Fe	
	2020	2021	2020	2021	2020	2021	2020	2021
SGM								
G1	665^{c}	690^{c}	87.6^{c}	92^{c}	396^{c}	413^{c}	1319^{c}	1375^{c}
G2	818^{a}	846^{a}	105.1^{a}	109^{a}	452^{b}	468^{b}	1492^{a}	1543^{a}
G3	738^{b}	774^{b}	97.2^{b}	101^{b}	469^{a}	487^{a}	1404^{b}	1459^{b}
ZF								
Z1	614^{c}	642^{d}	82.3^{b}	86.1^{b}	382^{b}	397^{b}	1215^{b}	1280^{b}
Z2	719^{b}	703^{c}	95.8^{a}	99.2^{a}	439^{a}	452^{a}	1389^{a}	1466^{a}
Z3	771^{a}	814^{ab}	100.6^{a}	106.2^{a}	458^{a}	482^{a}	1469^{a}	1575^{a}
Z4	791^{a}	838^{a}	101.8^{a}	105.8^{a}	456^{a}	472^{a}	1472^{a}	1501^{a}
Z5	757^{ab}	794^{b}	98.0^{a}	102.6^{a}	444^{a}	463^{a}	1418^{a}	1445^{a}
Z6	665^{c}	690^{c}	87.6^{c}	92^{c}	396c	413c	1319^{c}	1375^{c}

Means followed by the same letter(s) within a column do not differ significantly at 5% probability level by DMRT.

TABLE 9 Summer green manuring (SGM) and zinc fertilization (ZF) on Mn concentration (mgkg^{-1}) in different parts of grain and straw of basmati rice.

Mn concentration in different parts of grain and straw of *Basmati* rice								
	Husk		Bran		Milled rice		Straw	
	2020	2021	2020	2021	2020	2021	2020	2021
SGM								
G1	23.4^{c}	23.8^{c}	27.6^{c}	28.0^{c}	5.00^{b}	5.10^{b}	40.07^{c}	40.4^{c}
G2	25.5^{b}	26.0^{b}	29.3^{b}	30.0^{b}	5.59^{b}	5.70^{b}	42.3^{b}	42.7^{b}
G3	28.0^{a}	28.0^{a}	31.2^{a}	31.57^{a}	6.41^{a}	6.51^{a}	45.3^{a}	45.6^{a}
ZF								
Z1	25.5^{a}	25.8^{a}	29.3^{a}	29.6^{a}	5.56^{a}	5.60^{a}	42.4^{a}	42.8^{a}
Z2	25.1^{a}	25.4^{a}	29.0^{a}	29.2^{a}	5.43^{a}	5.53^{a}	42.1^{a}	42.4^{a}
Z3	25.6^{a}	26.0^{a}	29.5^{a}	30.0^{a}	5.71^{a}	5.81^{a}	42.6^{a}	43.0^{a}
Z4	26.0^{a}	26.2^{a}	30.0^{a}	30.0^{a}	5.88^{a}	6.00^{a}	43.0^{a}	43.2^{a}
Z5	25.4^{a}	25.7^{a}	29.2^{a}	29.5^{a}	5.56^{a}	5.66^{a}	42.3^{a}	42.7^{a}
Z6	26.0^{a}	26.2^{a}	30.0^{a}	30.0^{a}	5.85^{a}	5.95^{a}	43.0^{a}	43.20^{a}

Means followed by the same letter(s) within a column do not differ significantly at 5% level probability level by DMRT.

TABLE 10 Summer green manuring (SGM) and zinc fertilization (ZF) on Fe concentration (mgkg^{-1}) in different parts of grain and straw of basmati rice.

Fe concentration in different parts of grain and straw of *Basmati* rice								
	Husk		Bran		Milled rice		Straw	
	2020	2021	2020	2021	2020	2021	2020	2021
SGM								
G1	39.0^{c}	40.0^{c}	47.4^{c}	48.2^{c}	8.50^{b}	9.00^{b}	142.0^{b}	143.0^{b}
G2	44.4^{a}	45.2^{a}	55.0^{a}	55.4^{a}	10.2^{a}	10.4^{a}	148.0^{a}	149.3^{a}
G3	42.0^{b}	43.0^{b}	52.0^{b}	52.8^{b}	10.0^{a}	10.0^{a}	144.2^{b}	145.1^{b}
ZF								
Z1	40.1^{c}	41.0^{c}	50.0^{c}	50.5^{c}	8.00^{c}	8.10^{c}	144.1^{c}	145.0^{c}
Z2	40.0^{c}	41.0^{c}	50.0^{c}	50.3^{c}	8.00^{c}	8.00^{c}	142.1^{d}	143.0^{d}
Z3	42.3^{b}	43.1^{b}	52.0^{b}	52.6^{b}	10.05^{b}	10.3^{b}	145.1^{bc}	146.0^{bc}
Z4	43.2^{a}	44.0^{a}	53.0^{a}	53.5^{a}	11.0^{a}	11.1^{a}	147.3^{a}	148.3^{a}
Z5	42.1^{b}	43.0^{b}	51.6^{b}	52.5^{b}	10.0^{b}	10.0^{b}	143.5^{cd}	144.4^{cd}
Z6	43.0^{a}	44.0^{a}	52.5^{a}	53.3^{a}	11.0^{a}	11.0^{a}	147.0^{ab}	148.0^{ab}

Means followed by the same letter(s) within a column do not differ significantly at 5% probability level by DMRT.

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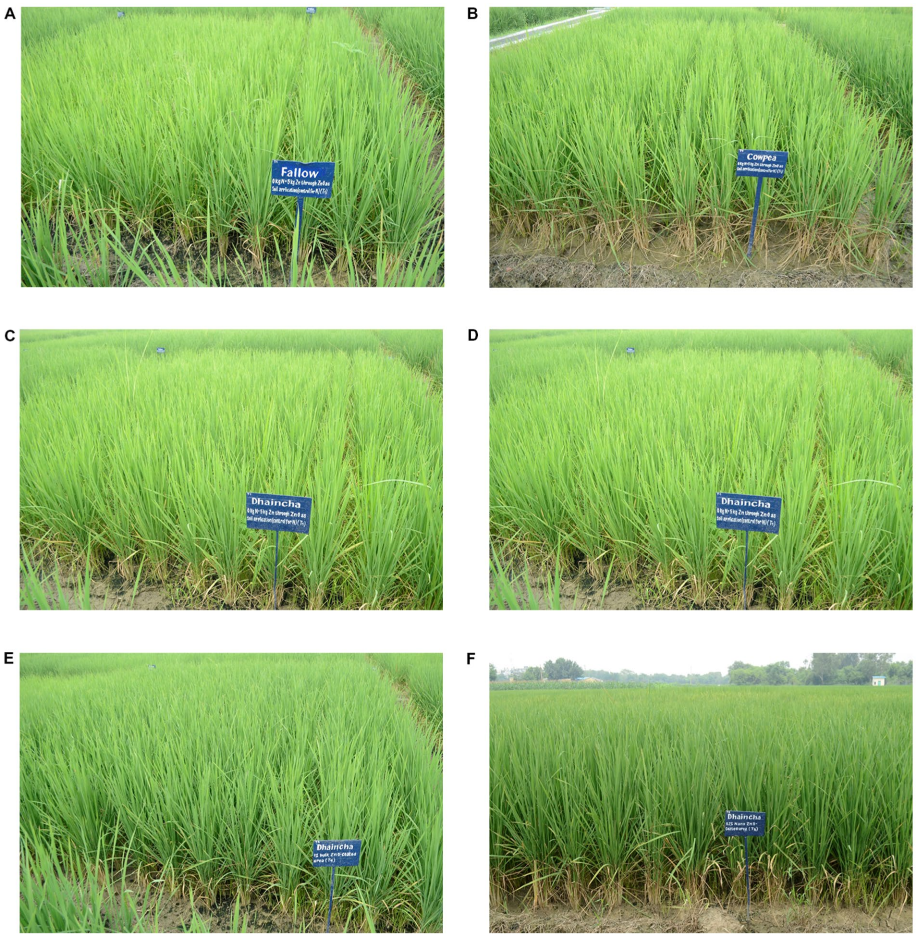

FIGURE 8
(A) Summer fallow +5kg Zn ha^{-1}- bulk ZnO. **(B)** Cowpea +5kg Zn ha^{-1}- bulk ZnO. **(C)** *Sesbania*+5kg Zn ha^{-1}- bulk ZnO. **(D)** *Sesbania*+5kg Zn ha^{-1}- bulk ZnO. **(E)** *Sesbania*+1% bulk ZnO-coated urea. **(F)** *Sesbania*+0.2% nano ZnO-coated urea.

3.3. Total micronutrient (Zn, Cu, Mn, and Fe) uptake

SGM and ZF had a substantial impact on basmati rice's total uptake of Zn, Cu, Mn, and Fe in both years (Table 8). Total micronutrient accumulation followed the pattern Fe > Zn > Mn > Cu. The effectiveness of SGM in terms of the total uptake of Zn, Cu, and Fe by basmati rice, as measured over a ZF treatment, followed the pattern *Sesbania* > cowpea > summer fallow. In both 2020 and 2021, the summer fallow had the lowest total micronutrient uptake. Cowpea, on the other hand, absorbed more manganese overall than any other green manure. In addition, the total uptake of micronutrients was dramatically enhanced by the use of zinc and urea in combination (both coating and separate application) as compared to the use of 5 kg Zn ha^{-1} applied to the soil. Over the summer fallow of 2020, we found an increase of 22.6% in total Zn, 18.4% in total Cu, 13.3% in total Mn, and 12.21% in total Fe, and in 2021, it was 23.0% for Zn, 19.97% for Cu, 14.14% for Mn, and 13.1% for Fe. In cowpea treated plot, the increment in total uptake was 12.17% in Zn, 10.97% in Cu, 17.9% in Mn, and 6.1% Fe during 2020 and 10.97% in Zn, 10.95% in Cu, 18.43% in Mn and 6.4% in Fe during 2021 compared to summer fallow.

3.4. Crude protein content and total crude protein yield

Research findings pertaining to crude protein content in different parts of the grain and total crude protein yield are given in Table 11. SGM and ZF significantly influenced the crude protein in the grain of *Basmati* rice during both years of experimentation. The highest protein content in *Basmati* rice grain was observed when it was grown after *Sesbania aculeata* (G2) residue incorporated plot during both the years and it decreased in the order: *Sesbania aculeata* (G2) > *Vigna unguiculata* (G3) > summer fallow (G1). During both years, crude protein content followed the pattern: bran > milled rice > hull. On average, the crude protein content of milled was 8.24 and 8.38% during 2020 and 2021, respectively. Averaged over SGM, the integrated application of zinc with urea significantly influenced crude protein content in milled rice which outperformed both sole urea application (Z2) and soil application of 5 kg Zn ha^{-1} through ZnO (Z1). The performance of ZF sources in enriching the crude protein content in milled rice was in the order of 0.2% NZCU = 1% BZCU>0.1% NZCU = N through prilled urea +5 kg Zn ha^{-1} ZnO soil application > N through prilled urea > soil application of 5 kg Zn ha^{-1} through ZnO.

TABLE 11 Effect of summer green manuring (SGM) and zinc fertilization (ZF) on crude protein content (%) in different parts of grain and crude protein yield (kgha^{-1}) of basmati rice.

Crude protein content in different parts of grain and crude protein yield of *Basmati* rice								
	Husk		Bran		Milled rice		Crude protein yield	
	2020	2021	2020	2021	2020	2021	2020	2021
SGM								
G1	3.68^{c}	3.91^{c}	10.17^{c}	10.35^{c}	8.00^{c}	8.00^{c}	257^{c}	273^{c}
G2	4.63^{a}	4.87^{a}	11.16^{a}	11.22^{a}	8.70^{a}	8.82^{a}	351^{a}	368^{a}
G3	4.16^{b}	4.40^{b}	10.73^{b}	10.85^{b}	8.29^{b}	8.41^{b}	304^{b}	321^{b}
ZF								
Z1	3.33^{d}	3.57^{d}	9.56^{d}	9.68^{d}	7.16^{d}	7.30^{d}	200^{d}	213^{d}
Z2	3.78^{c}	4.02^{c}	10.27^{c}	10.39^{c}	7.87^{c}	8.01^{c}	275^{c}	291^{c}
Z3	4.23^{b}	4.47^{b}	10.70^{b}	10.82^{b}	8.30^{b}	8.44^{b}	311^{b}	328^{b}
Z4	4.76^{a}	5.00^{a}	11.54^{a}	11.66^{a}	8.91^{a}	9.04^{a}	367^{a}	386^{a}
Z5	4.27^{b}	4.51^{b}	10.70^{b}	10.82^{b}	8.41^{b}	8.55^{b}	317^{b}	334^{b}
Z6	4.56^{a}	4.80^{a}	11.34^{a}	11.46^{a}	8.79^{a}	8.93^{a}	354^{a}	372^{a}
P value (SGM)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.0001
P value (ZF)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.0001

Means followed by the same letter(s) within a column do not differ significantly at 5% probability level by DMRT.

TABLE 12 Correlation matrix depicting correlation coefficients between Zn concentration and uptake vs. N concentration and uptake in basmati rice.

	NH	NB	NM	GNU	TNU	ZH	ZB	ZM	GZU	TZU
NH	-	0.92**	0.94**	0.94**	0.96**	0.82**	0.83**	0.88**	0.92**	0.92**
NB	0.92**	-	0.99**	0.94**	0.92**	0.82**	0.80**	0.85**	0.90**	0.87**
NM	0.94**	0.99**	-	0.96**	0.94**	0.80	0.80**	0.86**	0.92**	0.89**
GNU	0.94**	0.94**	0.96**	-	0.99**	0.78	0.78**	0.85**	0.95**	0.94**
TNU	0.96**	0.92**	0.94**	0.99**	-	0.76**	0.78**	0.86**	0.94**	0.96**
ZH	0.82**	0.82**	0.80**	0.78**	0.76	-	0.98**	0.92**	0.87**	0.85**
ZB	0.83**	0.80**	0.80**	0.78**	0.78	0.98**	-	0.93**	0.89**	0.87**
ZM	0.88**	0.85**	0.86**	0.85**	0.86	0.92**	0.93**	-	0.93**	0.92**
GZU	0.92**	0.90**	0.92**	0.95**	0.94	0.87**	0.89**	0.93**	-	0.96**
TZU	0.92**	0.87**	0.89**	0.94**	0.96	0.85**	0.87**	0.92**	0.96**	-

**Indicate significance at 1% ($p < 0.01$).
NH, N concentration (conc.) in husk; NB, N conc. in bran; NM = N conc. in milled rice; GNU, N uptake by grain; TNU, Total N uptake; ZH, Zn conc. in husk; ZB, Zn conc. in bran; ZM, Zn conc. in milled rice; GZU, Zn uptake by grain; TZU, Total Zn uptake.

3.5. Correlation analysis

The correlation coefficients between the Zn concentration in different parts of the grain, uptake by grain, and total uptake, and N concentration in different parts of the grain, uptake by grain, and total uptake revealed that the quantity of Zn loading in different parts of grain was significantly and positively correlated ($p < 0.01$) with the N content in different parts of grain during both years (Table 12).

4. Discussion

Leguminous green manure owing to a lower C/N ratio, decomposes quickly and results in net N mineralization (Khind, 1992). The incorporation of green manures adds a considerable amount of easily decomposable organic matter resulting buildup of organic carbon (Mandal et al., 2003), thereby promoting higher available N in the soil. Apart from the addition of nitrogen by green manuring, it contributed a significant amount of micronutrients thereby increasing the density of micronutrients in edible portions of food crops (Supplementary Table S1). Increased addition of micro-nutrient by summer green manuring compared to fallow promoted higher micronutrient and nitrogen-dense grain as reflected in our study. *Sesbania*-incorporated basmati rice outperformed cowpea in terms of increasing protein content and micronutrient density (except Mn) in rice grain factored by higher nitrogen and micronutrient addition associated with enhanced biomass production over both years. Our findings are consistent with Pooniya and Shivay (2013) who found that green manuring with *Sesbania aculeata* to basmati rice led to a higher accumulation of Zn, Fe, Cu and Mn and in the grain. Prior studies (Meena and Shivay, 2010) reported a positive effect of *Sesbania* on the agronomic profile of rice. The highest Mn content of cowpea green manured basmati rice can be attributed to significantly higher total Mn bioaccumulation in both root and shoot. Application of organic matter in the form of green manures leads to the release of decomposition products such as water-soluble organic ligands, e.g., citrate, oxalate, and malate (Jones et al., 2003) which have two-fold impacts (1) lowering of rhizosphere pH resulting in higher micronutrient bio-availability (2) complexing of these soil absorbed/fixed micronutrient thereby modulating their solubility (Evans, 1991). The incorporation of green manures *in-situ* may induce alterations in paddy rhizosphere like a more reducing environment driven by the addition of external carbon source, e.g., glucose and organic acids from the decomposed substrate which could accelerate the process of Fe^{+3} reduction, hence more bio-availability of Fe (Liu et al., 2013). A similar process might have promoted Mn availability and Mn-rich basmati rice grain. Organic matter also affects the activity and richness of soil microorganisms (Dhaliwal et al., 2019) consequently enhancing plant micronutrient uptake. Furthermore, organic matter improves soil structure, allowing plant roots to explore a wider soil volume (Mäder et al., 2002) and higher soil moisture retention facilitating nutrient transport to the plant roots. The deeper root penetration by summer green manuring than basmati rice might be another element that aided in the recycling of micronutrients from the subsurface layer to topsoil (Jat, 2010; Pooniya and Shivay, 2012).

The present report of increment in grain yield by application of nano-zinc oxide aligns with previous studies with rice (Bala et al., 2019; Jangid et al., 2019; Kheyri et al., 2019; Singh et al., 2019; Elshayb et al., 2021; Somaratne et al., 2021; Yang et al., 2021; Adhikary et al., 2022; Akmal et al., 2022; Sheykhzadeh et al., 2022; Waqas Mazhar et al., 2022) and with other crops like wheat (Babaei et al., 2017; Adrees et al., 2021; Sheoran et al., 2021; Adil et al., 2022). Conjoint application of nano zinc and urea has been shown to promote grain yield in rice (Kumar et al., 2022) and in wheat (Dimkpa et al., 2020; Asim et al., 2022; Beig et al., 2022). Previous agronomical studies have clearly demonstrated the beneficial effect of ZnO NPs in promoting enriched micronutrients, especially Zn, and improved quality parameters in grain of rice (Kheyri et al., 2019; Elshayb et al., 2021; Yang et al., 2021; Akmal et al., 2022) and wheat (Zhang et al., 2017; Munir et al., 2018; Zhu et al., 2020; Sheoran et al., 2021; Asim et al., 2022). This might be due to enhanced Zn uptake from ZnO NPs than bulk ZnO counterparts linked to the higher solubility of Zn in soil followed by preferential uptake of ZnO NPs by rice root. The Zn sufficiency in plant tissues from ZnO NPs can promote seed development and hence grain size and grain weight because Zn plays key roles in DNA polymerase activity as well as in cell division (Marschner, 2012). The higher Zn content in straw, hull, and bran compared to the rice kernel reflects low mobilization of Zn from different parts to the kernel, producing the lowest Zn content in the kernel indicating that when hull and bran are removed during hulling and milling, the grains lose a considerable proportion of their nutritional values. Interestingly, the dilution effect may account for the considerably reduced Zn concentration in several parts of rice grain and straw in lone urea-treated basmati rice (Table 6) compared to soil application of 5 kg Zn ha^{-1} using bulk ZnO (Jarrell and Beverly, 1981).

The improvement in nutrient content especially N (hence crude protein) in the grain of basmati rice by the application of ZnO NPs coated urea could be ascribed to enhanced nutrient mobilization in the soil by influencing the soil microbial population and extracellular enzymes secretion like urease in the soil (Raliya and Tarafdar, 2013), hence modulating available nitrogen in the soil. The application of 2% zinc-enriched urea outperformed other materials in raising N content which could be linked to synchronized released from the zinc-enriched urea (Shivay et al., 2008). In this context, it is worth noting that zinc content in edible parts of a grain of basmati rice was found to be highly correlated to N content in the grain. Since protein represents a sink for Zn due to the involvement of Zn in protein synthesis in the grain (Cakmak et al., 2010), an increase in crude protein content with Zn application is evident from our study. A possible explanation for the increased Zn concentration is the enhanced sink strength for Zn in grain caused by the increased N supply from summer green manure crops. Our findings corroborate with the findings of Hao et al. (2007) who found that N application promoted protein and accumulation of Zn content in grains. Our investigation has established that agronomic management using an appropriate combination of organic sources like green manuring and inorganic source like Zn-coated urea in basmati rice crop has the potential in modulating zinc levels in milled rice driven by synergistic nitrogen and zinc interaction can mitigate zinc malnutrition.

5. Conclusion

Our findings on basmati rice clearly demonstrated the effectiveness of these nano ZnO-urea assemblies in positively influencing and promoting the transportation of micronutrients and nitrogen into edible tissue. Notably, the yield response of the basmati rice treated with the 0.2% NZCU was comparable to those treated with a traditional fertilization strategy, i.e., 1% BZCU, despite the zinc rate being reduced by 20%. Since the aleurone layer is removed during milling, milled rice always has a lower mineral concentration. Thus, brown rice intake might be an alternative to milled rice for boosting micronutrient status in Asian diets. The delivery of micro-nutrients into plants using agronomic manipulations like summer green manuring and zinc-coated urea and the effective translocation of these nutrients into to edible portions of grain as demonstrated in the present study holds great promise for addressing the widespread micronutrient deficiency in plants and alleviating micronutrient malnutrition in human. Further, studies focusing on synergistic response factored by the integrated usage of green manure and nano ZnO coated urea for a variety of crop species, diverse cropping systems, and soil can be explored to fully understand the versatility and cost-effectiveness of these plant fertilization strategies. Our interventions could be advantageous for improving agri-food systems of rural households of India in combating protein-micronutrient malnutrition where the vast majority of the population relies largely on plant-based food like rice.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

KB, YS, and RP led the research work, planned, supervised, and conducted field experiments, and read and edited the manuscript. KB, SM, SN, and KR collected soil, plant samples and performed chemical analysis, also wrote the initial draft of the manuscript, and prepared figures and tables. RP, YS, DK, MS, DC, RK, and CS project supervision, reviewed, read, and edited the manuscript with significant contributions. BY performed the statistical analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1187717/full#supplementary-material

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Bioactivity and nutritional quality of nutgall (*Rhus semialata* Murray), an underutilized fruit of Manipur

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Introduction: Underutilized fruits plays a significant role in socio economic, cultural, nutritional and ethnomedicinal status of tribal people. However, scientific studies on the nutritional and other pharmaceuticals/biological activities of these fruits are meagre. Hence, the present study dealt with the quantification of nutritional quality and deciphering the bioactivity of nutgall (*Rhus semialata* Murray syn. *Rhus chinensis* Mill.), an underutilized fruit crop mainly found in foothill tracks of Eastern Himalaya, India, China, Japan, Korea and other South East Asian countries.

Methods: The *Rhus semialata* Murray fruits were collected from five different locations in Purul sub-division, Senapati district, Manipur, India. The nutritional composition of the fruit pulp was analysed. Further the fruit pulp was extracted in methanol and water. The methanol and water extracts were studied for bioactivity properties such as antioxidant, antihyperglycemic, antihypertensive, antihyperuricemia, anti-tyrosinase, and antimicrobial activity.

Results and discussion: The fruit was rich in essential fatty acids. The presence of linoleic and oleic acids, along with traces of docosahexaenoic acid and eicosapantaenoic acid, revealed the potential food value of the fruit. 59.18% of the total amino acid composition of the protein present was constituted by essential amino acids. The IC_{50} value of methanolic extract (MExt) and Water extract (WExt) of the fruit were recorded as 4.05 ± 0.22 and 4.45 ± 0.16 μg/mL, respectively, in the DPPH assay and 5.43 ± 0.37 and 11.36 ± 2.9 μg/mL, respectively, in the ABTS assay as compared to Ascorbic acid (3 and 5.4 μg/mL in DPPH and ABTS assay, respectively). The CUPRAC assay also showed a high antioxidant potential of MExt and WExt (1143.84 ± 88.34 and 456.53 ± 30.02 mg Ascorbic Acid Equivalent/g, respectively). MExt and WExt of the fruit were more active against α-glucosidase (IC_{50} of 1.61 ± 0.34 and 7.74 ± 0.54 μg/ mL, respectively) than α-amylase enzyme (IC_{50} 14.15 ± 0.57 and 123.33 ± 14.7 μg/mL, respectively). In addition, the methanolic fruit extract showed low to moderate pharmacological potential in terms of antihypertensive (Angiotensin converting enzyme-I inhibition), antihyperuricemia (xanthine oxidase inhibition), anti-tyrosinase, and antimicrobial activity. The IC_{50} values of angiotensin-converting enzyme I inhibition, xanthine oxidase inhibition and tyrosinase inhibition were recorded as 13.35 ± 1.21 mg/mL, 93.16 ± 4.65 mg/mL, and 862.7 ± 12.62 μg/mL, respectively. The study evidently indicates that nutgall fruit is a potential source of phytonutrients, bestowed with commercially exploitable, multifaceted health benefits

KEYWORDS

Rhus semialata Murray, nutrients, amino acids, fatty acids, antioxidant, antidiabetic, xanthine oxidase inhibition

Introduction

Fruits are known to be rich source of antioxidants, soluble fiber, nutrients, phenols, flavones, vitamins, and minerals. Low intake of fruits is connected with unhealthy diets and several chronic illnesses such as aging, cancer, and cardiovascular disease (1). Edible wild fruits or underutilized fruits serve an important role in augmenting people's diets, particularly in rural and tribal areas. Underutilized fruit crops (a neglected and hidden resource) are referred to the fruit crops that have potential but are not farmed commercially on a large scale (2). Because of their affordability and easy accessibility in ethnic food baskets, they have the potential to play a prospective role in attaining nutritional security and eradicating malnutrition, particularly in disadvantageous areas. Underutilized fruits are also wonderful source of phytonutrients and inadvertently contribute to control "hidden hunger" among the underprivileged communities.

Recognizing the significance of underutilized food crops, the International Centre for Underutilized Crops (ICUC) promotes their cultivation, usage, and marketing. These crops are also important for sustaining floral biodiversity (3). Furthermore, less economic input is required in cultivation because these crops require minimal maintenance and do not require much in the way of agro-inputs such as irrigation, fertilizers, pesticides, and so on. They are also well adapted to local agro-ecology and possess systemic resistance against biotic and biotic stresses.

Underutilized fruits are not only high in nutrients, but they are also believed to have a wide array of therapeutic properties such as antiaging, antibacterial, antidiabetic, anti-inflammatory, antihypertensive, cardioprotective, hypolipidemic, and anti-tyrosinase properties (4). Singh et al. (5), for example, revealed that *Viburnum mullaha* (Buch.-Ham. Ex D. Don), an underutilized wild edible fruit of the Indian Himalayan region, had anti-tyrosinase and antiaging activity. Antidiabetic properties have been identified in underutilized fruits found in Indian subcontinent such as *Syzygium cumini* L. (6) and *Spondias mangifera* (7). Similarly, Ibrahim et al. (8) found that five underutilized Nigerian fruits, *Ziziphus spinachristi, Ziziphus mairei, Parkia biglobosa, Detarium microcarpum,* and *Parinari macrophylla* inhibited intestinal maltase and sucrase in rats.

Consumption of fruit has been shown to significantly reduce blood pressure. Some underutilized indigenous fruits (*Trapa bispinosa, Phoenix sylvestris, Cicca acida, Achras sapota,* and *Averrhoa carambola*) have been shown to inhibit angiotensin converting enzyme I (9). Antibacterial activity has been observed for *Syzygium calophyllifolium* (10), *Garcinia gummi-gutta* (L.) N. Robson, and *Artocarpus lacucha* Buch.-Ham (11). Since time immemorial, traditional healers, as well as ayurveda, unani, and homeopathy, have employed many of the underutilized indigenous fruits or plant parts as medication (12). Because of these reasons, underutilized fruits have the potential to be employed as nutraceuticals and functional foods.

The Himalayan range is the home to the world's largest agro-ecosystem and the rarest crop biodiversity (13). In India, the Himalayan region is a rich reservoir of many underutilized fruit crops. However, comprehensive scientific research on nutritional and other pharmaceutical potential of these fruit crops has been sporadically undertaken. Nutgall (*Rhus semialata* Murray syn. *R. chinensis* Mill., *R. javanica* Linn.) is one such underutilized fruit crop found in the hills and valley regions of North East Indian Himalayan region. It is also wildly distributed in China, Japan, Java, North Korea, Malaysia, Indo-China Peninsula, Indonesia, Europe, and USA (14–16). The genus *Rhus* belongs to the Anacardiaceae family and consists of more than 250 species distributed in the tropics, subtropics and temperate regions. The fruits are edible and have a sharp, acidic taste. The fruits are spheroidal and drupe, slightly flattened, and are about 4–5 mm in diameter. It is separated into pulp and seed.

The fruits are nutrient-dense, including macronutrients, micronutrients, vitamins (vitamin C, B5, B6, and B9), and phytochemicals such as polyphenols, carotenoids, flavonoids, and others. It also includes vital fatty acids and amino acids, confirming the its nutritional excellence (17). Aside from its nutritional value, the fruit also demonstrated medicinal qualities. The infusion of Rhus fruits, for example, is traditionally used in traditional medicine practices to treat fungal, bacterial, and protozoal diseases (diarrhea and dysentery), food poisoning, and allergies in both people and animals (18). According to Bose et al. (19), the methanolic fruit extract of *R. semialata* demonstrated anti-diarrheal efficacy in rats by reducing gastrointestinal propulsion and fluid secretion. The fruit has also been made into digestion pills (20).

Given this backdrop, the current study sought to investigate the nutritional and biological activity of *Rhus semialata* Murray, an underutilized fruit collected from Manipur, India. There are reports in the literature on the nutritional (carbohydrate, fat, protein, mineral, etc.) and some medicinal qualities of this fruit. However, the current study focused on other aspects of the nutritional profile, such as amino acid content, important fatty acids, and so on. Furthermore, biological properties such as antioxidant, antihypertension, anti-hyperglycemic, antityrosinase, anti-gout, antibacterial activities and cytotoxicity were also assessed.

Methodology

Collection of samples and processing

Matured fruits were collected from five different locations in Purul sub-division, Senapati district, Manipur, India (25.37 N, 94.22E, 1,620 m above msl). About 2 kg of fresh fruit was collected from five different plants (400 g each plant) in each location and removed any unwanted non-fruit portions then dried overnight in the oven at 40–45°C. The dried fruit was hand crushed (using a sterile hand glove) to detach the pulp from the seed. Then the pulp was separated by winnowing and ground to a fine powder and stored in an air tide container at 4°C until further used.

Preparation of methanol extract (MExt)

Ten gram of fruit pulp powder was soaked in methanol (100 mL) at room temperature for 24 h with shaking at 180 rpm (Spinix orbital shaker, Tarson). Extraction was repeated for 48 and 72 h and filtered through Whatman no. 1 filter paper followed by drying at 45°C under low pressure (Rotatory evaporator, IKA, Germany). The dried sample was stored at −20°C until used for biological activity assay.

Preparation of water extract (WExt)

Ten gram of fruit pulp powder was soaked in deionized water (50 mL) at room temperature for 3 h with shaking at 180 rpm (Spinix orbital shaker, Tarson) then centrifuged at 10,000 rpm for 10 min. Extraction was repeated with the residue for another hour. The supernatants were pooled in a 250 mL flask and kept at −20°C for overnight. The sample was lyophilized at −80°C. The lyophilized sample was stored at −20°C until further use for biological activity assay.

Proximate and nutritional content

For proximate and nutrient analysis, fruit pulp sample was used. The parameters for proximate analysis *viz.* moisture (Method No. 934.01), crude protein (Method No. 2001.11), fat (Method No. 2003.05), total carbohydrate, ash content (Method No. 942.05) and micronutrients (Method No. 975.03) were determined according to AOAC (21). Total carbohydrate content was calculated by difference (22). The vitamin C was determined by the spectrophotometric method (23). For determination of ash and micronutrient analysis, 1 g dried sample was taken in a pre-weighed 15 mL crucible and digested at 550°C for 4 h in a muffle furnace till white residue remained. The residue was weighed and used for ash determination. For micronutrient analysis, the residue was dissolved in 10 mL HCl (2 N) using a glass rod and then filtered in Whatman No. 42 (ashless). The volume was made upto 50 mL using distilled water. The micronutrients (Ca, Fe, Zn, Mn, and Cu) were measured in an atomic absorption spectrophotometer (AAS) (Perkin Elmer, United States). Total phosphorous (P) was measured by spectrophotometer (450 nm) using ammonium vanadate-ammonium molybdate reagent and potassium was measured in a flame photometer (BioEra, India). The values were expressed in mg/100 g sample.

Amino acid analysis

The amino acid profile of *Rhus semialata* Murray fruit sample was determined using HPLC (Agilent, United States) equipped with a diode array and multiple wavelength detector. The fruit sample was hydrolyzed with 6 N HCl, and the hydrolyzed peptide samples were derivatized with OPA (o–phthalaldehyde for primary amino acids) and FMOC (9–fluorenylmethyl chloroformate for secondary amino acids) as reported by Sahoo et al. (24). The derivatized samples were analyzed for amino acid composition using a Zorbax Eclipse–AAA column (250 mm × 4.6 mm, L × ID, particle size 5 μm) (Agilent Technologies, Santa Clara, CA). The amino acid composition was expressed as the percent amino acid of the total protein content of the analyzed sample.

Fatty acid analysis

The total fat content of the moisture-free sample was extracted in Gerhardt Soxtherm fat analyzer [(21), Method No. 2003.06; (25)]. Fatty acids were analyzed by AOAC (21) (Method No. 996.06) and Shaik et al. (25) methods. The isolated fat was trans-esterified using 0.5 M methanolic KOH to form fatty acid methyl esters (FAME), which were then estimated by Gas Chromatograph (7890B of Agilent Technologies) equipped with a flame ionization detector and an Agilent-DB-FFAP column (nitroterephthalic-acid-modified polyethylene glycol (PEG) of high polarity for the analysis of volatile fatty acids). The temperature of the column was maintained at an initial temperature of 100°C for 5 min, then raised up to 240°C at the rate of 4°C/min. Nitrogen was used as carrier gas at a column flow rate of 1.0 mL/min. The detector temperature was maintained at 280°C. Standards used were 47,885-*U* Supelco® 37 Component FAME Mix (10 mg/mL in methylene chloride). Sample fatty acid composition was compared with standard fatty acid composition, and percentages were calculated by normalization of peak areas.

Antioxidant assay

2,2-diphenyl 1- picrylhydrazyl (DPPH) assay

0.1 mL of each of the five different concentrations of the extract was mixed with 1.9 mL DPPH reagent prepared in methanol ($A_{517} = 1.1 + 0.01$). The mixture was incubated for 30 min in the dark and then read at 517 nm. The radical scavenging activity (RSA) of each extract was calculated as follows (26).

$$RSA\% = \left(A_{control} - A_{sample}\right) / A_{control} \ X100\%$$

From the RSA%, 50 inhibitory concentration (IC_{50}) was calculated and expressed in μg/mL. IC_{50} is defined as the minimum amount of the extract required to scavenge half of the initial stable free radical in 30 min in 1 mL of reaction in the dark.

2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay

ABTS radical scavenging activity was performed as described by Singh et al. (26). The reaction mixture containing 0.1 mL of extract was mixed with 1.9 mL ABTS radical ($A_{734} = 1.0 \pm 0.01$) then incubated for 30 min in the dark, and the absorbance was read at 734 nm. RSA and IC_{50} of the extract were calculated as described above.

Ferric reducing antioxidant power (FRAP) assay

0.1 mL of each extract dissolved in methanol was reacted with 1.9 mL FRAP reagent and incubated for 5 min and the absorbance was read at 593 nm. $FeSO_4$ solution (5–30 µg/mL) was used as standard and ferric reducing antioxidant power was expressed as mM ferrous equivalent per gram of the extract (mM Fe Eq/g Ext.) (26).

CUPRAC (cupric ion reducing antioxidant capacity) assay

0.5 mL sample was mixed with 0.5 mL copper II chloride (10 mM) in a tube. 0.5 mL neocuporine solution was added, and followed by 0.5 mL ammonium acetate solution (1 M). The reaction mixture was incubated for 30 min at room temperature and the color developed was read at 450 nm. Ascorbic acid was used as a standard. Cupric ion reducing antioxidant capacity was expressed in milligram ascorbic acid equivalent per gram of extract (mg AAE/g extract) (27).

Phenolic and flavonoid assay

The total phenolic and flavonoid content of the fruit extract was analyzed by Folin Ciocalteu and aluminum chloride methods, respectively described in Singh et al. (26). Phenolic content was expressed in milligram of gallic acid equivalent per gram of extract (mg GAE/g), whereas flavonoid content was expressed in milligram of quercetin equivalent per gram of extract (mg QE/g).

Pharmaceutical property

Antihyperglycemic activity

α-amylase inhibitory assay

0.1 mL of the sample extract (5–50 µg) was taken in a 2 mL tube and mixed with 0.1 mL enzyme (1 U/mL, porcine pancreas), incubated at 37°C for 20 min. The enzyme reaction was initiated by the addition of 0.2 mL of starch solution (1%) and continued for 3 min at 37°C. After 3 min, 0.2 mL dinitrosalicylic acid (DNSA) reagent was added and kept incubated at 80°C, followed by 1.4 mL of distilled water. For the control reaction, instead of sample, the buffer was added, and a blank was prepared by the addition of DNSA before the addition of the enzyme. The color complex was read at 540 nm. Acarbose (1–10 µg) was used as a standard positive control. From the percentage inhibition, IC_{50} was calculated (28).

α-glucosidase inhibitory assay

0.1 mL of the sample extract (0.1–50 µg) was taken in a 2 mL tube and mixed with 0.2 mL enzyme (0.5 U/mL, α-glucosidase from *S. cerevisiae*), incubated at 37°C for 10 min. To the mixture solution, 0.1 mL 4-nitrophenyl α-D-glucopyranoside (2.5 mM) was added and incubated for 5 min at 37°C. The reaction was terminated by the addition of 0.2 mL sodium carbonate (20%) followed by the addition of 1 mL water. The color complex was measured at 405 nm against a reagent blank (29).

Xanthine oxidase inhibitory assay

0.1 mL sample of different concentrations (50–300 µg) was taken in a tube and the volume was made up to 0.6 mL with buffer (0.05 M, pH 7.5), and 0.1 mL enzyme (0.2 U/mL), followed by incubation for 15 min at 37°C. In the reaction mixture, 0.2 mL substrate (xanthine; 0.15 mM) was added and then incubated for 30 min at 37°C. After 30 min, 0.2 mL HCl (0.5 M) was added and then read at 293 nm against a reagent blank. Allopurinol was used as a positive control (30).

ACE (angiotensin-converting enzyme I) inhibitory assay

0.01 mL of enzyme was added to a tube containing 0.140 mL sample prepared in buffer (pH 8.3) and 0.05 mL sodium chloride (300 µM). To the reaction mixture, 0.5 mL substrate (hipuryl-his-leu) was added and incubated for 30 min. After 30 min, the reaction mixture was kept in a boiling water bath for 10 min. After cooling, absorbance was read at 385 nm. Captopril was used as a positive control (30).

Antityrosinase assay

0.340 mL of different concentrations of the sample (0.5–2 mg) was mixed with 0.02 mL enzyme (mushroom tyrosinase) and 0.360 mL substrate (16 mM DOPA) in a tube and then read at 480 nm spectrophotometrically for 3 min for every 30 s. For control (100% activity), instead of sample, 0.340 mL buffer was used. Kojic acid was used as a positive control (30).

Antibacterial assay

The Kirby-Bauer disk diffusion method (31) was used for the evaluation of the antibacterial activity of MExt. Four test organisms, *viz.*; *Salmonella typhimurium* (ATTC13311), *Escherichia coli* (MTCC 739), *Bacillus subtilis* (MTTCC 121), *Staphylococcus aureus* (ATCC 1026), were grown in Mueller-Hinton broth (MHB) at 37°C and 160 rpm and brought to its exponential phase. The sample was prepared by dissolving the MExt in 50% DMSO (at 25 mg/mL w/v concentration) and filtering through a (0.22 µm) syringe filter. Hundred microliter of each bacterial strain, equivalent to 0.5 McFarland turbidity were spread over sterile Mueller-Hinton agar (MHA) plate using a sterile swab. A 20 µL sample (0.5 mg) impregnated in a 6 mm sterile disc was seeded on the freshly spread MHA plates and incubated for 24 h at 37°C. Chloramphenicol 0.025 mg was used as a positive control. One reagent control containing the buffer and solvent used was also included in the experiment. The zone of inhibition was recorded using a Vernier caliper.

In vitro cytotoxicity assay

The cytotoxic activity of MExt was investigated using Sulforhodamine B colorimetric (SRB) assay against HeLa (human cervical cancer cell line). The cell was maintained in a Dulbecco's Modified Eagle Medium (DMEM). The cells were subjected to different concentrations of the extracts (5–100 µg/mL) for 48 h. Doxorubicin (5–100 µg/mL) was used as a positive control. The monolayer cells were then fixed with 10% Trichloroacetic acid (TCA) for 1 h at 40°C followed by washing with water. After drying, the cell plates were stained with SRB for 30 min and then washed with 1% acetic acid. The SRB dye bound to the protein was then dissolved by adding 10 mM Tris base (pH 10.5). The SRB dye released was quantified by taking absorbance at 510 nm in Varioskan Flash Multimode Reader (Thermo Fisher Scientific, United States) (32).

Statistical analysis

All the assays were performed with three replications, and each replication had 3 triplicates. The data are presented as mean ± standard deviation (SD). The IC_{50} values of assays were calculated using the Prism software (GraphPad, United States).

TABLE 1 Proximate constituent of *Rhus semialata* fruit pulp.

Parameter	g/100g
Moisture	11.32 ± 0.45
Crude protein	4.25 ± 0.04
Crude fat or oil	19.21 ± 0.76
Total carbohydrate	59.20 ± 1.68
Ash	4.02 ± 0.13
Vitamin C	95.24 ± 0.15
Micronutrient	**mg/100 g**
P	218.00 ± 6.8
Fe	19.92 ± 0.2
Mg	14.96 ± 1.2
Zn	2.43 ± 0.4
Cu	1.19 ± 0.08
Mn	0.93 ± 0.06

TABLE 2 Amino acid composition of *Rhus semialata* Murray fruit pulp.

Name of the amino acid	% composition
Threonine	27.21
Lysine	14.64
Glutamine	8.64
Cystine	7.80
Serine	5.63
Arginine	5.43
Alanine	4.71
Leucine	4.71
Hydroxy proline	3.64
Aspartate	3.11
Valine	2.88
Phenylalanine	2.82
Methionine	2.75
Histidine	2.25
Isoleucine	1.92
Glycine	1.12
Tyrosine	0.71

Results

Nutritional analysis

The nutritional contents of the *Rhus semialata* Murray are presented in Table 1. The fruit pulp was mainly comprised of moisture (11.32 ± 0.45%), total carbohydrate (59.2 ± 1.68%), crude fat (19.21 ± 0.76%), ash (4.02 ± 0.13%), and crude protein (4.25 ± 0.04%). The fruit also contained vitamins and minerals. The vitamin C content of the fruit was 95.24 ± 0.15 mg/100 g while the nutrients such as P, Fe, Mg, Zn, Cu, and Mn were 218 ± 6.8, 19.92 ± 0.2, 14.96 ± 1.2, 2.43 ± 0.4, 1.19 ± 0.08, and 0.93 ± 0.06 mg/100 g, respectively.

Amino acid composition

A total of 17 amino acids were detected in the fruit sample (Table 2). Of these, the essential amino acids, (EAAs) *viz.* histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine, constituted 59.18% of the total amino acid composition. Among the EAA, threonine (27.21%) was the highest, followed by lysine (14.64%) and leucine (4.71%), while isoleucine (1.92%) was the lowest. The two EAAs, threonine and lysine, constituted 41.85% of the total amino acid content. The non-essential amino acid content was in the range of 0.71–8.64%. Among the nonessential amino acids, glutamate (8.64%) was recorded as the highest followed by cysteine (7.80%), serine (5.63%), and Arginine (5.43%), while glycine (1.12%) and tyrosine (0.71%) were the lowest.

Fatty acid composition

The total fat content of the studied fruits was constituted by three different groups of fatty acids: polyunsaturated fatty acid (PUFA) (50.62%), saturated fatty acid (SFA) (35.13%), and monounsaturated fatty acid (MUFA) (12.54%), while 1.72% of fatty acids were unknown fatty acids (Table 3). The PUFA included 8 fatty acids, *viz.*, Linoleic acid methyl ester (C18:2n6c), y-linolenic acid methyl ester (C18:3n6), α-linolenic acid methyl ester (C18:3n), Cis-11,14,17 eicosatrienoic acid methyl ester (C20:3n3), Arachidonic acid methyl ester (C20:4n6), Cis-5,8,11,14,17-eicopentaenoic acid methyl ester (C20:5n3), Cis-13,16-docosadienoic methyl ester (C22:2) and Cis-4,7,10,13,16,19-docosahexaenoic acid methyl ester (C22:6n3). Saturated fatty acids (SFA) were obtained in the ranges of 0.08–28.46% of total fatty acids. The most abundant saturated fatty acid was palmitic acid (28.46%), followed by steric acid (C18:0) (2.92%) and butyric acid (2.38). Monounsaturated fatty acids were obtained in the range of 0.33–11.53%. The highest MUFA was recorded as oleic acid (11.15%), and followed by nervoinic acid (0.35%).

Antioxidant activity

The fruit exhibited significant free radical scavenging activity in the ABTS and DPPH assay. In the ABTS assay, the IC_{50} value for MExt and WExt was exhibited as 5.43 ± 0.37 μg/mL and 11.36 ± 2.91 μg/mL, respectively. While the IC_{50} value of standard ascorbic acid was recorded as 3.0 μg/mL. In the DPPH assay, the IC_{50} value for MExt and WExt was found to be 4.05 ± 0.12 μg/mL and 4.45 ± 0.06 μg/mL, respectively. While the IC_{50} value of standard ascorbic acid was recorded as 5.40 μg/mL.

The fruit extract also exhibited ferric and cupric ion-reducing antioxidant activity. The FRAP assay revealed that the MExt and WExt could reduce 4.89 ± 0.68 mM of Fe^{+3}/g Ext. and 1.23 ± 0.07 mM Fe^{+3}/g Ext, respectively. In the CUPRAC assay, the antioxidant activity of the fruit exhibited 1134.84 ± 88.06 mg AAE/g and 456.53 ± 30.02 mg AAE/g for MExt and WExt, respectively (Table 4).

TABLE 3 Fatty acid composition of *Rhus semialata* Murray fruit pulp.

Names of fatty acid	% composition
Saturated fatty acid (SFA)	**35.13**
Butyric acid (C4:0)	2.38
Myristic acid (C14:0)	0.15
Palmitic acid (C16:0)	28.46
Heptadecanoic acid (C17:0)	0.09
Stearic (C18:0)	2.92
Arachidic acid (C20:0)	0.20
Heneicosanoic acid (C21:0)	0.13
Behenic acid (C22:0)	0.72
Lignoceric acid (C24:0)	0.08
Monounsaturated fatty acid (MUFA)	**12.54**
Palmitoleic acid (C16:1)	0.33
Oleic acid (C18:1n9c)	11.53
Cis-11 eicosenoic acid (C20:1n9c)	0.33
Nervoinic acid (C24:1n9c)	0.35
Poly unsaturated fatty acids (PUFA)	**50.62**
Linoleic acid (C18:2n6c)	46.68
y-linolenic acid (C18:3n6)	2.61
α-linolenic acid (C18:3n)	0.49
Cis-11,14,17, eicosatrienoic acid (C20:3n3)	0.15
Arachidonic acid methyl ester (C20:4n6)	0.13
Cis-5,8,11,14,17- eicopentaenoic acid (C20:5n3)	0.27
Cis-13,16-docosadienoic acid (C22:2)	0.05
Cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n3)	0.24
Unknown	**1.72**

The bold value indicates the total value of the of the major fatty acid group SFA, MUFA, PUFA etc.) for example the total saturated fatty acid content is 35.13 which is further composed of individual fatty acids buytyric acid. . . . . . lignocric acid all the values of the individual fatty acid content will give the total value (i.e 35.13).

TABLE 4 Total phenolic, flavonoid content and antioxidant activity.

Antioxidant assay	Methanol extract	Water extract	Vitamin C (positive control)
ABTS (IC_{50} μg/mL)	5.43 ± 0.37	11.36 ± 2.91	3.00 ± 0.40
DPPH (IC_{50} μg/mL)	4.05 ± 0.12	4.45 ± 0.06	5.40 ± 0.30
FRAP (mM Fe Eq/g Ext)	4.89 ± 0.68	1.23 ± 0.07	–
CUPRAC (mg AAE/g Ext)	1134.84 ± 88.06	456.53 ± 30.02	–
Total phenolic (mg GAE/g)	310.37 ± 3.10	255.46 ± 4.40	–
Total flavonoid (mgQE/g Ext)	110.27 ± 20.23	83.02 ± 4.20	–

TABLE 5 Bioactivity of *Rhus semialata* Murray fruit pulp.

Bioactivity	IC_{50} μg/mL		
	Methanol extract	Water extract	Positive control
α-amylase inhibitory	14.15 ± 1.57	123.33 ± 14.70	Acarbose: 6.12 ± 0.68
α-glucosidase inhibitory	1.61 ± 0.34	7.74 ± 0.54	Acarbose: 404.00 ± 17.24
Xanthine oxidase inhibitory	93.16 ± 4.65	192.20 ± 1.90	Allopurinol 77.00 ± 0.08
ACE inhibitory	13.35 ± 1.21	34.57 ± 3.56	Captopril: 0.005 ± 0.0003
Tyrosinase inhibitory	862.70 ± 12.62	1026.00 ± 38.00	Kojic acid: 28.50 ± 2.00

Phenolic and flavonoid content

The phenolic content of MExt and WExt fruit extract was found to be 310.37 ± 3.1 and 255.46 ± 4.4 mg GAE/g extract, respectively. Whereas the flavonoid content of MExt and WExt fruit extract were recorded as 110.27 ± 20.23 and 83.02 ± 4.2 mg QE/g extract, respectively (Table 4).

Pharmaceutical activity

Antihyperglycemic activity

The fruit extracts demonstrated anti-hyperglycaemic activity by inhibiting α-amylase and α-glucosidase enzyme (Table 5). The IC_{50} value of MExt and WExt was found to be 14.15 ± 1.57 μg/mL and 123.33 ± 14.7 μg/mL, respectively. While the IC_{50} of standard acarbose was recorded as 6.12 ± 0.68 μg/mL. The fruit extract also inhibited the α-glucosidase enzyme. The IC_{50} value of MExt and WExt was found to be 1.61 ± 0.34 and 7.74 ± 0.54 μg/mL while the standard acarbose was 404 ± 17.24 μg/mL (Table 5).

Xanthine oxidase inhibitory assay

The methanolic fruit extract was found to inhibit the xanthine oxidase enzyme. The IC_{50} of MExt was 93.16 ± 4.65 μg/mL and 192.2 ± 1.9 μg/mL, respectively, while the standard drug allopurinol was 77 ± 0.085 μg/mL (Table 5).

Angiotensin-converting enzyme-I (ACE) inhibitory assay

The fruit extract showed inhibitory activity in Angiotensin-converting enzyme-I. The IC_{50} value of MExt and WExt was 13.35 ± 1.21 μg/mL and 34.57 ± 3.56 μg/mL, whereas the standard captopril was 0.00458 ± 0.0 μg/mL (Table 5).

Antityrosinase activity

The fruit extract displayed inhibitory activity against the tyrosinase enzyme. The IC_{50} value of MExt and WExt was

862.7 ± 12.62 μg/mL and 1,026 ± 38 μg/mL, respectively, whereas the IC_{50} of the standard kojic acid was found to be 27.04 ± 1.6 μg/mL (Table 5).

Antibacterial assay

The MExt also exhibited promising antimicrobial activity against *Salmonella typhi* and *Staphylococcus aureus*. However, it did not inhibit the growth of *Escherichia coli* and *Bacillus subtilis* organisms. The inhibition zone of *Salmonella typhi* and *Staphylococcus aureus were* 9.5 ± 0.4 mm and 12.39 ± 0.07 mm, respectively (Figure 1 and Table 6).

In vitro cytotoxicity assay

The inhibition of HeLa cell growth by the fruit extract was observed in a dose-dependent manner. The IC_{50} of the MExt against HeLa cells was recorded as 61.55 ± 3.37 μg/mL as compared to the standard anticancer compound Doxorubicin (2.37 ± 0.1 μg/mL) (Figure 2).

Discussion

The edible part of the *Rhus semialata* Murray fruit is its pulp. The pulp contributes 35.39% of the total weight of the fruit. Only the pulp portion was used for analysis in the present study. The proximate analysis of the present study revealed that the fresh fruit contained crude protein (4.25 ± 0.04%), crude fat (19.21 ± 0.76%), total carbohydrate (59.2 ± 1.68%), and ash (4.02 ± 0.13%). Higher amount of protein (8.13 ± 0.38%), lower fat (16.7 ± 0.18%), and carbohydrates (69.2%) content were reported by Loukrakpam et al. (17) in the same fruit, which might be due to the variation in the sample used for analysis (whole fruit including pulp and seed). The mineral composition of the fruit pulp suggested that the most abundant minerals were phosphorous (218 ± 6.8 mg/100 g), iron (19.92 ± 0.2 mg/100 g), and magnesium (14.96 ± 1.2 mg/100 g). These mineral nutrients are required by our bodies to stay healthy. The protein present in this fruit contained both essential and non-essential amino acids. Interestingly, the essential amino acid composition was higher (59.18%) than the nonessential amino acids (40.82%). Essential amino acids are those amino acids that cannot be synthesized by our body and must be present in the human diet. Moreover, the fruit showed significant variation in EAA content ranging from 1.92 to 27.21% of total protein content.

Generally, fruits contain less fat, however *Rhus semialata* Murray fruit was rich in fat (19.21 ± 0.76%). Shi et al. (33) reported a similar amount of fat content (19.68 ± 0.68 to 20.27 ± 1.33%) in *Rhus chinensis* Mill variety. Further analysis of the crude fat revealed that the fruit was rich in polyunsaturated fatty acids. Linoleic acid (C18:2n6c) constituted 92.21% of total PUFA. The essential fatty acids (EFA) such as n6 fatty acids (omega-6 fatty acid) and n-3 fatty acids (omega 3-fatty acid) constituted 49.42 and 1.15% of the total fat of the fruits, respectively. EFA and PUFA derivatives are important structural components of the cellular membrane and play an important role in signal transduction, particularly omega-6 fatty acids. Shi et al. (33) and Loukrakpam et al. (17) also demonstrated high percentage of unsaturated fatty acids in *Rhus chinensis* Mill, and high amount of linoleic acid (C18:2n6c) in *Rhus semialata, respectively.* Hence nutgall fruit is a potential source of unsaturated fatty acids. Unsaturated fatty acids find applications in the food (for formulation of dips and spreads) and pharmaceutical sectors. Most of the phytochemicals (both polar and nonpolar) are dissolved in methanol; hence it represents one of the most used organic solvents for extraction. However, extraction of bioactive metabolites from plants using water as a solvent has been attempted by many researchers. Moreover, the fruit, simply soaked in water, is used as medicine alone or mixed with other ingredients by local traditional healers, especially in Manipur, to treat various ailments. Hence, the fruit pulps were extracted in two solvents, i.e., methanol and water, and screened for biological activities (antioxidant, antihyperglycemic, xanthine oxidase inhibitory, antityrosinase, angiotensin-converting enzyme inhibitory, antibacterial and cytotoxicity of cancer cell lines). Both extracts showed antioxidant activity in all four antioxidant assays. In ABTS and DPPH assay, MExt showed a lower IC_{50} value, which indicates higher antioxidant activity than WExt. Zhang et al. (34) reported that IC_{50} of 80% methanol extract of the *Rhus chinensis* Mill. fruit was 3.72 μg/mL, which is slightly lower than in the studied sample (5.43 ± 0.37 μg/mL). For the FRAP and CUPRAC assay, antioxidant activity was expressed as mM Fe Eq/g Ext and ascorbic acid equivalent (AAE) respectively. The higher the value of mM Fe Eq or AAE, the higher the antioxidant activity. The MExt had higher antioxidant activity than the water extract. Similarly, Sharma et al. (35) reported that the crude methanolic extract of *Rhus semialata* exhibited an IC_{50} value of 5.31 ± 0.07 μg/mL and 5.84 ± 0.17 μg/mL in DPPH and ABTS assays, respectively. The high antioxidant activity of this fruit may be due to the presence of phenolic and flavonoids. These two phytochemicals are known to confer antioxidant activity of many

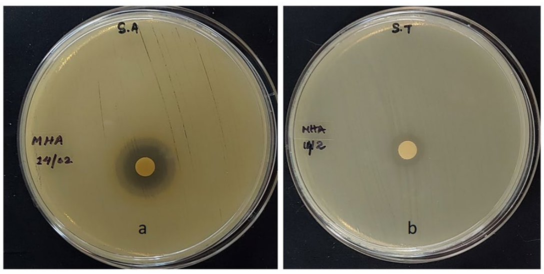

FIGURE 1
Antibacterial activity of methanolic fruit extract of *R. semialata* Murray **(a)** *Staphylococcus aureus* **(b)** *Salmonella typhi.*

TABLE 6 Antimicrobial activity of *Rhus semialata* Murray fruit pulp.

Sample	Inhibition zone (mm)			
	Bacillus subtilis	*Eschericia coli*	*Salmonella typhi*	*Staphylococcus aureus*
Methanolic extract	–	–	9.50 ± 0.40	12.39 ± 0.07
Chloramphenicol	30.20 ± 1.60	28.00 ± 2.20	30.00 ± 2.00	29.00 ± 1.00

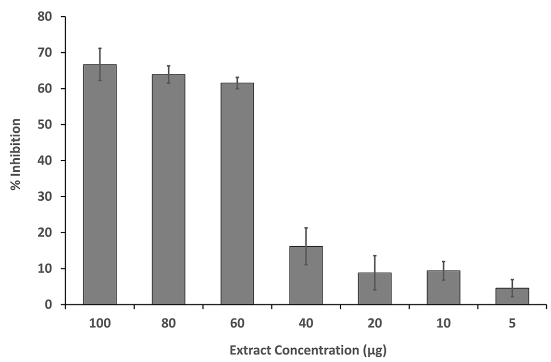

FIGURE 2
Dose-dependent inhibition of HeLa cells by methanolic fruit extract of *R. semialata* Murray.

plant extracts. Antioxidant activity is one of the desired properties for the formulation of nutraceuticals or functional foods.

Oxidative stress has been identified as a key factor in the development of diabetic complications (36, 37), and there was a significant association between antioxidant and anti-diabetic activities (38). After observing the potent antioxidant activity of fruit extracts, antihyperglycemic activity was also evaluated. The MExt of fruit showed higher α-amylase inhibitory activity than water extract. We noticed that the MExt and WExt of the fruit were more active against α-glucosidase than α-amylase enzyme. This result demonstrated that the fruit extract has the potential for anti-hyperglycaemic activity in terms of inhibition of α-amylase and α-glucosidase enzymes. Diabetes is closely related to increase production of ROS (reactive oxygen species) and their accumulation via various metabolic pathways (39). In diabetic conditions, as glucose uptake in insulin-dependent tissues (fat and muscle) is minimized, glucose uptake is elevated in insulin-independent tissues (40). This excessive intracellular glucose is converted to polyalcohol sorbitol, resulting in a decrease in the NADPH/NADP+ ratio and glutathione concentration. In addition, hyperglycemia leads to the activation of protein kinase C isoforms, induction of the hexokinase pathway, and overproduction of advanced glycation end products. All these effects of hyperglycemia are responsible for diminishing antioxidant agents and overproducing and accumulating reactive oxygen species, which ultimately leads to oxidative stress (King and Loeken, 2004; Vanessa Fiorentino et al., 2013). Hence, compounds having antihyperglycemic activity as well as antioxidant activity have the potential to be used in controlling diabetes. It has already been reported in the literature that natgall bark extract possesses antihyperglycemic properties. The two compounds responsible of α-glucosidase inhibitory activity have been identified as phloridzin and scopoletin (41). Similarly, Liu et al. (42) studied the inhibitory effect of free phenolic, esterified phenolic and insoluble-bound phenolic from *Rhus chinensis* Mill. on α-glucosidase and dipeptidyl peptidase-4 (DPP-IV) and formation of advanced glycation end (AGE) product. They observed that the free phenolic extract had higher α-glucosidase and dipeptidyl peptidase-4 inhibitory activity while the insoluble-bound phenolic extract exhibited higher inhibition on AGE product formation. The two phenolic compounds gvajaverin and quercitrin, made the most significant contributions to the inhibitory effects on α-glucosidase and DPP-IV, while trigalloyl glucose and its isomer may be the primary bioactive substances responsible for the suppression of AGE formation (43).

Xanthine oxidase enzyme inhibition is one of the targets for gout and other diseases that involve hyperuricemia (high levels of uric acid in the blood). Hyperuricemia is associated with chemotherapy and calcium oxalate kidney stones in patients. XO inhibitors are a therapeutic option for treating these types of disorders. Current drugs that are available on the market (allopurinol, febuxostat, and uricase, etc.) are associated with adverse side effects (44). Hence, research is ongoing to look for new, effective, and safer XO inhibitors of natural or synthetic origins for the treatment of the disease.

It is a widely known fact that people with diabetes frequently acquire high uric acid levels and vice versa. Desco et al. (45) reported that diabetes causes an increase in xanthine oxidase (XO) activity in the liver. XO catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid (46). Elevated uric acid production can aggravate insulin resistance and lead to diabetes. In addition to uric acid production, XO also plays an important role in generating free radicals, which may influence oxidative damage in diabetes (45, 47). Many antioxidants are also potent xanthine oxidase inhibitors.

In this regard, the *Rhus semialata* Murray fruit extracts were also screened for XO inhibitor activity. The extracts showed inhibitory activity against XO and their IC_{50} (MExt- 93.16 ± 4.65 µg/mL; WExt- 192.2 ± 1.9 µg/mL) was comparable with the standard inhibitor allopurinol (77 ± 0.08 µg/mL). Tsai et al. (48) reported that the n-hexane extract of *Rhus semialata* var. Roxburghiana leaves and stems exhibited xanthine oxidase inhibitory activity. The leaf extract showed higher activity as compared to the stem, as indicated by a lower IC_{50} value. The IC_{50} of leaf extract was recorded as 16.74 ± 0.74 µg/mL, while stem extract was recorded as 26.53 ± 0.54 µg/mL. As far as we know, there are no documentary evidences available on the XO inhibitory activity by fruit extract of *Rhus semialata* Murray.

Melanin is produced by the conversion of the amino acid L-tyrosine to 3,4-dihydroxyphenylalanine and its oxidation to dopaquinone (49, 50), the precursor of melanin formation. Tyrosinase catalyzes the conversion of L-tyrosine to melanin. Free radicals also participate in the biosynthesis of melanin and are involved in the catalytic conversion of L-DOPA to dopaquinone by tyrosinase (51). The tyrosinase-inhibiting and free radical scavenging effects of antioxidants inhibit melanin production. Tyrosinase inhibitors are used in the food, pharmaceutical, and cosmeceutical industries. Antioxidants such as vitamin C and E have been shown to have significant inhibitory actions against tyrosinase. Many antioxidant rich fruits have been reported to have antityrosinase activity. Antityrosinase compounds are identified from plant source as well as from microorganisms.

Given this context, the antityrosinase activity of *Rhus semialata* Murray fruit extracts (both MExt and WExt) was screened for the first time in the present study. The crude extracts showed tyrosinase inhibitory activity, though their activity is lesser than the standard kojic acid. It is one of the widely used commercial antityrosinase compounds obtained from different species of fungi and as a byproduct of the fermentation of certain foods (52). Nevertheless, the antityrosinase compound from *Rhus semialata* Murray will also be a potential candidate as a source of antityrosinase compounds.

Angiotensin-converting enzyme I (ACE) inhibitors are a class of drugs used to control high blood pressure. They're also used to treat other cardiovascular conditions such as heart failure, diabetes-related kidney disease, and more (53, 54). Significant routes leading to hypertension are ROS production in target tissues by the hypertensive agents. These agents stimulate the formation of superoxide through oxidases, uncoupled nitric oxide synthase, xanthine oxidase, and mitochondria (55). ROS can boost the activity of ACE. The oxidation of the sulfhydryl groups on ACE is one possible mechanism. As part of its route, ROS can also activate the renin-angiotensin system (RAS), which includes ACE. ACE is well-known for its twin functions of converting inactive Angiotensin I to active Angiotensin II and degrading active bradykinin, which play a crucial role in blood pressure regulation (56). An increase in angiotensin II production raises blood pressure. Again, several antioxidants possess ACE-inhibiting activities and aid in reducing hypertension.

Though *Rhus semialata* Murray is being used in traditional medicine and shows promising free radical scavenging activity, there is no report on the ACE inhibitory activity of this plant. Hence, the fruit extracts (MExt and WExt) of *Rhus semialata* Murray were assayed for ACE inhibitory activity. The extracts were found to be positive for ACE inhibitory activity, indicating that the fruit exhibits potential antihypertensive activity. The IC_{50} values of MExt and WExt was recorded as 13.35 ± 1.21 μg/mL and 34.57 ± 3.56 μg/mL, respectively. Hence the value-added functional food products prepared using *Rhus semialata* fruit may be used as therapeutic foods in controlling high blood pressure.

Numerous enzymes are responsible for oxidative stress, both directly and indirectly, and the resulting inflammation. For instance, xanthine oxidase increases the generation of superoxide. ROS often stimulates RAS. Several studies have demonstrated an over-activation of the RAS in diabetes problems (57), and the RAS pathway involves ACE. Free radicals are also involved in the tyrosinase-catalyzed conversion of L-DOPA to dopaquinone during melanogenesis. XO, ACE, α-amylase, α-glucosidase, and tyrosinase are therefore related in various ways and contribute to the onset of a number of chronic disorders. *Rhus semialata* Murray, which shows inhibitory capability against these enzymes, might be a significant treatment alternative for degenerative illnesses.

Rhus chinensis Mill. was reported to have antibacterial activity against a wide range of gram positive and gram-negative bacteria such as *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus, Streptococcus iniae, Vibrio ichthyoenteri* (58, 59). Similarly, the MExt. of *Rhus semialata* Murray fruit (present study) exhibited antimicrobial activity against *Salmonella typhi* and *Staphylococcus aureus*. However, the extract did not show antibacterial activity against *Bacillus subtilis* or *Escherichia coli*. The variation may be due to the difference in the solvent used for the extraction. Sreedharan et al. (60) also reported that the *Rhus semialata* seed extracted in different organic solvents such as petroleum ether, chloroform, and methanol showed antibacterial activity against at least one of the test organisms (*Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Penicillium* sp.). The 80% ethanolic extract of *Rhus javanica* exhibited antibacterial activity against three species of *Shigella, viz. S. sonnei, S. flexneri, S. boydii,* and *S. dysenteriae* (61). These findings indicate that *Rhus semialata* plant extract showed antimicrobial activity against a wide range of gram positive and gram negative pathogenic bacterial genera. However, there is a very limited report on the identification of antimicrobial metabolites from this plant. Hence, further study on the identification of metabolites responsible for antimicrobial activity and their mechanism of action is highly required to discover novel antimicrobial drugs.

Numerous plants have been shown to exhibit high ROS-scavenging activity, which is related with cytotoxicity or antiproliferative activity against cancer cells and might thus be employed as therapeutic and preventative agents (62, 63). The cytotoxicity of *Rhus semialata* against SW620 and HCT116 cell lines has also been reported in the literature. These two cell lines are responsible for colon cancer (64, 65). Taking into account the antiproliferative activity, the MExt of *Rhus semialata* Murray (present study) was evaluated for its *in vitro* cytotoxicity activity against HeLa cell line. It is the oldest and most commonly studied cell lines obtained from cervical cancer specimens. The inhibition of HeLa cell growth by the MExt was observed in a dose-dependent manner until 60 μg/mL concentration corresponding to 60% growth inhibition. Further increases in concentration did not improve the cell growth inhibition significantly. This might be due to an increase in undesired interactions between the compounds present in the extract at higher concentrations, which ultimately retards the activity of the extract. The IC_{50} of the MExt against HeLa cells was recorded as 61.55 ± 3.37 μg/mL. Similarly, Lalawmpuii et al. (66) reported that the methanolic extract of *Rhus javanica* L. fruit exhibited cytotoxicity activity against HeLa cells with an IC_{50} value of 98.28 μg/mL.

Conclusion

The present study presented thorough scientific evidence for the nutritional and therapeutic potential of *Rhus semialata* Murray grown in North-east Indian Himalaya. Our research showed that fruits are high in minerals and vitamin C. Interestingly, the fruits were also found to be a potential source of essential amino acids, unsaturated fatty acids, omega-6 and omega-3 fatty acids, all of which are vital for maintaining the normal function of the body. The MExt and WExt of *R. semialata* Murray fruits demonstrated multifaceted pharmaceutical properties. Fruit extracts showed antioxidant action equivalent to that of ascorbic acid. Furthermore, the fruit extracts inhibited the α-glucosidase and α-amylase enzyme, indicating antihyperglycemic activity. Besides, the dose-dependent inhibition of HeLa cell growth by the methanolic fruit extract indicated its anticancer potential. To the best of our knowledge, *R. semialta* Murray has been reported for the first time globally for its ACE inhibitory and anti-tyrosinase action. Additionally, the fruits extract exhibited low to moderate pharmacological activity in terms of xanthine oxidase inhibitory and antibacterial activity. The findings of our investigation will provide vital information about the food-value and bioactivity of *R. semialta* Murray fruits. The fruit might be recommended for regular consumption in order to maintain a healthy living. It may also be considered as a component in nutraceutical or functional foods to enhance quality of life or may be researched extensively for innovative value addition. Further investigation into the bioactive compounds responsible for these activities will give deeper insights about this potential fruit.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

TSS: experimental, data analysis, literature survey, and manuscript drafting. PK: literature survey, experimental, and manuscript drafting. AKD, PL, KT, TC, CD, TBS, SC, and YD: experimental. HS: experimentation and figure and table preparation. RA: literature survey and manuscript drafting. AK: experimental and manuscript drafting. SGS: experimental and language improvement. SKS: data analysis and interpretation. AD: manuscript editing. SR: conceptualization, supervision, interpretation, and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer HR declared a shared affiliation with the authors SR, TSS, PK, AKD, PL, KT, HS, RA, TC, CD, TBS, SC, YD, SKS, and AD to the handling editor at the time of review.

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Nutrition security, constraints, and agro-diversification strategies of neglected and underutilized crops to fight global hidden hunger

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Introduction: Neglected and underutilized crop species (NUCS) or forbidden crops offer tremendous potential to combat malnutrition, poverty, and global hidden hunger. Since overdependence on a few dominant cereal crops, *viz.,* rice, maize, and wheat, is insufficient to meet the global food energy intake, the identification, genetic improvement, and implementation of various policies for wenumerates comprehensive comparative analyses of the nutrient profile of staple crops vs. potent underutilized crops with reference to cultivation constraints and climate resilience with different agro-diversification strategies.

Methodology: The research databases Scopus, JSTOR, Web of Science, EBSCO, Google Scholar, ScienceDirect, PubMed, and Academic Search were searched using relevant research queries.

Result: Out of 2,345 hits, 99 articles pertinent to the subject domain showed that underutilized crops are nutritionally superior, contain health-promoting bioactive components, and are more climate resilient than cereal crops. However, several constraints hinder the efficient utilization of these crops.

Discussion: Despite underutilized crops' many health benefits, improved cultivation techniques for the large-scale production of these crops are still in their infancy. Most of the time, however, the scientific knowledge gleaned from various study domains stays within the scientific community. The most crucial need of the hour, therefore, is an efficient network structure connecting governments, farmers, researchers, and people in business. Moreover, care must be taken to ensure that the policies of governments and INGOs/NGOs are properly implemented within a NUCS framework.

KEYWORDS

nutrition security, neglected and underutilized crops, bioactive components, anti-nutritional factors, climate resilience

Introduction

An endeavor to improve global food security is being hindered by several intricate and interlinked challenges. Especially after the COVID-19 crisis, development across many domains is either being halted or reversed, worsening an already dire scenario that includes hunger and food insecurity. Even though the world has already fallen behind schedule in achieving the Sustainable Development Goals (SDGs) before 2020, the pandemic has accelerated this trend and had a disastrous impact on individual lives and livelihoods as well as global efforts to achieve the SDGs. The Food and Agriculture Organization (FAO) of the United Nations recently estimated the global hunger figure to be approximately 702 to 828 million people (1). Additionally, drastic food insecurity has increased significantly from 10% (2020) to 11.7% (2021) of total world population (1). According to the Global Hunger Index 2022, 44 countries are experiencing an alarming level of hunger issues, and 20 more countries showed a higher GHI score than in 2014, with moderate, serious, or alarming hunger levels, while 46 countries will fail to reduce hunger level by 2030. Among different countries, Chad, Madagascar, the Central African Republic, Yemen, Somalia, Syria, South Sudan, Congo, and Burundi showed GHI scores ranging from 37.2 to 45.1. A recent report by Statista 2022 revealed that nearly 23.2% of the population of Sub-Saharan African is experiencing malnutrition, while North America, Europe, and East Asia have the lowest (2.5%) share of population experiencing malnutrition (Figure 1). These differences could be attributed to inefficient food supply, low income, and poor health facilities in poor/developing countries as compared to developed countries. It is evident that more investment in the agricultural sector than non-agricultural sectors is highly effective in reducing poverty and global hunger. While more than 700 million hectares of global cropping areas are used to cultivate the five major cereal crops of maize, wheat, rice, barley, and sorghum, which alone supply 50% of the world's caloric intake (2), yield and grain quality have been plateauing in recent years, with a substantial reduction since 1960s (3). The dilemma of food insecurity cannot be met by focusing on just the productivity of the existing primary crops, which have been frequently chosen and developed under high-intensity agriculture. This could also render agriculture even more susceptible to future biotic and abiotic pressures. Over the past decade, research on potent alternate crops, *viz.*, neglected and underutilized crop species (NUCS), has gained considerable ground due to the focus on food quality, reduction of the risk of overreliance on a limited number of staple crops, preservation of cultural dietary diversity, and the potential of natural climate-resilient crops (4, 5). However, not every neglected and underutilized crop species (NUCS) is climate resilient and nutrient rich. The FAO (6) classifies NUCS as Future Smart Food (FSF) on the condition that they satisfy the following four criteria: they must be nutrient rich (improve nutrition), climate resilient (e.g., enhance climate change resiliency and environmentally sustainability by effectively reducing runoff and erosion), locally available or adaptable, and cost effective (e.g., produce income and lessen drudgery). In the present review article, we have provided a comprehensive comparative nutrient profile of staple crops vs. potent underutilized crops with reference to their climate resilience. Major bioactive components, bioavailability and anti-nutritional factors, and different constraints of NUCs were also briefly discussed. The detailed descriptions of the methodology and information sources are shown in Supplementary Note 1.

Identification and comparative nutritive values of neglected and underutilized crop species

NUCS, also known as forbidden or orphan crop species, are indigenous to a particular tribe, usually semi-domesticated or wild. NUCS are classically identified based on the following features described by Papoola et al. (7) and Chandra t al. (8):

1. Crops that have known native uses in specific localized areas with scientific or ethno-botanical evidence of their nutritional value.
2. Adaptation to agro-ecological niches or marginal areas and representation by ecotypes/landraces.
3. These crops must be grown less frequently as compared to traditional staple crops.
4. Adaptation and cultivation are based on indigenous knowledge and practices.
5. Rare depiction in the *ex situ* collection.
6. Grain supply networks are either underdeveloped or non-existent.

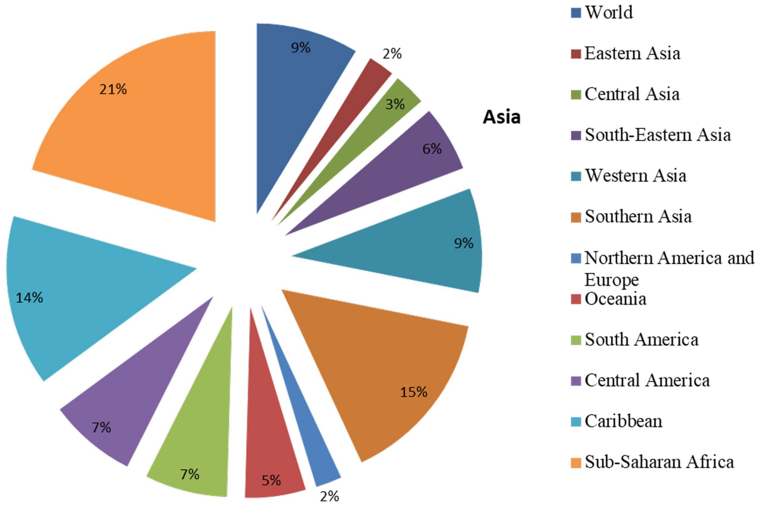

FIGURE 1
Region-wise share of population under malnutrition (Adapted from Statista 2022).

7. They have received scant attention from scientists, extension agents, farmers, policymakers, technologists, and consumers.
8. These crops might be extremely nutritive and/or possess therapeutic potential with other multiple uses.

NUCS include species from all forms of plants, *viz.*, herbs, shrubs, trees, crops, or vines. However, due to the wide range of species—and with the definition depending on the location, scale of reference, and possibility for enhanced use—compiling a comprehensive list of neglected and underutilized crops is not an easy process (9). The available lists as reported by the National Research Council of Africa (10), Li et al. (11), Wani et al. (12), and Hossain et al. (13) for Africa, Asia, and the Americas are subjected to regional preferences. A compiled list of potent NUCS has been shown in Supplementary Table 1. Among the different crops, the recent emergence of crops such as amaranth, buckwheat, yam, Colocasia, lemon, pumpkin, cassava, faba beans, millets, legumes, pulses, and traditional vegetables has diversified the food consumption profile of marginalized and neglected crop species. Although these crops are suitable for marginal areas, they still hold a significant value in the local food basket due to their sufficient nutrient content (14). A detailed nutrient profile of major staple crops and some specific underutilized crops has been shown in Table 1. Millets are highly nutritious and easily digestible due to their low glycemic index (17) compared to the staple cereals, and they are reported to be the sixth highest yielding crop in the world (18). While finger millets contain >10 times higher calcium content than polished rice (19), Quinoa (*Chenopodium quinoa*), a pseudocereal, is a rich source of protein and fiber (20), contains 14% protein, essential amino acids, a rich source of vitamins, fatty acids, and is free of cholesterol and gluten (20). In comparison to cereals, pseudocereals have significantly high-quality protein, which makes them appropriate for the functional food market. Pseudocereals are abundant in amino acids, including arginine, tryptophan, lysine, and histidine, which have been shown to be crucial for newborn and child health. This has led to the projection of pseudocereals as a suitable food supplement for child nutrition. The essential amino acid, *viz.*, lysine, content in buckwheat is much higher compared to rice, wheat, or maize. A comparative list of amino acid contents between cereals and NUCS is presented in Supplementary Table 2. Buckwheat is also a rich source of flavonoids such as rutin, quercetin, and alpha tocopherol (21). As per the FAOSTAT, 2018 report, France was reported to be the highest producer of buckwheat in the world (3,735 kg/ha). Grains of Amaranthus contain 64% starch, 10% fat, 4% protein, 2.5% ash, 16% fiber content, and essential amino acids such as lysine (22). The cultivation of underutilized pulses such as mung beans, pigeon peas, or lentils is confined to mostly Southeast Asian countries, and their production level is still considered insignificant compared to major cereals. While sprouted Adzuki beans (*Vigna angularis*) are a rich source of vitamin A, vitamin B, and folic acid with 19.9% protein content (23), jack bean is an excellent source of protein (23–34%) and carbohydrate (55%) (24). *Portulaca oleracea* contains 26.6 mg of ascorbic acid, 300–400 mg omega-3 fatty acids, 1.9 mg beta-carotene, 12.2 mg of α-tocopherol, and 14.8 mg of glutathione (25). Among many underutilized leafy vegetables, amaranth is widely grown in tropical regions such as Mexico, South America, Southern Asia, and Africa. Compared to cabbage, it is significantly more nutritious (26). Another nutrient- and mineral-rich underutilized plant is the drumstick, mostly cultivated in tropical Asia, Sub-Saharan Africa, Latin America, and the Caribbean. According to Chandrashekara and Kumar (27), root and tuber crops (RTCs) are the second highest source of carbohydrates after cereals. The fact that RTCs contribute significantly to global food security and are produced at >845 million tons on a global scale (6) demonstrates their significance. Yam is the fourth most produced RTC in the world, and it is primarily produced and consumed in Southeast Asia, West Africa, and the Caribbean (28). Sweet potato, cassava, yams, and aroids, rather than potatoes, account for 90% of production and consumption among the various underutilized roots and tuber crops. Currently, the largest producer of these crops is Asia, followed by America and Europe (28). Asia alone contributes more than 40% of global production. Cassava is a potent NUCS for more than 500 million people worldwide due to its distinctive nutritional components and high carbohydrate content (29). Recently, the concept of dietary fiber has attracted worldwide attention. Underutilized vegetables are considered reservoirs of various minerals. The nutritious pods of *Parkia roxburghii* and *Mucuna Pruriens* are considered among the most popular legumes in Northern India (30).

Major bioactive components of NUCS and their health benefits

Bioactive components in plants can be classified into four categories, *viz.*, phenolic acids, flavonoids (anthocyanidins, flavones, isoflavones, flavonols, and flavanones), stilbenes, and lignans. These naturally occurring antioxidants, especially flavonoids, have a wide range of biological activities, including anti-aging, anti-inflammatory, antiviral, antimicrobial, and anti-cancer properties. Different bioactive components and their health benefits have been shown in Figure 2. Several studies have reported that neglected and underutilized crops are rich sources of bioactive components (for example, bioactive flavonoids present in different parts of pseudocereal buckwheat, *viz.*, the root, flower, fruit, seed, sprouted seed, seedling, seed coat, seed husk, and processed food, establishing it as a highly treasured crop) (31). Rutin comprises 90% of total flavonoid phenolics, followed by quercetin (32, 33). High phenolic content imparts higher antioxidant activity in buckwheat than quinoa and amaranth (34). It has been reported that nitrogen-containing pigments such as betalains exist more abundantly in pseudocereals than in cereals. On the other hand, flavonoids and carotenoids are the major bioactive components present in fruits and vegetables. Some significant bioactive components found in the different underutilized crops, *viz.*, pseudocereals, fruits, vegetables, roots, and tubers crops, are listed in Table 2.

Climate-resilient crops: staple crops vs. NUCS

Global climate change has threatened the productivity of major staple crops such as rice, wheat, and maize. Cereal crops are sensitive to various biotic and abiotic constraints, *viz.*, salinity, drought, or heavy metal stresses. Under enduring climate change and variability conditions, underutilized crops are regarded as mitigation strategies for food insecurity (61). A considerable decrease in wheat output has

TABLE 1 Nutrient profile of selected staple cereal crops vs. neglected and underutilized crop species (NUCS) (11, 13, 15, 16).

Crop Categories		Scientific name	Nutrients composition (g/100g)						
			Common name	Energy	Protein	Carbohydrate	Fiber	Fat	Ash
Staple Crops	Cereals	*Oryza sativa*	Milled rice	345	6.8	78.2	4.1	3.6	0.8
		Oryza sativa	Red rice	362	7.5	76.2	3.6	2.4	1.5
		Triticum aestivum	Wheat	344	11.8	71.2	12.2	2.73	0.9
		Zea mays	Maize	366	9.4	63.6	7.3	4.7	1.78
Neglected and Underutilized Crop Species (NUCS)	Cereals and Pseudocereals	*Eleucine coracana*	Finger millets	328	7.3	75	20	1.3	1.9
		Setaria italic	Foxtail millet	331	12.3	69.9	4.25	4.3	1.9
		Pennisetum glaucum	Pearl millets	361	11.6	61.78	11.49	5	1.9
		Fagopyrum esculentum	Buckwheat	355	14.2	72.9	17.8	7.4	2
		Amaranthus caudatus	Amaranth	346	14.5	63	12.5	2.5	1.8
		Chenopodium quinoa	Quinoa	354	14.1	57.16	7	4.7	1.8
		Perilla frutescens	Perilla	544	17	44.1	3.2	51.7	3.7
	Root and tubers	*Colocasia esculenta*	Cocoyam	112	1.5	85.36	4.1	2	2.27
		Ipomoea batatas	Sweet potato	86	1.6	20.12	3	4.7	1.37
		Solanum tuberosum	Potato	95	2.63	21.4	2.3	0.13	1.3
		Manihot esculenta	Cassava	160	1.36	38.1	1.8	0.28	2.3
	Vegetables and pulses	*Amaranthus dubias*	Amaranth	49	4	46	2.87	0.2	2.3
		Brassica oleracea	Brassica	21	9	6	1	1	2.4
		Lablab purpureus	Hyacinth beans	344	23.9	60.74	25.6	1.69	0.7
		Vicia faba	Broad beans	341	26.12	58.59	25.0	1.53	1.7
		Parkia roxburghii	Tree bean	426	18.8	39.74	9.56	15.5	4.1
		Vigna umbellate	Rice beans	348	20.9	60.7	4.0	0.9	2.4
		Moringa oleifera	Moringa leaf	92	6.7	13.4	1.7	1.7	0.9
		Moringa oleifera	Moringa pod	26	2.5	3.7	0.1	0.1	4.8
		Vigna angularis	Adzuki beans	412	20	6.0	13.0	0.5	1.4
		Psophocarpus tetragonolobus	Winged beans	183	40	45	7	20	2.1
		Canvalia ensiformis	Jack beans	241	30	54.28	9.9	7.1	1.9
		Coccinia Grandis	Ivy guard	21	15	12.62	3.4	4	0.8
		Nelumbo nucifera	Indian lotus	350	15	65	1.9	2	1.5
		Sechium edule	Chow	19	0.82	4.51	1.7	0.13	0.9
		Citrullus lanatus	Watermelon	296	3.5	8	3.8	0.4	3.8
		Lagenaria siceraria	Bottle guard	14	0.62	3.7	0.5	0.02	0.5
		Solanum dulcamara	Nightshade	55	3	74	2.42	0.6	2.24
		Corchorus olitorius	Jews mallow	392	20.9	12.2	45.61	5.2	0.16
		Cicer arietinum	Chickpea	1,201	17.1	60.9	3.9	5.11	1
		Phaseolus vulgaris	Kidney beans	1,245	22.9	60.6	4.8	1.77	0.6
		Lens culinaris	Lentils	1,349	25.1	59	0.7	0.75	0.8
		Glycine max	Soybeans	1,597	43.2	20.9	3.7	19.42	1.2
		Vigna radiate	Green gram	1,363	24	56.7	4.1	1.14	0.9
		Pisum sativum	Pea	1,269	72	15.9	4	1.89	1
	Fruits and Nuts	*Phoenix dactylifera*	Dates	301	5.1	62.2	8.4	9	0.7
		Annona squamosal	Annona	113.65	1.25	18.65	21.62	3.78	0.6
		Passiflora edulis	Passion fruit	97	2.2	23.38	10.40	0.7	0.5
		Carica papaya	Papaya	32	0.6	7.2	2.6	0.1	0.2
		Artocarpus heterophyllus	Jackfruit	95	1.72	23.25	1.5	0.64	2.1

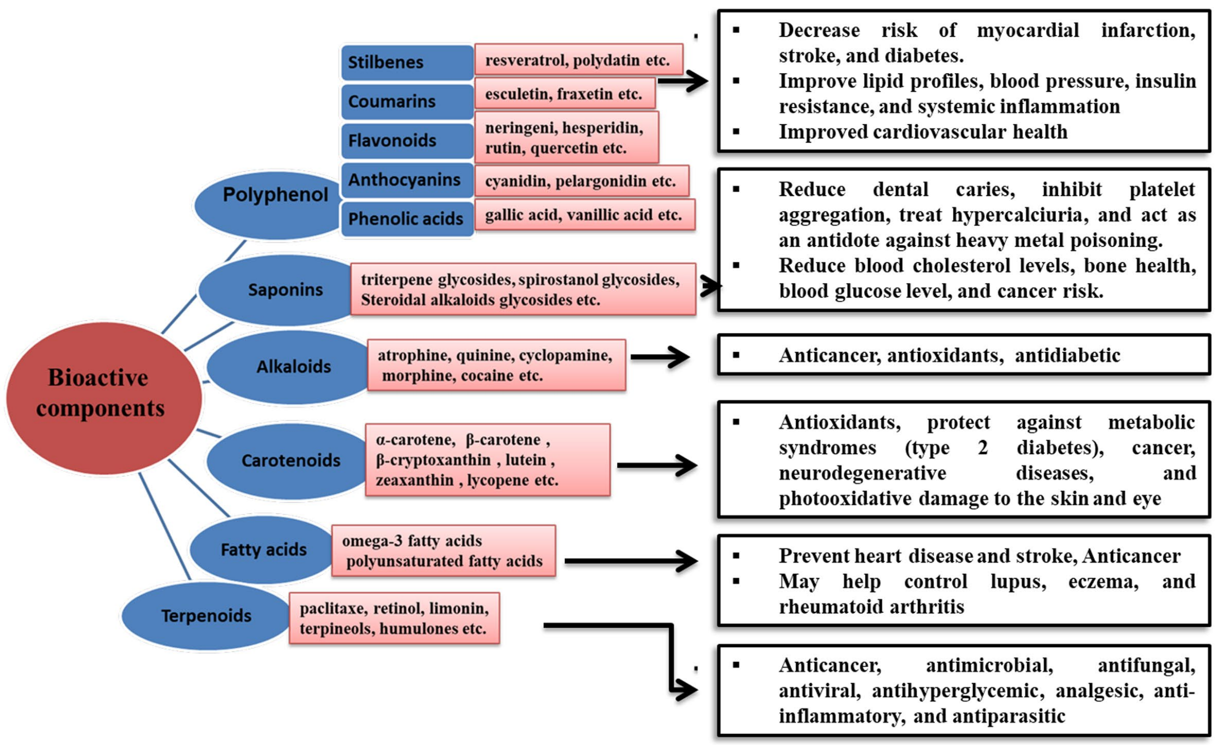

FIGURE 2
Schematic representation of bioactive components found in NUCS and their health benefits.

been anticipated to occur in temperate and tropical locations with every 2°C increase, as depicted by a meta-analysis of 1,700 published models (62). Similar climate modeling studies forecast a 6% drop in wheat yield, which translates to a potential 42 Mt./°C drop (63). Based on simulations using the regional calibrated crop model, Lu et al. also detected a reduction of 10–11% in rice production over the course of the past 50 years, provided that the crop's sowing dates remained constant (64). In contrast, NUCS such as millets, buckwheat, bambara groundnut, and cowpea can adapt to extreme weather (heat and drought stress) with fewer nutrient requirements (65), while cultivation of cereal crops as compared to underutilized crops requires a large agricultural input and contributes significantly to GHG emissions, which furthers global warming (66). Conversely, global warming has a number of negative repercussions on the viability of economies, ecosystems, and agriculture. Underutilized crops usually require less water and thus have high water-use efficiencies compared to staple crops (67). Additionally, they can be cultivated on severely degraded marginal land that is no longer appropriate for high-input commercial crops (67). In India, local finger millet genotypes such as Kurkuti, Lala, Ladu, Jhana, and Taya were shown to outperform other genotypes in terms of their photosynthetic capacity, water-use efficiency, and carboxylation efficiency (68). Pearl millet (*Pennisetum glaucum*) and foxtail millet (*Setaria italica*), mainly cultivated in African and Asian countries, are known for their high salinity and drought tolerance capacities (69), yet they have not been widely adopted due to their low yields and lack of local plant species. Pseudocereals such as buckwheat (*Fagopyrum esculentum* and *Fagopyrum tataricum,* quinoa (*Chenopodium quinoa*), and amaranth (*Amaranthus hypochondriacus*) have been reported to thrive in nutrient-poor soils and to be resilient to various biotic and abiotic stresses (70). Owing to their drought tolerance and ability to thrive in impoverished soil, many countries have started producing cassava (*Manihot esculentum*) and sweet potato (*Ipomoea batata*) as major food sources (71). Hyacinth bean (*Lablab purpureus* L.), Egyptian kidney bean, Indian butter bean, and lablab bean are extremely resilient to drought-prone areas, making them an efficient alternative for protein security (45). Underutilized crops showing different degrees of biotic and abiotic stress tolerance (72, 73) are listed in Supplementary Table 3.

Anti-nutritional factors in NUCS and abatement strategies

Anti-nutritional factors (ANFs) refer to the phytochemicals that can bind with different nutrients to impede their digestion, absorption, or utilization. If taken in large quantities, these substances can be harmful to human health. Apart from several pharmacognostic properties, some phenolic compounds have been reported to possess anti-nutritional effects on protein metabolism due to their ability to bind to digestion enzymes and protein substrates. ANFs can be broadly categorized into four groups: (1) compounds that affect protein utilization and digestion, such as tannins, lectins, and protease inhibitors; (2) compounds that affect mineral utilization, such as gossypol, phytates, and glucosinolates; (3) antivitamins; and (4) other compounds, such as mycotoxins, cyanogens, alkaloids, mimosine, and saponin. The major ANFs found in some of the selected NUCS are listed in Table 3.

TABLE 2 Bioactive components found in neglected and underutilized crop species (NUCS).

Scientific name	Common name	Bioactive components	References
Eleucine coracana, Setaria italic,Pennisetum glaucum	Finger millet, Foxtail millet,Pearl millet	Phenolic acids, anthocyanins, tannins, pinacosanols, catechin, epicatechin, quercetin, apigenin, hydroxybenzoic acid, protocatechuic acids, p-hydroxybenzoic acids, syringic acids, ferulic acid, and hydroxycinnamic acid	(35–37)
Fagopyrum esculentum	Buckwheat	Syringetin, dihydromyricetin, kaempferol, kaempferide, isorhamnetin, myricetin, quercetin, kaempferol, kumatakenin, fustin, laricitrin, morin, syringetin, isorhamnetin, afzelechin, hesperitin, naringenin, orientin, vitexin, homoorientin, and isovitexin	(38)
Amaranthus caudatus	Amaranth	Vanillic, 4-hydroxybenzoic, 4-syringic, caffeoylisocitric acids, rutin, isoquercitrin, α-tocopherol, β-tocotrienol, γ-tocotrienol, and δ-tocopherol	(39)
Chenopodium quinoa	Quinoa	Carotenoids (lutein, zeaxanthin, beta-carotene, and lutein) and phenolic acids	(39)
Perilla frutescence	Perilla	Rosmarinic acid, caffeic acid, and anthocyanins	(40)
Colocasia esculenta	Cocoyam	Alkaloids, tannins, flavonoids, saponins, polyphenols (flavonoids and phenolic acids), anthraquinones, and dioscorin and diosgenin	(41)
Ipomoea batatas	Sweet potato	Vitamins, amino acids and minerals, tocopherol, and beta-carotene	(42)
Solanum tuberosum	Potato	Polyphenols, anthocyanins, flavonoids, carotenoids, tocopherols, and vitamin C	(43)
Manihot esculenta	Cassava	Flavonoids, β-carotene, saponins, tannins, sitosterol, and stigmasterol	(44)
Lablab purpureus	Hyacinth beans	Phenols, steroids, essential oils, alkaloids, tannins, flavonoids, saponins, coumarins, terpenoids, glycosides, and anthocyanin	(45)
Vicia faba	Broad beans	Flavonoids, lignans, and terpenoids; protocatechuic acid, ferulic acid, vanillic acid, caffeic acid, sinapic acid, salvianolic acid, *cis*- and *trans*-*p*-coumaric acid, hydroxyeucomic acid, eucomic acid, caffeoylquinic acid, and dicaffeoylquinic acid	(46)
Macrotyloma uniflonum	Horse gram	Alkaloids, flavonoids, saponins, phenols, glycosides, tannins, terpenoids, quinones, mome inositol, hexadecanoic acid, methyl ester, octadecanoic acid, and gamma tocopherol (46)	(47)
Mucuna pruriens	Velvet beans	L-3,4-dihydroxyphenylalanine, lectin, isoflavanones, and some alkaloids, phenol, flavonoid, proanthocyanidin, and rutin	(48)
Moringa oleifera	Moringa leaf	Vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins	(49)
Fabaceae	Legumes	Polyphenols, alkaloids, saponins, carotenoids, terpenoids, omega-3 fatty acids, flavonoids, and anthocyanins	(50)
Coccinia Grandis	Ivy guard	Lupeol, β-sitosterol, β-amyrin, coccinioside-k, stigmast-7-en-3-one, flavonoid glycoside, phenol, benzofuranone, hexadecanoic acid methyl ester, β-sitosterol acetate, tocopherol, stigmasterol, ethisteron, and campesterol	(50)
Nelumbo nucifera	Indian lotus	Alkaloids, flavonoids, glycosides, triterpenoid, and vitamins	(51)
Sechium edule	Chow	C-glycosyl, O-glycosyl flavones, lutein, and β-carotene, tocopherol, myricetin, ferulic acid, chlorogenic acid, and (+)-catechin	(52)
Solanum dulcamara	Nightshade	Sugars, vitamin C, vitamin E, polyphenols, and flavonoids	(53)
Lens culinaris	Lentils	trypsin/protease inhibitors, lectins, defensins, polyphenols, flavonoids, triterpenoids, saponins, phytates, and phytosterols	(54)
Glycine max	Soybeans	Isoflavones, saponins, phytic acids, phytosterols, trypsin inhibitors, and peptides.	(54)
Phoenix dactylifera	Dates	Protocatechuic, gallic, caffeic, p-hydroxybenzoic, vanillic, ferulic, syringic, p-coumaric, o-coumaric acid, carotenoids, and flavonoids	(55)
Annona squamosal	Annona	Sodium benzoate, 4, 4-tert-butylcalix(4)arene, 4, 4-dimethylcholesterol, butyl octyl phthalate, stigmasterol acetate, and isoamyl acetate	(56)
Passiflora edulis	Passion fruit	C-glycosyl flavonoids vicenin, orientin, isoorientin, and vitexin	(57)
Carica papaya	Papaya	Alkaloids, flavonoids, polyphenols, and fatty acids	(58)
Artocarpus heterophyllus	Jackfruit	Phenolics, flavonoids that comprise prenylflavonoids, hydroxycinnamic acids, and glycosides, stilbenoids, triterpenoids, and steroids	(59, 60)

TABLE 3 Anti-Nutritional factors found in NUCS.

Scientific names	Common names	Anti-Nutritional factors	References
Amaranthus dubias	Amaranth	Betacyanins, chlorogenic acid, and caffeoyl iso citric acid	(74)
Fagopyrum esculentum	Buckwheat	Trypsin inhibitors, phytic acid, and tannins	(75)
Chenopodium quinoa	Quinoa	Saponins, phytic acid, oxalates, tannins, and trypsin inhibitors	(76)
Eleucine coracana	Finger millets	Tannic acid and phytic acid	(77)
Pennisetum glaucum	Pearl millet	Tannic acid and phytic acid	(78)
Phaseolus spp.	Pulses	Saponins, glycosides, tannins, alkaloids, phytic acid conjugates, and lectins	(79)
Vicia faba	Broad beans	Vicine and convicine	(80)
Pisum sativum	Peas	Lectin, tannins, and oligosaccharides	(81)
Glycine max	Soybean	Glycinin, lectin, phytic acid, and oligosaccharides	
Manihot esculenta	Cassava	Cyanoglucosides	(82)
Solanum tuberosum	Potato	Polanines and cyanogens	
Dioscorea	Yam	Dioscorine	
Phaseolus vulgaris	French beans	Pisatin and phaseottin	
Colocasia esculenta	Taro	Oxalate and oxalic acid	
Fabacea	Legumes	Antitrypsin factors, trypsin inhibitor, tannins, saponins, amylase inhibitors, protease inhibitors, phytic acids, and lectins	
Perilla frutescens	Perilla	Tannic acid and phytic acid	(83)
Solanum dulcamara	Nightshade	Oxalate, phytate, nitrate, and alkaloids	(84)
Annona muricata	Annona	Phytate and oxalate	(85)
Artocarpus heterophyllus	Raw Jackfruit	Tannins, phytate, oxalate, and trypsin inhibitor	(86)
Phoenix dactylifera	Dates	Oxalate and tannin	(87)

Although several NUCS-based food products have already been developed, an appropriate processing technology is still mandated to eliminate ANFs such as lectins, α-amylase inhibitor (αAI), and arcelins (Arc) (88). Heat treatment, however, improves their hydrolysis. Recently, an enzyme called VC1 has been characterized that converts GTP to vicine and convicine. Silencing of the VC1 gene or gene editing may be used to develop an anti-nutrient-free faba bean variety (89). The main strategy to remove the anti-nutrient factors could be either by targeting upstream genes or inhibiting the biosynthesis of particular anti-nutrients via different biotechnological routes. The ultrafiltration technique can also be used to remove and separate ANFs into different fractions; however, it does not inactivate ANFs. Considering the feasibility of the technological tools and economic conditions, the soaking of grains followed by heating is an effective strategy to boost protein digestibility and good sensory qualities. While conventional breeding techniques take several years to express desired traits, genome engineering techniques such as CRISPR/Cas9 can be employed to the targeted deletion of anti-nutritional factors of biosynthesis genes and to develop anti-nutrient-free cultivars in lesser time (90).

Constraints of NUCS: field to food basket

The domestication and cultivation of most neglected and underutilized crops are restricted to their native locations, and so cultivation strategies for large-scale production face many challenges linked to cultivation technology, infrastructure, and market linkage. The poor communication between the scientific community and governments and local farmers is another challenge for the proper utilization and implementation of knowledge. Some of the important constrains of NUCS are listed below:

1. Lack of knowledge and information on the nutritional value, consumption, and utilization of many underutilized plant products that are unpopular when compared to major staple crops.
2. Not enough people are aware of the financial advantages and market opportunities.
3. By using food processing at the village level, standard technology adds value.
4. No appropriate, higher-quality planting material is used, and no breeding or biotechnology efforts are made to shorten gestation times and increase fruit production.
5. The researchers, agriculturalists, and extension personnel showing less interest.
6. Poor producer interest and yield.
7. Losses during post-harvest and transportation.
8. For underutilized fruits, there is no infrastructure or marketing network.
9. The nation lacks proper credit, investment, and policy.
10. Insufficient scientific resources are available for evaluating, testing, and post-harvest management of various underutilized fruits.

Way forward to overcome challenges of NUCS

Several distinct approaches can be suggested as prospective solutions for the partial or complete overcoming of the major constraints of NUCS. Some of them are highlighted below:

1. Well-organized exploration programs and ecogeographic surveys must be carried out in order to create a database on the origin, distribution, habitat, agroclimatic requirements, advantages, and scientific application of potential underutilized crops.
2. In order to maximize the potential of underutilized crops, more focus must be given to developing suitable plant types with traits such as early emergence, photo insensitivity, high harvest index, lodging and shattering resistance, and determinate, bushy growth habit. Short-duration cultivars must be improved to work effectively in current farming systems and to thrive in unconventional seasons and locations.
3. Effective agronomic management is needed to incorporate underutilized crops into current agricultural systems. To ascertain the bundle of agricultural practices relating to sowing time and manner, seed quality, plant density and arrangement, irrigation, fertilization, and harvesting in various crops, well-prepared experiments are required.
4. More emphasis needs to be placed on in-depth research on nutritional quality, nutraceutical qualities, and anti-nutritional elements. Processing, value addition, product creation, and effective marketing strategies also require more attention.
5. To keep scientists, extension agents, and farmers informed of the most recent technological advancements pertaining to certain crops, training programs need to be held on a regular basis.
6. To create an efficient value chain to encourage the use of these potentially underutilized crops, close ties between growers, traders, processors, consumers, and other formal and informal sectors must be developed.
7. Priority should be given to adopting policies that will mainstream the use of neglected and underutilized crop species in food systems.
8. In order to give the essential impetus to research and development activities on underutilized crops, non-governmental organizations (NGOs) should be involved at the relevant levels.

Global/national efforts to improve NUCS productivity to fight global hidden hunger

While the majority of organizations and research and development NGOs and INGOs have primarily focused on the mandate cereal crops, very few initiatives have focused on NUCS. Recently, emphasis has been laid on the underutilized crops that have enormous potential, a range of nutrient statuses, and that require very little effort to incorporate into sustainable agriculture systems. NUCS have been considered under several projects by the SDGs, International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), biodiversity conservation plans, and the UN for sustainability issues, etc. These underutilized crops are now improving the sustainability of marginal local communities, as seen in India through the IFAD's kodo and millet program, in the Andes through its grain-centric program, in Mali by the bambara groundnut and fonio program, and in Guatemala through the tepary bean and Mayan spinach project (91). LI-BIRD, SAHAS, Helvetas, FAO, NARC, and Bioversity International have been working on a few of the neglected and underutilized crops such as amaranth, finger millet, buckwheat, beans, proso millet, foxtail millet, barley, yam, and turnip (92). The last decade was marked by a remarkable increase in support for NUCS from overseas development agencies (ODA), the IDRC, the Asian Development Bank, the European Commission, and several other donors joined by Germany and the United Kingdom in financing *ad hoc* projects and networks dealing with NUCS. Some of the important networks include BAMNET (International Bambara Groundnut Network), MEDUSA (Network on the Identification, Conservation and Use of Wild Plants in the Mediterranean Region), PROSEA (Plant Resources of South East Asia), UTFANET (Underutilized Tropical Fruit in Asia Network), and SEANUC (Southern and East Africa Network on Underutilized Crops) (93). The United States Department of Agriculture (94) has also implemented a project on climate-resilient orphan crops for increased diversity in agriculture. The project aims at reinforcing agro-biodiversity in distinct socio-economic and geographic locations with three major objectives: the promotion of six important underutilized arable crops, namely, oats, hull-less barley, triticale, buckwheat, faba bean, and lupin; the development of value chains for particular underutilized crops; and the analysis of the project result's socioeconomic effects. This concept is a cutting-edge, problem-driven strategy built on the promotion of underutilized crops in eco-sustainable cropping systems and locally sourced value chains (Figure 3).

In the current scenario, the world is facing three major intertwined challenges, *viz.*, climate change, food security, and sustainability. As discussed above, owing to their nutrient-dense and climate-resilient properties, expanding the use of these crops would boost agricultural biodiversity (in respect to genes, species, and ecosystems) to protect crops from climate change, pests, and diseases and would provide a wide range of high-quality food sources for ensuring food and nutritional security. For a household to be considered food secure, its members must always have access to adequate food to lead active and healthy lives. At a minimum, food security entails the immediate availability of nutrient-dense and safe foods and the assurance of being able to obtain appropriate foods in socially acceptable ways. While the conceptual framework of nutrition security strives to better comprehend whether dietary-related diseases and inequities coexist with food scarcity—specifically in the cases of people who belong to members of racial or ethnic minorities, those with lower incomes, and those who live in rural or distant areas—nutrition security involves having consistent access to, availability of, and affordability of foods and beverages that promote health and prevent disease. A total of 50% of the world's plant-based calorie intake supplied from only three crops (i.e., wheat, rice, and maize), the production of which covers around 40% of arable land. However, these crops alone cannot provide sufficient nutrients, and therefore, a diverse diet is required that can be accessible by the poorest. This is why it is time to look into some of the potential food crops (NUCS) that are thought to exist globally. The food system as we currently know must undergo a drastic revolution. In particular,

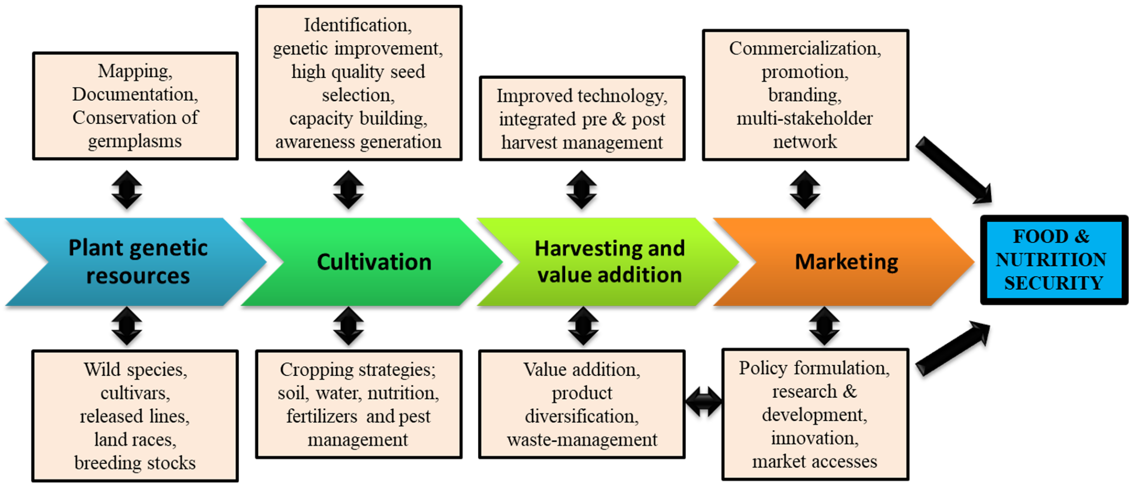

FIGURE 3
Value chain framework of NUCS.

underutilized plant-based foods can be excellent sources of macro and micronutrients; legumes abundant in protein, dietary fiber, and trace elements are only a few notable examples. Improvising in the agricultural extension of these crops would enhance the economic growth of countries and present a step forward to eradicate poverty. Under the SDGs, there are 17 aspirational "Global Goals" and 169 targets. Three of these objectives are specifically related to agriculture. These are "Climate Action," "Zero Hunger," and "No Poverty." Therefore, each country must focus on the three key goals of food, nutrition, and healthcare. The debate surrounding underutilized crops frequently centers on how well suited these crops are to places of sparse output, with a special focus on such crops in developing countries. In this regard, it is claimed that if promoted in certain regions, they may have a favorable effect on food production. As a result, household earnings, food security, and nutrition would all increase. Considering this, it becomes apparent that underutilized crops may be able to serve certain SDGs. Therefore, acceptance and enhancing the agro-diversification of these food crops (NUCS) could be one of the answers to achieve zero hunger and fulfill the SDGs more quickly.

Concluding remark and policy implications

Regardless of agricultural and technological advances, malnutrition, hunger, and food insecurity remain serious issues in the present world. Limited dietary diversity and persistent malnutrition are mostly caused by an overreliance on basic crops. Although NUCS have the potential to fight hidden hunger and malnutrition, underutilized crops are still ignored, specifically NUCS. Future smart food crops have four distinct advantages over staple crops that could help the world to achieve zero hunger: nutrition, climatic resilience, local relevance, and economic feasibility. Different studies on the nutraceutical and pharmacognostic properties of NUCS have revealed presence of good proteins, dietary fibers, minerals, polyphenols, vitamins, and other bioactive compounds. The bioactive compounds in NUCS have the potential to combat chronic ailments such as cancer, neurological disorders, cardiovascular diseases, hypertension, or diabetes (15, 16, 95, 96). Despite their several nutrition-rich qualities, improved cultivation strategies for the mass production of these crops are still underdeveloped. Meanwhile, the scientific knowledge acquired from different research fields mostly remains within the scientific community. Therefore, an effective network system between farmers, researchers, entrepreneurs, and governments is very crucial. Additionally, the proper execution of NUCS-based INGOs/NGOs and government policies still requires focus. Some of the important global/national events that have fostered NUCS are listed in Supplementary Table 4.

It is evident that most agricultural and food policies are based on a limited number of staple crops. Therefore, it is recommended that every country should have a particular policy to ensure the promotion and usage of underutilized crops. Although the National Department of Agriculture, Forestry and Fisheries (DAFF) Strategic Plan for 2016–2020, the National Policy on Food and Nutrition Security (97), and the National Plan on Integrated Growth and Development Planning (IGDP-2010) are all in sync with the strategy for promoting NUCS, the challenge of the proper execution of these policies with reference to NUCS has remained ambiguous. Therefore, policies that facilitate the mainstreaming of the use of underutilized crops in food systems require special attention as follows:

1. Given their excellent nutritional value, it is important to encourage children to eat NUCS-based food products, especially as part of their midday meals.
2. Farmers should be granted the proper subsidies to encourage them to grow underutilized crops. Reducing the price of high-quality seed is necessary, and the initial procurement of their output must be guaranteed.
3. To promote the spread of an already well-known but underutilized crop, local political and administrative support must be built. In order to cultivate these crops, governing bodies must be persuaded through appropriate discussion. By giving those rewards and subsidies, farmers should be encouraged to plant the underutilized crops.
4. In order to give the essential impetus to research and development activities on NUCS, non-governmental organizations (NGOs) should be involved at the relevant levels.

5. To strengthen research and development programs on NUCS, adequate resources, both financial and manpower, must be allocated.
6. The free exchange policies of climate-resilient germplasms within or between nations should be emphasized.

To achieve these goals, policy packages should be designed in a coherent manner, each with a clear objective and a set of targets that emphasize accountability for execution. In India, a notable approach taken in Chhattisgarh state, under the Pradhan Mantri Poshan Shakti Yojana, is the adoption of millet-based foods as a midday meal for school children. The implementation of this policy is needed in other states as well. The Ministry of Agriculture and Farmers Welfare, India, under different central schemes such as ATMA, AGMARKNET, Pradhanmantri Krishi Sinchayee Yojana, mKisan, Pradhan Mantri Fasal Bima Yojana, and Kisan Call Center, has targeted the improvement of crops and agro-diversification of nutraceutical crops. The inclusion of NUCS under these schemes for mass cultivation would improve the nutrition security of the country. MOVCD-NER (Mission Organic Value Chain Development for North East Region) is another central sector scheme under the National Mission for Sustainable Agriculture (NMSA), launched by the Ministry of Agriculture and Farmers Welfare for implementation in the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura. This program aims to support the growth of the entire value chain, from inputs and distribution of seeds to the certification and establishment of facilities for collection, aggregation, processing, marketing, and branding. It also aims to develop certified organic production in a value chain to connect farmers and consumers. Currently, the center is working on an improved roadmap to promote the cultivation of crops, particularly okra, gourd, ginger, pineapple, and red rice. Similarly in Africa, the National Food and Nutrition Security Policy 2014 (97), which aims to ensure food availability, accessibility, and affordability, could be achieved by integrating NUCS, which are locally available, nutritious, and the cheapest source of macro and micronutrients. Incorporating NUCS into the policies of the 2014–2019 National Climate Change and Health Adaptation Plan has proven helpful in maintaining socioeconomic and environmental resilience due to their sustainability and health benefits.

In conclusion, since most of the available research projects on NUCS are typically dispersed and difficult to assemble in one research article, this article attempts to address the background information on the proper identification of NUCS and their nutrient content, nutraceutical properties, and major constraints. Furthermore, we enumerated potential strategies to overcome these challenges and global/national policies for improvising NUCS to eradicate global hidden hunger.

Author contributions

AA and BB conceived the concept of the manuscript. AA wrote the manuscript. BB supervised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1144439/full#supplementary-material

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Evaluation of millets for physio-chemical and root morphological traits suitable for resilient farming and nutritional security in Eastern Himalayas

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Introduction: Millets are nutritionally superior and climate-resilient short-duration crops and hold a prominent place in cropping sequences around the world. They have immense potential to grow in a marginal environment due to diverse adaptive mechanisms.

Methods: An experiment was conducted in an organic production system in the North Eastern Himalayan foothills of India for 3 consecutive years by evaluating high-yielding varieties (HYVs) of different millets, viz., finger millet, foxtail millet, little millet, barnyard millet, proso millet, and browntop millet, along with local landraces of finger millets (*Sikkim-1* and *Sikkim-2; Nagaland-1* and *Nagaland-2*) to identify stable, high-yielding, and nutritionally superior genotypes suited for the region.

Results: Among the various millets, finger millet, followed by little millet and foxtail millet, proved their superiority in terms of productivity (ranging between 1.16 and 1.43 Mg ha^{-1}) compared to other millets. Among different varieties of finger millets, cv. VL Mandua 352 recorded the highest average grain yield (1.43 Mg ha^{-1}) followed by local landraces**,** *Nagaland-2* (1.31 Mg ha^{-1}) and *Sikkim-1* (1.25 Mg ha^{-1}). Root traits such as total root length, root volume, average diameter of roots, and root surface area were significantly higher in finger millet landraces *Nagaland-1*, *Nagaland-2*, and *Sikkim-1* compared to the rest of the millet genotypes. The different millets were found to be rich sources of protein as recorded in foxtail millet cv. SiA 3088 (12.3%), proso millet cv. TNAU 145 (11.5%), and finger millet landraces, *Sikkim-1* and *Nagaland-2* (8.7% each). Finger millet landrace *Sikkim-2* recorded the highest omega-6 content (1.16%), followed by barnyard millet cv. VL 207 (1.09%). Barnyard millet cv. VL 207 recorded the highest polyunsaturated fatty acid (PUFA) content (1.23%), followed by foxtail millet cv. SiA 3088 (1.09%). The local finger millet landraces *Sikkim-1* and *Sikkim-2* recorded the highest levels of histidine (0.41%) and tryptophan (0.12%), respectively. *Sikkim-1* and *Nagaland-2* recorded the highest level of thiamine (0.32%) compared to the HYVs.

Conclusion: These findings indicate that finger millet has great potential in the organic production system of the North Eastern Himalayan Region (NEHR) of India, and apart from HYVs like VL Mandua 352, local landraces, *viz.*, *Nagaland-2 and Sikkim-1*, should also be promoted for ensuring food and nutritional security in this fragile ecosystem.

KEYWORDS

millets, physio-chemical traits, root architecture, nutritional security, destabilized soil ecosystem

1. Introduction

The rising concern for food and nutritional security and environmental sustainability is creating tremendous pressure on mankind for judicious resource allocation and conservation (1). Soil degradation, malnourished human population, and poverty are some of the major concerns of the twenty first century (2). These global issues must be addressed by shifting toward lower energy and more resilient intensive cultivation practices (3, 4). For maintaining proper human health and physical wellbeing, the nutritional quality of food is the most important factor (5). Millets have many nutritional and health benefits (6) and are considered a superfood (7, 8). Most millets are extraordinarily superior to other cereals, such as rice and wheat. Millets are climate-resilient and sustainable crops that can be grown with a minimum amount of input (9, 10). Small and marginal farmers of the North Eastern Himalayan Region (NEHR), India, are facing a variety of problems that will intensify in the era of climate change (3, 11). Millets are small-seeded cereal crops belonging to the family *Poaceae* and are considered the world's sixth most important cereal grain crop, feeding more than one-third of the world's population (12, 13). They are pre-green-revolution crops cultivated traditionally by many generations (9). Millets are considered to be ancient grains of mankind that can grow from coastal regions of Andhra Pradesh to moderately high altitudes in the Himalayan Region, namely, the states of Uttarakhand and NEHR of India, which is indicative of their wide capacity for adaptation (14). Cultivated and consumed in over 50 countries around the globe including India, millet is central to the cultural ethos of indigenous communities in the Eastern Himalayas and other parts of India (15). Grown as dual-purpose crops (food and fodder), millets occupy an integral part of subsistence agriculture by providing food and livelihood security to millions of inhabitants including small and marginal farmers of remote rain-fed/hilly areas of the NEHR, India (16). Millets are short-duration, non-exhaustive crops that can be grown with minimum input requirements (17, 18) and thus fit well in organic farming (18). Generally grown as rain-fed crops, they require very low amounts of water to complete their life cycle (19, 20). India is the largest producer of millets in the world with a share of 41%, followed by African countries like Niger (11%) and Nigeria (7%) (21). They contain a high amount of carbohydrates (60–70%), dietary fibers (10–12%), protein (6–9%), fat (1.5–5%), and a considerable amount of minerals (2–4%) (22). They stand out from other cereals because of the high calcium and polyphenol content in the grains (23). Millets are great for boosting the nervous system (8, 17). Consumption of millets controls blood sugar levels and cholesterol and enhances the immune system (10). They can be consumed by people having type 2 diabetes and are good for heart ailments.

At the moment, the fragile and marginal ecosystem in the NEHR of India is one of the most significant factors that act as a barrier to optimum crop production (18). Among the different millets, minor millets such as finger millet, followed by foxtail millet and barnyard millet, are the major and most versatile millets in the NEHR of India (18). Owing to their high nutritional content (18, 22), good yield potential, availability of seed, storage, and utilization technology, millets could significantly contribute to the food and nutritional security of the region's inhabitants (24). In northeast India, millets have been an integral part of the farming system for a long time, and local cultivars are grown in the *Jhum* fields along with other crops such as paddy (25). They are not commonly grown as commercial crops but are mainly cultivated by tribal farmers as a part of subsistence farming (14, 26). Small millets especially foxtail millet, finger millet, and barnyard millet are confined to the NEHR of India in Nagaland (Phek, Tuensang, and Kiphire districts) (27), Meghalaya (Khasi Hills and West Garo hills), Manipur (Churachandpur and Senapati districts), and hilly areas of Arunachal Pradesh, Sikkim (28) and Mizoram (Mara tribe) (14). In Manipur, ethnic tribes such as the Thadou Kuki and Paite tribes cultivate millets in *Jhum* agriculture and traditionally make cake from millets as an offering to their ancestors. Raishan (*Digitaria cruciata* var. *esculenta* Bor) is an indigenous cold-tolerant millet crop, endemic to the Khasi hills of Meghalaya, that is cultivated for both food and fodder (24, 29). "Tsiinyi" or millet festival celebrated by the Angami Naga tribe of Nagaland signifies the importance of millets in their traditions. Tribal people from some areas of Sikkim prepare "*kodoko Jaanr*" from seeds of finger millet (30). The cultural utility of minor millets such as proso millets is high in Arunachal Pradesh. They are cultivated in *jhum* fields and provide various indigenous food items for use in traditional ceremonies and occasions. Brown top millet and little millet are concentrated in specific parts of the NEHR and are grown in hilly terrain (31). The elderly tribal population of the NEHR grew up having them as "staple food." Although millets were known as the poor man's food, increased consumer awareness and the high market price of millets in recent times have opened a new avenue to grow them as cash crops even in hills (16). Low-productive cereal and mixed agricultural cropping patterns are prevalent in the NEHR hills, particularly on sloppy and *jhum* terrain of India (11). Low-input, resource-efficient crops, such as suitable millets (nutri-cereals), are gaining popularity as prospective solutions for assuring food and nutritional security. Most of the farmers in the region do not use any synthetic fertilizer or pesticides, and hence, they are organic by default (5, 32, 33). The NEHR generates 2.55 million tons of agricultural biomass and has 2.98 million bovines, encouraging organic crop production through recycling for valuable nutrient management inputs (5). In the past few decades, there has been a rising emphasis on using organic production systems to increase soil quality, crop productivity, and nutritional security vis-a-vis maintaining environmental quality.

Identification of suitable and resource-efficient millets with better adaptive mechanisms (better root architecture with enriched biochemical properties) for the marginal and destabilized ecosystem is of the utmost importance to integrate suitable millet into the cropping systems. Small millets have a high production potential under ideal conditions, and millets have a diverse set of adaptation mechanisms that allow them to grow and survive in environments that are relatively marginal and destabilized. The relevance of root design for water and nutrient uptake has been extensively documented in both monocots and dicots, and it could be employed effectively for trait-specific genetic enhancement of roots. Millets have fibrous root systems in which distinct root types contribute toward improving resource use efficiency (water, nutrients etc.) (34). Significantly large variations in root properties were identified for minor millets including local germplasms of finger millets from Himalayan foothills, indicating a potential capacity to incorporate minor millets on a large scale in this fragile ecosystem. There is also a need to identify nutritionally superior millets suitable for these areas.

Through proper awareness programs and focused research, millets can be popularized as a potential cash crop with organic certification in this ecosystem of the NEHR of India and similar other agroecological regions of the world. Both the nutritionally rich local landraces and high yielding varieties (HYVs) of millets must be selected, conserved, and promoted for cultivation by farmers. Furthermore, this highly nutritious grain crop is mostly limited to particular patches and should be promoted to the majority of the population residing in the region. Keeping these points in view, the study "Evaluation of millets for physio-chemical and root morphological traits suitable for resilient farming and nutritional security in Eastern Himalayas" was conducted.

2. Materials and methods

2.1. Experimental site and treatment details

An experiment was undertaken with different varieties/landraces of finger millet, foxtail millet, little millet, brown top millet, proso millet, and barnyard millet for evaluating their suitability under the organic production system in the NEHR of India (Table 1). Altogether, 13 different germplasms of millets were evaluated for 3 consecutive years in the *kharif* seasons of 2018–2020 in organic upland terraces of the Agronomy field, ICAR Research Complex for the NEHR of Umiam, Meghalaya (25°30'N latitude and 91°51'E longitude) situated at 980 m ASL. Apart from HYVs of different minor millets, the local landraces of finger millets from the NEHR of India (*viz., Nagaland-1*, *Nagaland-2*, *Sikkim-1*, and *Sikkim-2*) were also collected and evaluated. The temperature in this region is moderate throughout most of the year except for the few months of winter. The maximum temperature ranges from 26 to 29 °C from March to October. In the winter, the minimum temperature rarely goes below 5°C. The region receives a good amount of rainfall (∼ 2,400 mm annually), but the majority of it occurs from April to October (Supplementary Figure 1). The maximum relative humidity of the region generally ranges above 80%, while the minimum relative humidity rarely goes below 50%, with mean annual evaporation is approximately 850 mm. A collection of practices used for growing millets under organic conditions in this study's experiment is given in Table 2.

2.2. Physiological observations

2.2.1. Leaf chlorophyll analysis

Chlorophyll index as a reflection of chlorophyll density per unit area was determined using the acetone extraction method (80:20 acetone and water mixture) to reflect different components of leaf chlorophyll in terms of its sub-components, *viz.,* chlorophyll a, b, total chlorophyll, and carotenoids content (35). A known quantity of fresh leaf sample (0.5 g) was grounded and homogenized with 15 ml of 80:20 acetone: water mixture solution. The leaf extract was then filtered through the Whatman No. 42 filter paper after which the volume was made up to 25 ml with the same solvent mixture solution. Later, the required aliquot of the final extract was taken in a cuvette, and absorbance was measured at 648, 652, and 663 nm using a Spectronic-20 UV spectrophotometer to assess chlorophyll "a," chlorophyll "b," and total chlorophyll of each sample in mg g^{-1} of fresh tissue. The required calculations were performed using the following formulae (35, 36). Similarly, leaf carotenoid was also calculated through the same extraction protocol.

$$Chla\,(mg\,g^{-1}) = (12.72 \times A_{663} - 2.58 \times A_{645}) \times (V/W) \times (1/1000) \quad (1)$$

$$Chlb\,(mg\,g^{-1}) = (22.87 \times A_{645} - 4.67 \times A_{663}) \times (V/W) \times (1/1000) \quad (2)$$

$$Chla + b\,(mg\,g^{-1}) = (8.05 \times A_{663} + 20.29 \times A_{645}) \times (V/W) \times (1/1000) \quad (3)$$

$$Carotenoids = (A_{480} + 0.114 \times A_{663} - 0.638 \times A_{645}) \times (V/W) \times (1/1000) \quad (4)$$

where "V" refers to the total volume of the extract, "W" refers to the weight of tissue taken for pigment measurements, and A_{663}, A_{645}, and A_{480} are the optical absorbance values recorded by the Spectronic-20 UV spectrophotometer at 663, 645, and 480 nm, respectively.

TABLE 1 Millets used in the experiment with their common English name and characteristics.

Millet	Common name	Scientific name	Germplasms	Duration (days)	Av. yield (Qha^{-1})	Agronomic characteristics
Foxtail millet	Kangni/Kakun	*Setaria italica*	SiA 3088	70–75	20–25	Short duration, non-lodging, suitable for double cropping
Little millet	Kutki/Shavan	*Panicum sumatrense*	OLM 203	105–110	10–11	Blast and grain smut resistant
Browntop millet	Korale millet	*Urochloa ramose*	Local	95–100	14–18	Nutritionally superior, hardy crop
Barnyard millet	Sanwa	*Echinochloa frumentacea*	VL 207	85–100	16–20	High nutritional value and can withstand biotic and abiotic stresses
Proso millet	Chena/Barri	*Panicum miliaceum*	TNAU 145	70–72	18–20	Superior grain quality for value addition, suitable for limited crop growth period areas because of its short duration
Finger millet	Ragi/Mandua/Nachani	*Eleusine coracana*	VL Mandua 324	105–135	20–25	Blast resistant
			VL Mandua 352	90–100	25–30	High yielding, moderately resistant to blast, and is also used for contingent crop planning
			VL Mandua 172	80–95	13–17	Medium duration
			VL Mandua 347	< 100 days	20–22	Short duration and moderately resistant to blast disease Suitable for higher hills (or areas where crop growth period is limited) because of its short duration
			Sikkim-1	85–95	15–20	Stress tolerant, faster growth, suitable for hilly areas
			Sikkim-2	85–100	12–17	Stress tolerant, faster growth, suitable for hilly areas
			Nagaland−1	90–100	13–16	Pest and disease resistant
			Nagaland−2	90–100	20–25	High yielding

10.3389/fnut.2023.1198023

TABLE 2 Package of practices of millets grown under organic conditions in North Eastern Hill Region (NEHR), India.

Varieties	Finger millet	HYVs like VL Mandua 324, 352, 172, 347, HR 374, local landraces of Sikkim (*Sikkim-1,2*) and Nagaland (*Nagaland-1, 2*)
	Foxtail millet	SiA 3088
	Proso millet	TNAU 145
	Little millet	OLM 203
	Barnyard millet	VL 207
	Browntop millet	Local
Land preparation	Field was prepared by two cross plowings followed by two harrowing and planking. Well-decomposed farm yard manure (FYM) @ 5 t/ha was added and mixed in the soil before sowing.	
Seed rate	8–10 kg seeds per hectare.	
Sowing time	The crop was sown from mid-June to the first week of July.	
Sowing method	Line sowing was followed.	
Spacing	A row-to-row spacing of 25–30 cm and plant-to-plant spacing of 8–10 cm was maintained.	
Nutrient management	7.0–8.0 t/ha FYM, or 5.0 t/ha FYM + 1.0 t/ha vermicompost, 400–500 kg lime, and 150 kg/ha rock phosphate were mixed and applied in furrows before seed sowing.	
Water management	No irrigation was applied, as NEHR gets plenty of rainfall from May to October (more than 1,500 mm).	
Intercultural operations	Thinning and gap filling	Thinning was performed after 2 to 3 weeks of germination to maintain the desired spacing. Gap filling was practiced in case of the death of seedlings.
	Weeding	First-hand weeding was carried out at 25–30 DAS and second weeding using a wheel hoe at 50 DAS.
Insect pest and disease management	Spraying of neem oil 0.03% @ 3 ml/liter of water twice at 10-day intervals for control of pink stem borer and Bihar hairy caterpillar. Disease-free seeds were selected, and proper sanitation practices were followed. Bio-control agents such as *Pseudomonas* spp. and *Trichoderma* spp. were used for seed and soil treatment.	
Harvesting	The crop matured in approximately 70–120 days depending on altitude and variety, and was ready for harvest by the first week of October.	
Yield	Millets have a grain yield potential of 1.30–1.50 t/ha under organic cultivation	

2.2.2. Gas exchange measurements

Photosynthetic parameters, *viz.,* photosynthetic rate (A), stomatal conductance (GH_2O), transpiration rate (E), leaf temperature (°C), and intracellular CO_2 (Ci) were recorded at the flowering stage using a portable infrared gas analyzer (IRGA-GFS Walz-3000 Model, Germany) in the fully expanded (third leaf from top) leaf of randomly selected representative plants. The IRGA mainly consists of a main console that comprises separate detectors for two different apparent hetero-atomic molecules CO_2 and H_2O involved during photosynthesis, an internal air supply unit, a leaf chamber to clamp the leaves, and the necessary software for the computation of gas exchange parameters. It is equipped to measure the light intensity in the PAR range using a point quantum sensor, relative humidity using a thermocouple, and temperature of the air using a thermostat. Butyl rubber tubing is used to carry air from the leaf chamber into the IRGA. Instantaneous water use efficiency was calculated using the formula Pn/E (net photosynthetic rate over leaf transpiration) (37, 38).

2.2.3. Determination of stomatal attributes

As leaves of millets have the amphistomatic type of stomatal distribution in both surfaces (39), stomatal counting was carried out on both the abaxial and adaxial surfaces by smearing with nail polish followed by shade drying (40). The smeared leaf was cut into 2.0–2.5 cm^2 dimensions, and the layer of nail polish impression was gently removed. With the help of forceps, the same was placed on microscopic slides with a few drops of water and covered with a glass cover slip. Stomatal numbers on every sample surface were counted in three different microscopic fields of 10X and 40X magnifying lenses of a compound microscope (BX-50F, Olympus, Japan). Other related observations such as stomatal length, breadth, number of guard cells, and stomata in the particular microscopic field were recorded using user-friendly software.

$$Stomatal\ frequency = Numbers\ of\ stomata\ per\ unit\ area\ (No.\ mm^{-1}) \quad (5)$$

$$Stomatal\ index = \frac{Numbers\ of\ stomata}{Number\ of\ stomata + Number\ of\ epidermal\ cell} \times 100 \quad (6)$$

2.2.4. Root architecture analysis

Plants from different treatments were carefully uprooted without disturbing the intact root system, and the same roots were washed with a smooth flush of water (2, 38). After shade drying for 1 or 2 h, intact turgid roots (full root system) were evenly spread on a transparent fiber tray (30 cm × 20 cm) without overlapping, and the same was scanned for a two-dimensional root image at a resolution of 200 dpi (dots per inch) using an Epson v 700 perfection scanner (Regent Instrument Canada Inc., Quebec, Canada). The resulting images were acquired and processed using the WinRHIZO professional software program. Every root system image was analyzed for total root length (TRL), root surface area (RSA), root volume (RV), average diameter (RD), and number of tips (N Tips). Both Regent's non-statistical method and Tennant's statistical method were chosen to perform root morphology measurements (41) in WinRHIZO. To avoid a high scanning density, the large root sample was subdivided into smaller sub-samples before scanning (2, 42, 43).

2.2.5. Measurement of shoot traits

The fully expanded and matured leaves (fourth leaf from the top) were used for the measurement of leaf thickness (LT). LT was measured using an absolute digital vernier caliper (Mitutoyo Corp., Japan) at the broadest part of the leaf excluding major veins with an accuracy of ±0.01 mm and expressed in µm. The LT was measured as direct reading with a gentle pressing of the caliper to avoid overestimation and any injury to the intact leaf (44). For recording the shoot and root dry weight, the fresh and air-dried root and shoot samples were oven dried at 72°C for 48 h or until reaching constant weight and expressed in terms of g/plant. The root-to-shoot ratio was calculated by dividing root dry weight (RDW) by shoot dry weight (SDW). Total dry matter (TDM) was derived by adding SDW and RDW and expressed on a single-plant basis.

2.3. Yield attributes and yield

The grain, as well as straw yields of millets from the experimental fields, was measured from net plot areas. Net grain and straw yields were reported at a moisture content of 13%. The unit grain weight was obtained for 1,000 grains as test weight (g). The harvest index (HI) and production efficiency were calculated by the following formula:

$$HI = \frac{Economic\ yield}{Biological\ yield} \times 100 \quad (7)$$

$$Economic\ yield = Grain\ yield\ in\ Mg\ ha^{-1} \quad (8)$$

$$Biological\ yield = Total\ plant\ biomass\ (seed\ yield + stover\ yield + root\ biomass)\ in\ Mg\ ha^{-1} \quad (9)$$

2.4. Grain nutritional quality analysis

Grain nutritional traits were estimated based on near-infrared spectroscopy (NIRS) with validated calibration models. Grains of each genotype were threshed, cleaned, and ground to flour of <1 mm particle size, using a CM290 Cemotech™ laboratory grinder (FOSS, Hillerød, Denmark). The flour samples were then stored in 50-mL conical polypropylene falcon tubes at 4°C until scanning with NIR instruments. Prior to scanning, the samples were dried at 50°C for 16 h and cooled to room temperature. The samples were then scanned using a benchtop NIR spectrometer DS2500 flour analyzer from FOSS (FOSS-DS2500; FOSS Electric A/S, Hillerød, Denmark). For obtaining the spectral sample signature from the FOSS-DS2500, each flour sample was transferred to the standard circular ring cup (inside diameter∼6 cm, FOSS sample cup) and scanned three times at room temperature (∼26°C). The sample was mixed before each scan. The NIR spectral absorbance, with a range of 400–2498 nm, was recorded as the logarithm of reciprocal reflectance (1/R) with 2 nm intervals, using SCANISI and predicted using Solo Mosaic analytical software (v4.4, InfraSoft International LLC, PA, USA) and calibration models for various traits [(45); https://fern-lab.github.io/].

2.5. Statistical analysis

All the data obtained from the study were statistically analyzed using the ANOVA and the "F test" for testing their significance ((46), 35). Standard error of means (SEm±), as well as least significant difference (LSD), was calculated at a 5% level of significance for all the parameters studied to know the differences between treatment means. The R program was used to perform the principal component analysis (PCA) (47).

3. Results

3.1. Physiological parameters

Among the root morphological attributes, the finger millet landrace, *Nagaland-1*, recorded the highest average root diameter and total root length with values of 1.87 mm and 284.53 cm respectively, whereas the landrace, *Nagaland-2*, recorded the highest root surface area (189.60 cm^3) followed by *Nagaland-1* (166.42 cm^2) (Figure 1). The finger millet landrace, *Sikkim-2*, obtained the highest root volume (5.22 cm^3), which was statistically at par with the finger millet landrace, *Nagaland-1* (5.11 cm^3) (Figures 1, 2). The highest stomatal frequency was recorded in finger millet landrace, *Sikkim-2* (471 cm^{-2}), and little millet cv. OLM 203 observed the highest stomatal size with a value of 1338.7 μm^2 followed by local landrace, *Sikkim-1* (1138.7 μm^2) and *Sikkim-2* (1104.1 μm^2) (Figure 3). Among the different millets, foxtail millet cv. SiA 3088 recorded the highest Chl*a* content (3.69 mg g^{-1} FW) followed by finger millet cv. VL Mandua 352 and local landrace, *Sikkim-1* (3.55 mg g^{-1} FW each) (Table 3). Similarly, the highest Chl*b* (2.10 mg g^{-1} FW) was recorded for foxtail millet cv. SiA 3088, which was followed by finger millet landrace, *Sikkim-2* (2.08 mg g^{-1} FW) and cv. VL Mandua 352 (2.06 mg g^{-1} FW). However, the highest ratio of Chl*a/b* was recorded for foxtail millet cv. SiA 3088 (1.76) followed by local landraces of finger millet *Nagaland-2* (1.78 mg g^{-1} FW) and *Sikkim-1* (1.74 mg g^{-1} FW). Significantly, the highest carotenoids were recorded for foxtail millet cv. SiA 3088 (149.10 mg g^{-1} FW) followed by little millet cv. OLM 203 (136.70 mg g^{-1} FW) and finger millet landrace, *Sikkim-1* (125.10 mg g^{-1} FW). The highest stomatal conductance-GH_2O (286.10 mmol m^{-2} s^{-1}) was recorded under the local landrace

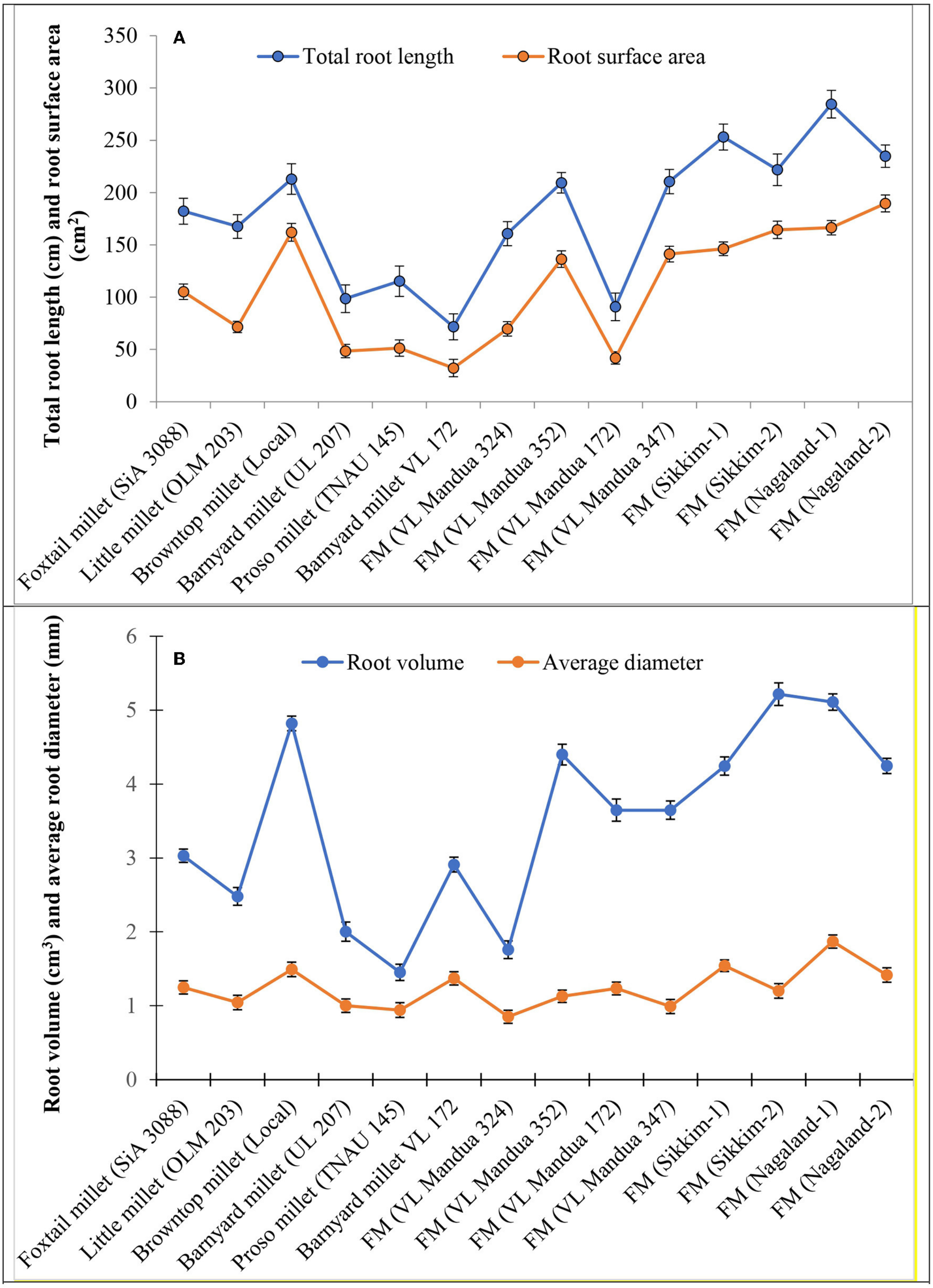

FIGURE 1
Root morphological attributes of millet genotypes. **(A)** Total root length and root surface area. **(B)** Root volume and average of diameter. Vertical bars (both way) represent standard error ($p = 0.05$).

of browntop millet (Table 3 and Figure 3). Among the different germplasms of finger millets evaluated, landrace *Sikkim-1* recorded the highest leaf temperature (41.1°C) followed by *Nagaland-2* (41.0°C) (Table 3).

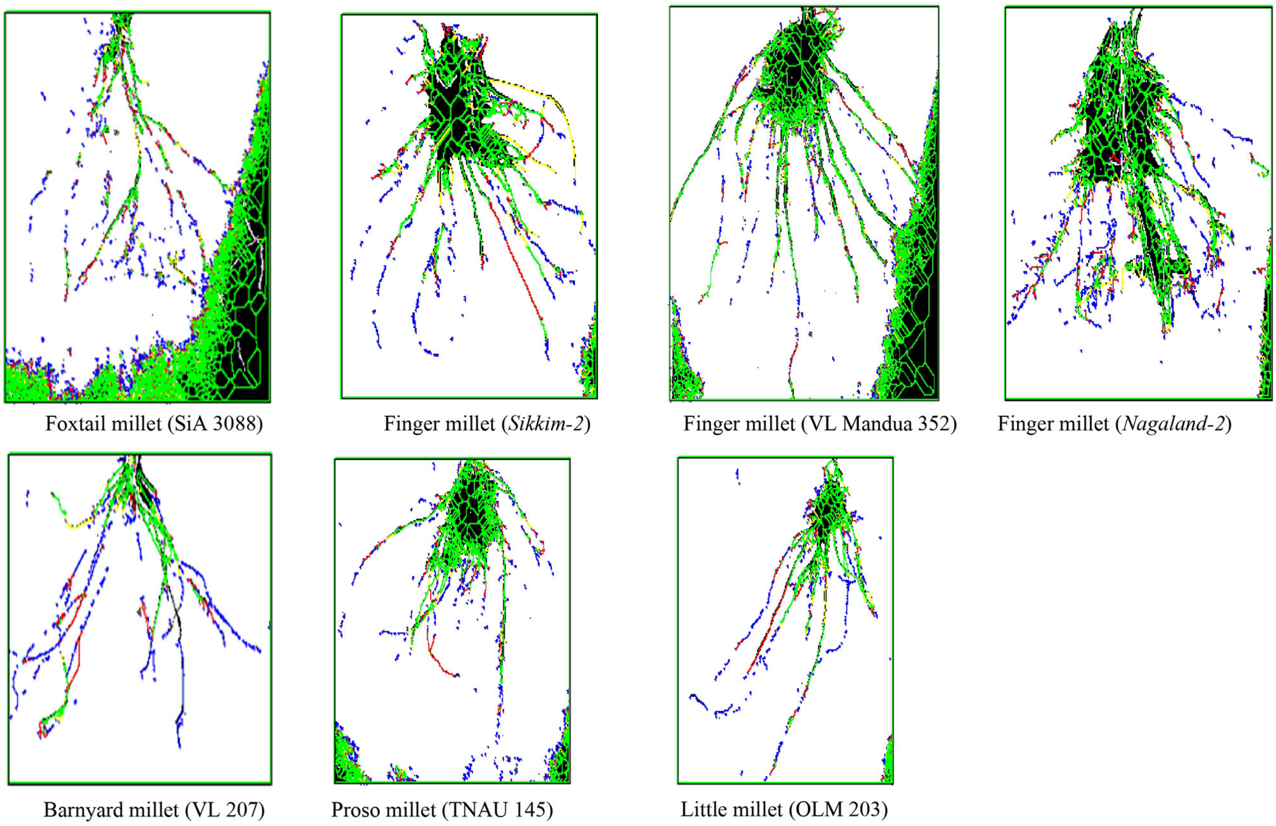

FIGURE 2
Images from root architecture analysis of millets.

3.2. Yield

Finger millet recorded the highest average grain yield (0.73−1.43 Mg ha^{-1}) followed by foxtail millet (1.17 Mg ha^{-1}) and little millet (1.16 Mg ha^{-1}) (Table 4). The lowest yield was observed for proso millet (0.29 Mg ha^{-1}). Among the different finger millet germplasms, the highest average grain yield was recorded for cv. VL Mandua 352 (1.16 Mg ha^{-1}) followed by local landraces, *Nagaland-2* (1.31 Mg ha^{-1}) and *Sikkim-1* (1.25 Mg ha^{-1}). The highest HI was recorded for finger millet cv. VL Mandua 352 (27.3%) and cv. VL Mandua 172 (27.2%) (Table 3). Among the local landraces of finger millet evaluated over 3 years, the highest HI was recorded for *Sikkim-1* (22.6%). Significantly, the highest test weight (weight of 1,000 seed weight) was reported in barnyard millet cv. VL 207 (3.61 g) followed by finger millet cv. VL Mandua 172 (3.20 g).

3.3. Nutritional quality

The experiment of different types of millets including different lines of finger millet over the years showed that there were significant variations in nutritional properties in grains such as protein content, amylose, amylopectin and starch content, and profile of amino acids and fatty acids (Table 5 and Figure 4). Foxtail millet cv. SiA 3088 recorded the highest protein content (12.30%) followed by proso millet cv. TNAU 145 (11.50%) (Figure 4). The local landraces of finger millet, *Sikkim-1* recorded the highest amylose content (17.20%) followed by *Nagaland-2* (16.80 %) compared to HYVs. Similarly, finger millet landrace, *Nagaland-1*, obtained the highest amylopectin content (17.10%) among all germplasms of millets. The starch content was found to be highest in finger millet landrace, *Nagaland-1*, with a value of 30.90 % followed by cv. VL Mandua 352 (30.20%) as shown in Figure 4. The finger millet landraces were also found to be superior in different nutritional properties content as shown in Table 4. For most of the nutritional parameters, local landraces of finger millet, *Sikkim-1 and Sikkim-2* and *Nagaland-1 and Nagaland-2,* outperformed the HYVs (Table 4 and Figure 4). The finger millet landrace, *Sikkim-2*, recorded the highest omega-6 content (1.16%) followed by barnyard millet cv. VL 207 (1.09%). cv. VL 207 also recorded the highest polyunsaturated fatty acid (PUFA) content (1.23%) followed by foxtail millet cv. SiA 3088 (1.09%). However, among the different germplasms of finger millets evaluated, local landraces, *viz., Sikkim-2* (1.01%) followed by *Nagaland-2* (0.79%) recorded significantly higher PUFAs against the best HYVs such as VL Mandua 324 (0.63%). Saturated fat concentration ranged from 0.30% in finger millet landrace, *Sikkim-2*, to 1.3% in foxtail millet cv. SiA 3088. The different germplasms of millets were also found to contain significant amounts of essential amino

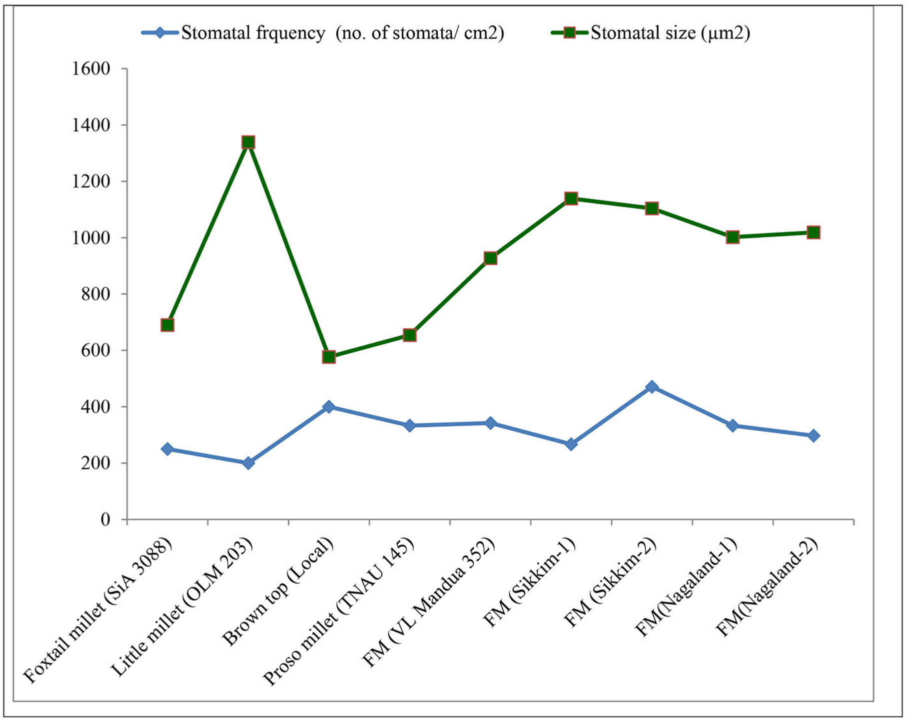

FIGURE 3
Stomatal attributes of millets grown under organic conditions.

acids as shown in Table 4. For sulfur-containing amino acids (methionine and cysteine), the highest values of methionine were observed in finger millet cv. VL Mandua 172 and 347 (0.19%). The finger millet landraces, *Nagaland-2* and *Nagaland-1*, also recorded significantly higher values of methionine (0.19 and 0.17%, respectively) compared to other millets.

While the landraces of browntop millet (local) and finger millet, *Nagaland-2,* recorded the highest values for cysteine (0.23%), the highest amount of glutamic acid content was found for local browntop millet (3.62%). Finger millet landraces, *viz.*, *Nagaland—2* and *Sikkim-1*, also recorded higher values of glutamic acid (3.19 and 3.08%, respectively) compared to most of the millets evaluated. The histidine content was recorded to be maximum in finger millet landrace, *Sikkim-1* (0.41%) followed by *Sikkim-2* and little millet cv. OLM 203 (0.36%). Both the landraces of finger millets, *Sikkim-1* and *Nagaland-2,* recorded the highest values for thiamine (0.32% each). The finger millet landrace, *Nagaland-2*, contained the highest amount of tryptophan (0.12%) followed by *Nagaland-1*, *Sikkim-1*, barnyard millet variety, VL 207, and proso millet cv. TNAU 145 (each with a value of 0.11%).

3.4. Principal component analysis

PCA revealed that the first three principal components (PC1, PC2, and PC3) explained 66% of the total variation. PC1 accounted for 26% of the total variation, whereas both PC2 and PC3 accounted for 22% of the total variation, respectively (Table 6). Within PC1, chlorophyll a, chlorophyll b, amylose, amylopectin, starch, saturated fat, histidine, and thiamine showed positive loadings, while the rest of the parameters showed negative loadings (Figure 5 and Table 5). In PC2, Chl a/b, grain yield, glutamic acid, cysteine, and thiamine showed positive loading, and the rest of the variables revealed negative loadings (Figure 5 and Table 5). The factor map (component plot) and clustering of all the variables (Figure 5), which reveal the distance between the variables and the origin and assessed the quality of the variables, are both depicted. Positively associated variables are clustered together, while variables with negative correlations are placed on the opposing sides of the plot's origin.

4. Discussion

4.1. Physiological attributes

There was a need to study the adaptation mechanism of millets in harsh stress environments for their optimum performance and tolerance to stresses (48). The physiological attributes of a plant always play a major role in determining the yield potential and nutritional content in a particular climate and stress condition (49). The root morphological characteristics of millets, such as average root diameter, total root length, and root surface area, play a very important role in essential nutrient uptake from soil and transporting them to the shoots for further help in photosynthesis (48, 50). Among the different millets evaluated in the NEHR of India under organic conditions, the local landrace of finger millet, *Nagaland-1*, recorded the highest average root diameter, root surface area, and root volume, which were statistically at

Germplasms	Varieties/landraces	Chl*a* (mg g^{-1} FW)	Chl*b* (mg g^{-1} FW)	Carotenoids (µg/gFW)	Chl a/b	Leaf temperature (°C)	Stomatal conductance-GH_2O (mmol m^{-2} s^{-1})
Foxtail millet	SiA 3088	3.69	2.10	149.10	1.78	39.00	147.00
Little millet	OLM 203	3.33	2.01	136.70	1.66	39.90	236.40
Browntop millet	Local	3.21	1.86	121.80	1.73	38.40	286.10
Barnyard millet	VL 207	3.47	1.73	117.30	2.01	39.20	234.70
Proso millet	TNAU 145	3.08	2.05	96.50	1.50	39.10	253.60
Finger millet	VL Mandua 324	3.32	1.96	112.70	1.69	38.50	162.50
	VL Mandua 352	3.55	2.06	116.50	1.72	38.60	280.30
	VL Mandua 172	3.38	1.98	102.60	1.71	39.40	217.10
	VL Mandua 347	3.26	1.94	91.90	1.68	40.40	159.70
	Sikkim-1	3.55	2.04	125.10	1.74	41.10	168.20
	Sikkim-2	3.45	2.08	105.70	1.66	40.40	239.40
	Nagaland-1	3.17	1.84	107.90	1.72	40.60	192.10
	Nagaland-2	3.52	1.98	98.00	1.76	41.00	238.70
	SEm±	0.04	0.05	2.59	0.04	SEm±	0.80
	CD (P=0.05)	0.12	0.14	7.57	0.13	CD ($P = 0.05$)	2.34

TABLE 4 Yield of different millets under organic production system (average of 3 years data).

Germplasms	Varieties/landraces	Grain yield (Mg ha^{-1})	Harvest index (%)	Test weight (g 1,000 $seed^{-1}$)
Foxtail millet	SiA 3088	1.17	25.0	3.14
Little millet	OLM 203	1.16	24.2	3.09
Browntop millet	Local	1.02	17.5	2.80
Barnyard millet	VL 207	1.09	26.5	3.61
Proso millet	TNAU 145	0.29	18.9	3.17
Finger millet	VL Mandua 324	1.16	22.8	2.84
	VL Mandua 352	1.43	27.3	2.99
	VL Mandua 172	1.21	27.2	3.20
	VL Mandua 347	1.22	26.7	2.99
	Sikkim-1	1.25	22.6	2.93
	Sikkim-2	0.73	20.2	2.79
	Nagaland-1	0.97	20.3	2.81
	Nagaland-2	1.31	19.6	2.69
SEm±		0.050	0.55	0.07
CD ($P = 0.05$)		0.13	1.60	0.20

par with landraces *Sikkim-1*, *Sikkim-2*, and cv. VL Mandua 352. The higher root length, root volume, and surface area of the millet germplasms, VL Mandua 352, *Nagaland-2*, *Sikkim-1*, etc. determined the overall distribution and functioning of roots and promoted water and nutrient acquisition from the soil, thereby enhancing the crop productivity. There is a need to study the root system architecture of landraces in comparison with high-yielding varieties for improved adaptation to a particular ecosystem with minimum input application (51). This will also help plant breeders to develop suitable varieties by mixing the traits of high-yielding varieties with local landraces for various stress situations (52).

Among different millets evaluated under the organic production system, significantly higher Chl*a*, Chl*b*, and *Chla/b* ratios and carotenoids were recorded for foxtail millet cv. SiA 3088 and finger millet landraces, *Sikkim-2, Sikkim-1,* and *Nagaland-2,* respectively (Table 3). These may be due to the enhancement of photosynthetic efficiency of the millet germplasms as reflected by the improvement of concentration of carotenoids, Chl*a,* and Chl*b* in millet leaves (49). The enhancement of Chl*a*, Chl*b*, and carotenoids is also known to stimulate chlorophyll biosynthesis and subsequently delay the process of senescence of leaves, thereby prolonging the photosynthetic period of plants (53). Table 3 also depicts the stomatal conductance and leaf temperature of different millets grown under organic farming practices. Chatterjee et al. (54) reported that speciation resulted in a steady increase in stomatal conductance (anatomical, *gmax*) in different *Oryza* species. This reduces water loss by transpiration and indicates a good physiological capacity for stomatal regulation (55, 56). Leaf temperature under drought stress conditions has been recognized as an indicator of plant water status (57, 58). The highest stomatal frequency was recorded in finger millet landrace, *Sikkim-2* (471cm^{-2}), and little millet cv. OLM 203 observed the highest stomatal size with a value of 1338.7 μm^2 (Figure 3).

4.2. Yield of millets

Among the different millets, finger millet germplasms, *viz.*, VL Mandua 352, *Nagaland-2*, *Sikkim-1*, and foxtail millet cv. SiA 3088 recorded a significantly higher grain yield compared to the rest of the germplasms (Table 3). Better root architecture, higher photosynthetic attributes, and uptake of nutrients and water may have paved for higher photosynthesis (59, 60). The increase in grain yield might be due to the increased photosynthetic activity, which resulted in a higher accumulation of photosynthates and their translocation to sink due to better source and sink channel (61, 62). The increase in grain yield with increased nutrient supply could be explained on the basis of their beneficial effects on yield-attributing characteristics (63). The increase in yield may be due to genetic and environmental factors (64). Pareek and Shaktawat (65) in a study on pearl millet and Munirathnam et al. (66) in a study on foxtail millet have also reported similar findings. Higher HI (Table 3) in selected millet germplasms might be due to dry matter partitioning along with an increased level of nitrogen as reported by Reddy et al. (67).

4.3. Nutritional quality parameters

The quality attributes of the millets in this study, as shown in Table 4 and Figure 5, varied across the millets and their germplasms. Generally, millets are reported to contain high concentrations of minerals, essential amino acids, antioxidants, and vitamins, which keep them nutritionally superior compared to other cereals such as rice, wheat, and maize (51, 68). The root is a very sensitive part of plants (here in millets) and responsible for the uptake of water and macro- and micronutrients from the soil (69, 70). Millet lines have better root architectural design (higher root

TABLE 5 Profiling of fatty acids and amino acids in different millets under an organic production system.

Germplasms	Varieties/ landraces	Omega 6 (%)	PUFA (%)	Saturated fat (%)	Glutamic acid (%)	Cysteine (%)	Histidine (%)	Thiamine (%)	Methionine (%)	Tryptophan (%)
Foxtail millet	SiA 3088	1.04	1.09	0.13	2.88	0.13	0.35	0.29	0.13	0.10
Little millet	OLM 203	0.79	0.72	0.08	3.01	0.16	0.36	0.23	0.14	0.07
Browntop millet	Local	0.55	0.61	0.07	3.62	0.23	0.33	0.26	0.14	0.03
Barnyard millet	VL 207	1.09	1.23	0.04	2.17	0.12	0.17	0.17	0.12	0.11
Proso millet	TNAU 145	0.50	0.55	0.07	1.53	0.08	0.34	0.32	0.18	0.11
Finger millet	VL Mandua 324	0.55	0.63	0.11	3.44	0.21	0.34	0.27	0.14	0.05
	VL Mandua 352	0.49	0.57	0.07	3.52	0.22	0.30	0.30	0.18	0.06
	VL Mandua 172	0.54	0.53	0.08	3.52	0.22	0.31	0.30	0.19	0.06
	VL Mandua 347	0.53	0.56	0.06	2.44	0.13	0.32	0.31	0.19	0.07
	Sikkim-1	0.68	0.74	0.08	3.08	0.21	0.41	0.32	0.13	0.11
	Sikkim-2	1.16	1.01	0.09	2.97	0.22	0.36	0.27	0.11	0.09
	Nagaland-1	0.61	0.70	0.09	2.19	0.11	0.34	0.29	0.17	0.11
	Nagaland-2	0.89	0.79	0.03	3.19	0.23	0.23	0.32	0.19	0.12
SEm±		0.02	0.02	0.00	0.07	0.00	0.01	0.01	0.00	0.00
CD (P=0.05)		0.06	0.05	0.00	0.21	0.01	0.02	0.02	0.01	0.01

PUFA, Polyunsaturated fatty acids.

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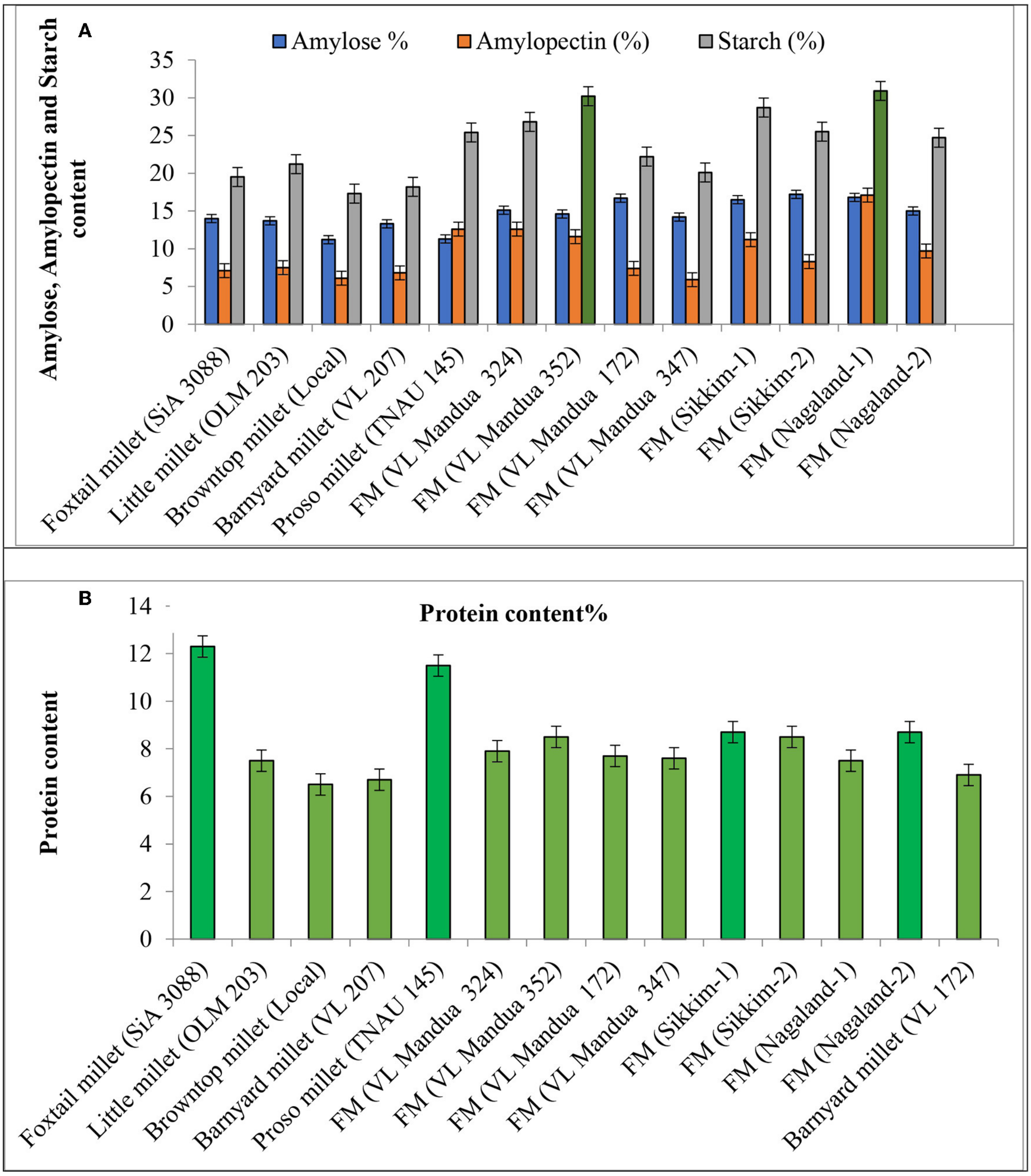

FIGURE 4
Effect of different lines of millets on quality parameters. **(A)** Amylose amylopectin and starch content. **(B)** Protein content. Vertical bars (both way) represents standard error ($p = 0.05$).

length, root surface area, and diameter, etc.), have better chances to survive under stress conditions, such as flooding, drought, and deficiency of nutrients, and produce nutritionally superior grains compared to others (51). This was proved by nutritionally superior grains with high values of protein, amylose, amylopectin, and starch content of local landraces of finger millets compared to HYVs. Foxtail millet and proso millet are known to contain a relatively higher amount of protein compared to other millets and non-millet cereals (71). Apart from that, the local landraces of finger millets also recorded high amounts of essential amino acids and fatty acids. Lipids are an important source of essential fatty acids, and local landraces of finger millet, foxtail millet, and barnyard millet were found to be excellent sources of omega-3 and polyunsaturated fatty acids (PUFA). Similar findings for essential amino acids in millets were also reported by Amadou et al. (6). The local landraces of finger millet showed significant amounts of essential nutrients compared to other varieties. The experiment also revealed interesting data on the concentrations of essential amino acids, such as histidine, tryptophan, lysine, and methionine. Most of these essential amino acids were found to be significantly higher

TABLE 6 Factor loadings of biochemical parameters along with the percentage of variance and cumulative variance accounted for each component.

Parameters	PC1	PC2	PC3
Chlorophyll a	0.27	−0.25	0.07
Chlorophyll b	0.36	−0.16	0.12
Carotenoids	−0.02	−0.21	0.38
Chl a/b	−0.37	0.12	−0.17
Grain yield	−0.19	0.20	0.28
Harvest index	−0.25	0.071	0.21
Test weight	−0.18	−0.26	−0.07
Amylose	0.13	0.05	0.10
Amylopectin	0.30	0.06	−0.19
Starch	0.30	0.14	−0.09
Omega 6	−0.13	−0.34	0.06
Saturated fat	0.23	−0.12	0.30
PUFA	−0.16	−0.38	0.05
Glutamic acid	−0.06	0.25	0.42
Cysteine	−0.03	0.25	0.31
Histidine	0.35	0.01	0.23
Thiamine	0.24	0.27	−0.09
Methionine	0.01	0.34	−0.27
Tryptophan	0.07	−0.30	−0.28
Standard deviation	2.25	2.07	1.82
Proportion of variance	0.26	0.22	0.17
Cumulative proportion	0.26	0.49	0.66

PUFA, Polyunsaturated fatty acids.

in local finger millet landraces such as *Sikkim-1* and *Nagaland-2* compared to their corresponding HYVs like VL Mandua 352 or VL Mandua 324. Better uptake of nutrients and higher translocation of photosynthates from source to sink lead to higher protein content in seeds and also higher accumulation of carbohydrates for the local landraces of finger millets (64, 72). These observations corroborate those made by Sharer et al. (73), Chauhan et al. (74), and Nandini and Sridhara (75).

4.4. Principal component analysis

Understanding the link between variables can be aided by multivariate statistical analyses such as PCA. These could be useful in clarifying the nature of defining attributes and simplifying the data collection process. The PCA confirmed our findings (Table 5 and Figures 5a, b), with strong and positive correlations among chlorophyll a, chlorophyll b, amylose, amylopectin, starch, saturated fat, histidine, and thiamine in PC 1, while strong and positive correlations were found among Chl a/b, grain yield, glutamic acid, cysteine, and thiamine in PC 2. While the HYVs have high yield potential, they are inferior in nutritional content compared to local millet landraces. In addition, local landraces are adapted to the NEHR, India, and are comparatively more resistant to drought and heavy rainfall than HYVs. This indicates the possibilities for using the local landraces in breeding programs for the production of high-yielding and nutrient-rich varieties.

5. Conclusion

The northeastern part of India is organic by default, creating a huge scope for organic millet production. From the above results, it can be concluded that millets can be an important alternative to diversify low-productive mixed farming and supply nutritious food to the people of the northeastern region of India. Apart from nutritional benefits, millets are remarkable crops due to their ability to survive under marginal soil conditions, especially in sloppy and shifting cultivated areas of the NEHR. These features of resilience for the climate-smart crop ensure stable production, enabling local farmers to cultivate millets, which can be a great alternative to rice cultivation in the NEHR. Among the different minor millets studied and evaluated, the foxtail millet cv. SiA 3088, little millet cv. OLM 203, and finger millet germplasms performed well in terms of yield. Along with the HYVs of finger millet, VL Mandua 352 and 347, local landraces of the NEHR, *Nagaland-2* and *Sikkim-1*, showed significantly higher yield and even performed better in biochemical and root traits. The local landraces of finger millets, *viz.*, *Nagaland-2* and *Sikkim-1*, were also found to have superior nutritional quality compared to the HYVs. Therefore, apart from HYVs of finger millets, *viz.*, VL Mandua 352 and VL Mandua 347, local landraces such as *Nagaland-2* and *Sikkim-1* should be encouraged to grow among the tribal and hill farmers of the NEHR of India under organic conditions for higher yield and nutritional quality.

6. Policy implications

Recent studies have proven that regular consumption of millets along with rice/wheat/maize help in better digestion, nutrition, and reduction in various diseases such as diabetes, arthritis, and heart disease. To popularize millets in the Indian NEHR and to increase its production, several NGOs and local organizations such as the North East Slow Food & Agrobiodiversity Society (NESFAS) and the North Eastern Council (NEC) of the Government of India are constantly working. To remove malnutrition among children and women, our government should give importance to millets in the Midday Meal Scheme, Integrated Child Development Scheme (ICDS), and Public Distribution System (PDS). For enhancing the production as well as consumption of millets along with creating awareness among the masses for food and nutritional security, the United Nations has declared the year 2023 as the International Year of Millets. Research is also needed for using local landraces of millets in breeding programs to produce low-nutrient-demanding and climate-specific millet varieties for food and nutritional security across different ecosystems.

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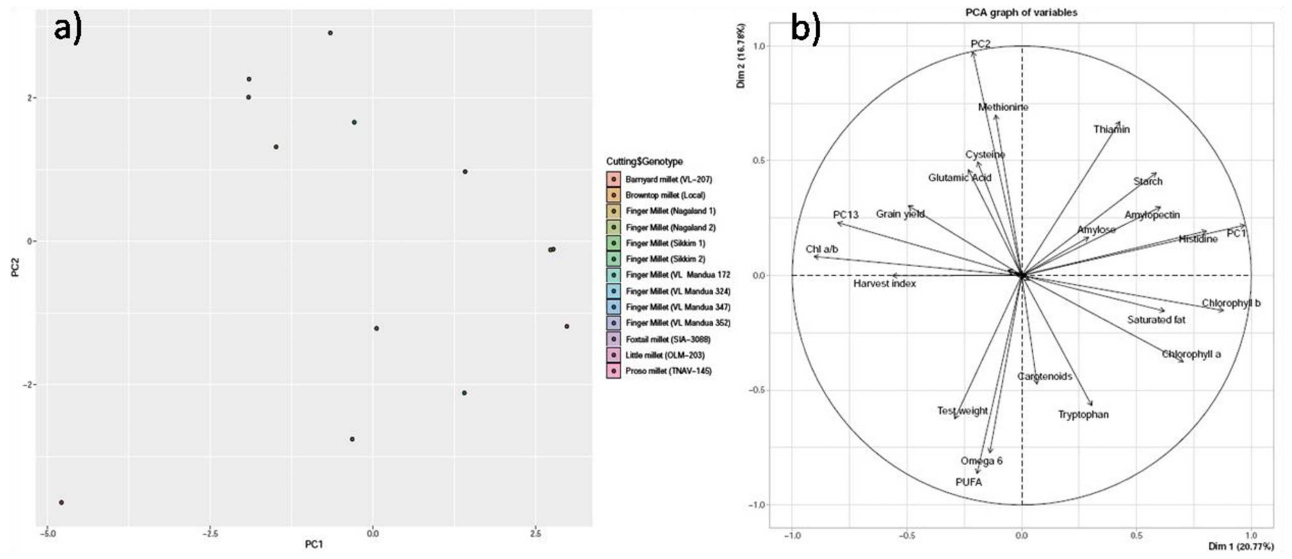

FIGURE 5
Principal component analysis (PCA) of biochemical parameters. **(a)** Scatter plot and **(b)** Bi-plot. The PCA achieved is not rotated such that each succesfull axis captured as much variance as possible.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

JL: conceptualization, methodology, investigation, monitoring, data curation, and writing of original and final draft. KR: data curation, review, writing, and editing. AD: monitoring, data curation, review, and editing. MA: data analysis, writing of original and final draft, review, and editing. SC, NRaj, SP, AK, SD, SB, MT, and NS: data analysis, review, and editing. VM, NRav, SK, and SH: review, editing, and project administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1198023/full#supplementary-material

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Do agronomic approaches aligned to regenerative agriculture improve the micronutrient concentrations of edible portions of crops? A scoping review of evidence

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Regenerative Agriculture (RA) is used to describe nature-based agronomic approaches that aim to build soil health and crop resilience, minimize negative environmental outcomes, and improve farmer livelihoods. A benefit that is increasingly attributed to crops grown under RA practices is improved nutritional content. However, we do not know the extent to which RA influences crop nutritional quality and under what management approaches and context, can such effects be realized. A scoping review of recent literature (Web of Science, 2000–2021) was carried out to assess the evidence that RA approaches improve crop micronutrient quality. Papers included combinations of agronomic approaches that could be defined as Regenerative: "Organic Inputs" including composts and manures, cover crops, crop rotations, crop residues and biochars; "Reduced Tillage", "Intercropping", "Biostimulants" e.g. arbuscular mycorrhizal fungi; plant growth promoting bacteria, and "Irrigation", typically deficit-irrigation and alternate wetting and drying. The crop types reviewed were predetermined covering common sources of food and included: Tomato (*Solanum lycopersicum L.*), Wheat (*Triticum aestivum L.*), Rice (*Oryza sativa L.*), Maize (*Zea mays L.*), Pulses (Fabaceae), Alliums (*Allium spp.*), and "other" crop types (30 types). This scoping review supports a potential role for RA approaches in increasing the concentrations of micronutrients in the edible portions of several crop types under specific practices, although this was context specific. For example, rice grown under increased organic inputs showed significant increases in grain zinc (Zn) concentration in 15 out of 16 studies. The vitamin C concentration of tomato fruit increased in ~50% of studies when plants were grown under increased organic inputs, and in 76% of studies when plants were grown under deficit irrigation. Overall, the magnitude and reproducibility of the effects of RA practices on most crop nutritional profiles were difficult to assess due to the diversity of RA approaches, geographical conditions, and the limited number of studies for most crops in each of these categories. Future research with appropriate designs, improved on-farm surveillance and nutritional diagnostics are needed for better understanding the potential role of RA in improving the quality of food, human nutrition, and health.

KEYWORDS

alliums, beta-carotene, iron, rice, tomato, vitamin C, wheat, zinc

Introduction

Regenerative Agriculture (RA) is widely used to describe agronomic approaches based on the principles of improving soil health and sequestering carbon. Giller et al. (1) provide a comprehensive overview on RA from an agronomic perspective. Their review highlights that—ever since the RA term entered regular usage in the 1980s—RA is an evolving conceptual area which spans across agronomic, biophysical, and social justice dimensions. RA is typically framed in terms of agricultural systems which: (1) minimize the external impacts of agriculture beyond the farm; (2) minimize energy and other inputs into the farm; (3) sequester carbon, improve nutrient cycling and wider ecosystem services, (4) increase biodiversity, and (5) promote social justice. Soil conservation is considered as the entry point for most agronomic approaches informed by RA, although universally accepted formal definitions and inclusion criteria for RA are lacking (1).

Agronomic approaches within the scope of RA fall broadly into the following, non-exclusive, categories: (1) increased use of organic inputs[1] for nutrition and soil cover, including animal manures, green manures/mulches/cover crops, crop rotations, other composts, and biochars; (2) reduced soil tillage, and (3) increased plant diversity (e.g., intercropping, more diverse rotations, agroforestry). The role of agrochemicals and genetically modified organisms (GMOs) within RA approaches remains contested. Some proponents of RA consider that the judicious use of these conventional and/or novel technologies can be consistent with the principles of RA. Other authors consider RA to align more closely with the principles of "organic" agriculture, although there are no current standards or certification for RA. Under conditions of zero tillage, for example, weed control using herbicides would be considered essential, whereas tillage would be used to control weeds in certified organic systems. Other agronomic strategies which are potentially consistent with RA principles include the use of biostimulants, such as incorporation of arbuscular mycorrhizal fungi (AMF), plant growth-promoting bacteria (PGPB), humic acids, and other beneficial bioactive compounds. The use of RA approaches such as organic inputs and reduced tillage to improve the functional attributes of soil microbiota indirectly, should also be considered (2). Similarly, water conservation techniques such as deficit irrigation systems can improve soil structure and reduce greenhouse gas losses in some cropping systems. This therefore reduces pressure on water availability in the landscape, and thereby be considered to fall within the scope of RA. Holistic RA systems which include grazing livestock can also be consistent with RA principles, including as a source of organic inputs. However, this area is contested due to the contributions of livestock to greenhouse gas emissions (1). From a nutritional yield perspective, the contribution of livestock to protein and micronutrient supply into food systems is still critical in many food system contexts. Recent evidence showed a high prevalence in selenium (Se) deficiency in cattle in Ethiopia, which negatively affects the health and productivity of livestock and consequently the soil-feed-livestock-human nexus (3). Detailed interactions between RA principles, soil health and nutritional quality are presented in Figure 1.

Giller et al. (1) define the two main challenges for RA as needing to: (1) restore soil health, including the capture of carbon to mitigate climate change, and (2) reverse biodiversity loss. There are implicit human health outcomes arising from climate change mitigation and improved biodiversity. Similarly, reductions in the intensive use of agrochemical inputs such as fertilizers and pesticides can also deliver potential health benefits on-farm during handling and application, and beyond the farm-gate. This can be directly, during processing and consumption; and through decreased potential for environmental pollution of landscapes (soil, water and atmosphere).

A further linkage between RA and human health can arise if these agronomic approaches lead to improvements in crop nutritional quality. Nutritional quality is the value of the product for the consumer's physical health, growth, development, reproduction and psychological or emotional well-being (4). Here, we use the term crop nutritional quality to represent the nutritional value of the edible portions of crops. Crop nutritional quality is important because of the widespread global risks of micronutrient deficiencies (MNDs) which are likely to affect more than two billion people worldwide (5, 6). These MNDs, also known as "hidden hunger," remain a major challenge for achieving the United Nations' Sustainable Development Goal 2 (SDG2, zero hunger) by 2030 (7). Causes of MNDs include the inadequate dietary intakes of micronutrients, for example, calcium (Ca), iron (Fe), magnesium (Mg), iodine (I), Se, zinc (Zn) and vitamin A. Although MNDs can affect all people in all countries, the risks of MNDs are greater in low- and middle-income countries (LMICs) than in high-income countries [HICs; (5, 8, 9)]. In LMICs, access to foods from plant and animal sources that are richer in micronutrients is often limited and diets are dominated by cereals which typically have smaller micronutrient concentrations per unit energy (10). Although the bran and embryo fractions of cereal grains are often removed during milling, cereal grains also contain large concentrations of anti-nutritional compounds such as phytates (inositol phosphate compounds), which inhibit the absorption of Ca, Fe, Mg and Zn in the human gut (5). The consequences of MNDs include impaired physical and mental development and performance, and increased risks of communicable and non-communicable disease and mortality (7).

Effects of different agronomic management practices on crop nutritional quality

The intercropping studies of Fusou Zhang and colleagues provide a detailed insight of the effects of an RA approach on crop nutritional quality, albeit generally from a crop nutrition, rather than a human nutrition perspective [reviewed by Zuo and Zhang (11)]. Intercropping

1 Not limited to certified organic agriculture practices.

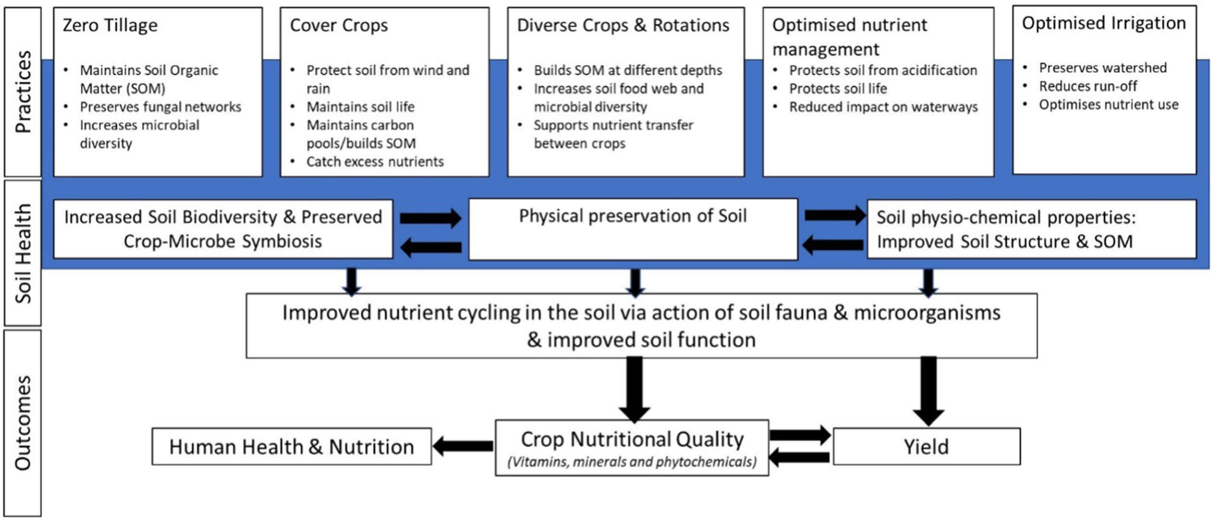

FIGURE 1
Interaction between Regenerative Agriculture practices, soil health, and nutritional quality.

is a production system in which two or more crop species are grown together in the same field, and contrasts with the more common monocropping systems where a single crop is grown. Intercropping systems such as peanut (*Arachis hypogaea* L.)/maize (*Zea mays* L.), wheat (*Triticum aestivum* L.)/chickpea (*Cicer arietinum* L.), and guava (*Psidium guajava* L.)/sorghum [*Sorghum bicolor* (L.) Moench] or maize, have been shown to improve Fe and Zn nutrition of crops leading to improved growth (11). The scientific principles behind this effect of intercropping are that "graminaceous" cereal crops can mobilize soil Fe and Zn through the release of siderophores and other compounds from their roots, which in turn will increase the availability of soil Fe and Zn to their neighboring "non-graminaceous" crop plant [reviewed for Fe, by Dai et al. (12)]. However, there is much still to learn about the role of specific compounds and their interactions with soil micro-organisms (microbiomes) in the rhizosphere and wider bulk soil, including how this might translate into agronomically appropriate combinations of crop types to yield nutritional benefits.

More recently, surveys of cereal grain quality among maize-based (13, 14) and wheat-based (15) smallholder farmers in sub-Saharan Africa (SSA) reported that use of animal and green manures led to nutritionally significant increases in grain Fe and Zn concentrations in maize. Similarly, augmented use of Integrated Soil Fertility Management (ISFM) approaches, including the use of organic inputs of leaf litter and cattle manure and micronutrient fertilizers improved grain Zn concentration in field-grown maize and cowpea (16, 17). These increases in grain Fe and Zn quality were likely greater than would have been expected from the direct effects of the additional inputs of Fe and Zn from the organic inputs into the system and are likely due to improvements in soil structure and retention/plant availability of micronutrients. The mechanisms by which soil organic carbon (SOC) affects crop Fe and Zn availability for uptake is not yet known, although it can help in terms of more effective micronutrient management of crops. For example, increased SOC supports better soil structure, including water and soil nitrogen (N) retention and provisioning to crops (18). Crops of better N status are likely to produce greater amounts of Fe- and Zn-chelating compounds such as nicotianamine synthase (NAS) and therefore support enhanced remobilization of Fe and Zn from crop leaves into grains, which is where Zn-protein co-localization occurs (19–23). Improved N nutrition of field-grown crops has been linked to increases in grain Zn concentrations in maize and cowpea [*Vigna unguiculata* (L.) Walp; (17)] and to increases in grain Fe concentration in field-grown finger millet [*Eleusine coracana* Gaertn; (24)]. However, synergies between crop N nutrition and grain Fe and Zn concentration are not seen consistently across all crop types under field conditions. This implies that complex interactions between yield improvements due to N and potential "dilution" effects on grain micronutrient concentration are likely to arise.

In a recent study from the US, a "paired-farm" approach was adopted to compare the nutritional quality of different crops [pea, *Pisum sativum* L.; sorghum; maize/corn; soybean, *Glycine max* (L.) Merr.] grown on RA farms with a proximal non-RA farm (25). There was evidence that crops grown on the RA farms exhibited greater concentrations of a range of vitamins and minerals in general than crops from non-RA farms, which corresponded with improvement in SOC on the RA farms. For example, maize, soybean, and sorghum grown on RA farms had 17, 22, and 23% more Zn than the same crops from a non-RA farm, albeit from a single contrast. The authors also noted potential decreases in the Zn concentration of cabbage (*Brassica oleracea* L. var. *capitata*) and pea, and, more importantly, that the paired-farm study design lacked statistical power to test for effects on individual crops. In the same paper, the use of cover crops vs. traditional fallow with regular herbicide use on no-tilled wheat resulted in significant increases in grain mineral micronutrient concentrations in wheat from the cover cropped field. A 48, 29, and 56% increase in grain Ca, Mg and Zn concentration was also reported, respectively. However, this was based on technical replication derived from a single sample (25).

Soil health and crop nutritional quality

Relationships between soil health and food nutritional quality have recently been reviewed by Bourne et al. (26). There was no evidence of increased wheat grain protein concentrations under no-till conditions compared to conventional tillage from longer term studies. From shorter-term studies, there was considerable year-to-year variation and results from wheat studies were inconclusive. The authors noted that increases in SOC and soil organic N did not always result in increased grain N/protein concentration. Furthermore, there was some evidence of decreased grain protein concentration which could arise due to soil cultivation increasing soil N mineralization and availability to the crop. In crop rotational systems using legumes, increases in wheat grain protein concentration were observed, likely due to enhanced mineralization of N from residues from the preceding legume crop, and subsequent availability for uptake. For mineral micronutrients in wheat grain, the results were inconsistent across both tillage and rotational studies. However, several studies reported positive linkages between increased SOC, soil total N, and crop Zn uptake. This may be due to increased synthesis of Zn chelating compounds in crops and/or increases in soil cation exchange capacity and retention of Zn in plant available forms. However, Bourne et al. (27) noted these processes are complex. Furthermore, increased wheat grain Zn concentrations were associated with crop rotations that increased colonization of wheat roots by AMF and other fungi, for example by using clover (*Trifolium* spp.) or flax/linola (*Linum usitatissimum* L.) rather than canola (*Brassica napus* L.). Many studies on tomato (*Solanum lycopersicum* L.) quality lacked data on soil properties and yield, and focused more on secondary compounds (e.g., beta-carotene, lycopene, phenolics, vitamin C) rather than mineral micronutrients, in contrast to most wheat studies. Few consistent effects of production systems on mineral micronutrients and secondary compounds were noted, and these were likely influenced by genotypic differences between tomato varieties. Overall, few studies contained sufficient relevant information to provide evidence of linkages between metrics of soil health and crop nutritional quality of relevance to human health (26). Taken together, the evidence on linkages between agronomic practices which fall within the scope of RA and crop nutritional quality is currently limited. The authors of all these studies have noted the challenges in synthesizing data due to the many different input variables and geographies represented by these studies.

Agricultural systems and crop nutritional quality

Dangour et al. (28) conducted a systematic review on the nutritional quality of crops grown in organic production systems. They found little evidence for differences in the nutritional quality of organic foods compared to conventionally produced foods. From an initial screen of 52,471 articles, and a shortlist of 162 studies (137 crops and 25 livestock products), only 55 articles were of satisfactory quality for a comparison of nutritional quality between organically- and conventionally-produced food. Conventionally produced crops had a significantly higher content of N; organically produced crops had a significantly higher content of phosphorus (P) and higher titratable acidity. There was no evidence of further differences in nutritional quality of the remaining 8 of 11 crop nutrient categories. A subsequent systematic review (29), comprising 223 studies, came to a similar conclusion, although a further review and meta-analysis of 343 studies by Barański et al. (30) reported greater concentrations of several secondary compound micronutrients, notably polyphenols. A general challenge of synthesizing data from "organic vs. conventional" comparisons is that crops will have been grown in different geographical locations, and with multiple differences in nutrient inputs and other confounding factors.

A review by Montgomery and Bikle (31), starts to pull apart the controversy around organic vs. conventional, and suggests looking at the effect of specific farming practices on soil health and nutritional content, rather than attempting to attribute improvements in nutrient density to highly variable and complex systems.

Two systematic reviews have recently reported linkages between general agronomic approaches and tomato fruit micronutrient quality. In a meta-analysis on the effects of N supply on tomato yield, water use efficiency and fruit quality, Cheng et al. (32) assessed 1,096 data pairs from 76 publications. Under N supply rates sufficient for optimal yield, vitamin C increased by 19%, whereas lycopene decreased by 11% and nitrate content of fruit increased by 60%, compared to low N-input conditions. The second study was a meta-analysis on the effects of deficit irrigation on tomato quality (33). They assessed 2,369 data pairs, from 83 publications. Under deficit irrigation compared to full irrigation conditions, vitamin C increased by 14% and lycopene increased by 10%, whereas beta-carotene decreased by 11%. This was driven by a single study and the effect on beta-carotene was not seen when the study was excluded. However, the influence of deficit irrigation on nutritional quality was highly dependent on soil properties including texture and bulk density. For example, vitamin C improved more on course soils than medium textured soils whereas lycopene concentrations were larger on medium than course textured soils (33).

Study aims

The review by Montgomery and Bikle (31), gives a broad overview of practices beyond defined systems that have been linked to observed changes in nutritional and phytochemical profiles across a range of crops and highlights the types of studies, potential mechanisms as well as the challenges of accounting for the factors affecting crop growth and health. Due to the complexity of different cropping systems and agronomic approaches, and a lack of explicit definitions of RA approaches, we adopted a wide literature search to ensure inclusion of papers on an agreed set of different RA practices. In contrast to the Bourne et al. (26) study, we considered practices intended to improve "soil health" and "landscape health" therefore included water management practices, rather than those which explicitly reported only soil health indicators.

We hypothesize that agronomic practices aligned to RA principles improve nutritional quality of edible portions of crops. The aim of this review was to identify where RA approaches can significantly improve crop micronutrient quality in the edible portion of field grown crops. We aimed to identify important food crops where there is robust evidence for positive nutritional effects linked to specific classes of practices. The study focused on a selection of mineral and secondary metabolites (vitamins) with well-established benefits to human health. It was guided by the following specific objectives:

1. To conduct a scoping review to generate the strength of evidence on effects of RA on crop nutrition.
2. To identify research gaps and weaknesses of current studies reporting on effects of RA and crop nutrition.
3. To encourage and recommend further research in RA and crop nutrition.

Methods

To review the evidence on whether RA approaches can improve crop micronutrient quality, search terms were developed following discussions between co-authors, to define the scope of this study. We based the review on the Web of Science (Clarivate) data base, using the publication period of 2000–2021 (searches conducted in October 2021). We considered different RA approaches as "input" terms, using a range of keywords linked with the "or" Boolean operator. We adopted the same approach for "Crop Type," based upon crops commonly consumed and used in food manufacturing. We considered crop micronutrient quality as an "output" term. These three terms were linked using the "AND" Boolean operator. The full search term string was:

("conservation agricultur" or "crop rotation" or "soil type" or "permaculture" or "agroforestry" or "agro-forestry" or "intercropping" or "inter-cropping" or "monoculture" or "mono-culture" or "agro-ecolog*" or "pixel farming" or "strip farming" or "soil microbial diversity" or "soil bacteria" or "soil fungi" or "mycorrhizal fungi" or "irrigation" or "fertigation" or "fertigation management" or "agroecolo*" or "manure" or "regenerative agricultur*" or "integrated soil fertility management" or "zero tillage" or "minimum tillage" or "soil organic matter" or "soil organic carbon") AND ("wheat" or "tomato" or "carrot" or "rice" or "onion" or "lentils" or "pulses" or "beans" or "cereal*" or "grain legumes") AND ("crop nutrient content" or "micronutrien*" or "vitami*" or "tocopherol" or "riboflavin" or "folate" or "zinc" or "iron" or "ferritin" or "magnesium" or "potassium" or "fibre" or "crop nutritional quality" or "selenium" or "calcium" or "beta carotene" or "ascorbic acid" or "iodine" or "mineral composition" or "trace elements").*

Truncated words (i.e., conservation agricultur*, agro-ecolog*, agroecolo*, agricultur*) were used to include words such as conservation agriculture, conservation agricultural practices, agro-ecology, agroecological region, agroecological zones, agroecologies, agriculture, agricultural practices, agricultural technologies, respectively. Articles were imported from Web of Science into Zotero (version 5.0.96.4; Roy Rosenzweig Center for History and New Media, 2016; www.zotero.org), using the RIS format which captured Author, Title, Source, Abstract and Meta Data (publication date, volume, and issue number, DOI, etc.). A wide literature search was based on three selection steps: (1) an initial search in which a set of RA-related agronomic practices and a crop micronutrient quality outcome was reported in the abstract or keywords of a paper in the Web of Science (Clarivate) data base, for a set of pre-determined crop types, for the period 2000–2021; (2) a manual review of all of the abstracts returned from this initial search, using the same criteria; (3) a manual assessment of the reported outcome of experimental studies conducted under "field" conditions, in which two or more treatment factors relevant to RA and a crop micronutrient quality measurement in the edible portions of the crop was reported. Each abstract was read by one of three co-authors (MGM-K, MRB and RML), with abstracts allocated according to the alphabetical position of the first author's name on the paper to avoid crop-specific biases. Abstracts that were considered within the scope of an RA approach were then copied into a Zotero sub-folder according to primary crop-type: "alliums" (*Allium* spp.), "maize," "other," "pulses" (*Fabaceae* family), "rice" (*Oryza sativa* L.), "tomato," "wheat." Other studies were placed into various "excluded" sub-folders.

Results

Screening output

A total of 4,463 papers were returned from this search (Figure 2; https://www.zotero.org/groups/4466584/unilever_regenerative_agriculture/library). Whilst these search terms were not comprehensive in terms of RA approaches, crop types, or nutritional quality metrics, the sample size was considered sufficiently representative to enable a robust analysis of the evidence. The first (non-conservative) screen of RA approaches and crop micronutrient quality resulted in 575 abstracts being retained (Figure 2; https://www.zotero.org/groups/4500243/crop_type_screening_for_unilever_regenerative_agriculture). These abstracts were then subjected to a second round of review (MGM-K and MRB). During this second round, we removed abstracts reporting non-RA approaches such as agronomic biofortification (see Discussion), fertigation, liming, and the use of contaminated/saline wastewaters and sewage sludges as the primary objective of the study, also studies whose primary focus was to reduce the transfers of contaminants (e.g., heavy metals) into crops. For tomato, we removed abstracts in which crops had been grown in hydroponics or other soil-less systems. This second round of review reduced the number of abstracts to 342 for further analysis; 341 of these were secured in full-paper .pdf format from library subscriptions or inter-library loans. Where data for more than one crop type was reported in a study, the study was copied across multiple crop sub-folders in Zotero, providing a total of 367 records.[2] The 367 records were combinations of RA agronomic approaches ["Organic Inputs" (including animal and green manures, cover crops, crop rotations, crop residues, composts, biochars; excluding those studies whose primary focus was on contaminated soils or amendments), "Tillage," "Intercropping," "Biostimulants" (e.g., AMF; plant

2 https://www.zotero.org/groups/4531670/unilever_scoping_analysis/library

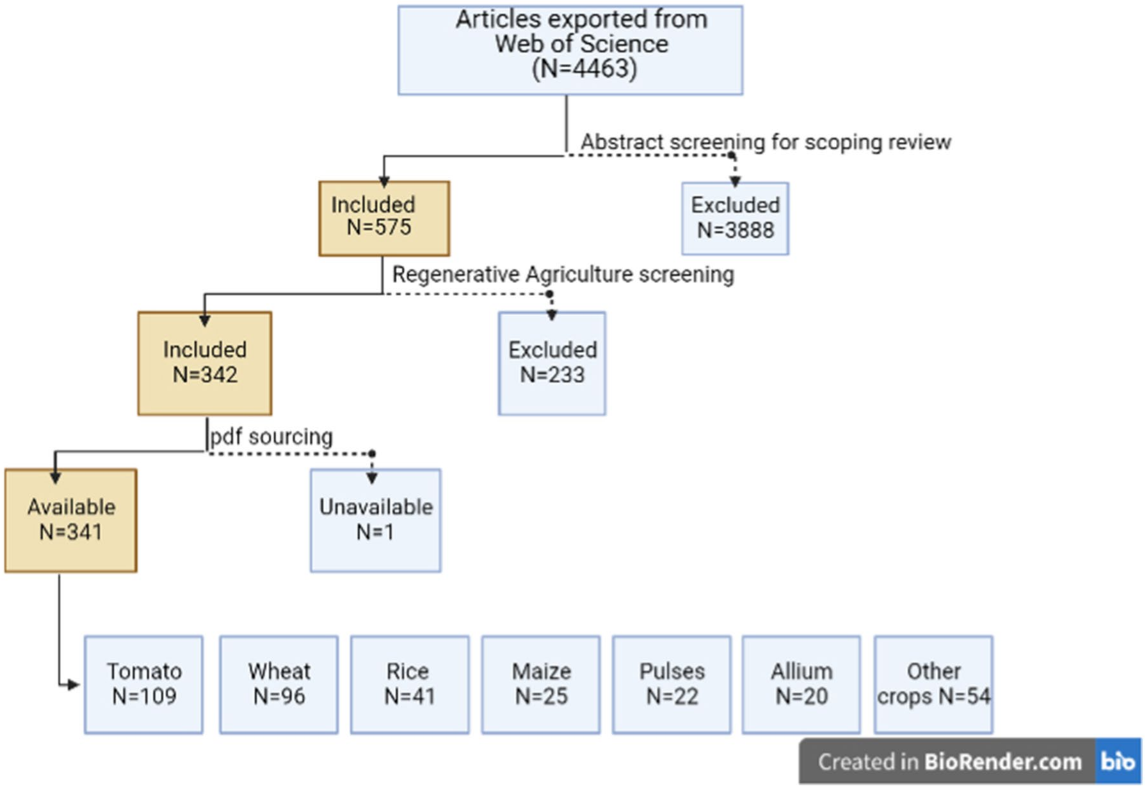

FIGURE 2
Flow chart on scoping review process. Dash line denotes exclusion. Created using BioRender (https://app.biorender.com/).

growth promoting bacteria, PGPB), and "Irrigation" (typically deficit-irrigation systems)]. The records were then allocated as: "tomato" (*n* = 109), "wheat" (*n* = 96), "rice" (*n* = 41), "maize" (*n* = 25), "pulses" (*n* = 22), "alliums" (*n* = 20), and "other" crop types (30 types, representing 54 studies (Figure 2).

A full review of these 367 records was conducted (EJMJ, MGM-K and MRB). We retained the same inclusion criteria and categorized the type of RA approach adopted as: "Organic Inputs" (including animal and green manures, cover crops, crop rotations, crop residues, composts, biochars; excluding those studies whose primary focus was on contaminated soils or amendments), "Intercropping," "Tillage," "Biostimulants" (e.g., AMF, PGPRs), and "Irrigation" (typically deficit-irrigation systems for horticultural crops; excluding studies whose primary focus was on contaminated/toxic/saline/wastewater irrigation). Studies whose primary focus was on experimental interventions outside of these five categories were excluded. Studies conducted in the field were included; studies conducted in pots or containers were excluded. For tomato, protected field crops were included when conducted in open soil environments. Studies reporting micronutrient concentrations in crop edible tissue were included; studies reporting micronutrient concentrations only in non-edible shoot tissues were excluded. Where possible, RA approaches were compared to "conventional" treatments which typically involved recommended applications of NP and potassium (K) mineral fertilizer. Studies were excluded if a statistical analyses of a "control" versus an "RA" input condition was not reported explicitly. Reviews and surveys were excluded, as were studies published in a language other than English.

The evidence for an effect of RA approach on the micronutrient concentration of the edible portion of the crop was recorded, for each micronutrient reported within a study, as: (1) evidence of a statistical significant increase (↑) or decrease (↓) in the micronutrient concentration of the crop, under one or more of the RA/control contrasts reported in the study; (2) no evidence of a statistical significant change in the micronutrient concentration of the crop, in any of the RA conditions reported in the study (↔); (3) evidence of both a statistical significant increase and a statistical significant decrease in the micronutrient concentration of the crop, under one or more RA conditions reported in the study (↕). If a study reported a statistical significant increase or decrease in the micronutrient concentration of the crop in a subset of treatment levels, or years, and no statistical significant changes in another subset of years or treatment levels, then this was recorded as evidence of a significant increase (↑) or decrease (↓).

Results are reported in order of numbers of studies reviewed in full for each crop type, i.e., tomato, wheat, rice, maize, pulses, alliums, and other crops.

Tomato

A total of 109 papers on tomato were read in full. There were 51 studies whose primary focus was on Organic Inputs; 35 studies on Irrigation; 10 studies on Biostimulants; 2 studies on Intercropping. Details of the 109 studies are given in Supplementary Table 1. The micronutrient with the greatest coverage among tomato studies is vitamin C/ascorbic acid, which was reported in 74 of the papers, followed by lycopene ($n = 34$), beta-carotene/carotenoids ($n = 21$), polyphenols/phenolics ($n = 3$), flavonoids ($n = 2$), and tocopherols (n = 2). Fewer tomato studies reported mineral micronutrient concentrations compared to vitamins and secondary nutrient compounds: Ca (n = 16), Mg (n = 12), Fe ($n = 8$), Zn ($n = 9$), Cu ($n = 7$), Mn ($n = 7$), Se ($n = 1$).

Fifty-two of the 109 studies on tomato were excluded from this scoping review: 28 pot studies (i.e., closed soil systems) which comprised: 11 Organic Inputs studies; 8 Irrigation studies; 6 Biostimulant studies; 2 non-RA studies. From tomato studies conducted in open soil ("field") conditions, which could be either protected (e.g., polytunnel) or non-protected systems, 10 papers were excluded due to being non-RA which comprised: 3 Irrigation studies based on physical interventions (e.g., installation of improved drainage systems); 3 conventional fertilizer studies; 2 wastewater studies; 1 seedling (pre-transplant) study; 1 study which used compositing to increase atmospheric carbon dioxide (CO_2) in a protected field system. A further 4 field studies were excluded due to no fruit micronutrients being reported (e.g., (34) and Asri (35), reported leaf/shoot micronutrients). A further 10 exclusions comprised: 2 studies compared organic and non-organic product batches, confounded by location; 4 review articles; 4 non-English language papers. Of the 57 included studies, Organic Inputs ($n = 29$), Irrigation ($n = 23$), Biostimulants ($n = 3$), Intercropping ($n = 2$), were represented among the RA approaches (Table 1).

Among the studies on tomato whose primary focus was Organic Inputs, there was some evidence that fruit vitamin C concentrations increased (Table 1; also see Supplementary Table 1); 11 studies reported an increase, 12 studies reported no significant change, and 2 studies reported a decrease. Among the (deficit) Irrigation studies on tomato, there was strong evidence that fruit vitamin C concentrations increased: 13 studies reported an increase, 2 studies reported no significant change, and 2 studies reported a decrease in fruit vitamin C concentrations. Among the studies on biostimulants (using PGPBs) on tomato, 2 studies reported an increase, and no studies reported a decrease, or a

TABLE 1 Studies reporting effects of regenerative agriculture on vitamin C concentration in tomato.

Primary regenerative ag. strategy reported	Micronutrient [evidence of direction of change, ↔,↑,↓,↕]	Number in included studies	References
Organic Inputs	Vitamin C [increase, ↑]	11	Yu et al. (36), Abduli et al. (37), Song et al. (38), Wang et al. (39), Dinu et al. (40), Özer (41), Jin et al. (42), Duan et al. (43), Zhang et al. (44), Guo et al. (45), Nabaei et al. (46)
	Vitamin C [decrease, ↓]	2	Petropoulos et al. (47), Huang et al. (48)
	Vitamin C [no significant change, ↔]	12	Tuzel et al. (49), Ece and Uysal (50), Polat et al. (51), Rady (52), Ceglie et al. (53), Majkowska-Gadomska (54), She et al. (55), Mukherjee et al. (56), Qahraman et al. (57), Rosa-Martinez et al. (58), Wu et al. (59), Turhan and Ozmen (60)
	Vitamin C [increases and decreases, depending on treatment, ↕]	0	n.a
Irrigation	Vitamin C [increase, ↑]	13	Chen et al. (61), Helyes et al. (62), Shao et al. (63), Abdel-Razzak et al. (64), Nangare et al. (65), Du et al. (66), Guida et al. (67), Wang et al. (68), Marti et al. (69), Cui et al. (70), Samui et al. (71), Al-Selwey et al. (72), Wu et al. (73)
	Vitamin C [decrease, ↓]	2	Helyes et al. (74), Turhan et al. (75)
	Vitamin C [no significant change, ↔]	2	Helyes et al. (76), Al-Harbi et al. (77)
	Vitamin C [increases and decreases, depending on treatment, ↕]	0	n.a
Biostimulants	Vitamin C [increase, ↑]	2	Tiyagi et al. (78), Le et al. (79)
	Vitamin C [decrease, ↓]	0	n.a
	vitamin C [no significant change, ↔]	0	n.a
	Vitamin C [increases and decreases, depending on treatment, ↕]	0	n.a
Intercropping	Vitamin C [increase, ↑]	1	Liu et al. (80), (with garlic)
	Vitamin C [decrease, ↓]	0	n.a
	Vitamin C [no significant change, ↔]	0	n.a
	Vitamin C [increases and decreases, depending on treatment, ↕]	1	Demir and Polat (81) (with lettuce)

n.a. = not applicable.

TABLE 2 Studies reporting effects of regenerative agriculture on carotenoids, flavonoids, lycopene, and phenolics contents in tomato.

Primary regenerative ag. strategy reported	Carotenoids, *Flavonoids, Lycopene and Phenolics [evidence of direction of change, ↔,↑,↓,↕]	Number in included studies	References
Organic Inputs	Carotenoids [increase, ↑]	2	Dinu et al. (40), Turhan and Ozmen (60)
	Carotenoids [decrease, ↓]	1	Ceglie et al. (53)
	Carotenoids [no significant change, ↔]	1	Rosa-Martinez et al. (59)
	Carotenoids [increases and decreases, depending on treatment, ↕]	0	n.a
	Lycopene [increase, ↑]	3	Wang et al. (39), Mukherjee et al. (56), Turham and Ozmen (68)
	Lycopene [decrease, ↓]	1	Petropoulos et al. (47)
	Lycopene [no significant change, ↔]	3	Wu et al. (58), Huang et al. (48), Rosa-Martinez et al. (59)
	Lycopene [increases and decreases, depending on treatment, ↕]	0	n.a
Irrigation	Carotenoids [increase, ↑]	1	
	Carotenoids [decrease, ↓]	0	n.a
	Carotenoids [no significant change, ↔]	1	
	Carotenoids [increases and decreases, depending on treatment, ↕]	0	n.a
	Lycopene [increase, ↑]	7	Helyes et al. (82), Helyes et al. (62), Helyes et al. (83), Pék et al. (84), Turhan et al. (75), Du et al. (66), Samui et al. (71)
	Lycopene [decrease, ↓]	1	Liu et al. (85)
	Lycopene [no significant change, ↔]	4	Helyes et al. (74, 76), Martí et al. (56), Wu et al. (73)
	Lycopene [increases and decreases, depending on treatment, ↕]	1	Wang et al. (68)
	Phenolics [increase, ↑]	4	Helyes et al. (74), Helyes et al. (62), Helyes et al. (83), Pék et al. (84)
	Phenolics [decrease, ↓]	0	n.a
	Phenolics [no significant change, ↔]	0	n.a
	Phenolics [increases and decreases, depending on treatment, ↕]	0	n.a
Biostimulants	Carotenoids [increase, ↑]	0	n.a
	Carotenoids [decrease, ↓]	0	n.a
	Carotenoids [no significant change, ↔]	0	n.a
	Carotenoids [increases and decreases, depending on treatment, ↕]	1	Le et al. (79)
	Lycopene [increase, ↑]	0	n.a
	Lycopene [decrease, ↓]	0	n.a
	Lycopene [no significant change, ↔]	0	n.a
	Lycopene [increases and decreases, depending on treatment, ↕]	1	Le et al. (79)

*Increase [↑] in flavonoids reported in one irrigation study (83). n.a. = not applicable.

non-significant change in fruit vitamin C concentrations. Among the Intercropping studies on tomato, 1 study reported an increase, and 1 study reported significant increases and decreases in fruit vitamin C concentrations within the same study (81).

For fruit lycopene, 3 Organic Inputs studies reported an increase in concentrations; 3 studies reported no significant changes; 1 study reported a decrease (Table 2). Among Irrigation studies on tomato, 7 reported an increase in fruit lycopene concentrations; 4 studies reported no significant changes; 1 study reported a decrease; 1 study reported significant increases and decreases in fruit lycopene concentrations within the same study. Among the Biostimulants studies (PGPBs) on tomato, 1 reported both significant increases and decreases in fruit lycopene concentrations within the same study (79).

For fruit beta-caroteine/carotenoids, 3 Organic Inputs studies reported an increase in concentrations; 1 study reported no significant changes; 2 studies reported a decrease (Table 2). Among the Irrigation studies on tomato, 1 study reported an increase in fruit beta-caroteine/carotenoids concentrations; 4 studies reported no significant changes; no studies reported a decrease. Among the Biostimulants studies (PGPBs) on tomato, 1 reported significant increases and decreases in fruit beta-caroteine/carotenoids concentrations within the same study (79). For fruit tocopherols and polyphenols/phenolics, there was one study under Organic Inputs, for each nutrient, that reported an increase in fruit concentrations. Among the Irrigation studies on tomato, 1 reported significant increases and decreases in fruit tocopherol concentrations within the same study (74); 4 studies reported increases in fruit polyphenols/phenolics concentrations; 1 study reported increases in fruit flavonoid concentrations (83).

There were many fewer studies on tomato fruit mineral micronutrient concentrations under RA approaches than for secondary micronutrients, all of which were in the Organic Inputs category. For fruit Fe and Zn concentrations, 2 studies reported an increase (51, 86); 1 study reported no significant changes; no studies reported a decrease in either micronutrient. For fruit Ca concentration, 2 studies reported an increase; 2 studies reported no significant changes; 1 study reported a decrease. For fruit Mg concentration, increases were reported in 1 study; 2 studies reported no significant changes; no studies reported a decrease. There was 1 study which reported an increase in fruit Se concentration (48), and one study which reported an increase in fruit copper (Cu) concentration (51).

Wheat

A total of 96 papers on wheat were read. There were 43 studies whose primary focus was on Organic Inputs; 10 studies on Biostimulants; 5 studies on Tillage; 1 study on Intercropping; and 6 Surveys. There were 4 studies reporting other non-RA techniques. Details of the 96 reviewed studies are given in Supplementary Table 2.

Fifty-two of these 96 studies were excluded from this scoping review: 13 pot studies; 6 survey studies; 4 non-RA field studies; 19 studies in which only micronutrient concentration data for non-edible portions were reported; 2 studies not in English language; 1 conference abstract; 1 review paper; 2 studies with no quantitative data; 1 study with no comparator for the RA treatment; 1 study not on wheat; 2 studies with potassium data only. Of the 44 included studies, Organic Inputs ($n=34$), Biostimulants ($n=6$), Intercropping ($n=1$), and Tillage ($n=5$) were represented among the RA approaches (Supplementary Table 2), noting that Woźniak (87) tested Organic Inputs and Tillage treatments while Shivay et al. (88) tested Organic Inputs and Biostimulants approaches.

Among the 34 studies on wheat whose primary focus was Organic Inputs and reporting grain mineral concentrations, there was some evidence for increases in grain micronutrients. For Zn, 11 studies reported increases in grain Zn concentration; 3 studies reported decreases in grain Zn concentration; 8 studies did not report a significant change in grain Zn concentration; 1 study reported significant increases and decreases in grain Zn concentration (89). Increases in grain Zn concentration were attributed to co-application of Organic Inputs with crop residues and mineral N fertilizer [i.e., urea-CH_4N_2O; e.g., (90, 91)]. Decreases in grain Zn concentration were reported when organic farming management followed grass/clover as a pre-crop (92) and under long term biochar application (93). Gondek (89) reported an increase in grain Zn concentration of wheat when sewage sludge was applied and a decrease in grain Zn concentration when swine farmyard manure and compost from plant and biodegradable waste were applied in the 3rd year of experimentation. For Fe, 4 studies reported increases in grain Fe concentration; 2 studies reported decreases in grain Fe concentration; 12 studies did not report a significant change in grain Fe concentration. For Mg, 2 studies reported increases in grain Mg concentration; 2 studies reported decreases in grain Mg concentrations; 4 studies did not report a significant change in grain Mg concentration. For Ca, 1 study reported increases in grain Ca concentrations; no studies reported decreases in grain Ca concentration; 5 studies did not report a test of significance or reported no significant changes in grain Ca concentration.

Among the 6 studies on wheat whose primary focus was Tillage, there was no evidence for changes in grain Zn and Fe concentrations. For Zn, 0 studies reported increases in grain Zn concentrations; 0 studies reported decreases in grain Zn concentrations; 2 studies did not report a test of significance or reported no significant changes in grain Zn concentration; and 1 study reported significant increases and decreases in grain Zn concentration (87). For Fe, 0 studies reported increases in grain Fe concentrations; 0 studies reported decreases in grain Fe concentrations; 4 studies did not report a test of significance or reported no significant changes in grain Zn concentration.

Among the 6 studies on wheat whose primary focus was Biostimulants, there was some evidence for increases in grain Zn concentrations but mixed evidence for Fe. For Zn, 4 studies reported increases in grain Zn concentrations; 0 studies reported decreases in grain Zn concentrations; 2 studies did not report a test of significance or reported no significant changes in grain Zn concentration. For Fe, 2 studies reported increases in grain Fe concentration; 1 study reported decreases in grain Fe concentration; 1 study did not report a test of significance or reported no significant changes in grain Fe concentration.

Rice

A total of 41 papers on rice were read. There were 25 studies whose primary focus was on Organic Inputs; 9 studies on Irrigation; 2 studies on Biostimulants; 1 study on Tillage; 2 Surveys. There were 2 studies reporting other non-RA techniques. Details of the 41 reviewed studies are given in Supplementary Table 3.

18 of these 41 studies were excluded from this scoping review: 7 pot studies; 2 surveys; 3 non-RA field studies; 3 field studies in which rice grain micronutrient concentration data were not reported; 3 field studies in which only micronutrient

concentration data for non-edible portions of rice were reported. Of the 23 included studies for rice, Organic Inputs ($n = 16$) and Irrigation ($n = 7$) were represented among the RA approaches (Supplementary Table 3).

Among the 16 studies on rice whose primary focus was Organic Inputs, there was strong evidence for increases in grain Zn and Fe concentration. For Zn, 14 studies reported increases in grain Zn concentration; 1 study reported no significant changes in grain Zn concentration (94); 1 study reported significant increases and decreases in grain Zn concentration (95). For Fe, 5 studies reported increases in grain Fe concentration; 2 studies reported decreases in grain Fe concentration; 3 studies reported significant increases and decreases in grain Fe concentration [e.g. (96), who also reported increases and decreases in grain Ca and Mg concentration under different treatments].

Among the 7 studies on rice whose primary focus was Irrigation (deficit techniques, including alternate wetting and drying, AWD), the results were less conclusive than for studies on Organic Inputs. For Zn, 3 studies reported increases in grain Zn concentration; 2 studies reported decreases in grain Zn concentration; 1 study reported no significant changes in grain Zn concentration (97). For Fe, 3 studies reported decreases in grain Fe concentration, and none reported an increase. For Se, 1 study reported an increase in grain Se concentration (98) and 1 study reported a decrease in grain Se concentration (99).

Maize

A total of 25 papers on maize were read. There were 11 studies whose primary focus was on Organic Inputs; 6 studies on Intercropping; 2 studies on Tillage; 1 Survey. There were 5 studies reporting other non-RA techniques. Details of the 25 reviewed studies are given in Supplementary Table 4.

Fifteen of these 25 studies were excluded from this scoping review: 5 pot studies; 1 survey study; 4 non-RA field studies; 2 field study in which micronutrient concentration data were not reported; 3 field studies in which only micronutrient concentration data for non-edible portions were reported. Of the 10 included studies, Organic Inputs ($n = 4$), Intercropping ($n = 5$), and Tillage ($n = 1$) were represented among the RA approaches (Supplementary Table 4).

Among the 4 included studies on maize whose primary focus was Organic Inputs, there was evidence for significant increases in mineral and vitamin micronutrient concentration (Supplementary Table 4). For Fe and Zn, all 4 studies reported increases in grain concentration. One of these studies also reported an increase in grain Ca and Mg concentrations (100). Another of these studies, on baby sweetcorn, reported an increase in vitamin C concentration (101).

Among the 5 included studies on maize whose primary focus was Intercropping, two studies reported an increase (102–104) and one study reported a decrease (105) in grain Fe concentration. One study reported an increase (102, 103), and two studies reported a decrease (102, 103, 105), in grain Zn concentration. One study reported an increase in grain Ca concentration and a decrease in grain Mg concentration under intercropping (106). In the Tillage study, there was no significant change in grain Zn concentration (107).

Pulses

Of the 22 studies read which included a pulse crop, there were 7 studies on *Phaseolus vulgaris* (common bean, snap bean, pinto bean, green/yellow bean), 5 studies on *Arachis hypogea* (peanut, groundnut), 3 studies on *Pisum sativum* (pea), 2 studies on *Vigna unguiculata* (cowpea), and 2 studies on *Vicia faba* (faba bean). There were 12 studies whose primary focus was on Organic Inputs; 7 studies on Intercropping; 1 study on Biostimulants; 1 study on Tillage; 1 Survey. Details of these 22 reviewed studies are given in Supplementary Table 5.

Fifteen of these studies were excluded from this scoping review: 4 pot studies; 1 review; 1 variety trial (cowpea) on reduced tillage; 1 field study with no control comparison; 1 field study in which micronutrient concentration data were not reported; 7 field studies in which only micronutrient concentration data for non-edible portions were reported. Of the 7 included studies, Organic Inputs, Intercropping, and Biostimulants were represented among the RA approaches (Supplementary Table 5).

Among the 5 included studies on pulse crops whose primary focus was Organic Inputs (Supplementary Table 5), 2 studies reported an increase in mineral micronutrient concentration (copper-Cu; Fe; manganese-Mn; Zn), whereas 3 studies reported no significant changes for the concentration of these mineral micronutrients; one of these studies also reported no significant changes for vitamin C concentration. There was no evidence of decreased concentration of micronutrients in seeds of pulses under Organic Inputs. The 1 pulse study on Intercropping (pea with oat; *Avena sativa*) that was included reported no significant changes in seed Ca or Mg concentration. The 1 pulse study whose primary focus was Biostimulants (AMF inoculations; Supplementary Table 5) reported an increase in vitamin C concentration.

Alliums

Of the 20 studies read which included an *Allium* crop, there were 13 studies on onion, 3 studies on garlic, 2 studies on leek, 1 study on shallots, and 1 study which reported micronutrient data for both onion and garlic. There were 14 studies whose primary focus was on Organic Inputs; 2 studies on Biostimulants; 1 study on Intercropping; 1 study on Irrigation; 1 study on Fertigation; 1 Survey. Details of these 20 reviewed studies are given in Supplementary Table 6.

Eight of these 20 studies were excluded from this scoping review: 1 pot study; 1 survey study; 1 fertigation study; 2 field studies in which micronutrient concentration data were not reported; 1 field study in which only micronutrient concentration data for non-edible portions were reported; 1 field study lacking a non-regenerative control; 1 non-English language study. Of the 12 included studies, only Organic Inputs and Biostimulants were represented among the RA approaches (Supplementary Table 6).

Among the 10 included studies on *Allium* crops whose primary focus was Organic Inputs (Supplementary Table 6), three studies reported an increase in vitamin C concentration in their edible portions, whereas two studies reported no significant changes. One *Allium* crop study reported an increase in mineral micronutrient

TABLE 3 Species of other crops represented in studies included in the scoping review.

Species	Number of studies
Cereal crops	
Barley (*Hordeum vulgare*)	4
Oat (*Avena sativa*)	2
Pearl millet (*Pennisetum glaucum*)	3*
Sorghum (*Sorghum bicolor*)	3
Non-cereal crops	
Acacia mearnsii	1
Broccoli (*Brassica oleracea* var. *italica*)	1
Brussels sprout (*Brassica oleracea var. gemmifera*)	1
Cabbage (*Brassica oleracea var. capitata*)	5
Carrot (*Daucus carota*)	11
Cauliflower (*Brassica oleracea var. botrytis*)	3
Cocoyam (*Colocasia esculent*)	1
Cucumber (*Cucumis sativus*)	1
Elephant foot yam (*Amorphophallus paeoniifolius*)	1
Ice plant (*Mesembryanthemum crystallinum*)	1
Jute mallow (*Corchorus olitorius*)	1
Korean ginseng (*Panax ginseng*)	1
Lettuce (*Lactuca sativa*)	7
Melon (*Cucumis melo*)	1
Mulberry (*Morus alba*)	1
Mustard (*Sinapis alba*)	1
Oilseed rape (*Brassica napus*)	2
Pepper (*Capsicum annuum*)	5
Potato (*Solanum tuberosum*)	4
Red clover (*Trifolium pratense*)	1
Squash (*Cucurbita*)	1
Strawberry (*Fragaria × ananassa*)	1
Sunflower (*Helianthus annuus*)	1
Sweet cherry (*Prunus avium*)	1
Sweet potato (*Ipomoea batatas*)	1
Various species (one temporal survey and a review)	2

*Pearl millet counted twice in Bana et al. (108), under organic inputs and intercropping.

concentrations, but five studies reported no significant changes and two reported a decrease in mineral micronutrient concentrations. One *Allium* crop study reported an increase in flavonoid and phenolic concentrations, and one reported no significant changes. Among the three included studies on *Allium* crops whose primary focus was Biostimulants (AMF inoculations; Supplementary Table 6), one study reported an increase in vitamin C concentration and two studies reported no significant changes. All three *Allium* crop studies whose primary focus was Biostimulants (AMF) reported an increase in one or more mineral micronutrient concentrations.

Other crops

Of the 54 papers read which included 'other', crop types, the most common horticultural crop types were carrot (*Daucus carota*; $n=11$), lettuce (*Lactuca sativa*; $n=7$), pepper (*Capsicum annuum*; $n=6$), cabbage (*Brassica oleracea* var. *capitata*; $n=5$), and potato (*Solanum tuberosum*; $n=4$). The most common cereal crop types were barley (*Hordeum vulgare*; $n=4$), then sorghum (*Sorghum bicolor*; $n=3$) and pearl millet (*Pennisetum glaucum*; $n=3$). The number of species in this category are given in Table 3; these add to 69 studies because some of the 54 papers included multiple crop types. There were 44 studies whose primary focus was on Organic Inputs; 14 studies on Intercropping; 6 studies on Biostimulants; 3 studies on Irrigation; 2 studies on Fertigation. Details of these 69 reviewed studies are given in Supplementary Table 7.

Thirty-nine of the 69 studies were excluded from this scoping review: 7 non-RA studies; 17 pot studies; 2 field studies in which micronutrient data were not reported; 8 field studies in which only micronutrient concentration data for non-edible portions were reported; 2 survey studies; 1 review paper; 1 non-English language study; 1 study could not be accessed through library (109). Of the 30 included studies, Organic Inputs ($n=21$), Intercropping ($n=6$), Irrigation ($n=2$), and Biostimulants ($n=1$) were represented among the RA approaches (Supplementary Table 7).

Among the 21 included studies on Other Crops whose primary focus was Organic Inputs, there was little evidence for significant changes in mineral or vitamin micronutrient concentration (Supplementary Table 7). For Fe and Zn, 8 studies reported no significant changes in concentration. One study (pearl millet) reported an increase in Fe and Zn concentration (108) and 1 study (pepper) reported an increase in Zn concentration (110). One study (barley) reported a decrease in Fe concentration (111). For Ca and Mg, there were no increases in concentration in 13 and 11 studies, respectively; 1 of these Ca studies (carrot) reported a decrease in Ca concentration (112). For vitamin C, there were no significant changes in concentration in 6 studies; 1 study (strawberry) reported an increase in concentration (113). For beta-carotene, there were no significant changes in concentration in 2 studies.

Among the 6 included studies on Other Crops whose primary focus was Intercropping, 1 study (pearl millet) reported an increase in Fe and Zn concentration (108); 1 study (cabbage) reported no significant changes in Fe concentration (114). For Ca and Mg, 1 study (carrot) reported an increase in concentration (115); 1 study (oat) reported a decrease in concentration (116) and 1 study (cabbage) reported no significant changes in concentration (114). For vitamin C, one study (lettuce) reported significant increases and decreases in concentration within the Intercropping study (81). The two included Irrigation studies on Other Crops, whose primary focus was Intercropping, had no effect on carotenoid concentration. The one included Biostimulant study (potato) showed an increase in vitamin C concentration.

Summary of results by regenerative agriculture practice and crop type

To summarize the results from this scoping review, we focused on Zn, Fe, and vitamin C, as examples of mineral and secondary micronutrients with the greatest coverage. For each combination of crop and RA category, we considered the strength of the evidence on the following arbitrary categories based on the proportion of studies that showed a significant increase (or decrease) in micronutrient content in a RA treatment vs. control: >66% = "Good evidence"; 33–66% = "Some evidence"; <33% = "Little/no evidence." This evidence is summarized and presented for any increase and/ or decrease in Zn, Fe and vitamin C in all crop types in Table 4. Percent change values (evidence of increase or decrease in a nutrient) for different crops was calculated by dividing the total number of studies reporting a significant increase (or decrease) in a particular nutrient by the total number of studies reporting the nutrient within a particular RA approach (i.e., Organic Inputs). This value was then multiplied by 100 (Excel Supplementary file).

$$\% \ change = \frac{\begin{array}{c} Number\ of\ studies\ reporting\ a\ significant \\ increase\ or\ decrease\ in\ a\ nutrient \end{array}}{\begin{array}{c} Total\ number\ of\ studies\ reporting\ the \\ nutrient\ within\ a\ particular\ RA\ approach \end{array}} x\,100.$$

Evidence of increases in the micronutrient concentrations of crop edible portions under different categories of RA practices

Organic Inputs

All crop types were represented in this category of RA practice, which comprised a wide range of organic inputs including the use of animal and green manures, crop rotations, cover crops, and various composts; typically compared to a control of conventional mineral fertilizer inputs. There is good or some evidence that the use of organic inputs increases Zn concentration in the grains/seeds of most field crops (i.e., rice = 94% of studies, wheat = 48%, maize = 100%, pulses = 40%; Table 4). Grain Fe concentration increased in 80% of rice studies, 22% of wheat studies, 100% of maize studies and 33% of studies on pulses. Similarly, some evidence of organic inputs effect on increasing Zn and Fe concentration in tomato was reported (67%). There is little/no evidence that organic inputs increase Zn concentration in the edible portions of alliums (20%), or other crops (18%). Similar evidence was reported for Fe concentration in alliums (20%) and other crops (10%). There is little/no evidence of organic inputs decreasing Zn or Fe with the exception of rice where a decrease in grain Fe concentration was reported in 50% of the studies, some of which focused on effects of poultry and vermicompost and 17-years continuous application of pig manure and straw on rice grain Fe concentration. There is some evidence that the use of organic inputs increases vitamin C concentration in the edible portions of wheat grown in rotation with a legume (100%, $n = 1$ study), tomato grown with biochar, mulch and vermicompost (44%) and alliums grown with green and animal manure (60%), with little/no evidence reported in other crops (14%). There is little/no evidence of any decreases in the vitamin C concentration in tomato or other crop types, noting there are few studies reporting vitamin C concentration under organic inputs for other crop types.

Irrigation

Rice, tomato, and other crops were represented in this category, which generally focused on reduced/deficit irrigation and alternate wetting and drying (AWD) compared to conventional practices. Altered irrigation is likely to have large effects on soil nutrient availability due to changes in redox conditions and associated changes in pH and other geochemical properties. There is some evidence that alternative irrigation strategies increased (50%) or decreased (33%) the Zn concentration in the edible portion of rice, which was the only crop represented in this category (Table 4). In contrast, good evidence of decrease in grain Fe concentration (100%) was reported. This was based on findings from three studies reporting grain Fe concentration in rice grown under alternative irrigation strategies. There is good evidence that alternative irrigation strategies increase the fruit concentration of vitamin C in tomato (76%), which was the only crop represented in this category. There is little/no evidence that alternative irrigation practices decrease the fruit concentration of vitamin C concentration in tomato (12%).

Tillage

Wheat and maize were the only crops represented in this category, which focused on reduced tillage practices compared to a conventionally tilled control. There is some evidence that alternative tillage strategies employed over 2 years, increased Zn concentration in the grains of wheat (33%) with little/no evidence reported in maize (0%; Table 4) grown under a zero-tilled field over a 17-year period. Similarly, there was some evidence that alternative tillage strategies decreased Zn concentration in the grains of wheat (33%) with no evidence reported in maize (0%). Contrary to tillage effects on Zn, no evidence of such effects was reported in all four studies measuring grain Fe concentration in wheat (evidence of 0%). None of the tillage studies reported grain vitamin C concentrations.

Biostimulants

All crops, except for rice and maize, were represented in this category of RA practices, which typically comprised AMF, PGPBs, bacterial strains (*Bacillus*, *Pseudomonas*, *Arthrobacter*, and *Azotobacter* species) and amino acids, although the number of studies captured in this review are small. There is some evidence that biostimulants increased Zn concentration in the edible portions of wheat (67%) and alliums (33%) with similar evidence of 60 and 67% increase in Fe concentration reported in wheat and alliums, respectively (Table 4). A 20% evidence of decrease in wheat grain Fe concentration with use of biostimulants was reported. No studies measured the effect of biostimulants on Zn or Fe concentrations in the edible portions of tomato, pulses, or other crops. There is good evidence that biostimulants increased the concentration of vitamin C in the edible portions of tomato (100%), pulses (100%), and other crops (potato; 100%), with some evidence of this effect in alliums (33%). There is little/no evidence that biostimulants decreased the vitamin C concentration in the edible portions of different crop types, in any crop type.

Intercropping

There is good or some evidence that intercropping increased the grain Zn concentration of maize (33%) and other crops (100%; Table 4). There was no evidence of intercropping effects on increasing

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TABLE 4 Percentage of studies reporting increases (and decreases) in zinc, iron and vitamin C with respect to a specific Regenerative Agriculture practice.

	Regenerative practice	i. Evidence of increase (%)							ii. Evidence of decrease (%)						
		Wheat	Rice	Tomatoes	Alliums	Pulses	Maize	Other crops	Wheat	Rice	Tomatoes	Alliums	Pulses	Maize	Other crops
Zinc															
	Organic inputs	48 (12/25)	94 (15/16)	67* (2/3)	20* (1/5)	40* (2/5)	100* (4/4)	18 (2/11)	16 (4/25)	6 (1/16)	0	0	0	0	0
	Irrigation		50 (3/6)							33 (2/6)					
	Biostimulants	67 (4/6)			33* (1/3)				0			0			
	Intercropping	0					33* (1/3)	100* (1/1)	0					67* (2/3)	0
	Zero tillage	33* (1/3)					0		33* (1/3)					0	
Iron															
		Wheat	Rice	Tomatoes	Alliums	Pulses	Maize	Other crops	Wheat	Rice	Tomatoes	Alliums	Pulses	Maize	Other crops
	Organic inputs	22 (4/18)	80 (8/10)	67* (2/3)	20* (1/5)	33* (1/3)	100* (4/4)	10 (1/10)	11 (2/18)	50 (5/10)	0	20 (1/5)	0	0	10 (1/10)
	Irrigation		0							100* (3/3)					
	Biostimulants	60* (3/5)			67* (2/3)				20* (1/5)			0			
	Intercropping	100* (1/1)					67* (2/3)	50* (1/2)	0					33* (1/3)	0
	Zero tillage	0							0						
Vit. C															
		Wheat	Rice	Tomatoes	Alliums	Pulses	Maize	Other crops	Wheat	Rice	Tomatoes	Alliums	Pulses	Maize	Other crops
	Organic inputs	100* (1/1)		44 (11/25)	60* (2/5)			14 (1/7)			8 (2/25)				
	Irrigation			76 (13/17)							12 (2/17)				
	Biostimulants			100* (2/2)	33* (1/3)	100* (1/1)		100* (1/1)							
	Intercropping			100* (2/2)				50* (1/2)			50* (1/2)				50* (1/2)
	Zero tillage														

Key:
1. Vit. C = Vitamin C.
2. Orange = Some evidence/inconclusive (33-66%).
3. Blue = Good evidence for positive effects (>66%).
4. Gray = Little/No evidence (<33%).
5. Numbers in parenthesis denote the number of studies reporting an increase/decrease over the total number of studies reporting the nutrient within a specific Regenerative Agriculture (RA) practice.
6. * denotes small number of studies (i.e., <6).
7. Blank = Significant research gap.
8. Cells are blank where no data were identified for the nutrient-RA practice combination.
9. Values are "0" where studies reported no increase or decrease in nutrient concentration.
10. Figures are percentages of studies showing a significant increase and/or decrease in the nutrient. Percentages may sum to >100 because some studies reported significant increases and decreases for the same nutrient-RA practice combination, e.g. at different sites, or for different specific treatments.

grain Zn concentration in wheat (0%). Good evidence of intercropping effects on increasing grain Fe concentration was reported in wheat (100%) and maize (67%) with some evidence of 50% reported in other crops. No studies measured effects of intercropping on the concentration of Zn or Fe in the edible portions of the other specific crop types. There is little/no evidence that intercropping decreased Zn or Fe concentrations in the edible portions of wheat and other crop types, but good or some evidence in maize Zn concentration (67%) and Fe concentration (33%) was reported. There is good evidence that intercropping increased the vitamin C concentration in the edible portions of tomato (100%) and other crops (50%). There is some evidence that intercropping decreased the vitamin C concentration in the edible portions of tomato (50%) and other crops (50%). No studies measured effects of intercropping on vitamin C concentrations in the edible portions of the other specific crop types.

Discussion

Agronomic approaches have a role to play in crop nutrition

Findings from this scoping review showed some good evidence of increasing micronutrient concentration in crops with agronomic approaches encompassing RA. Organic inputs improved nutritional composition of crops and similarly biostimulants and intercropping, albeit based on small numbers. Examples of biostimulants with beneficial influence on crop nutrition from this study included a microbial consortium of AMF *Glomus intraradices* BEG72, *Glomus mossae* and *Trichoderma atroviride* MUCL 45632 and bacterial strains of the *Bacillus Pseudomonas* and *Arthrobacter* sps (88, 117). Evidence of increases in micronutrient concentration due to biostimulants were alluded to enhanced mycorrhizal colonization with bacterial inoculation, and improved seedling establishment (including crop rooting and vigor) which enhances micronutrient uptake (117). Intercropping increased Zn, Fe and vitamin C concentration in edible portions of cereals (maize and wheat), tomatoes and other crops. This is due to increased nutrient availability from release of compounds (including siderophores) from graminaceous cereals crops which consequently benefits the neighboring non-graminaceous crop (11, 12). Alternatively, increases in micronutrients in intercropping systems could be attributed to improved soil structure from the leguminous crop which improves retention/plant availability of micronutrients. Alternative tillage strategies employed over 2-years improved grain Zn concentration of wheat (118). However, implementation of alternative tillage strategies over a longer period did not have any effects on grain Zn concentration of wheat and maize, although significant effects were reported for heavy metals (107). Future studies could focus on effects of long-term tillage strategies (often accompanied by residue incorporation) on soil physio-chemical properties such as P accumulation, which might negatively influence uptake of essential micronutrients including Zn. Fewer studies on the effects of deficit irrigation strategies on Zn or Fe concentrations in the edible portions of most crops were reported in this study. However, there was some evidence of increases in grain Zn, and decreases in grain Fe concentration, in rice, likely reflecting complex effects of soil water content on soil micronutrient availability. Future studies could focus on impacts of irrigation strategies (i.e., alternate wetting and drying) complimented with increased organic inputs on micronutrient concentration, especially Fe which decreased in 100% of the studies.

Strengths and weaknesses of this scoping review study

We consider that a strength of this study is the scope of the literature search, and the large numbers of papers returned during stage 1 of the search ($n = 4,463$). The choice of crop types and micronutrients included in the search were made following discussions between co-authors, representing academic and private sectors, but these terms were established *a priori* to avoid any further selection bias arising. We therefore consider this scoping study to be representative, notwithstanding the potential for selection bias in terms of the original publications.

A general weakness of this study is that we do not report the magnitude of the effects of different RA practices on crop nutritional quality for each study, nor have we used formal meta-analysis techniques. There are two main reasons: (1) for each combination of crop and RA category, the number of studies is generally small, with many studies being underpowered to detect relatively small effect sizes, and (2) where the number of studies in a category is larger (e.g., tomato under deficit irrigation; wheat with organic inputs), the diverse conditions between studies, including input types, and landscape/soil factors, mean that direct comparisons of studies would have little value. These weaknesses are discussed further in the following section.

Potential underpowering of studies in terms of detecting small effect sizes

Relatively small improvements in the micronutrient quality of staple crops can be impactful in terms of micronutrient provisioning in food systems (14). However, effect sizes and the statistical quality of each study were not assessed within this scoping review. Notably, we did not see a power analysis reported in any of the studies. However, many studies will be underpowered to detect small effect sizes due to a small number of replications. When nutritional outcome indicators are reported from human/animal studies, a power analysis/registered trial report would be an ethical requirement of any trial design.

Two studies have recently discussed the issues of small effect sizes in terms of nutritional quality responses of crops under different agronomic treatments, even when large amounts of a micronutrient are added in the form of Zn fertilizers (119, 120). For example, in Pakistan, Figure 3A illustrates that ~8 replicates would be needed to detect a 25% effect size in terms of wheat grain Zn concentration, with an ~80% experimental power, when Zn fertilizers are applied to soils. In Malawi, Figure 3B illustrates that ~10 replicate blocks, would be needed to detect a 10% difference in maize grain Zn concentration at a similar power, again, when Zn fertilizers are applied to soils. Notably, these studies were based on data from trials conducted in research

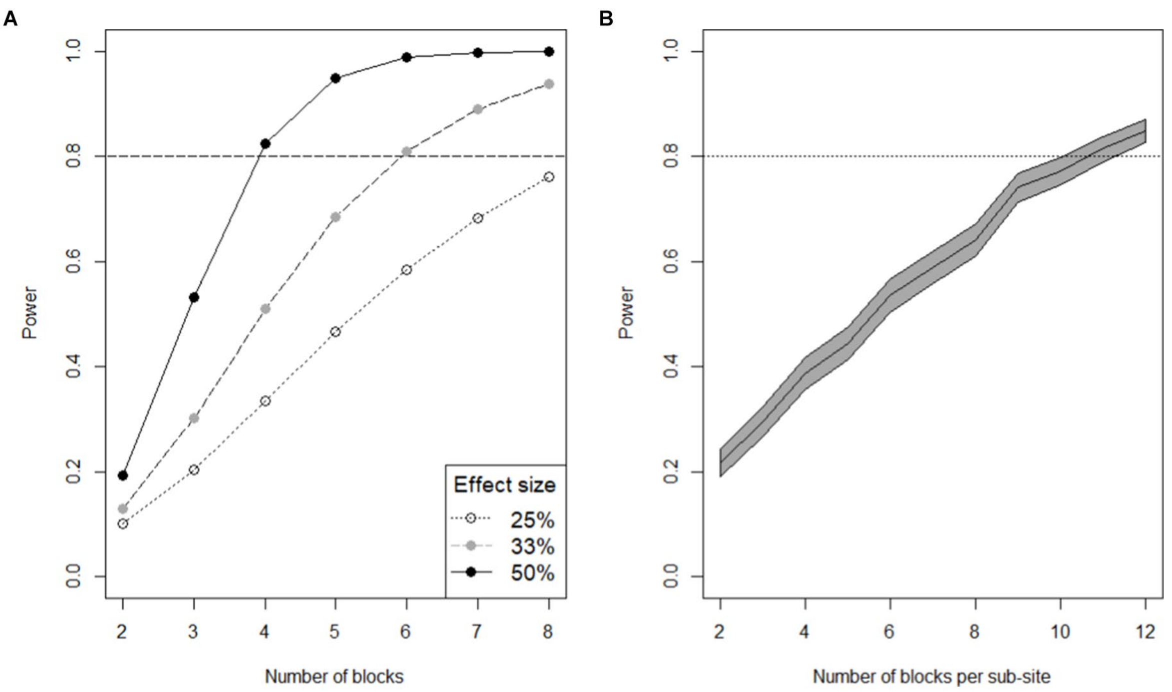

FIGURE 3
(A) Power analysis for a simple control/treatment experiment for an effect size of 25, 33% or 50% under soil-applied Zn fertilizers. Data are based on a treatment mean wheat grain Zn concentration of 36.9 mg kg^{-1} and a residual mean square of 35.1, as observed at a site in Pakistan; Zia et al. (120).
(B) Power to detect a 10% effect size of soil-applied Zn fertilizer treatment on maize grain Zn concentration in Malawi; Botoman et al. (119). The grey band shows the 95% confidence interval for estimated power to detect fertilizer effects for differing numbers of blocks per sub-site (i.e., replicates). The central line is the estimated power (119).

station settings. For on-farm type designs, influenced by additional landscape covariates including soil type, climate, etc., appropriate replications for RA interventions may need to be larger, especially given the magnitude of an RA intervention on grain quality is often likely to be smaller than direct application of micronutrient fertilizers (14).

The challenges of comparing crop responses across different environments and experimental conditions

Recent studies have reported large geographical differences in the micronutrient concentration (including Zn and Fe) of staple cereal grains, which is important in the context of generating robust evidence on the influence of RA approaches on crop nutritional quality. For example, in the Amhara region of Ethiopia, there was spatially correlated variation in cereal grain Zn concentration with wheat grain Zn concentration varying by more than 30% (~20–27 mg/kg) between districts (121). In Malawi, maize grain Zn concentration showed similar evidence of spatially correlated variation (27). Some of this variation was associated with soil and environmental covariates. What this means is, beyond localized sources of variation (e.g., crop variety, agronomic practices, etc.), the geographical location of a household can sometimes be the largest factor influencing the dietary intake of Zn from cereals. Combining outputs of experiments involving RA approaches, across widely different geographical locations and without considering geospatial variation, would therefore be difficult to justify.

Conclusion

Evidence from this scoping review showed potential of RA practices to increase the concentrations of micronutrients in the edible portions of most crops. However, detecting changes in crop nutritional quality due to RA agronomic approaches is inherently challenging, due to potentially small effect sizes and the inherent variation in crop micronutrient composition due to other factors (e.g. crop yield and variety, soil type, and other landscape factors). Whilst the effect size of an RA agronomic approach is relatively small, the population-level health benefits through improved dietary micronutrient supply could potentially be large. This study was limited due to a lack of a formal meta-analyses to statistically quantify the magnitude of the effects of different RA practices on crop nutritional quality. This is largely due to small sample sizes within each RA and crop (+ nutrient) category and diverse conditions (i.e. input types and environmental factors), impeding a more direct comparison of studies. Future research should include appropriate experimental designs to test RA-informed interventions from which potential crop nutritional co-benefits (or trade-offs) might arise. Improvements in on-farm surveillance and nutritional diagnostics and a greater appreciation of the value of including crop nutritional quality metrics, potentially plays a key role in understanding linkages between RA, human nutrition, and human health. Additionally, linkages to potential nutritional outcomes within agronomic studies could be established, particularly when combined with other food system interventions, including biofortification of crops through breeding and/or the use of micronutrient-based fertilizers.

Author contributions

MGM-K, RML, SR, AE, and MRB contributed to the conceptualization of the paper. MGM-K, RML, and MRB conducted the initial screening of abstracts. MGM-K and MRB conducted the second screening of abstracts and wrote the initial draft of the manuscript. MGM-K, EJMJ, and MRB conducted the full review of 367 records included in the review. All authors contributed to the article and approved the submitted version.

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Conflict of interest

SR and AE are employees of Unilever.

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Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1078667/full#supplementary-material

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Nutritional, bioactive components and health properties of the milpa triad system seeds (corn, common bean and pumpkin)

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The milpa system is a biocultural polyculture technique. Heritage of Mesoamerican civilizations that offers a wide variety of plants for food purposes. Corn, common beans, and pumpkins are the main crops in this agroecosystem, which are important for people's nutritional and food security. Moreover, milpa system seeds have great potential for preventing and ameliorating noncommunicable diseases, such as obesity, dyslipidemia, type 2 diabetes, among others. This work reviews and analyzes the nutritional and health benefits of milpa system seeds assessed by recent preclinical and clinical trials. Milpa seeds protein quality, vitamins and minerals, and phytochemical composition are also reviewed. Evidence suggests that regular consumption of milpa seeds combination could exert complementing effect to control nutritional deficiencies. Moreover, the combination of phytochemicals and nutritional components of the milpa seed could potentialize their individual health benefits. Milpa system seeds could be considered functional foods to fight nutritional deficiencies and prevent and control noncommunicable diseases.

KEYWORDS
milpa system seeds, bioactive components, corn, common beans, health properties, nutritional potential

1. Introduction

Milpa is an agro-productive system used in Mesoamerica, especially in Mexico, since pre-Hispanic times; its etymological origin comes from the Nahuatl language. The words ("*milli*" means "planted plot" and "*pan*" means "above") (1). The milpa system (MS) is a traditional polyculture method, which constitutes a dynamic space for genetic resources. Corn (*Zea mays* L.) is the main species in the MS and is accompanied by other edible species, such as common beans (*Phaseolus vulgaris* L.), pumpkin (*Cucurbita pepo* L.), chili (*Capsicum annum* L.), and tomato (*Solanum lycopersicum* L.) (1–3). The MS is considered the first organized agricultural system in the Americas and was the base of the feeding of the Mesoamerican civilizations. However, the MS has lost popularity and has been replaced by monoculture practices (2–4).

The combination of corn, common beans, and pumpkin is known as "the Mesoamerican triad" or "milpa triad." The MS is more efficient in using natural sources (such as lower water demand, soil, space, and light) compared to traditional monocultures (2–5). Moreover, the MS offers various food products compared to conventional agriculture (5, 6). In the same way, MS polyculture helps the crops' resilience to the adverse effects of climate change (7). In an eco-friendly future scenario, the MS offers one of the most sustainable alternatives to producing food with low environmental impact (6, 8–11).

The milpa system-derived food products represent an important source of nutritional components (protein, complex carbohydrates, fiber, lipids, vitamins and minerals) and phytochemical compounds (phenolics, saponins, phytosterols, carotenoids, polyunsaturated fatty acids). Several investigations highlight milpa seeds' nutritional and health benefits due to their chemical composition (12–17). Regular consumption of corn, common beans, and pumpkin seeds have been related to preventing and mitigating noncommunicable diseases, such as cancer, type 2 diabetes, hypertension, obesity, among others (12–17).

Nutritional and pharmacological evaluations of the milpa seeds have been performed on seeds cultivated in monoculture. Besides, most of these studies were made on the seeds individually. The evaluation of the combined milpa seed's nutritional and biological potential has not been explored (4). This work reviews and analyzes the nutritional and health benefits of milpa system seeds assessed by recent preclinical and clinical trials. A comprehensive evaluation of the potential nutritional and health benefits of the milpa triad seeds diet incorporation was performed.

2. Materials and methods

2.1. Bibliographic review

The bibliographic search was carried out using the following keywords: "milpa system," "*Phaseolus vulgaris*," "*Zea mays*," and "*Cucurbita pepo*"; using Scopus,[1] PubMed,[2] Elsevier,[3] Google Scholar,[4] ResearchGate,[5] Web of Science,[6] and ScienceDirect[7] as the main database; those articles not older than 5 years were selected.

2.1.1. Nutritional quality estimation

The combination of milpa seed proteins in the diet could be a strategy to reach the recommended daily dose for adequate nutrition (FAO/WHO/UNU) (18, 19). Corn, common bean, or pumpkin seed proteins show a deficiency in one or more essential amino acids. To estimate the value of the proteins from the milpa seeds, the following protein quality parameters were calculated:

- Amino acid score (AAS) of seed proteins was calculated using the FAO/WHO/UNU (19) reference pattern and using the following equation:

$$AAS = \frac{mg\ of\ amino\ acids\ in\ 1\ g\ of\ total\ protein}{mg\ of\ amino\ acids\ in\ requirement\ pattern} \times 100$$

- Essential amino acid index (EAAI) was estimated using the following equation comparing the amino acid composition of the whole egg protein as standard (20):

$$EAAI = \sqrt[9]{\frac{(Lys \times Thr \times Val \times Met \times Ile \times Leu \times Phe \times His \times Trp)a}{(Lys \times Thr \times Val \times Met \times Ile \times Leu \times Phe \times His \times Trp)b}}$$

- where "a" represents the amino acid content specified in the formula in seed or grain protein samples and "b" is the content of the same amino acids in standard egg protein (%), respectively.
- Predicted biological value (BV) was calculated using the following equation (21):

$$BV = (1.09 \times EAAI) - 11.7$$

- Protein efficiency ratios (PER) were calculated from the amino acid composition of grains samples based on the following five equations (21):

$$PER_1 = -0.684 + 0.456(Leu) - 0.047(Pro)$$

$$PER_2 = -0.468 + 0.454(Leu) - 0.105(Tyr)$$

$$PER_3 = -1.816 + 0.435(Met) + 0.780(Leu) + 0.211(His) - 0.944(Tyr)$$

$$PER_4 = 0.08084(Thr + Val + Met + Ile + Leu + Phe + Lys) - 0.1094$$

$$PER_5 = 0.06320\begin{pmatrix} Thr + Val + Met + Ile + Leu \\ +Phe + Lys + His + arg + Tyr \end{pmatrix} - 0.1539$$

3. Main findings

3.1. Milpa system

The milpa system is a polyculture-diversified food production and is considered by the Food and Agriculture Organization (FAO) as an "important system of world agricultural heritage." This system guaranteed the perpetuation of the culture and covers the basic food needs of peasant families. The MS favors the production of foods due

1 www.scopus.com
2 https://pubmed.ncbi.nlm.nih.gov/
3 https://www.elsevier.com
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5 https://researchgate.net/
6 https://www.webofknowledge.com/
7 www.sciencedirect.com

to the morphological differences between the roots of common beans, corn, and pumpkin, which promote the absorption of nutrients. The entanglement of the common bean plant in the corn canes favors sheltering beneficial insects. It decreases the development of weeds and generates a microenvironment that conserves humidity in times of drought. Moreover, the common beans promote the fixation of atmospheric nitrogen. Besides, the pumpkin covers the soil, reducing the appearance of pests and weeds, and reducing weeding, thus increasing the fertility of the land for prolonged periods (Figure 1) (22–24).

This type of agricultural food production system in Mexico has decreased over time due to the introduction of monocultures with high economic impact and the loss of farmers' financial support. Another important factor is the change in traditional diets due to industrialization in the food sector and changes in lifestyles that have promoted the introduction of highly processed foods with high calories and poor nutritional value, resulting in the appearance of diet-related diseases (25).

3.2. Nutritional composition

Milpa system plants are considered crops of great importance worldwide. Their nutritional importance is related to their carbohydrate, lipid, and protein content, as well as vitamins and minerals (Table 1) (12, 15, 26–38).

4. Macronutrients

The major macronutrient of cereals, pulses, and pumpkin seeds is carbohydrates (54–80%), which consist of structure and reserve materials, such as fiber and starch, respectively (39). The carbohydrate content in corn ranges between 67.8–74.9%, mostly composed of starch and dietary fiber (2–11.2%), such as hemicellulose and pectin (26–30). In common beans, carbohydrates range between 43 and 65%, mainly composed of starch, resistant starch, and dietary fiber (30–33). In pumpkin seeds, carbohydrates range between 10.6–25%, mainly composed of starch, and dietary fiber (3–6.5%), such as hemicellulose and lignin; the content of carbohydrates and fiber varies with the consumption of whole seed or only the kernel seed (12, 34–37).

Lipids in corn and common beans present a proportion not higher than 5%, but their chemical composition is especially rich in mono- and polyunsaturated fatty acids (38). The lipid content in corn varies between 2.7–4.4% and is composed mainly of unsaturated (oleic acid), polyunsaturated (linoleic acid), and saturated (palmitic acid) fatty acids (26–29). In common beans, lipids vary between 0.3–2.5%, composed mainly of polyunsaturated fatty acids, such as linoleic and linolenic acids (30–34). In pumpkin seeds, lipids are about 41.4–50.5%, showing a profile of polyunsaturated fatty acids (mainly linoleic acid) (12, 34–37).

Protein content is an important criterion for determining the quality of seeds. Corn has a protein content of 9.3–15.8%, mainly composed of zeins and globulins (26–29). Common beans present a protein content of 19.1–28.3%, including albumins, phaseolins, and glutelins (29–33). Pumpkin seeds are rich in protein with 21.3–37.7%, mainly constituted of globulins, glutelins, and albumins (12, 34–37).

4.1. Nutritional properties of protein in milpa system seeds

4.1.1. Amino acid score

The amino acid score (AAS) is based on the calculation of limiting amino acids (Table 2). The AAS is a complement for calculating protein digestibility and should be corrected for incomplete protein digestibility and the unavailability of individual amino acids (40). The AAS of corn, common bean, and pumpkin seeds ranged from 90 to 170.9%, 107.9–173.1%, and 32.1–160.1%, respectively. The three seeds showed maximum values above 100%, which means that the consumption of certain varieties of these seeds could be above the amino acid intake recommended by the FAO/WHO (40). Ranges in AAS could be associated with phenotypic characteristics derived from environmental factors influencing the genotype of the crops evaluated. Several varieties of milpa seeds are grown under different latitudes, which could influence the chemical composition of these seeds

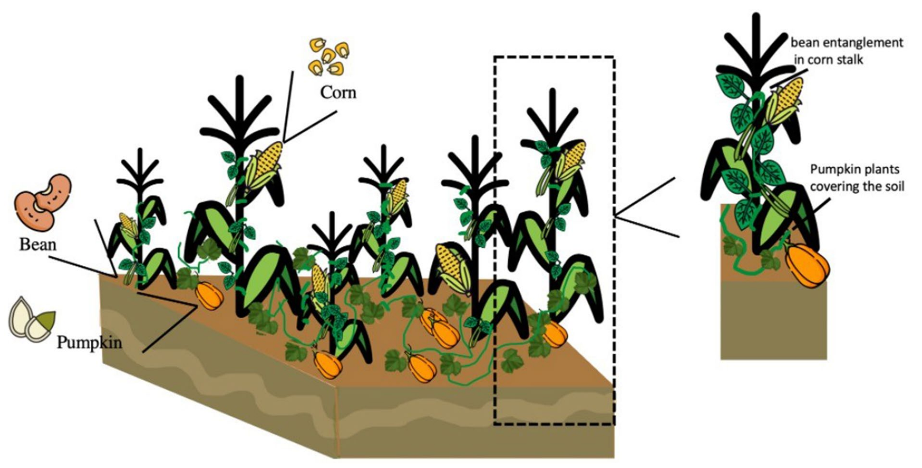

FIGURE 1
Interaction of the plants of the main milpa system components (corn, common beans, pumpkin).

TABLE 1 Proximate composition of the seeds of the milpa triad system.

Component	Corn (%)	Common bean (%)	Pumpkin seed (%)	References
Protein	9.32–15.75	19.06–28.32	21.31–37.70	(12, 15, 26–37)
Lipids	2.70–4.43	0.33–2.50	41.40–49.3	(12, 15, 26–38)
Carbohydrates	67.76–74.88	43.00–65.00	10.59–25.00	(12, 15, 26–37)

TABLE 2 Protein quality parameters of milpa triad system.

Parameters	Corn	Common bean	Pumpkin seed
AAS (%)	90.01–170.90	107.87–173.14	32.09–160.07
EAAI (%)	61.82–123.41	81.47–151.36	2.18–46.37
BV (%)	55.69–122.82	77.10–153.28	9.32–38.85
PER_1	2.53–6.38	2.53–3.43	0.32–4.83
PER_2	2.72–6.51	2.57–3.44	0.49–4.62
PER_3	4.25–11.19	2.42–3.81	0.51–5.19
PER_4	1.72–3.31	1.67–2.58	0.57–3.25
PER_5	1.60–3.15	1.83–2.62	0.59–3.08

AAS, amino acid score; EAAI, essential amino acid index; BV, biological value; PER, protein efficiency ratio. Based on amino acid profiles reported by Eleazu et al. (27), Amin et al. (34) and USDA (https://fdc.nal.usda.gov/).

(26–33, 39). However, according to the amino acid profile of common bean seeds showed the best AAS among the MS triad grains compared to egg protein reference (20).

4.1.2. Essential amino acid index

The essential amino acid index (EAAI) is the geometric mean of the ratios of the essential amino acids in the food protein relative to their content in a highly nutritious reference protein vs. whole egg (Table 2) (41). The EAAI estimated was 61.8–123.4% for corn, 81.5–151.4% for common beans, and 2.2–46.4% for pumpkin seeds. Values of EAAI over 90% are assumed as good nutritional quality (20). Corn and common beans could be considered that meet this qualification. Common beans presented the highest EAAI values due to their high protein content and the relatively high proportion of essential amino acids, compared with cereals (i.e., oat flour and oat protein concentrate present 44.55 and 45.92%, respectively) and non-legume seeds (i.e., canola meal show 80% and 32.8–32.9% for amaranth flours) (42, 43).

4.1.3. Biological value

The EAAI not only integrates all essential amino acids into the calculation but also allows estimating the effect of amino acid or protein supplementation on biological value (BV, nitrogen with potential to be absorbed from food for tissue formation) (Table 2) (41). As EAAI, values of BV over 70% may be considered good nutritional quality products (20). The BV of corn, common bean, and pumpkin seeds were estimated at 55.7–122.8%, 77.1–153.3%, and 9.3–38.9%, respectively. Similarly, the highest BV values were found for common beans.

4.1.4. Protein efficiency ratios

The protein efficiency ratio (PER) is an index of nutritional quality represented by values ranging from 0 to up to 2 (from low to high protein quality) (21). According to the amino acid profile and PER criteria for estimating MS seeds, there is a wide range of PER values, especially in pumpkin seeds (Table 2). These disparities could be related to the amino acid content variation among the seeds. For example, the limiting amino acids in corn are Lys and Trp, sulfur amino acids Cis and Met in common beans, and Lys and Thr in pumpkin seeds. However, consuming these seeds in combination could complement the amino acid deficiencies of individual seed proteins (44, 45).

5. Micronutrients

Micronutrients are small amounts of vitamins and minerals required by the body for most cellular functions. Novel research highlights the relevance of the consumption of seeds from the MS due to their high content and diversity of micronutrients (46).

5.1. Vitamins

Numerous studies have demonstrated that milpa crops present a wide diversity of vitamins (Table 3). Vitamin content and diversity depend strongly on the growth conditions, and it varies among the milpa seed species, i.e., corn: $B_4 > C > B_3 > B_5 > B_6 > B_1 > E > B_2 > B_9 > A > K$; common beans: $B_4 > E > B_3 > B_1 > B_5 > B_6 > B_2 > C > K > B_9$; and pumpkin seeds: $B_4 > B_3 > C > E > B_5 > B_1 > B_2 > B_6 > B_9 > K > A$ (16, 17, 47, 48).

Vitamin A or retinol is present in corn (9 µg/100 g), and pumpkin seeds (16 µg/100 g), reaching 1 and 1.8% of the retinol recommended dietary allowance (RDA), respectively. This vitamin is important for development and growth, as well as good vision and immune system maintenance (49). Vitamin A deficiency or hypovitaminosis A is related to growth and development disorders and increased susceptibility to severe infections, common in children and women of reproductive age from developing countries (50).

The vitamin B complex is a group of 8 vitamins; 6 are found in MS seeds, and all are related to cellular metabolism (51). Vitamin B_1 or thiamine is reported in corn, common beans, and pumpkin seeds at

TABLE 3 Micronutrient profile of the milpa triad system.

Micronutrients	Content in Milpa Seeds (mg/100g of dry weight)			
Vitamins	**Corn**	**Common beans**	**Pumpkin seeds**	**RDA**
Retinol (A)	<0.01	<0.01	0.02	0.12
Thiamine (B_1)	0.16–0.39	0.88	0.27	1.2
Rivoflavin (B_2)	0.01–0.02	0.19–0.50	0.15–0.20	400
Niacin (B_3)	1.77–3.63	2.10–2.49	4.40–4.99	35
Pantothenic acid (B_5)	0.42–0.72	0.85	0.75	5
Pyridoxine (B_6)	0.09–0.62	0.49–0.50	0.10–0.14	1.3
Folate (B_9)	0.04	<0.01	0.06	900
Choline (B_4)	23.00	99.40	15.87	200
Ascorbic acid (C)	6.80	0.01	0.27–1.9	75
Tocopherol (E)	0.07	2.50	1.03	15
Phytonadione (K)	<0.01	0.01	0.02	0.2
Minerals				
Potassium (K)	77.2–327.3	73.6–249.0	579.0–788.0	2000
Phosphorous (P)	25.0–165.5	30.0–106.7	332.0–1570.0	700
Calcium (Ca)	3.2–64.7	0.1–99.8	15.0–52.0	800
Magnesium (Mg)	20.0–141.3	10.0–74.6	156.0–569.0	375
Iron (Fe)	0.4–4.2	183.0–1207.0	2.3–10.6	14
Zinc (Zn)	0.4–0.9	41.0–624.0	2.2–11.3	10
Manganese (Mn)	0.3–0.8	2.1–263.0	1.3–4.9	2
Copper (Cu)	0.1–0.2	4.0–140.0	0.4–1.5	1

RDA, recommended dose allowance. Adapted from Amin and Thakur (66), Anstalt (67), Bressani et al. (47), Espinoza-García et al. (64), FAO (68), Gouveia et al. (7), Haytowitz et al. (48), Novotny et al. (11), Syed et al. (17), and USDA (65).

0.2–0.4, 0.9, and 0.3 mg/100 g, respectively, equivalent to 13.3–32.4% of RDA in corn, 73.3% in common bean and 22.5% in pumpkin. Thiamine (Vitamin B_1) is an essential micro-nutriment. Its deficiency is related to the development of beriberi and Wernicke-Korsakoff syndromes, as well as behavioral circumstances in the nervous system (such as irritability, depression, poor memory, and ability to concentrate, lack of mental dexterity, palpitations at the cardiovascular level), and heart hypertrophy (52). Vitamin B_2 or riboflavin was quantified in 0.01–0.02, 0.2–0.5, and 0.15–0.2 mg/100 g, respectively, for corn, common bean, and pumpkin seeds, representing an RDA for corn of 0.4–0.8%, 7.6–20% for common beans and 6–8% for pumpkin seeds. Riboflavin is necessary for the metabolism of lipids, carbohydrates, and proteins. Additionally, this vitamin participates in energy metabolism to maintain the integrity of the skin and mucous membranes (including the cornea) (53). Vitamin B_3 or niacin has been reported in 1.8–3.6, 2.1–2.5, and 4.4–5.0 mg/100 g in corn, common beans, and pumpkin seeds, respectively. Niacin's RDA for corn is 5.1–10.4%, 6–7.1% in common beans, and 12.6–14.3% in pumpkin seeds. Niacin contents in corn, common beans and pumpkin seeds represent 5.1–10.4%, 6–7.1% and 12.6–14.3% of the RDA, respectively. Niacin is an essential nutrient that acts as a coenzyme, forming nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), playing a key role in energy transfer reactions in the metabolism of glucose, fat, and alcohol (54). Vitamin $B_{5,}$ or pantothenic acid, is related to the oxidation of carbohydrates and fatty acids, synthesizing amino acids, fatty acids, ketone bodies, phospholipids, acetylcholine, and other neurotransmitters, steroid hormones, antibodies, and cholesterol (55, 56). Vitamin B_6 or pyridoxine has been reported in corn, common beans, and pumpkin seeds in amounts of 0.09–0.6, 0.5, and 0.1–0.14 mg/100 g, respectively. Pyridoxine RDA is about 1.3 mg, corn provides 6.4–47.7%, while common beans give 37.7–38.5%, and pumpkin seeds offer 7.7–10.8%. This vitamin synthesizes neurotransmitters as a cofactor in enzymes related to amino acid synthesis (57). Vitamin B_9 or folate in corn, common beans, and pumpkin seeds is quantified in 42, 4.1–4.6, and 57–58 μg/100 g, respectively, representing 10.5%, 1.04–1.16% and 14.3–14.5% of the RDA for this vitamin. Folate is a coenzyme in the synthesis of purine and pyrimidine nucleotides, and it is also involved in erythropoiesis (58).

Vitamin B_4 or choline is estimated at 23 mg/100 g in corn, 99.4 mg/100 g in common beans, and 15.9 mg/100 g in pumpkin seeds. This vitamin is the most abundant of the three MS seeds. Its RDA is also high (200 mg), corn contributes 11.5%, common beans 49.7%, and pumpkin seeds 7.9%. Choline is an essential micro-nutrient in producing neurotransmitters, such as acetylcholine, and methyl donors, such as *S*-adenosylmethionine. Its deficiency is rare in humans but may produce muscle damage and non-alcoholic fatty liver disease (59).

Vitamin C or ascorbic acid is quantified in corn, common beans, and pumpkin seeds in 6.8, 0.01, and 0.3–1.9 mg/100 g, respectively, contributing to the RDA of vitamin C with the 9.1, 0.01%, and 0.4–1.9%. Ascorbic acid is a potent antioxidant and an essential micro-nutrient in humans. Play a key role in growth and development, such as in synthesizing collagen and carnitine. It also maintains endothelial integrity and lipoprotein metabolism. Its deficiency is related to scurvy (60).

Vitamin E or tocopherol has been reported in corn, common beans, and pumpkin seeds in amounts of 0.7, 2.5, and 1 mg/100 g, respectively, which represents the 0.5, 16.7, and 6.9%, respectively, of the tocopherol RDA. Tocopherol (α- and γ-tocopherol forms) is an essential micro-nutriment related to beneficial effects on the immune system: It also minimizes and delays the aging process, and benefits endothelial integrity, lipoprotein metabolism and protects against the development of cancer, dementia, and cardiovascular diseases. Its deficiencies cause neurological disorders due to poor conduction of nerve impulses, and also some individuals may have problems absorbing fats in the gastrointestinal tract (61).

Finally, vitamin K or phytonadione abundance in corn, common beans, and pumpkin seeds has been registered at 0.3, 10, and 17.7 μg/100 g, respectively. Its RDA is about 200 μg. Corn represents 0.3%, common beans contribute 8.2%, and pumpkin seeds 14.5%. Vitamin K acts as a cofactor for activating proteins related to liver coagulation factors, prothrombin, and factor X, among others. Its deficiency is associated with coagulation problems, calcium fixation difficulties, and arteriosclerosis (62).

5.2. Minerals

The ash of corn, common bean, and pumpkin seeds is constituted by various minerals (Table 3). The mineral content depends on several factors, such as genotype and environmental conditions during cultivation. The mineral profile is variable among the milpa triad (corn: K > P > Mg > Ca > Fe > Zn > Mn > Cu; common beans: Fe > Zn > K > P > Mg > Cu > Mn > Ca; pumpkin seeds: P > K > Mg > Ca > Zn > Fe > Mn > Cu) (11, 17, 47, 48, 63–68).

Potassium (K) is the most abundant mineral in corn (77.2–327.3 mg/100 g), the second more abundant in common beans (73.6–249 mg/100 g), and the third in pumpkin seeds (579–788 mg/100 g). Potassium main role in the body is to help maintain normal fluid levels inside the cells (69). Its RDA is 2,000 mg. Moderate consumption of MS seeds is enough to reach this amount (Table 3).

Phosphorous (P) is the most frequent mineral in pumpkin seeds (332–1,570 mg/100 g), while in corn (25–165.5 mg/100 g) is the second most important. Phosphorous has a key role in forming bones and teeth, in the metabolism of carbohydrates and fats, and in making processes of proteins for the growth, maintenance, and repair of cells and tissues (69). Its RDA is about 700 mg, meaning less than 50 g of pumpkin seed reaches the RDA of P.

Calcium (Ca) is present in lower amounts than K and P in the three seeds (corn: 3.2–64.7 mg/100 g; common beans: 0.1–99.8 mg/100 g; 15–52 mg/100 g). The consumption of moderated Ca is associated with keeping healthy bones and teeth, muscle contraction, nervous functions, and regulating normal heart rhythms and blood clotting (69). Calcium RDA is 800 mg, and its content in MS seeds will not exceed its permitted levels in a moderated consumption.

Magnesium (Mg) is a major element in pumpkin seeds with a content of 156–569 mg/100 g, while its content in corn is 20–141.3 mg/100 g and 10–74.6 mg/100 g in common beans. Magnesium (Mg) is an important element for regulating muscle and nerve functions, blood sugar levels, and blood pressure, as well as making proteins, bones, and DNA, assisting more than 300 enzyme reactions (69). Mg′s RDA is about 375 mg, which means that a moderated consumption of these seeds may provide the daily dose recommendation for adults.

Iron (Fe) is the major mineral in common beans, ranging from 183 to 1,207 mg/100 g, and 0.4–4.2 and 2.3–10.6 mg/100 g for corn and pumpkin seeds, respectively. The body needs Fe for hemoglobin and some hormone-making. Iron is also part of myoglobin and is important for healthy brain development and children's growth (69). Moderate intake of common beans may provide more than the Fe′s RDA (14 mg), but corn and pumpkin seeds present a lower content.

Zinc (Zn) is an important element in common beans with a content of 41–624 mg/100 g, also in pumpkin seeds with 2.2–11.3 mg/100 g, but only contains 0.4–0.9 mg/100 g in corn. Zn is an important trace mineral necessary for almost 100 enzymes. It also participates in the DNA and protein synthesis, growth of cells, healing of damaged tissue, and supporting the proper functionating of the immune system (69). A portion of 100 g of corn does not exceed the RDA of Zn (10 mg). On the other hand, 100 g of common beans or pumpkin seeds exceed the RDA of this mineral.

Manganese (Mn) is a minor mineral in corn and pumpkin seeds, with 0.3–0.8 and 1.3–4.9 mg/100 g, respectively, but this element ranges from 2.1 to 263 mg/100 g in common beans. Mn is important for multiple body functions, such as carbohydrate metabolism, Ca absorption, regulating brain and nerve functions, forming connective tissue, sex hormones, bones, and blood clotting factors (69). Manganese RDA (2 mg) could be exceeded with common beans and pumpkin seeds.

Copper (Cu) is reported in common beans in a range of 4–140 mg/100 g, while in corn and pumpkin seeds, this represents a content of 0.1–0.2 and 0.4–1.5 mg/100 g, respectively. In nutritional terms, Cu is considered an essential trace mineral for its key role in assisting several enzymes and enzymatic systems related to the chemical energy production in the body and the breaking down and absorption of Fe. Also, Cu is involved in the red blood cells, collagen, connective tissue, and brain neurotransmitter building (69). The RDA for Cu is about 1 mg, which means that consumption of a few amounts of common bean easily meets the demand for this trace mineral.

Despite the diversity and content of minerals in corn, common beans and pumpkin seeds. To reach the RDA for those minerals, it is necessary to consider their chemical properties, bioaccessibility, and bioavailability to fulfill their bioactive and nutritional potential.

The MS has a great diversity of high-quality macro- and micro-nutrients. Together, the cultivation and consumption of these three seeds could complement the nutritional limitations in amino acid, vitamin, and mineral profiles. They provide, in combination, a good balance of protein, fat, carbohydrates, vitamins, and minerals in quantities that contribute to meeting the daily RDAs (2, 3).

5.3. Bioactive compounds

The three seeds part of the Mesoamerican triad are also known to contain a wide diversity of bioactive components, such as polyphenols, phytosterols, saponins, fiber, bioactive peptides, and carotenoids (Table 4) (70). Many of these components are synthesized by plants mainly as defense and adaptation mechanisms against environmental conditions. Also, form part of macronutrients, including complex carbohydrates (resistant starch, cellulose, fiber, etc.) and protein-derived peptides (from dipeptides to polypeptides) (71). On the other hand, there are anti-nutritional components that could exert health benefits. In adequate concentrations and suitable conditions of bioaccessibility and bioavailability, the anti-nutritional components found in MS seeds could be considered bioactive compounds with beneficial health effects galactooligosaccharides, phytic acid, tannins, and enzyme inhibitors, among others (71, 72).

5.3.1. Phenolic compounds

Phenolic compounds or polyphenols are organic compounds whose molecular structures contain at least one aromatic ring attached to a hydroxyl group (phenol) group (73). They are the result of the secondary metabolism of plants in response to environmental stimuli (73). It is known that these types of compounds are present in corn, common beans, and pumpkin seeds (74–76). The main phenolics in these seeds are hydroxybenzoic acids: gallic, vanillic, and protocatechuic acids; hydroxycinnamic acids: coumaric, caffeic, and ferulic acids anthocyanins: glycosylated and acylated forms of delphinidin, petunidin, cyanidin, malvidin, pelargonidin, and peonidin; flavonols: quercetin, myricetin, naringenin, catechin, hesperidin, and kaempferol; and isoflavonoids: daidzein and genistein (74–76).

Free and bounded derivatives from cinnamic and benzoic acids are easily found in milpa seeds. Bounded phenolic compounds can be released by acid, alkali, and enzyme hydrolysis from seed bran, endosperm, and coat (77). Phenolic compounds are the main bioactive compounds in corn. Their content varies between 1,377–1,421 mg GAE/100 g, including gallic acid, coumarin, quercetin, catechin, kaempferol, and anthocyanins, mainly in colored varieties. While in common beans, phenolic compounds concentrations range from 600 to 2,624 mg GAE/100 g, conformed by gallic acid, coumarin, quercetin, catechin, kaempferol, and many others. Besides, in pumpkin seeds, phenolic compounds concentration ranged from 31.9–224.6 mg GAE/100 g (Table 4) (12, 16, 30, 37, 78–87).

The role of polyphenols from Mesoamerican triad seeds on human health has been thoroughly investigated individually. Research has shown that the polyphenols in these seeds can scavenge free radicals found in cells and tissues by donating electrons or a single proton in oxidation–reduction reactions. Still, they can chelate ionic metals that could harm cell structures and other biological molecules (73, 88). Additionally, phenols can inhibit the lipid peroxidation of cell membranes from converting them into stable compounds. Moreover, polyphenols could modulate enzymes involved in the antioxidant process, i.e., by induction of the expression of Nfr2 to produce antioxidant molecules such as superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) (84). In this way, polyphenols could, directly and indirectly, neutralize oxidizing agents in the human body. Phenolic compounds have also been shown to participate actively in other physiological processes. These molecules can decrease the oxidative state by reducing proinflammatory molecules, and controlling signaling pathways, including inflammatory cytokines such as interleukins 1, 6, 8, growth factors such as TNF-α and Interferon-γ, the latter indicating that the consumption of these compounds has an antiinflammatory potential (89).

5.3.2. Carotenoids

Carotenoids are organic pigments responsible for providing orange and red coloration to vegetables. The general structure of the carotenoid is a polyene chain consisting of 9–11 double bonds (possibly ending in rings). This conjugated double-bond structure leads to a high reducing potential, or the ability to transfer electrons through the molecule (90). Carotenoids such as lutein, zeaxanthin, astaxanthin, or lycopene could be found in the milpa triad seeds (91, 92). Carotenoids found in corn are present in concentrations of 9.2–19.8 mg/100 g, mainly zeaxanthin, α-cryptoxanthin, lutein, and β-carotene. Common beans carotenoids concentration ranged from 0.2 to 9.2 mg/100 g, primarily β-carotene. At the same time, pumpkin seeds carotenoids are found at a concentration of 7–35.2 mg/100 g, mainly β-carotene and cryptoxanthin (Table 4) (12, 16, 27, 31, 78–83, 85–87, 93, 94).

Similar to polyphenols, carotenoids function as electron donors to reactive species due to their structure and the delocalization of electrons along their chain, presenting high antioxidant potential. The hypocholesterolemic potential of carotenoids has been related to their ability to inhibit the oxidation of LDL cholesterol and the blockage of the enzyme HMG-CoA reductase, which is involved in cholesterol synthesis (Table 4) (95).

5.3.3. Saponins

Saponins are glycosides of steroids or triterpenoids, made up of a lipid-soluble element (the steroid or triterpenoid) and a water-soluble component (sugar) (96). Saponins are found in plants; their name is due to their ability to form foams. It is known that corn, common bean, and pumpkin seeds contain triterpene-type saponins derived from mevalonic, betulinic, and olean acids, which can form various glycosides depending on the union with sugars. The saponins are reported in amounts of 0.9–6.2, 44–148, and 5–7.4 mg/100 g in corn, common beans, and pumpkin seeds, respectively (Table 4) (27, 31, 37, 78–83, 85, 86).

TABLE 4 Profile of groups of bioactive compounds found in the triad system grains.

Bioactive Compound	Corn	Common Bean	Pumpkin Seeds	References
Total Phenolic content (mg GAE/g)	1,377–1,421	600–2,624	31.90–224.61	(12, 16, 27, 31, 37, 121–76)
Carotenoids (mg/100 g)	9.24–19.78	0.20–9.20	6.95–35.20	(12, 16, 27, 31, 37, 121–72, 74–76, 82, 83)
Saponins (mg/100 g)	0.92–6.19	44.00–148.00	5.02–7.40	(17, 31, 37, 121–72, 75, 76)
Total Phytosterols (mg/100 g)	112.36–362.08	242.00–350.00	782.10–805.20	(16, 27, 31, 37, 121–72, 74–76, 82)
Fiber (%)	2.00–11.18	25.80–39.20	3.00–6.50	(26–28, 30–34, 36, 37)

mg GAE/g, mg of gallic acid equivalents/g.

Saponins have multiple benefits, including inhibiting lipases, which prevent the absorption of lipids in the intestinal lumen. This function has been related to the decrease in cholesterol and triglyceride levels. Besides, saponins have been associated with reducing adipogenesis by regulating the AMPK pathway, increasing AMPK phosphorylation, and activating peroxisome proliferator-activated receptors (PPARs) and sterol regulatory element-binding proteins (SREBP). Resulting in decreasing the accumulation of triglycerides and preventing the growth of preadipocytes (Table 5) (97–101).

5.3.4. Phytosterols

Phytosterols are natural sterols present in fruits, seeds, and leaves. Structurally, they share characteristics with cholesterol. Phytosterols' structure is a fused polycyclic molecule composed of high variable carbon side chains and/or the presence or absence of a saturation (double bond) (102). These compounds have been reported in some oilseeds, cereals, and legumes, such as soybean, beans, and corn, as well as in Cucurbitacea seeds (16, 103). The total phytosterol content in corn, common beans, and pumpkin seeds have been estimated at 112.4–362.1, 242–350, and 782.1–805.2 mg/100 g, respectively (Table 4) (16, 27, 31, 37, 78–83, 85–87, 93).

Principal phytosterols are α-sitosterol, campesterol, and stigmasterol. These represent approximately 98% of these molecules. The consumption of phytosterols has been related to reducing cholesterol in the blood. This effect can be connected to the similarity between both molecules. The reduction is due to the competition for the absorption of dietary cholesterol in the intestinal lumen, reducing transport to the liver and preventing its metabolism (104).

5.3.5. Fiber

Dietary fiber is the edible part of plants or analogous fibers resistant to digestion and absorption in the small intestine, with partial or complete fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, and lignin (105, 106). It is known that corn contains high amounts of insoluble fiber, mainly due to the presence of cellulose and hemicellulose, starch, and low soluble fiber content. On the other hand, common beans and pumpkin seeds present high soluble and insoluble fiber contents (13, 15, 17).

Dietary fiber has been related to cardioprotection due to its ability to reduce the absorption of lipids in the gastrointestinal tract, reduce appetite and promote satiety in individuals. Moreover, the intestinal microbiota uses polysaccharides as a substrate, generating short-chain fatty acids (propionate, butyrate, acetate) as metabolites. These

TABLE 5 *In vivo* and clinical healthy properties of some bioactive compounds present in the seeds of the milpa triad system.

Bioactive Compound	Healthy properties	Mechanism	Reference
Polyphenols	Metabolic syndrome CardioprotectiveanticancerAntioxidantAntiinflammatoryCo-adjuvant in the treatment of diabetesDecrease in cholesterol and triglycerides	Negative regulation of ERK/PPAγ/γ-adiponectin; increase of SOD and GSH; decreased inflammatory cytokines and inflammatory infiltrate; increased insulin secretion; decreased peroxidation and hepatic lipid synthesis; HDL-PON1 inhibition	(114, 115, 73, 77, 78)
Carotenoids	Anti-obesity potentialDecrease in cholesterol and triglyceridesAnorexigenic potentialAntiinflammatoryAntioxidant	Decreased cholesterol synthesis; regulation of HMG-CoA reductase and acyl-CoA; inhibition of β-oxidation of fatty acids; HDL increase; leptin regulation; decrease in inflammatory cytokines and inflammatory infiltrate; ROS decrease	(84)
Saponin	Anti-dyslipidemic potential	Decreased cholesterol and triglycerides; SOD increase; increased bile salts; decreased expression of HMG-CoA reductase and SREBR-1c; AMPK activation; PPARγ decreased	(86–91)
Fiber	Anti-dyslipidemic potentialAnti-obesityAntihyperglycemic	SREBR-1c decreases; GPAT decreased; decreased triglycerides; reducing the absorption of lipids and glucose in intestine; enhance colon health; reducing the oxidative stress in β-pancreatic cells	(96, 144, 147)
Bioactive peptides	Anti-dyslipidemic antioxidant potentialAnti-diabetes.	Residues of tyrosine and phenylalanine free radical scavengers; TNF-α decreased; increased lipoprotein lipase; anorexigenic effect; HMG-CoA reductase inhibition; inhibition of α-amylase and α-glucosidase	(97–103)
PUFA	AntihypertensiveAnti-obesity	Reducing systolic blood pressure, LDL levels, arterial stiffness; decreasing body weight and waist circumference; increases HDL levels	(127–129)

ERK/PPAγ, phosphorilation of extracellular signal-regulated kinase activated by peroxisome proliferator-activated receptors; SOD: superoxide dismutase; GSH, hormone L-g-glutamil-L-cisteinil-glicine; HDL, high-density lipoprotein; ROS, reactive oxygen species; HMG-CoA, β-hydroxy β-methylglutaryl-coenzyme A; SREBP: sterol regulatory element-binding proteins; AMPK, AMP-activated protein kinase; PPAR: peroxisome proliferator-activated receptors; GPAT, glycerol phosphate acyltransferase; TNF-α: tumor necrosis factor alpha.

short-chain fatty acids are absorbed in the colon and metabolized in the liver, preventing hepatic cholesterol synthesis and lowering blood cholesterol levels (Table 5) (106).

5.3.6. Bioactive peptides

Bioactive peptides are obtained from dietary proteins or derivatives of precursor proteins. Several peptides with biological potential related to the modulation of markers of diseases such as type 2 diabetes, hypertension, oxidative stress, and inflammation have been studied in common beans. Also, bioactive corn peptides exert antiinflammatory, antioxidant, hepatoprotective, antihypertensive, and antimicrobial potential (107).

Moreover, the health benefits of bioactive peptides from the milpa triad seeds are associated with multiple mechanisms of action. These mechanisms include the modulation of inflammatory cytokines, enzymatic inhibitions such as HMG-CoA reductase, and increased expression of proteins, such as lipoprotein lipase, as well as increased bile salt excretion (Table 5) (108–110).

Díaz-Gómez et al. (111) reported that zein-derived peptides from corn (≤5 kDa) demonstrated antioxidant (against HepG2 and Caco2 cells exposed to high oxidative stress), antihypertensive (in spontaneously hypertensive rats), hepatoprotective (in rats with liver damage induced by exposure to lipopolysaccharides from *Bacillus* Calmette-Guérin), anticancer (on HepG2 cells and H22-tumor bearing mice model) and antimicrobial potential (on *Caenorhabditis elegans* in an *in vivo* study). On the other hand, common bean bioactive peptides (LVTTTVDL, QTSTPLFS, VELVGPK, and TRGVLG) biological potential was related to reducing the inflammatory process, inhibition of dipeptidyl peptidase-4 (DPP-IV) and angiotensin-converting-enzyme inhibitor (ACE), and promoting glucose uptake (112). In a recent report of several edible Cucurbitaceae species, it was reported the *in vitro* antioxidant, antiinflammatory, α-amylase inhibitory potential of some bioactive peptides (<15 kDa) (113). However, more studies on pumpkin protein hydrolyzates are needed to investigate their biological potential.

5.3.7. Fatty acids

A fatty acid is a carboxylic acid joined to an aliphatic chain, which could be saturated or unsaturated. Fatty acids are the basic units of fat in the human body and foods. Milpa seeds have a diverse profile of saturated and unsaturated fatty acids. The lipid profile of corn, common beans, and pumpkin seeds are composed of at least 16, 21, and 9 different fatty acids, respectively (Table 6) (114–124).

Linoleic (C18:2, ω-6), a polyunsaturated omega-6 fatty acid, Oleic (C18:1) and linolenic acid (C18:3) are poly unsaturated fatty acids (PUFA) in corn, common beans, and pumpkin seeds with proportions of 54.5–59.41%, 24.1–67.0%, and 43.68–52.61%, respectively (114–124). Oleic acid (C18:1), another PUFA, was the second fatty acid more abundant in corn and pumpkin seeds with values of 23.74–30.83% and 18.14–42.07%, respectively, while linolenic acid (C18:3), another PUFA, has a content of 6.24–46.0% in common bean. Palmitic acid (C:16), a saturated fatty acid (SFA), was reported as the third most abundant in the three seeds, with contents of 10.32–12.0%, 5.0–13.9%, and 10.21–16.01%for corn, common bean, and pumpkin seed, respectively. In total, more than 70% of the lipid profile of the three seeds is made up of PUFA.

Fatty acids, especially PUFA, positively impact human health and development (125). The evidence shows a strong role in preventing and treating some chronic degenerative diseases. PUFA present in corn and pumpkin seed oils have demonstrated antihypertensive effect in clinical trials (126, 127). At the same time, the fatty acids from pumpkin seeds also have shown antiobesity effects in human trials (128).

Milpa triad seeds bioactive compounds (polyphenols, carotenoids, saponins, phytosterols, or proteins/peptides) have demonstrated individually high potential to modulate molecular markers related to noncommunicable diseases (16, 129, 130). However, there are still research opportunities in order to understand the biological potential of the milpa seeds. The opportunities include the validation of the mechanisms of action observed in biochemical and *in vitro* assays compared to preclinical and clinical trials and the evaluation of the potential synergistic effect of milpa seeds on health when grown and consumed together. However, preclinical and clinical evaluations using extracts, purified and isolated compounds, or groups of compounds from these seeds provide important information related to the potential of milpa seeds as functional ingredients in the treatments or control of noncommunicable diseases.

5.4. Milpa system seeds health properties in preclinical and clinical trials

Growing evidence highlights the role of a balanced diet in reducing risk factors for developing noncommunicable diseases such as cardiovascular diseases, type 2 diabetes, obesity, cancer, and inflammatory conditions (131). Recent studies have reported the health benefits of MS seeds consumption (Table 7; Figure 2).

5.4.1. Antihypertensive properties

Cardiovascular diseases are the lead cause of death globally, with >17.9 million per year, an estimated 32% of all deaths worldwide (132). These are a group of heart and vascular tissue disorders and are strongly related to behavioral risk factors such as physical inactivity, tobacco, and alcohol use, as well as an unhealthy diet (132). Antihypertensive potential of milpa seeds has been evaluated by several authors through *in vivo* and clinical trials. In a preclinical trial, Sugiyanta et al. (133) observed a reduction in the diastolic blood pressure (79.87 mmHg) in induced hypertensive mice (by deoxycorticosterone acetate) supplemented with an aqueous extract of coffee:corn (50:50). The antihypertensive effect of the mix was compared through the concentration of F2-isoprostane, a stable end product of lipid peroxidation present in tissues and biological fluids, whose levels were negatively correlated to the blood pressure reduction (414.33 pg./mL). Authors attribute this effect to ferulic acid, a potent antioxidant compound commonly found in coffee and corn, and as a degradation product of other phenolic compounds such as flavonoids and tannins (134).

In a study selected from the Brisighella Heart Study (a population-based longitudinal epidemiological investigation initiated in 1972 in Bisighella, Italy, still active) cohort subjects who were not treated with antihypertensive drugs and reported with certainty their daily mean intake of dietary fats in cooking and seasoning, the patients who used corn oil as the main source of dietary oil presented low blood pressure (systolic 134.8 mmHg, 72.5 mmHg), arterial stiffness (94.8 mmHg), and cholesterolemia (total cholesterol 212.4 mg/dL, LDL-C 138.6 mg/dL, HDL-C 50.1 mg/dL, triglycerides 110.9 mg/dL). All these values were lower when compared to several vegetable and animal oil sources, except extra-virgin olive oil (126). The high content of

TABLE 6 Fatty acid profile in milpa triad system.

Fatty acid*	Corn	Common beans	Pumpkin seeds
Caproic acid (C6:0)	0.11	0.01	ND
Caproic acid (C10:0)	0.02–0.16	ND	ND
Lauric acid (C12:0)	0.01	0.49	ND
Trydecanoic acid (C13:0)	ND	0.49	ND
Myristic acid (C14:0)	0.1–0.8	0.25–0.1	0.1–0.18
Pentadecanoic acid (C15:0)	ND	0.14–0.44	ND
Palmitic acid (C16:0)	10.32–12.0	5.0–13.9	10.21–16.01
Palmitoleic acid (C16:1)	0.13–0.2	0.1–0.47	0.16
Margaric acid (C17:0)	0.04	0.1–1.76	ND
Stearic acid (C18:0)	1.77–2.07	0.46–4.6	4.45–10.44
Oleic acid (C18:1)	23.74–30.83	1.7–18.7	18.14–42.07
Linoleic acid (C18:2)	54.5–59.41	24.1–67.0	43.68–52.61
Linolenic acid (C18:3)	0.48–1.62	6.24–46.0	0.2–1.27
Arachidic acid (C20:0)	0.28–1.39	0.1–0.7	0.28–0.43
Eicosenoic acid (C20:1)	0.14–0.3	4.40	ND
Heneicosanoic acid (C21:0)	ND	0.1–1.43	ND
Behenic acid (C22:0)	0.1	0.4–0.7	ND
Erucic acid (C22:1)	ND	ND	0.76
Docosadienoic acid (C22:2)	ND	0.1–0.4	ND
Eicosatrienoic acid (C22:3)	0.1	0.3–0.7	ND
Tricosylic acid (C23:0)	ND	0.15	ND
Lignoceric acid (C24:0)	0.2	0.4–2.97	ND
Pentaosanoic acid (C25:0)	ND	0.04	ND
SFA	13.96–15.38	7.7–20.18	27.06
MUFA	26.08–30.62	7.3–19.16	19.06–43.68
PUFA	55.49–58.63	40.9–58.82	42.23–52.88

*Proportion of fatty acids in the seeds' oil fraction. SFA, saturated fatty acids; MUFA, monousaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, non-detected. Adapted from Bhagya et al. (120), David et al. (121), Iwuagwu et al. (123), Kawakami et al. (115), Lee and Ahn (116), Lee et al. (118), Opapeju et al. (114), Słowik-Borowiec et al. (119), Sutivisedsak et al. (121), Vujasinovic et al. (124) and Zamaninour et al. (117).

TABLE 7 Beneficial effects of consumption of milpa seeds in preclinical and clinical models.

Seed	Model	Food presentation	Dose	Beneficial effect	References
Corn and common bean	C57BL/6J mice	Baked snack	0.5–2.0 g/day	Lowering of lipid serum	(145)
Common bean	Adult men and women (aged 18–40 years, BMI 25.0–29.9 kg/m^2)	Baked snack	32 g/day	Reduction of apolipoprotein B-100	(141)
Common bean and oats	Hypertriglyceridemic women	Snack bar	50 g/day	Reduction of hypertriglyceridemia	(149)
Common bean	Adult male BALB/c mice	Cooked common bean flour	346 g/d	Improve gut health and modulate the composition and function of microbiota	(102)
Pumpkin	56 men (aged 56–75 years) with prostatic surgery or other invasive intervention	Oil-free extract	500 mg	Reduction of benign prostatic hyperplasia symptoms	(156)
Pumpkin	Healthy participants (aged 39–63 years)	Oil	1,000 mg	Reduction of LDL and diastolic blood pressure levels; increase HDL levels.	(128)

BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

monounsaturated and PUFA in corn oil are the responsible compounds for these effects.

In an *in vivo* experiment, Guo et al. (135) found that a high-dose corn germ peptide (1,000 mg/kg body weight) significantly reduced the systolic blood pressure by acute oral (10.12%) and long-term intragastric (15.89%) administration tests. In a consecutive study, the corn germ peptide also inhibited the ACE activity in the kidney (22.28%), lung (24.53%), and heart (12.93%) of rats. It regulated the level of endothelium-derived vasoconstrictor and relaxing factors in serum (136). A biopeptide identified by bioinformatic tools (SKFDNLYGCR, 1,258 Da), found in corn silk tea, reduced systolic blood pressure levels (36.78 mmHg) in spontaneously hypertensive rats and inhibited the ACE activity (IC_{50} 44.11 µM). Molecular docking analysis showed that this peptide directly interacted with ACE residues (Asn277, Gln281, Thr282, Thr302, His353, Asn374, His513, Ser516, Ser517, and Tyr523) (137). However, further studies are required to validate these *in silico* outcomes.

Ribeiro et al. (138) described a dose-dependent antihypertensive effect in spontaneously hypertensive Wistar rats treated with common bean protein extract (<3 kDa). Which was related to the presence of short peptide sequences related to cardioprotective activities (i.e., VSE, EV, AVT, DF, ELL, SF, AF, VLL, PLL, and GF). The main activity of these peptides was directly related to vasorelaxation and reducing arterial pressure and resistance in aortic vascular beds. In another study, a fraction of 3–10 kDa of Alcalase common bean hydrolysate showed *in vivo* antihypertensive potential in naturally hypertensive rats after 2 h of 4 mg/kg intraperitoneal administration (139).

Moreover, a common bean-baked snack consumed by people with overweight or abnormal blood lipid levels reduced apolipoprotein B-100 levels (56.6 mg/dL) (140). The consumption of the snack did not affect other blood indicators, such as lipids or glucose ($p > 0.05$). The authors did not attribute this effect to any particular compound.

In a study by Majid et al. (127), the effect of pumpkin seed oil on total cholesterol was evaluated. 1,000 mg per day during 3 months showed a significant reduction in the final values of LDL and diastolic blood pressure along with an increase in HDL. These results suggest a hypolipidemic and antihypertensive potential of pumpkin seeds due to the content of unsaturated fatty acids and plant sterols.

The antihypertensive effect of milpa seeds is related to different compounds (mainly phenolic compounds, bioactive peptides, and fatty acids). Vasomodulatory effect could be one of the principal pathways of the antihypertensive potential of the MS seed bioactive components. Principally related to the potential of these molecules to block oxidative systems and modulate enzymes related to vasoconstriction and vasorelaxation. These preclinical and clinical assays show evidence of the potential of the milpa seeds for cardioprotective benefits.

5.4.2. Antihyperglycemic and antiobesity properties

Diabetes is a chronic-metabolic disease characterized by elevated glucose levels in the blood (141). Over 420 million people globally live with diabetes and about 1.5 million deaths are attributed to this disease each year (142). Type 2 diabetes (T2D), the most common, is characterized by insulin resistance and low or null production of insulin (141). Obesity is characterized by a body mass index above 30 kg/m^2 in adults (142). A healthy lifestyle and a balanced diet are effective strategies to prevent and delay the development of obesity and T2D.

Milpa seeds have the potential to modulate biological markers related to obesity and T2D. In a 14-day *in vivo* assay, type 2 diabetes-induced male and female rats were fed with rice analog (formulated with mocaf, corn, pigeon pea, and seaweed, 71:21:7:1, w/w/w/w) and showed better morphological characteristics in islets of Langerhans and an increase in insulin production, since rice analog presented a low glycemic index (47.36) compared with the control diet, based in commercial Broiler-1 product (66.35) (143). Additionally, serum glucose levels decreased in male rats up to 60.79 mg/dL, which was lower compared to female rats (88.98 mg/dL). Authors found that resistant starch and fiber contained in rice analogs might release bioactive compounds during digestion, which could reduce oxidative stress in β-pancreatic cells of diabetic rats (143).

Corn silk extract showed antidiabetic potential in a high-fat diet/streptozotocin-induced rat model (144). After 4-weeks, the treatment effectively prevented the weight loss of diabetic mice and showed a significant reduction in fasting blood glucose and enhanced glucose tolerance. Also, hyperlipidemia was alleviated since total cholesterol, triglycerides, and low-density lipoprotein-C (LDL-C) values decreased and, at the same time, HDL-C increased (high-density lipoprotein). Moreover, the oxidative stress was reduced by decreased malondialdehyde and elevated SOD activity. Besides, hepatic lipid accumulation decreased and prevented liver tissue morphological change (144).

Domínguez-Uscanga et al. (145) reported the beneficial effect of baked snack with 70% corn and 30% of common beans in the reduction of lipid serum by the inhibition of PPAR-γ and SREBP2 in a high-fat diet murine model. The study suggests the reduction of obesity, dyslipidemia, and non-alcoholic fatty liver. In another preclinical study, Gomes et al. (146) showed that the administration of common bean flour at a dose of 346 g per day improves colonic health and the microbiome compared to the high-fat diet-fed group. Results suggest that a diet high in fiber and bioactive compounds from MS seeds could be a strategy to improve consumers' health.

A clinical trial reported by Escobedo et al. (140) shows the reduction of apolipoprotein b-100 blood levels after a daily 32 g consumption of common bean baked snack. This reduction could have a preventive effect on developing dyslipidemia. Similar to previous reports, Ramírez-Jiménez et al. (147) elaborated a snack bar with common beans and oats to reduce hypertriglyceridemia markers in Mexican women. They found a reduction of triglycerides and glucose by inhibiting adipogenesis after consuming the snack bar.

On the other hand, pumpkin seeds showed antihyperglycemic and antihyperlipidemic effects in albino rats (148). A significant decrease in blood glucose level (128.33 mg/dL), total plasma cholesterol (88.43 mg/dL), triglycerides (69.79 mg/dL), and low-density lipoprotein cholesterol (21.45 mg/dL) were found in the rat groups fed with 15 g pumpkin seed powder. Authors conclude that pumpkin seeds have a high potential to be used in the human diet to manage noncommunicable diseases such as diabetes and hypercholesterolemia. In another study, Indian women (aged 30–50 years) diagnosed with metabolic syndrome received 5 g of pumpkin seeds for 60 days (128). Anthropometric measurements (body weight 72.20 kg, waist circumference 90.62 cm), biochemical parameters (HDL cholesterol 42.24 mg/dL, non-HDL cholesterol 142.05 mg/dL, LDL cholesterol 120.22 mg/dL,), and systolic blood pressure (122 mm Hg) showed statistically significant improvement ($p < 0.05$) compared to

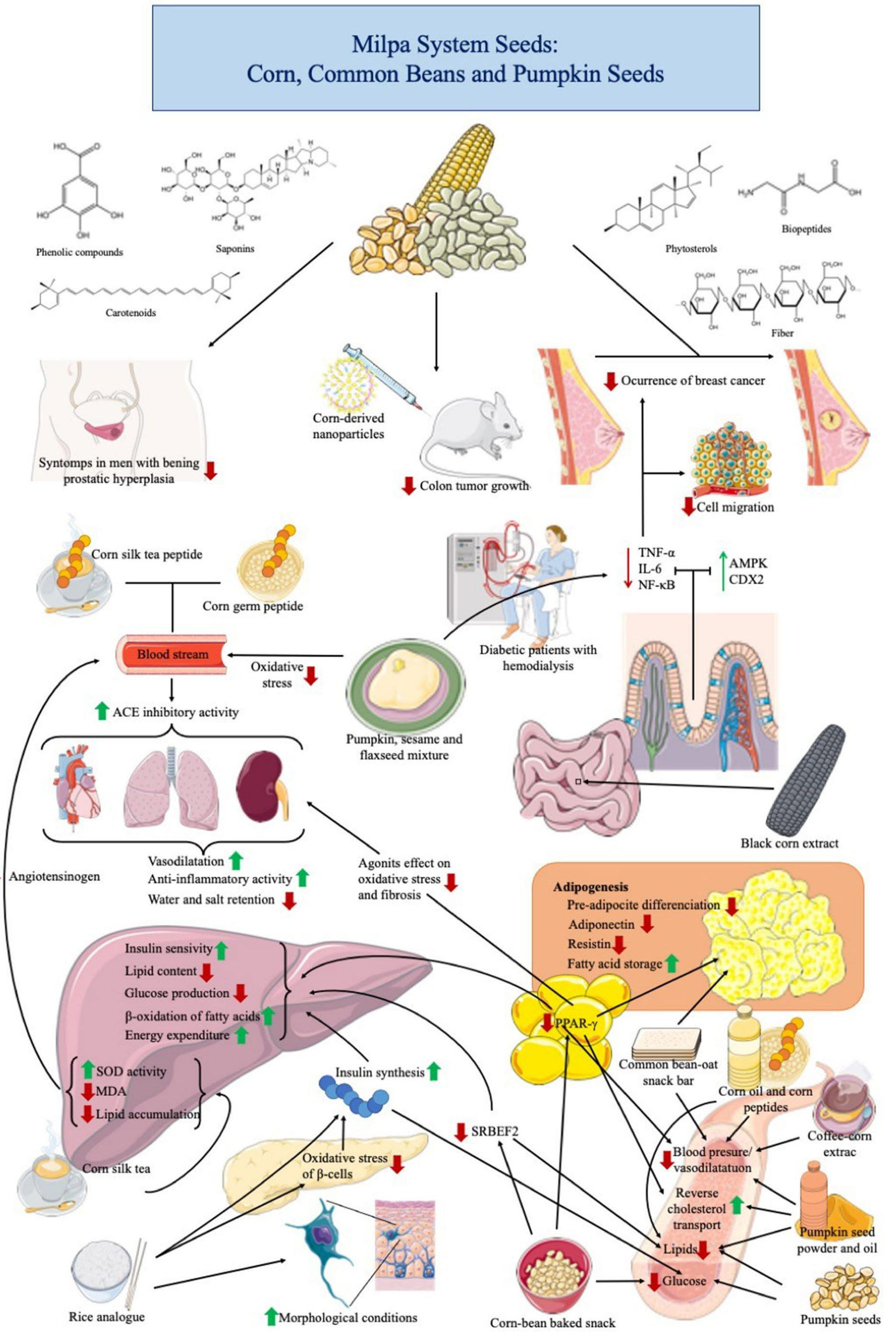

FIGURE 2
Health properties of milpa system grains in preclinical and clinical trials on chronic-degenerative diseases.

control group (patients without pumpkin seed). The authors found some bioactive compounds (fatty acids, propyl piperidine, and benzene derivatives) in pumpkin seeds with antidiabetic and antiobesity potential effects.

Milpa system antidiabetes and antiobesity potentials have been associated with their bioactive components. Several molecular pathways against these diseases indicated that milpa seeds could reduce glucose levels and lipid accumulation. Including milpa seeds in the diet could protect insulin-producing cells from oxidative stress, activate endogenous molecular regulators of lipids in serum, prevent the development of dyslipidemia, and regulate adipogenesis, among other molecular mechanisms of action.

5.4.3. Anticancer properties

Cancer is a group of diseases that can appear in any part of the body (149). This is characterized by the abnormal and rapid growth of altered cells that may adjoin other tissues and organs (metastasis). In 2020, cancer represented >10 million deaths worldwide. Some of its causes are related to tobacco and alcohol consumption, lack of physical activity, and low plant-based food intake (149). Some research related to the anticancer effect of milpa seed products has been conducted. A recent study demonstrated that regular consumption of corn, legumes, and vegetables was negatively related to the occurrence of breast cancer in postmenopausal women from Northern Mexico. In contrast with women of the same region and biological condition, with a regular diet based mainly on red and processed meats and foods high in fats and sugars (150).

In tumor-bearing mice (151), corn-derived nanoparticles (covered with polyethylene glycol) exhibited significantly higher serum concentrations and lower liver accumulation compared to not-covered corn-derived nanoparticles. Corn-derived nanoparticles were accumulated in the colon of mice, delaying tumor growth without hepatoxicity or nephrotoxicity. In a study performed by Leibbrand et al. (152), the reduction of prostatic hyperplasia symptoms in men using non-fat pumpkin seed extract was evaluated. These subjects were administered for 3 months a dose of 500 mg/day, equivalent to 10 g of seed, showing a decrease in symptoms between 8 and 12 weeks after the intervention. Dietary inclusion of milpa seed could be an effective alternative in preventing cancer. Preclinical and clinical evaluations are scarce; however, innovative treatments based on nanomedicine and nanopharmacology, which use isolated compounds from milpa seeds, have shown promising results.

5.4.4. Antiinflammatory properties

The inflammatory response is a mechanism of the body that activates the immunological system to react against external agents or an injury (153). Some disorders in the activation of the inflammatory response are related to the development of chronic diseases (153). In many cases, lifestyle factors, including diet, could promote antiinflammatory effects. A soluble extract from black corn showed antiinflammatory potential in an animal model (154). This effect was determined by down-regulating the gene expression of tumor TNF-α, interleukin-6, and NF-κB. The extract increased the Goblet cell size and number in the intestine villi and Paneth cell number in the crypt. The epithelial physical barrier was strengthened by up-regulating intestinal biomarkers AMP-activated protein kinase (AMPK) and caudal-related homeobox transcriptional factor 2 (CDX2). The authors concluded that this extract promotes intestinal antiinflammatory responses and enhances intestinal barrier function.

A mixture of seeds, including pumpkin, sesame, and flaxseed, was administered in hemodialysis patients to evaluate the antiinflammatory potential and the decrease in markers related to hypertriglyceridemia and diabetes. Results showed a decrease in the levels of TNF-α, interleukin-6, c-reactive protein, triglycerides, glucose, and insulin. These results suggest that the intake of PUFA in these seeds favors the improvement of the oxidant state and a hypotriglyceridemic effect (155). A study showed that pumpkin seed oil-loaded niosomes displayed a decrement in *in vivo* hair loss of 44.42%, representing 1.4-fold higher than commercial treatments (156). Authors also evaluated the cellular antiinflammatory activity of the experimental material, finding that functionalized niosomes inhibited 5α-reductase activity and hindered IL-6 activity in DU-145 and RAW 264.7 cellular models, respectively (156). Results indicate the great potential of pumpkin seed oil to reduce hair loss induced by inflammation.

Soluble compounds from milpa seeds may modulate the immune response by regulating gene expression and activating endogenous antiinflammatory mechanisms. At the same time, it is possible to identify other effects related to noncommunicable diseases when patients consume milpa seeds. Indicating that the intake of these seeds may exert multi-beneficial effects against chronic diseases.

In vivo, preclinical, and clinical trial studies reveal an increasing interest in milpa seeds as a source of macronutrients, micronutrients, and bioactive components for nutrition and health. Different phytochemical and bioactive components in the MS seeds could exert antihypertensive, antidiabetic, antiobesity, anticancer, and antiinflammatory potential. Evidence shows that milpa seeds can improve health through several metabolic pathways. However, most of the studies have been performed on the seeds separately or in combination with two seeds or with other foods.

Preclinical and clinical trials evaluating the combination of the three seeds to assess the possible synergistic effects on health are needed. Several molecular markers related to noncommunicable diseases have been associated with milpa seed bioactive components. Including reduction of blood pressure/vasoconstriction, reduction of glucose and lipid levels, modulation of the expression of fat storage/metabolism biomarkers, reducing oxidative stress, enhancing the expression of tumor suppressor genes, and downregulating cancer markers, among many others. Nanomedicine, *in silico* simulations, omics (epigenomic, genomic, transcriptomic, proteomic, etc.), and human trials are little-explored strategies to validate the milpa seeds potential effects to prevent and treat deficiencies in nutrition and chronic-degenerative diseases.

6. Conclusion

The milpa system has socioenvironmental, economic, cultural, nutritional, and healthy implications that must be deeply studied. There are several benefits that the MS presents for small farmers, especially related to the diversity of foods that could be obtained from relatively small spaces. The combined consumption of milpa seeds could help to meet macro- and micro-nutrient requirements and provide important phytochemicals and bioactive components with beneficial effects for human health.

However, there is limited information related to the effect of the combined cultivation of corn, common beans, and pumpkin seeds on their nutritional and phytochemical composition. Moreover, consuming these seeds in combination could exert health benefits beyond the nutritional requirements. The consumption of combined milpa triad system seed could promote a synergistic effect in the prevention or adjuvants in the treatment of noncommunicable diseases. However, comprehensive and well-designed studies are needed to validate the health benefits of the combined consumption of milpa triad seeds as health promoters. Novel and innovative explorations in biomedicine, nanotechnology, nutrigenomics, and other recent omics fields could be used to guide these multidisciplinary investigations and support the biological potential of this ancient agro-productive system.

Author contributions

LM, EV-T, and DL-V: conceptualization. LM, EV-T, and JC: methodology. LM, JC, DL-V, NM-H, EV-T, and JF-M: validation. OS-V: formal analysis. EV-T, LM, and OS-V: writing–original draft. OS-V, DL-V, NM-H, JC, EV-T, JF-M, and LM: writing–review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

AAS	amino acid score
ACE	angiotensin-converting-enzyme
AMPK	AMP-activated protein kinase
BV	biological value
CAT	catalase
CDX2	Homeobox protein encoded by the CDX2 gene
DPP-IV	dipeptidyl peptidase 4
EAAI	essential amino acid index
FAO	Food and Agriculture Organization
GAE	gallic acid equivalent
GPAT	glycerol phosphate acyltransferase
GPx	glutathione peroxidase
GR	glutathione reductase
HDL	high-density lipoprotein
HMG-CoA	β-hydroxy β-methylglutaryl-coenzyme A
LDL	low-density lipoprotein
MDA	malondildehyde
MUFA	monousaturated fatty acids
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PER	protein efficiency ratio
PPARs	peroxisome proliferator-activated receptors
PUFA	polyunsaturated fatty acids
RDA	recommended daily allowance
SFA	saturated fatty acids
SOD	superoxide dismutase
SREBP	Sterol regulatory element-binding proteins
TNF-α	tumor necrosis factor alpha
Wnt	Wingless and Int-1 signaling pathway

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Improving the nutritional and livelihood security of landless laborer through the Backyard Farming System

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Introduction: India is home to around 140 million landless laborers who live below the poverty line and are the most vulnerable group in terms of food and nutritional security. The three critical problems faced by the poor laborer families are poverty, hunger, and malnutrition. To address these problems, a backyard farming system was conceptualized and evaluated with an aim to ensure the nutritional security of landless laborers. The main objective of this work was to utilize the homestead area of 100-150 m^2 for ensuring year-round food availability.

Methods: Integration of vegetables, pulses, fruits, spices, fishes, and ducks was done in the available area. Technological interventions with the integration of ducks (Khaki campbell), fish (carps and Self-recruiting species), pulses, and leafy vegetables can help in improving nutrient consumption.

Result and discussion: A total of 1400 kg of vegetables (including root vegetables and leafy vegetables) can be produced annually from an area of 150 m^2 that can fulfil 30-70% of the vitamins (RDA of 70 % of B1 and 30% of B3) and mineral requirements (RDA of 45% of Iron and 30% of Ca) of the average family, as well as providing an annual saving of INR 25,000/annum and an extra income of INR 10,000/annum that can be earned from selling the extra produce. Herbs (Coriander, Mint, and Fenugreek) and spices (Ginger, Turmeric, and Chilli) with antioxidants ranging from 2-13 millimol/100 gm can help in developing a good immune status. Integration with ducks, pond dyke utilization with cucurbits, spices and herbs, and the introduction of self-recruiting species in a composite fish culture system can further enhance the income by INR 14,000/annum after family consumption. Year-round cultivation made the optimum use of the available resources. A net return of INR 30,000 from 300 m^2 could be obtained with a B:C ratio of 2.98 as well as generating an employment of 136 mandays. The food produced from the system can contribute to the nutritive requirements throughout the year and lead to a greater diversity in the food consumption pattern of the family.

KEYWORDS

landless laborers, food, nutrition, livelihood, employment

1. Introduction

Food, nutritional, and livelihood security are the parameters that mark the criteria for a healthy and secured life and is a right of every individual. The FAO defines Food security as "a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life."

India is home to 140 million landless laborers who live below the poverty line and are the most vulnerable group in terms of food and nutritional security. The three critical problems faced by poor laborer families are poverty, hunger, and malnutrition. Landless laborers and small and marginal farmers are deprived of nutritional security primarily because the diversity of food a person or a family should be utilizing is reduced because of monocropping. In addition, rice and wheat are the only grains that are distributed in Public Distribution Systems from the government. Other food items like vegetables, fruits, fresh foods, eggs, and milk are expensive items for a poor landless laborer. The average earning of a laborer is INR 300–500/day depending on their skill, with 100–150 days of employment in a year. So, farming families do not have the resources to meet their nutritional requirements.

The Champions of Nutrition Programs give due emphasis to the consumption of locally available fruits and vegetables through the establishment of a household kitchen garden. A kitchen garden/ Nutritional Garden is an area where food like fruits and vegetables can be grown to achieve a continuous supply for meeting the daily needs of the family utilizing primarily domestic discards. In a study conducted in Kerala, it was shown that a nutritional garden helps to meet the complete requirements of fruits and vegetables for a family throughout the year (Sheela et al., 1998). So if a family can grow its own fruits and vegetables, they can fulfil their own requirement by converting them into nutrients and energy. An average daily vegetarian diet needs to consist of 300 gms vegetables, 85 g fruits, 85 g pulses, and 475 g of cereals (Prathiba, 2012).

In the state of Tripura, where this work was carried out, there are 11 lakhs laborers out of a population of 36 lakhs. The average family size is 5–6 members. The family in the study only possessed a small house with 100–400 m^2 land adjacent to it. The area is largely utilized for drying clothes and as a sitting area but is not utilized for producing food items like vegetables, spices, or fruits or animal-based items like eggs and fish. These and other available areas were targeted to create a model for addressing their food and nutrition. This program was conceptualized based on the "Backyard farming System." The main stakeholders identified for this system were the women of the family and the main criteria that were taken into consideration were the available area and the time a woman can spare after her normal household work (Figure 1). Previous work (Akhter et al., 2010; Shukla and Rajkumari, 2012; Patalagsa et al., 2015; Ghimire, 2019) has also stated the significance of women in maintaining the homestead garden with the collective support of other family members.

The main objective of the program was:

(1) To ensure year-round availability of fruits, vegetables, and eggs for ensuring food and nutritional security of the landless family.
(2) To make the family livelihood secure by generating a surplus amount in the system.

FIGURE 1
Schematic representation of the concept of the Backyard Farming System.

2. Materials and methods

2.1. Study location and area developed

The study was conducted in the ICAR Research Complex for North Eastern Hill Region, Tripura Centre, India (Latitude is 23.90^0 and Longitude is 91.31^0). An area of 300 m^{2}was developed (150 m^2 land area and 150 m^2 pond area) in the form of the Backyard Farming System (Figures 2, 3).

2.2. Fish species and stocking density

For the study, 150 fingerlings of Indian major carps like *Labeo rohita* (Hamilton, 1822), *Catla catla* (Hamilton, 1822) and *Barbonymus gonionotus* (Bleeker, 1849) were introduced along with 2 Kg of small indigenous self-recruiting species (SRS) like *Amblypharyngodon mola* (Hamilton, 1822) (Mola) and *Puntius* (Puti). Mola and Puti breed in the pond and therefore enable poor households to increase their consumption of fish. All standard pond management practices like management of aquatic weeds and liming were practiced. No fertilization was done since the system was integrated with ducks.

2.3. Animal component

Along with fishes, 12 Khaki Campbell cross ducks (2 male and 10 female birds) were also integrated in the system for which a shed was constructed at one corner of the pond. In this system, a free-range system of duck rearing was followed. The ducks were allowed to scavenge for feed in the day time and were allowed to enter the shed during the night. For the night, extra provision for feed and water was made in the shed. They had access to pond water through a bamboo-made sloppy run.

2.3.1. Legumes, vegetable, and fruit component

Tree-type vegetables like Drumstick (*Moringa oleifera* Lam.) (1 plant) and Jackfruit (*Artocarpus heterophyllus* Lam.) (1 plant) were planted. Cucurbits like pumpkin (*Cucurbita moschata* Duchesne), bottle gourd (*Lagenaria siceraria* Moilina), ash gourd (*Benincasa hispida* Thunb.) and bitter gourd (*Momordica charantia* L.) were planted at the four sides of the pond and above the compost pit on bamboo platforms (Figure 4). Different varieties of beans were planted in the fencing of the household to ensure sufficient availability year-round. In addition, 100 Pigeon pea plants were planted in the boundary of the system. Tuber crops like Colocasia, elephant foot yam

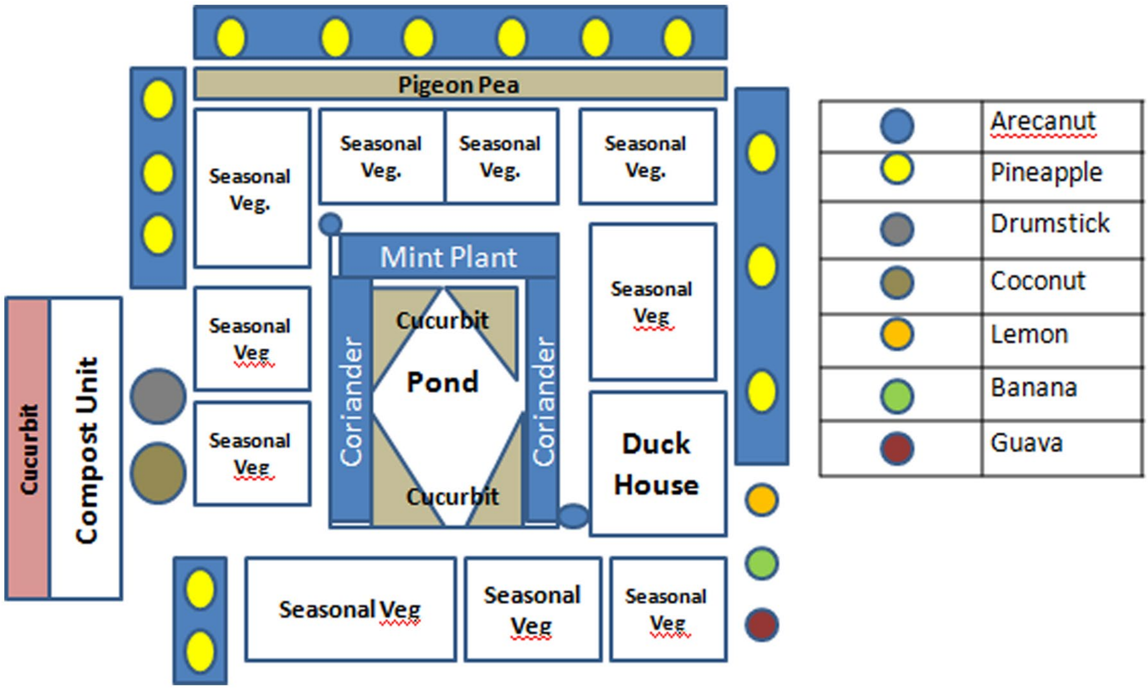

FIGURE 2
Schematic diagram of the Backyard Farming System.

FIGURE 3
View of Backyard Farming System.

[*Amorphophallus paeoniifolius* Dennst. (Nicolson)], and dioscorea yam were also incorporated. The vegetables introduced were lady finger (*Abelmoschus esculentus* L.), brinjal (*Solanum melongena* L.), chilies (*Capsicum sps*), leafy vegetables, cabbage (*Brassica oleracea* L.), cauliflower (*Brassica* spp.), radish (*Raphanus sativus* L.), vegetable pea (*Pisum sativum* L.), mustard (*Sinapis spp.*), maize (*Zea mays* L.), and tomato (*Solanum lycopersicum* L.). For fruit, Papaya (*Carica papaya* L.), Guava (*Psidium guajava* Linn.) (1 no), Banana (1 vegetable variety and 2 fruit variety (*Musa* spp.), Lemon (*Citrus limon* L.) (2 plants), were incorporated. A single plot was used for the production of some traditional medicinal plants like tulsi (*Ocimum sanctum* Linn.) and thankuni (*Centiella asiatica* L.).

2.4. Spices

Coriander (*Coriandrum sativum* L.) and mint (*Mentha spicata* L.) were planted in pond dykes to ensure sufficient availability of

FIGURE 4
Production of different components in the system.

FIGURE 5
Different cropping patterns followed in the system.

moisture. Turmeric (*Curcuma* spp.) and ginger (*Zingiber officinale* Rosc.) were planted in the shaded part of the backyard without disturbing the main plots.

2.5. Cropping pattern

An 150 m^2 area was divided into 15 plots of 10 m^2 each where vegetables were planted in a staggered manner to ensure daily production of vegetables. The seeds were sown a few days prior to the standing crops reaching their production stage. Crops of different types were grown through inter-cropping, mixed cropping, or relay cropping (Figure 5). Seeds of all crops were initially procured from local sources and thereafter maintained for future use.

2.6. Compost pit

For on-farm recycling of nutrients, two compost pits were dug behind the duck house. The wash offs of the duck house were diverted to the compost pits or the fishponds through a valve. To prevent direct sunlight from affecting the pits, bamboo platforms were built over the compost pit and cucurbits were grown over those.

2.7. Economics of the system

The data on different inputs and outputs were recorded regularly. The requirement of food components for the family was taken from data repositories of the Indian Council of Medical Research (ICMR). The gains in nutrition were calculated from the data of the ICMR-National Institute of Nutrition (NIN). Economics was calculated as per (Das et al., 2021). Gross return was calculated by multiplying the production of the components with the prevailing unit market price. Benefit Cost ratio (B:C Ratio) was estimated by dividing the gross return with the cost of production. The net return was divided by 365 to obtain the System economic efficiency (SEE). This farming system used a single laborer for 3 h in a day. The purpose was to make it achievable by a woman in the household.

2.8. Equivalent yield and cropping intensity

The production of the components was converted to Rice Equivalent Yield (REY) by the formula: REY=Production (Kg) of component X Price per Kg of Component/Price per Kg of rice (De Wit, 1960).

Protein equivalent yield (PEY) was calculated by the formula: PEY (Kg/ha) = Production of component X Protein content per Kg of that component (Manay and Shadaksharaswamy, 1987).

Man-days were calculated on the basis of working hours: 1 man-day = 8 h

Cropping intensity was calculated by the formula:

$$\text{Cropping intensity} = \left(\begin{array}{l}\text{Sum of all area under all}\\ \text{components / Net land area}\end{array}\right) \times 100$$

2.9. Data analysis

The data obtained was analyzed using MS Excel Program. Standard data on food and nutritional components were obtained from the ICMR-National Institute of Nutrition (NIN) and then used for comparative analysis of the component/system in MS Excel. All the graphs were prepared in MS-Excel and then exported.

3. Results

3.1. Fish production, egg and meat production, and vegetable production in pond dykes for nutrition and income

From an area of 150 m^2, 60 kg of fish could be harvested (50 kg of carps and 10 Kg of SRS). The ICMR recommends consumption of 25 g of fish per day for a person (Figure 4). So, the annual requirement for a family of five is 45 kg. After meeting the family's requirement, a surplus of 15 kg is available from the system that can be sold at the market for around INR 2250/− (Table 1).

In addition, the four corners of the pond were utilized for growing cucurbits like Ash-gourd and bottle gourd which are good sources of vitamins, minerals, and fibers. From a total of 16 plants, 480 kg of fruits could be harvested in one cycle. Two hundred kilograms can be used for family consumption, leaving a surplus of 280 kg for livelihood generation (in a year 2–3 cycles of gourds could be grown) of INR 5600/− that could be earned from the extra produce (Table 1).

Additionally, the incorporation of ducks was done in the system. Ten female ducks and two male ducks were introduced in the system. One thousand one hundred eggs were surplus after family consumption of 910 eggs; the surplus eggs can be used for income generation of INR 6600/− for the family (Table 2; Figure 4). Ten kilograms of meat can be produced that can be used to meet the nutritional requirements of the family.

In the pond dykes and the slopes, spices like cilantro and mint were grown throughout the year.

3.2. Production of legumes and vegetable from the system

The mean monthly vegetable production ranged from around 40 kg to around 140 kg/month (Figures 4, 6). The maximum vegetable production was reported in the month of February and the least in the month of July. The average vegetable production from the system was 18 kg/week. This vegetable production is exclusive of the cucurbits that were produced from the pond dykes. One hundred kilograms of pigeon pea could be harvested from 100 plants that were grown in the boundary of the system (Table 2). Seventy-three kilograms is required for family consumption, leaving a surplus of 27 kg that can be used for generating income (~INR 2700/− can be earned by selling 27 kg of pigeon pea).

3.3. Recycling of nutrients in the system

The left-over crop biomass and the excreta of ducks were used in the compost pit for recycling nutrients in the system on the concept of farm bio-resource flow. Two compost pits were made, and the production ranged from 70 to 100 kg/month (Table 3). The integration of ducks in the system made the system more sustainable because of the nutrient-rich duck manure. On average, 12 ducks generated 30–40 kg of droppings which was diverted to the compost pit. The wash outs of the house were diverted to the ponds. Vegetable residue was produced in the range of 20–60 kg.

3.4. Economics and nutrition gain from the system

The backyard farming system model generated a REY of 18.05 t/ha with a PEY of 72.84 kg/unit/year. Cropping intensity was 150%, generating an additional employment of 136 man-days (Table 4).

3.4.1. Change in consumption pattern and nutrition gain of the farm family

The RDA provided by the ICMR was considered as the basal level for ensuring food and nutritional security of the family. Figure 7 shows that the developed model in the small area available could contribute

TABLE 1 Fish production, egg and meat production, and vegetable production in pond dykes for nutrition and income.

Sl. No.	Components	Production	Family Consumption	Surplus Production	Income (INR)
1	Fish	60 kg	45 kg	15 kg	2,250/−
2	Duck eggs	2,000 nos	910 nos	1,100 nos	6,600/−
3	Duck meat	10 kg	10 kg	Nil	Nil
4	Vegetables from pond dyke	480 kg	200 kg	280 kg	5,600/−
				Total	14,450/

a sufficient amount of vegetable and eggs along with pulses, spice, and fresh foods that could lead to a significant change in the consumption patterns of the household. According to the ICMR-RDA, a family needs 1,000 kg of vegetables and 900 eggs to maintain nutrient requirements. The production from the system was 1,400 kg of Vegetables and 2000 eggs. The production of pulses was 100 kg against the requirement of 73 kg. Sixty kilograms of meat like fish and duck were produced against the requirement of 91 kg. Because of these interventions, the expected gains in nutritional improvement were met. Protein consumption could was by 80%, and micronutrients like calcium by 60%, Iron by 40%, and Thiamine by 70% (Figure 8). An average family of five needs INR 75,000/− to fulfil their food requirements (Table 5). In the system developed after fulfilling the food requirement, a net return of INR 30,410/− was achieved with a BC ratio of 2.98. The cost of cultivation was calculated to be INR 15,290/−. The system economic efficiency was calculated to be 83.3 (Table 4).

4. Discussion

The landless laborers and the poorer section of society suffer from lack of nutrition. The Public distribution system of the Government of India provides rice/wheat and sugar, although these alone do not meet the nutritional needs of people. In this context, the backyard farming system can help in improving the food and nutritional security of laborer families. This can also lead to livelihood security of the family. A landless laborer has a house and a small adjoining area of 100–400 m^2 in his possession. These areas were targeted to be effectively utilized so that the food requirement of the family can be addressed. In the case of small and marginal farmers, kitchen gardens form an integral part of the households but they are done in a very casual and subsistence level, a level at which the food and nutrition demand cannot be attained. The availability of diverse foods to meet nutritional requirements does not currently happen locally at affordable prices. The prices of vegetables, fruits, and other protein sources fluctuate, and the prevailing market price is unaffordable by the laborers. Through this system, not only the landless laborers but also small and marginal farmers can have access to a diverse year-round supply of food and nutrition along with income generation. The key stakeholders will be women who can contribute 2–3 h daily after their household chores to maintaining the garden. In a study that was conducted in the state of Kerala, a family of four could have an assured year-round supply of vegetables, milk, and eggs by integrating livestock as a component with homestead farming in an area of 0.2 ha (John, 2014).

The local pattern of consumption of poor households in Tripura is rice and fermented fish; hence there is a gap in meeting the nutritional requirement of the body. In a survey by the International Food Policy Research Institute (IFPRI), it has been found that in the state of Tripura, stunting in children below 5 years ranges from 20 to 32%, anemia in women of reproductive age is 50% and wasting ranges from 14 to 24% (Kohli et al., 2017). Nationally, malnutrition in Indian children is at a higher level with 42.3% underweight, 59% stunted, and 11% wasted (Indumathi et al., 2012). Access to food and nutrition is a human right, but only a small percent of people have access to nutritious food and a huge chunk of the population live on a subsistence diet which is nutritionally imbalanced (Karim et al., 2021). This leads to malnutrition, stunting, anemia, and other physical and cognitive health problems. As per the ICMR, an Indian food platter should constitute 300 g of Vegetables, made up of 50 g of Green Leafy Vegetables, 50 g of Roots & Tubers, and 200 g of other vegetables.

TABLE 2 Performance of pigeon pea in the boundary of the system.

Items	Production	Price (INR)
100 pigeon pea plants	1–1.5 kg/plant total yield from system: 100 kg	INR 100/kg ie. INR 10,000/−
Family consumption for nutrition (Protein, fiber, Vitamins, and minerals)	73 kg	
Surplus for income generation	27 kg	INR 2,700/−

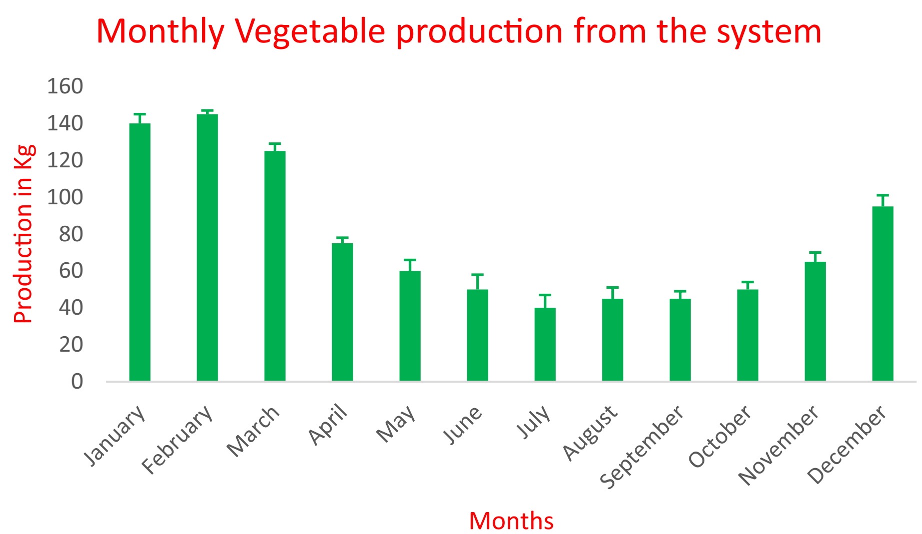

FIGURE 6
Monthly Vegetable production from the system.

TABLE 3 Compost pit for nutrient recycling.

Components	Production/Month
12 ducks	30–40 kg
Vegetable residue	20–60 kg
Total production from 1 pit/month	70–100 kg
Requirement/month	100–110 kg

TABLE 4 Economics and different sustainable parameters of the system.

Economics of the system	
Expenditure (INR)	15,290/−
Gross return (INR)	45,700/−
Net return (INR)	30,410/−
B: C ratio	2.98
SEE	83.3
Equivalent yields, Cropping intensity, and employment generation	
REY (t/ha)	18.05
PEY (kg/unit/year)	72.84
Cropping intensity (%)	150
Man-days	136

Additionally, each individual should also consume 100 g of fresh fruits regularly and, in case of pregnancy, the consumption of leafy vegetables should be increased to 100 g to cater to the higher need for folic acid and iron. To fulfil the recommended dietary allowance, a family of five needs 1,000 kg of vegetables annually. A total of 1,400 kg of vegetables (including root vegetables and leafy vegetables) could be produced from an area of 150m^2 that can fulfil 30–70% of the vitamins (RDA of 70% of B1 and 30% of B3) and mineral requirements (RDA of 45% of Iron and 60% of Ca) of the family. Herbs (Coriander, Mint, and Fenugreek) and spices (Ginger, Turmeric, and Chilli) with antioxidants ranging from 2–13 millimol/100gm (Benzie and Wachtel-Galor, 2011) can help in developing a good immune status for the family. Many local plants have anti-oxidative compounds and anti-mutagenicity and anti-inflammatory properties (Chavasith, 2012) and, globally, the North Eastern States of India are recognized as being rich repositories of aromatic and medicinal plants (Lahiri et al., 2017). After family consumption of around 1,000 kg, a surplus of 400 kg will be available to be sold in the market, thereby generating an income of INR 10,000/− with an annual saving of INR 25,000/− that could otherwise be spent for procuring these items. In this system, from a small area, increased vegetable production could be attributed to enhanced availability of nutrients leading to good growth (Yadav et al., 2013). The application of recycled duck manure and vegetable biomass from compost pits aided in improving the physical, chemical, and biological properties of soil that led to high vegetable production (Das et al., 2017). The most notable technologies were utilizing the pond dykes, utilizing the pond corners, and the staggered method of vegetable cultivation by dividing the area into 10m^2 plots which could be easily managed by a woman. Different types of vegetable peas and beans, like French beans and yardlong beans, were planted seasonally in plots as a vegetable component. To further utilize the available spaces in a more efficient way along with adding to the protein requirement of the family, 100 pigeon pea plants were planted in the boundary of the system. 100 kg of pigeon pea could be harvested. An income of INR 2700/− could be generated after family consumption of 73 kg. The incorporation of pulses in the north eastern region of India is very important because this can lead to nutritional security if the deficit in these crops can be addressed (Layek et al., 2021). These nutrient-dense crops not only contributed to the protein requirement of the family, but also enriched the soil for the next crops. Leguminous crop can lead to nitrogen improvement of the soil because of fixation in root nodules and contribute to manure after composting (Ali and Venkatesh, 2009). In this system, the local preference and diversity in vegetables and other crops can be additionally addressed. This will further increase vegetable consumption in that area.

The recommendation for protein, fat, vitamins, and minerals through animal sources is 1 kg per week for a family of five. To fulfil this requirement, ducks and animals were integrated into the system. For fish production, a composite fish culture system was adopted. The species that were incorporated were *Labeorohita*, *Catlacatla*, *and Barbonymus gonionatus*. Along with these three species, two self-recruiting species, mola *Amblypharyngodon mola*) and puti (*Puntius sophore*), were also incorporated. Sixteen self-recruiting species of fish have been identified (Felts et al., 1996) in India. Among these, mola (*Amblypharyngodon mola*) and puti (*Puntius sophore*) are important because of their high nutritional value. The culture of these species with major carps may contribute to the diet of the rural poor. In addition, the inclusion of these indigenous species in composite systems can improve the nutritional security and add to the income enhancement of farm families as they fetch higher market prices (Debnath et al., 2014). Policy makers worldwide have been emphasizing the utilization of smaller water bodies for aquaculture because they are largely underutilized and, if properly managed, can be a source of nutrition and income for farmers (Debbarma et al., 2020). In the present work, 150 fingerlings of *Labeorohita*, *Catlacatla*, and *Barbonymus gonionatus* were stocked in a pond of size 150 m^2 along with 2 kg of SRS like Mola and Puti. An overall production of 50 kg of carps and 10 kg of SRS could be achieved. The SRS can be harvested easily by the women with the use of traditional traps. In the Indian family scenario, the women of the household eat after all the family members have had their share of food. If bigger fish are cooked, they may or not have a share, but in the case of small fishes it is likely they are assured of a share. The intra-household distribution of fish, depending on the kind of fish cooked, is another important dimension that upholds the involvement of farm women to take up fish culture as they are assured of the adequate nutrition for their family. In case of small species, unlike the larger ones, they will be marketed locally within the villages, especially by women and children. The significance of SRS has been felt in the last decade or so and some works have cited their significance in uplifting the nutritional status and economy of the rural poor (Roos, 2001; Amilhat et al., 2005). Debnath and Sahoo (2020) have evaluated *Esomus danricus* as a potential SRS in aquaculture that in enhances the benefit ratio of composite fish culture by 4.5%. Further, Pond dyke utilization with cucurbits, spices, and herbs can further enhance the income by INR 5000/annum. In a different study by Debnath et al. (2015), an additional benefit of INR 5000/ha could be obtained through the integration of crops and fruits in pond dykes. Pond dyke utilization with vegetables and integration of poultry with aquaculture is 850% more profitable than a single enterprise that is conventionally practiced (Babu et al., 2019).

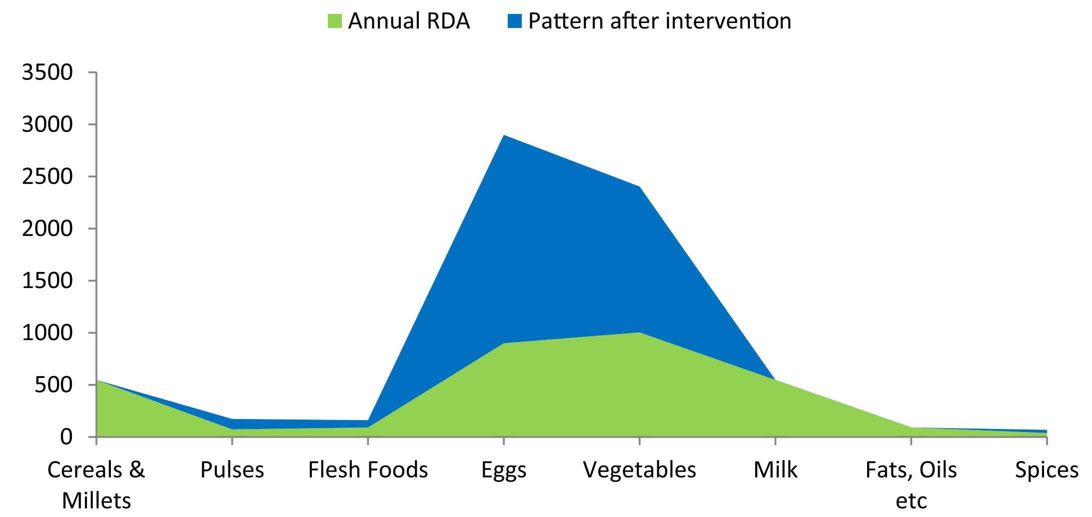

FIGURE 7
Expected changes in food consumption pattern through the backyard farming system (Inner ring represents annual RDA (Indian Council of Medical Research India, 2020) for a family of five, and the outer ring represents the food consumption pattern after intervention).

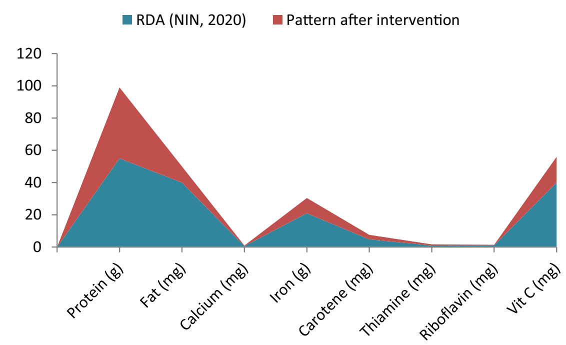

FIGURE 8
Expected gains in nutrient improvement.

Twelve ducks were introduced in the system. Ducks are hardy and relatively more resistant to diseases than poultry birds (Panda et al., 2022). So, they were chosen over poultry birds to make the woman more independent in raising the animals; they also earn more money in market than poultry birds. A duck house was constructed at one corner of the pond and a wash out was made for the droppings to go directly to the pond. A valve was made in the wash out so that if the pond gets over-enriched, the droppings can be diverted to the compost pit. The compost pit was built behind the duck house. This integration of ducks in the system can provide an additional component of meat and eggs which are preferable protein sources for people of North-Eastern India. It generates production of additional food and income to forthe farmer. Chauhan et al. (2022) have emphasized the significance of livelihood diversification through multiple sources of income generation for improving farmers' livelihood. Duck manure is a rich source of nutrients and has traditionally been integrated with aquaculture. It contains 0.9% Nitrogen and 0.4% Phosphorous, which act as good organic manure for producing phytoplankton and zooplankton in the pond (Saikia et al., 2020). Planktons are natural fish food organisms and the availability of sufficient planktons is beneficial for the growth and health of fish. Apart from this, ducks act as natural aerators, helping in the oxygenation of the pond. As a result, dependency on outside feed in the pond is reduced and 60% of operational expenditure is saved, which would have otherwise have been spent on fish feed (Tripathi and Sharma, 2005; Das et al., 2017). Approximately 40–50 kg of organic waste is converted into 1 kg of fish (Kumar et al., 2012; Saikia et al., 2020) and can be a viable option for natural biodiversity (Banerjee et al., 2014).

Two compost pits were constructed behind the duck house. In addition to duck manure and vegetable residue, aquatic weeds were also added to the pits. A production of 70–100 Kg of manure could be produced from a single pit. As this system was integrated with the ducks, the nutritionally rich duck manure helped in producing good-quality organic manure. This helped in manuring the plots, thereby complementing the recycling of nutrients in the system. Recycling of waste followed by field application improves the soil health and reduces the family's dependency on extraneous inputs, thereby reducing the cost of production (Yadav et al., 2013; Das et al., 2013a). The cucurbits planted in the bamboo-raised structure that was made over the compost pits provided shade to the pits, thereby preventing nutrient losses from the manure. Additionally, these cucurbits can provide food and income to the family. The whole plant is consumed in North East India as the leaves and flowers are also enjoyed by the people.

An integrated farming system is a set of systems where waste is utilized as inputs to generate food, which not only makes the system eco-friendly but also economic (Ansari et al., 2014). An integrated farming system through farm diversification is necessary to address multiple concerns like food and nutritional security, poverty alleviation, generation of employment, and improvement of the environment with prudent utility of natural resources (Kumari et al., 2019). As per ICMR recommendations, the requirement of weekly vegetables and fruits, eggs, meat, and pulses are 14 kg, 38 eggs, 1 kg, and 1.4 kg, respectively, for a family of five. Through this system, a production of 26 kg of vegetables, 38 eggs, 1.1 kg of meat, and 2 kg of pulses per week could be generated. The pattern of availability of pulses increased by 37%, Eggs by 119%, and vegetables by 40% in comparison to the RDA, thus enhancing the nutritional requirement of protein by 80%, energy by 60% and vitamins and minerals by

TABLE 5 Livelihood assessment for food and nutritional security (ICMR).

Sl. No.	Particulars	Individual Requirement (kg/year)	Family Requirement (kg/year)	Budget (INR)
1	Cereals	157	785	23,550/–
2	Pulses	14.6	73	8,760/–
3	Oil	8 lit	40 lit	6,400/–
4	Vegetables (including GLV, Roots, and tubers) and Fruits	18.25	92	2,300
5	Meat	10	50	7,500
6	Egg	182	910	5,460
7	Milk	55 lit	275 lit	16,500
8	Sugar	12	60	2,700
9	Spices	2	10	1,000
10	Salt	3	15	300
	Total			INR 74,470/–

30–60%.This indicates that sufficient availability of different components of food for consumption and income generation. In an earlier study (Devendra and Thomas, 2002), it was reported that an integrated farming system is an important means for meeting the protein needs of a family through meat, eggs, and milk for small and marginal farmers. Success of any integrated farming system is determined by quantifying physical indicators of sustainability based on system productivity, profitability, and employment generation (Singh et al., 2016). In this system, all the physical indicators like productivity, profitability, and employment generation could be achieved. Rice equivalent yield of 18.05 t/ha was observed from this system. This was because of higher production of vegetables and eggs from the system. An 190% increase in REY has been observed in a duck-based farming system in a previous study (Das et al., 2013b). A protein equivalent yield of 78 kg was observed in this single unit in a year. This indicates that this system can generate adequate protein to meet the nutritional requirements of the family. A net return of INR 30,410/with a B-C ratio of 2.98 was generated from the system. The inclusion of ducks and year-round vegetable cultivation and utilization of vertical farming in pond dykes and above compost pits led to higher system production and thereby high income. An SEE of 83.3 and cropping intensity of 150% indicates that it is a self-sufficient system and such a small area can also lead to income if used judiciously. This indicated that through this system, the women of the household, who otherwise do not have any access to finance, could earn an amount of INR 30,000/year with 136 days per year of gainful employment. This also will improve the mental satisfaction of the women of the household as they will be the key generators of food. They could harvest one or the other components depending on her choice throughout the year. The food produced from the system can contribute to the nutritive requirements throughout the year as well as leading to a greater diversity in the food consumption pattern of the family. Moreover, the vegetables, ducks, and fish produced in the system will be largely organic in nature because, as a member of the family, the farmer will adopt all good management practices to assure good health for her family. She could also sell the produce at a high price in the market because of ever-increasing demand for good health and quality food (Singh et al., 2021).

5. Conclusion

The main objective of this study was to ensure the food and nutrition of a poor landless laborer utilizing the available area he has and utilizing his own family labor. A small are of 150m^{2}land and 150m^{2}water was developed for a backyard farming system integrating vegetables, fruits, fish, and ducks. This system can produce year-round vegetables, eggs, and fish that are sufficient to meet the needs of a five-member family. Leguminous crops were incorporated to make the family protein sufficient. Moreover, the surplus vegetables and eggs can be used to improve the livelihood of the family. As the laborer will be occupied with daily labor engagement, the woman of the household was targeted, who, after completing her household chores, has 2–3 h of available time that she can use for generating food for her family. A net return of INR 30,000 and an annual saving of INR 50,000/– from 300 m^2 with a BC ratio 2.98 suggests that this system is economically profitable and can also be adoptable by the landless laborers.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

LS: conceptualization of the project, designing of experiment, data collection, analysis of data, and writing the manuscript. BK: conceptualization of the project and designing of experiment. AD: conceptualization of the project, designing of experiment, and management of crop component. CD: data collection and analysis of data. VS: management of duck component. HD: management of vegetable and fruit component. HB: pond management. JP: provided self recruiting species analysis of data and writing the manuscript. AS and JD: assistance in data collection. BD: guidance in manuscript preparation.

VM: monitoring, facilitation, and overall guidance in project works. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Elucidating the interactive impact of tillage, residue retention and system intensification on pearl millet yield stability and biofortification under rainfed agro-ecosystems

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Micronutrient malnutrition and suboptimal yields pose significant challenges in rainfed cropping systems worldwide. To address these issues, the implementation of climate-smart management strategies such as conservation agriculture (CA) and system intensification of millet cropping systems is crucial. In this study, we investigated the effects of different system intensification options, residue management, and contrasting tillage practices on pearl millet yield stability, biofortification, and the fatty acid profile of the pearl millet. ZT systems with intercropping of legumes (cluster bean, cowpea, and chickpea) significantly increased productivity (7–12.5%), micronutrient biofortification [Fe (12.5%), Zn (4.9–12.2%), Mn (3.1–6.7%), and Cu (8.3–16.7%)], protein content (2.2–9.9%), oil content (1.3%), and fatty acid profile of pearl millet grains compared to conventional tillage (CT)-based systems with sole cropping. The interactive effect of tillage, residue retention, and system intensification analyzed using GGE statistical analysis revealed that the best combination for achieving stable yields and micronutrient fortification was residue retention in both (wet and dry) seasons coupled with a ZT pearl millet + cowpea–mustard (both with and without barley intercropping) system. In conclusion, ZT combined with residue recycling and legume intercropping can be recommended as an effective approach to achieve stable yield levels and enhance the biofortification of pearl millet in rainfed agroecosystems of South Asia.

KEYWORDS

conservation agriculture, nutrient biofortification, pearl millet, system intensification, residue retention, zero tillage

1. Introduction

Malnutrition, lower productivity, and recurrent crop failures due to insufficient soil moisture are the predominant challenges in the water-deficit agroecologies across the globe. The twin catastrophes of climate change and the COVID-19 pandemic have further aggravated these problems in ecologically and economically fragile regions like South Asia and Africa (1). The restrictions imposed due to the pandemic have impeded food production, distribution, and trade, exacerbating the already dismal situation caused by climate-related catastrophic events. As weather patterns shift and ecological systems undergo physiological adaptations, the productivity of these regions has been severely impacted, leading to increased malnutrition and compromised food security (1, 2). Transformative changes in major food systems are necessary to build resilience and ensure equitable access to nutritious food for all (3). Micronutrient deficiencies, particularly zinc (Zn) and iron (Fe), rank among the leading causes of illness and disease in developing economies (4, 5). Globally, ~17.3% of the population suffers from zinc deficiency alone (6). Over 828 million people are estimated to be undernourished worldwide.[1] To tackle these challenges, new fortification and system resilience approaches are needed in addition to cultivating nutrient-rich and high-yield-producing food crops (4). Micronutrient malnutrition remains a pressing issue due to low nutrient content and bioavailability in staple food grains, especially in arid and semi-arid regions where diverse and nutritious food options are limited (4, 5, 7–9). Biofortification of major food crops through climate-smart ecological approaches is a promising pathway to ensure affordable nutrition (4, 7, 8, 10, 11). Food fortification offers the advantage of delivering nutrients to large populations without drastic changes in food consumption patterns and minimal production costs (11, 12).

In rainfed regions of India, Africa, and Latin America, marginalized communities and small farmers heavily rely on millet and millet-based cropping systems for nutritional security (8, 13). Pearl millet [*Pennisetum glaucum* (L.) R. Br.], a major cereal crop of rainfed drylands, covers ~30 million hectares in Asia and Africa, with India accounting for around 30% of the total area (14). It exhibits inherent resilience to climate change and water stress and contains relatively higher levels of Zn, Fe, and proteins as compared to other cereals, such as wheat, rice, and maize (8, 9, 15). Additionally, it contains phenols and antioxidant compounds that are essential for human immune health (8). Among the leading production systems of semi-arid ecologies of the Indian sub-continent, the pearl millet-Indian mustard [*Brassica juncea* (L.) Czern & Coss] cropping system (PMCS) is predominant. This production system is practiced over a million-hectare area, mainly in tropical regions of India characterized by undulating light- to medium-textured soils, water scarcity, and poor soil fertility (16). This system faces challenges such as intermittent hydrothermal stresses, sub-optimal nutrient utilization, unstable productivity, and below-par quality of economic produce (14).

CA has been advocated as a solution to overcome these issues in rainfed dry-land regions (9, 13, 14). CA helps conserve soil moisture through crop residue retention (CRR), reducing evaporative water losses (9, 14), moderating thermal effects (14, 15), altering weed flora (17), and improving soil health (7). Despite the global advocacy for CA in diverse ecologies, research in India has primarily focused on irrigated agro-ecosystems, neglecting rainfed farming systems. There is a lack of systematic information on the various aspects of CA in the PMCS, including nutrient and moisture dynamics in the soil-plant system, yield stability, and crop quality. Diversification with legumes and cereals within CA and a system intensification approach could provide effective alternatives for climate resilience, higher productivity, and nutritious food (15, 18).

The association of legumes with their natural colonizing microorganisms appears to be a powerful combination for a sustainable and eco-friendly approach to cope with climate change effects on crops and improve plant nutrition (19). Legumes play a vital ecological role in improving the chemical and biological functions of the soil-plant-atmospheric continuum, in addition to their rich nutritional value (18). Crop management practices such as tillage, crop rotations, legume inclusion, and CRR enhance crop quality and nutrient assimilation in plant parts (20). The improvement in micronutrient content under ZT, in particular, has been associated with enhanced microbial activity and nutrient release during the decomposition process of crop residues (1, 21). Legume-imbedded systems fix more N with the addition of sufficient biomass with a narrow C:N ratio (22). This expedites the biomass decomposition with more C-sequestration and more micronutrient acquisition (23). The resultant soil organic matter (SOM) assists in the synthesis of organic acids in the rhizosphere, which in turn behave as micronutrient chelates, influencing the translocation and remobilization of micronutrients. It is hypothesized that the interaction between tillage, residue retention, and system intensification can sustain higher crop productivity through moisture conservation, less weed infestation, higher water use efficiency (WUE), and greater nutrient recycling while augmenting the micronutrient and protein content in the edible portion of the crops. The independent effects of these three factors are known to positively impact soil moisture conservation, weed infestation, and nutrient acquisition by plants. Additionally, this study focuses on providing integrative solutions for climate change and food security by utilizing the innovative approaches of sustainable intensification, tillage configuration, and cropping system-based diversification to achieve food biofortification and yield stability in prevalent millet systems. We also hypothesized that intercropping under ZT and CRR could enhance crop yield, micronutrient uptake, and grain quality. These practices are expected to enhance soil structure, increase soil organic matter content, and subsequently lead to significant improvements in crop yield stabilization, biofortification, and grain quality.

This study aims to investigate the effects of contrasting tillage systems, residue management options, and system intensification on pearl millet fatty acid and micronutrient contents, yield stability, and grain quality. By exploring diverse intensification alternatives, the research will provide a theoretical basis for selecting suitable cropping systems and tillage practices in rainfed areas. Furthermore, the study examines the impact of crop residue retention on yield stability, grain quality, fatty acid contents, and micronutrient profiles of pearl millet within the pearl millet-mustard production system in a cropping system mode.

1 WHO (2022). https://www.who.int/news/item/06-07-2022-un-report--global-hunger-numbers-rose-to-as-many-as-828million-in-2021 (accessed June 28, 2023).

2. Materials and methods

2.1. Experimental site and climatic conditions

A 2-year field experiment (2020–2021 and 2021–2022) was conducted at the ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi (28°35′ N latitude, 77°12′ E longitude, 229 m altitude). The experimental site is located in a sub-tropical semi-arid climate characterized by hot and dry summer and cold winter seasons, with a mean annual precipitation of ~652 mm. The soil of the experimental site is sandy-loam in texture and taxonomically classified as a Typic Haplustept of Gangetic alluvial origin (24). The description of the initial soil characteristics of the experimental field is given in Table 1, while the weather conditions (rainfall, minimum, and maximum temperatures) of the *Kharif* and *Rabi* seasons are presented in Figure 1.

2.2. Crop and soil management

As rainfed crops, pearl millet, cluster bean, and cowpea were sown during the *Kharif* (July–October) season. Winter season crops (Indian mustard, chickpea, and barley) were also grown as rainfed crops; however, a pre-sowing irrigation of 50 mm depth was applied to ensure uniform germination and crop establishment. The triplicate experiment was laid out in a split-plot design with cropping systems and tillage configurations as the main plot (six treatments) and crop residue management as the sub plot treatments (three). Field layout details are explained in Supplementary Figure 1. CA practice encompasses not tilling the soil, rotating crops over the years, and leaving crop residues on the surface (35). In ZT plots, no preparatory tillage operations were carried out. CT is totally different from ZT practice. In CT plots, one deep plowing, two passes of harrowing, and planking were performed to have a uniform seedbed of fine tilth, and crop residues were incorporated by the rotavator. Mustard crop residues (2 Mg ha^{-1}) from the previous crop were incorporated into CT and retained in ZT plots. For each sub plot (48 m^2), 9.6 kg of crop residues (required rate of 2 Mg ha^{-1}) were weighed, chopped, and applied. The pearl millet cultivar *"Pusa 443"* and the mustard cultivar *"Pusa mustard 28"* were sown with a seed rate of 4 kg ha^{-1} for each crop. A detailed description of the treatments is given in Table 2. The timeline (sowing and harvesting) is shown in Supplementary Figure 2. The row-to-row and plant-to-plant spacing of 50 and 30 cm, respectively, was adopted. The sowing was performed using a seed-cum-fertilizer drill in CT plots and a 9-tyne zero-till planter in ZT plots. The fertilizer application was made based on the soil test values of the experimental plots. The pearl millet and mustard crops received fertilizers at the rates of 60:40:40 and 80:40:40 kg ha^{-1} of N:P:K, respectively. The sources of nutrients were urea (46% N), single superphosphate (SSP, 16% P_2O_5), and muriate of potash (MOP, 60% K_2O). In the *Kharif* season, two-thirds of the nitrogen (N) and total doses of P_2O_5 and K_2O were applied as basal. The remaining one-third N-dose was applied 5–6 weeks after sowing, based on the soil moisture status of the experiment field. The crop residue nutrient contents (N, P, and K) have been provided in Supplementary Table 1.

2.3. Crop harvesting and yield estimation

Each year, the pearl millet, cowpea, and cluster bean crops were harvested manually at physiological maturity. The final grain yield was calculated by taking the grain moisture content at 12%. To compare different treatments under intercropping systems, the crop yields were converted to pearl millet equivalent yield (PEY) (36). Minimum support prices (MSP) for pearl millet, cowpea, and cluster bean as fixed by the government of India (data provided in Supplementary Table 2) were used to convert these crops' yields to PEY and summed up for system PEY. MSP is the minimum price set by the government for certain agricultural products, at which the products would be bought directly from the farmers if the open market prices were less than the cost incurred. Eq. 1 was used to estimate PEY as follows:

$$\text{PEY (Mg ha}^{-1}) = \text{Pearl millet yield} + [(\text{CY} \times \text{Cp}) + (\text{CLY} \times \text{CLp})]/\text{Pp} \quad (1)$$

where CY is the yield of cowpea (Mg ha^{-1}), CLY is the yield of cluster bean (Mg ha^{-1}), Cp is the cowpea MSP (INR kg^{-1}), CLp is the cluster bean MSP (INR kg^{-1}), and Pp is the MSP of pearl millet (INR kg^{-1}).

2.4. Plant chemical analysis

To estimate N-concentration and crude protein content in plant parts, the plant samples were air-dried and oven-dried at 60 ± 2°C. Representative plant samples (0.5 g each) were digested with 10 ml of analytical-grade concentrated sulfuric acid combined with a digestion mixture ($CuSO_4 + K_2SO_4$ + Selenium powder + Mercury oxide) and analyzed using Kjeldahl's apparatus as per the procedure described by Rana et al. (37). For micronutrient analysis in pearl millet grains and stover, the samples were taken at the crop harvest stage. The 0.5 g of finely ground (1 mm sieve) plant samples were taken and digested with the solution of concentrated HNO_3 and $HClO_4$ acids (in a 9:4 v/v ratio) in conical flasks. The flasks were kept on a digestion plate for heating up to 3.5 h, or until a colorless residue was left in the digestion vessel. After being cooled, the remaining substance was combined with a 0.1 N solution of H_2SO_4 and diluted to a final volume of 100 ml. The digested samples were taken to estimate micronutrients (Fe, Zn, Mn, and Cu) using atomic absorption spectrometry (AAS PLUS, Motras Scientific, India) as per the procedure described by Rana et al. (37). The resultant micronutrient content of grain and straw was converted to micronutrient uptake (g ha^{-1}) using yield data from this study using Equations (2) and (3) as follows:

$$\text{Micronutrient content (mg to g kg}^{-1}) = \text{ppm} \times 0.001[\because 1\,\text{g} = 1,000\,\text{ppm}] \quad (2)$$

$$\text{Micronutrient uptake (g ha}^{-1} = \text{micronutrient content (g kg}^{-1}) \times \text{yield (kg ha}^{-1}) \quad (3)$$

TABLE 1 Initial soil properties of the experiment field.

Soil properties		CT		ZT	Method
Soil chemical properties					
Available N (kg ha^{-1})		164.8		212.7	Alkaline $KMnO_4$ (25)
Available P (kg ha^{-1})		14.9		15.1	Bray's No. 1 method (26)
Available K (kg ha^{-1})		172.4		183.2	Neutral NH_4OAc (27)
Available S (kg ha^{-1})		17.6		19.2	(28)
Soil pH		7.2		6.7	(29)
Soil EC		0.16		0.24	(30)
Available micronutrients (mg kg^{-1}) soil	Fe	4.32	Fe	4.92	DTPA extraction (31)
	Zn	0.47	Zn	0.65	
	Mn	4.12	Mn	5.16	
	Cu	1.14	Cu	1.36	
Soil biological properties					
SMBC (μg g^{-1} soil)		176.8		194.8	(32)
Dehydrogenase (μg TPF g^{-1} soil day^{-1})		26.2		35.4	(33)
Alkaline phosphatase (μg p-NPP g^{-1} soil h^{-1})		67.3		84.7	(34)

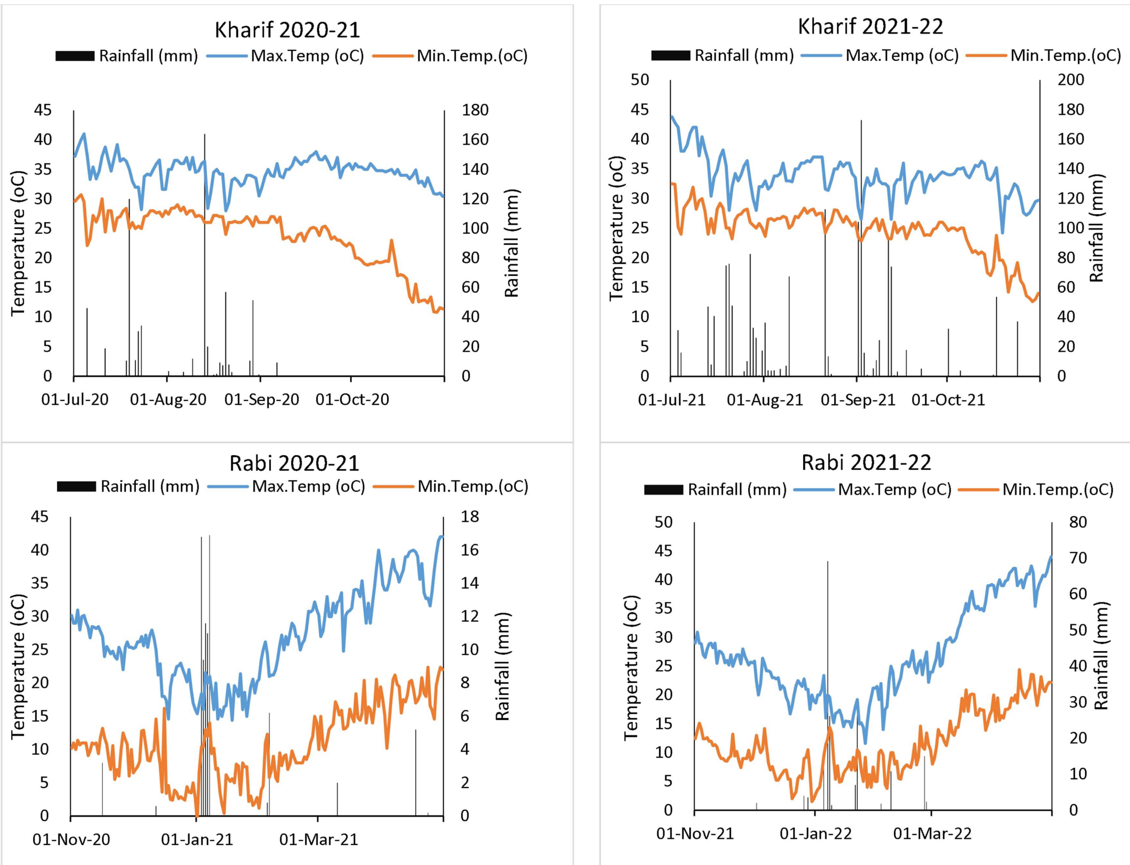

FIGURE 1
Mean monthly weather parameters recorded during the crop seasons of experimental years 2020–2021 and 2021–2022.

TABLE 2 Treatment details of the experiment.

No.	Treatment	Description		Short-term
Cropping system and tillage configuration (main plot)				
1	Conventional tillage (CT)-based pearl millet-mustard cropping system	Crop residues were applied and incorporated through one deep plowing, two harrowings, and planking.		CT (P – M)
2	Zero tillage (ZT)-based pearl millet—mustard	No plowing during both years; zero-till planter sowing, pearl millet and mustard as monocrops.		ZT (P – M)
3	ZT-based (pearl millet + cowpea – mustard + barley)	No plowing during both years; zero-till planter sowing. Pearl millet + cowpea – [1:1] and mustard + barley – [4:3 row ratio]		ZT (P + C – M + B)
4	ZT (pearl millet – mustard + chickpea)	No plowing; zero-till planter sowing [pearl millet (100% plant population)] and mustard + chickpea [1:1 row ratio]		ZT (P – M + CP)
5	ZT (pearl millet + cluster bean – mustard)	No plowing, zero-till planter sowing. Pearl millet: cluster bean [1:1 row ratio]		ZT (P + CL – M)
6	ZT (pearl millet + cowpea – mustard)	No plowing, zero-till planter sowing [pearl millet: cowpea – 1:1 row ratio]		ZT (P + C – M)
Residue intensity (sub plot)				
1	*Kharif* season crop residue-retention	Mustard crop residue was applied and incorporated with conventional tillage treatment and surface retention in zero tillage treatment	Mustard crop residue-retention (2.26 Mg ha^{-1})	KR
2	*Rabi* season crop residue-retention	Pearl millet residues were applied and incorporated with conventional tillage treatments, and the surface was retained in zero tillage treatments	Pearl millet crop residue (2.0 Mg ha^{-1})	RR
3	Both season crop residue-retention	Crop residues of both pearl millet and mustard crops were used under the treatments CT (incorporated) and ZT (surface residue retention)	Mustard residue (*Kharif* season) 2.0 Mg ha^{-1} and pearl millet residue (*Rabi* season) 2.0 Mg ha^{-1})	BR

Interaction treatment symbols (GGE analysis)

Treatment (main plot)	CT (P – M)	ZT (P – M)	ZT (P + C – M + B)	ZT (P – M + C)	ZT (P + CL – M)	ZT (P + C – M)	
Sub plot	KR	1	2	3	4	5	6
	RR	7	8	9	10	11	12
	BR	13	14	15	16	17	18

For quality control, blank and replicated samples were run with each batch of samples and calibrated with standard solutions (0.2, 0.4, 0.6, 0.8, and 1.0, 5, 10 mg L^{-1} of mineral nutrients). The resultant precision was verified by analyzing three replicated samples. Additionally, glassware and flasks were thoroughly washed using strong oxidizing agents to prevent contamination. Certified standards, such as the 1,000 mg L^{-1} concentration of Zn, Fe, Mn, and Cu Certipur® standard solution from Merck KGaA, EMD Millipore Corporation, Germany, were used for this purpose. The non-destructive method of oil estimation in whole seeds was carried out using a NIR transmittance grain analyzer (FOSS InfratecTM 1241) operating in the near-infrared region. This instrument was employed to determine the oil content of the seed samples.

2.5. Soil sampling and analysis

Soil samples were collected on July 2020 randomly from a well-established ZT field by taking five cores at 0–15 cm depth using a 5 cm diameter core sampler. The collected soil cores were mixed thoroughly, sieved (<2 mm), and divided into three sub-samples. One of the sub-samples was stored at 4°C before analysis of alkaline phosphatase activity, while another sub-sample was air-dried and analyzed for pH (37), organic carbon (37), oxidizable N (38), microbial biomass C (32), available P (39), K (27), and micronutrient content (32). The third sub-sample was used to determine the bulk density and soil moisture. Bulk density determination was done by the core method (37). Soil pH (water) was measured using a pH 700 Bench Meter (Eutech Instruments) at a soil:water ratio of 1:2.5. Soil samples were oven-dried and analyzed for nutrient contents as per the method described by Rana et al. (37). Total N was measured using the Kjeldahl method (38). Microbial biomass C was determined by chloroform fumigation and extraction (40). Soil total P and plant-available P content were determined by perchloric acid ($HClO_4$) digestion (39) and the 0.5 M $NaHCO_3$ extraction method (26), respectively, using a spectrophotometer. Dehydrogenase activity (DHA) was estimated by releasing triphenyl formazan and reducing 2,3,5-triphenyl tetrazolium chloride (33). Alkaline phosphatase activity was determined as described by Tabatabai (34).

TABLE 3 ANOVA for pooled data over year, cropping system (A), residue management (B), and replication (Rep) and their interaction.

Source	Year	Rep	A	Year*A	A*Rep.	B	Year*B	B*Rep	A*B	Year*A*B
DF	1	4	5	5	20	2	2	8	10	10
Grain yield	0.02**	0.203**	0.244**	0.009**	0.002	0.439**	0	0.002	0.002	0.004*
Straw yield	43.61**	0.83**	1.1**	0.01	0.03	1.86**	0.13**	0.02	0.03	0.02
Grain Zn content	345**	9**	36**	2**	0	55***	17**	0	1	1
Grain Fe content	2097**	245**	164**	7	8	338**	24	6	19	39**
Grain Mn content	365***	18**	36**	2*	1	0	30**	2	2*	2*
Grain Cu content	11**	2***	11***	1***	0	4***	6***	0*	0**	0
Straw Zn content	3,220**	5.3***	22.2**	2.4***	0.4*	4.2**	7.6**	0.3	0.5*	0.5*
Straw Fe content	6,151.4**	532.7**	725.6**	15.2	10.7	195.2**	44.5	5.1	30	43.5**
Straw Mn content	421**	29.1**	36.5***	4.8*	1.8	12**	53.2***	1.4	4.5*	5.7**
Grain Zn uptake	15,404,675**	39,510**	26,948**	18,406**	177	39,921**	26,152**	198	498	484
Grain Fe uptake	12,970**	3,489**	2,528*	52	53	6,596**	209*	39	98	243**
Grain Mn uptake	2,655**	950**	1,556	65**	11	1,496**	199**	19	11	25*
Grain Cu uptake	24**	68**	161**	4**	1**	198**	33**	1**	2**	1
Straw Zn uptake	19,390,512**	44,426**	89,615*	63,330**	1,912*	82,043**	69,358**	2,675**	1,542*	1,606*
Straw Fe uptake	20,846**	42,536**	64,383**	501	908	55,309**	5,844**	94*	1,497**	2,602**
Straw Mn uptake	144,769**	8,645**	14,172**	238	222	15,658**	5,129**	279	545*	500*
Straw Cu uptake	21,257**	781**	1,696*	8	47*	4,763**	6	60*	61**	81**
Oil content (%)	0.03**	0	0.08**	0.02**	0*	0.01**	0.01	0.02**	0.01**	0.01**
Protein content (%)	0.1	0.09	3.42**	0	0.08	0.04	0.39**	0.1	0.06	0.12

Values are mean squared (MS). DF: degree of freedom, yield in Mg ha^{-1}, nutrient content (mg kg^{-1}), nutrient uptake (g ha^{-1}); $^{*}p = 0.05$, $^{**}p = 0.05–0.01$, $^{***}p < 0.01$. Cropping system (A), residue management (B), and replication (Rep).

2.6. Fatty acid profiling

For the fatty acids profiling of pearl millet grains, samples were esterified individually with methanol in the presence of concentrated sulfuric acid. Fatty acid esters were extracted with hexane from the reaction mixture and concentrated using a rotary evaporator (Heidolph, Germany). The fatty acid profile of the samples was analyzed in gas chromatography-mass spectrometry (GC-MS) using an 8010C GC (Agilent Technologies, USA) equipped with an HP-5MS column (60 m × 0.25 mm;/0.25 mm, Agilent Co., United States), which was directly connected to a triple-axis HED-EM 5975C mass spectrometer (Agilent Co., United States). The injection volume was 1 µl with flow mode in split control at 1:20. The carrier gas flow was set at 1.00 ml/min helium. Helium (high purity of >99.99%) was used as a carrier gas at a head pressure of 10 psi. The oven temperature was initially held at 80°C, and then increased with a ramping rate of 5°C/min until it reached 150°C and was held for 1 min. Again, the temperature was elevated with a gradient of 7°C/min to get 220°C. Finally, the temperature was raised to 320°C with an increment of 10°C/min. The MS acquisition parameters were as follows: ion source (150°C), electron ionization (70 eV), full scan mode (50–550 mass units), transfer line temperature (220°C), and EM voltage (1,250 V). Fatty acid esters were identified by matching their respective mass spectra from the NIST (National Institute of Standards and Technologies) mass spectral library (41).

2.7. Statistical analysis

Tukey's HSD test was used to identify variations in nutrient content (NC), uptake, and yield traits. The normality of the response variables was tested by the Bar graph method, and all the variables were found to be normally distributed. The significance and interactions between treatments were evaluated using a two-way ANOVA in a split plot design. All the effects were fixed effects used in the model. Differences between treatment means were compared using Tukey's HSD at a 5% probability level ($p = 0.05$). The SAS 9.3 statistical software package was used to analyze the data. R Studio version 2022.12.0 was used for the analysis of multivariate stability statistics (GGE biplot) (28, 42). GGE biplot analysis was computed using the "GGE Biplot GUI" package (28), with support from the helper application "RStudio" in the R statistical software. GGE biplot analysis was used to visually assess the presence of genotype × environment interaction, rank genotypes based on stability and mean in each treatment, and identify optimally performing combinations (43–45).

TABLE 4 Effect of cropping systems and residue management on yield and grain quality parameters of pearl millet (2-year pooled data).

Treatments	PEY (Mg ha^{-1})	Pearl millet grain yield (Mg ha^{-1})	Stover yield (Mg ha^{-1})	Grain protein (%)	Oil content (%)	Oil yield (kg ha^{-1})	Protein yield (kg ha^{-1})
Cropping system and tillage configuration (main plot)							
CT (P – M)	2.04^{d}	2.04^{c}	6.42^{d}	10.65^{d}	4.97^{d}	101.5^{d}	217.2^{d}
ZT (P – M)	2.37^{c}	2.37^{a}	7.06^{a}	11.00^{c}	4.96^{d}	117.7^{c}	261.2^{c}
ZT (P + C – M + B)	3.46^{b}	2.20^{b}	6.70^{c}	11.43^{b}	5.05^{bc}	174.9^{b}	398.5^{b}
ZT (P – M + C)	2.25^{cd}	2.25^{b}	6.83^{c}	10.98^{c}	4.99^{cd}	112.7^{cd}	247.7^{cd}
ZT (P + CL – M)	3.89^{a}	2.25^{a}	6.93^{a}	11.56^{ab}	5.07^{b}	197.34^{a}	450.3^{a}
ZT (P + C – M)	3.54^{b}	2.33^{a}	7.07^{a}	11.82^{a}	5.13^{a}	182.1^{b}	419.3^{ab}
Residue intensity (sub plot)							
KR	2.99^{a}	2.23^{b}	6.90^{b}	11.22^{a}	5.018^{a}	150.8^{a}	338.7^{a}
RR	2.86^{b}	2.13^{c}	6.58^{c}	11.22^{a}	5.053^{a}	144.8^{a}	323.6^{b}
BR	2.93^{ab}	2.35^{a}	7.02^{a}	11.28^{a}	5.02^{a}	147.6^{a}	333.3^{ab}

Different letters within the same columns are significantly different (Tukey's HSD). Treatment codes are given in Table 2.

3. Results

3.1. Effects of tillage, CRR, and system intensification on crop productivity

The zero-tillage (ZT) system, particularly the ZT pearl millet + cluster bean – mustard cropping system (P+CL–M) and ZT pearl millet + cowpea – mustard + barley cropping system (P + C – M + B), gave the highest productivity ($p = 0.036$) and superior quality of pearl millet compared to other treatments (Tables 3, 4). Treatment ZT-based pearl millet + cluster bean – mustard cropping system [ZT (P + CL – M)] produced the highest pearl millet equivalent yield (PEY) of 3.89 Mg ha^{-1} (pearl millet yield + PEY of intercrop), which was 90.9% higher than the lowest yield of 2.04 Mg ha^{-1} in sole cropped CT-based pearl millet-mustard cropping system [CT (P – M)]. On average, various ZT and system intensification treatments enhanced 49.7% PEY compared to CT (Table 4). In terms of grain yield of pearl millet, the highest yield was obtained with the ZT pearl millet + cowpea – mustard cropping system [ZT (P + C – M)], which was 12.5% higher than CT (P – M), followed by ZT (P + CL – M) > ZT (P – M) (Table 4). The interaction effect analysis highlighted that the ZT-pearl millet – mustard + chickpea cropping system [ZT (P – M + CP)] resulted in the highest grain yield when combined with *Kharif* season crop residue retention (KR), followed by *Rabi* season crop residue retention (RR) practice (Figure 2A). Other cropping systems were statistically at par (Figure 2A and Table 3). ZT (P + C – M) gave a maximum stover yield (7.07 Mg ha^{-1}), which was ~10.3% higher than the lowest stover yield of 6.41 Mg ha^{-1} in CT (P – M). On average, the ZT treatments brought ~10.8% improvement in stover yield compared to CT. These results elucidate the significant impact of the interaction between the cropping system and residue intensity on pearl millet grain yield.

3.2. Effects of tillage, CRR, and system intensification on grain protein and oil content

The non-significant but highest grain protein content (11.6%) was observed in the ZT (P +CL – M) system, which was 8.1% higher than the lowest content of 10.6% in the CT (P – M) system (Table 4). On average, the CA enhanced the protein content by 5.3% relative to the conventional system of crop establishment. The highest grain protein percentage was observed in the ZT (P + C – M) system among all the treatments, and the difference ranged from 2.2–9.9% compared to the best treatment. Overall, the residue treatments resulted in ~0.7% increase in protein content compared to each other. Therefore, ZT (P + C – M) can be considered the best treatment for achieving high grain protein content. The ZT (P + CL – M) produced the highest protein yield (450.3 kg ha^{-1}), whereas the lowest protein yield (217.2 kg ha^{-1}) was recorded when the CT (P – M) system was followed. In terms of oil content, a statistically significant difference was observed between CT (P – M) and all other treatments, whereas ZT (P – M + C) and ZT (P + CL – M) were not significantly different from each other ($p = 0.001$). The sub plot treatment of *Rabi* season residue (RR) had the highest oil content at 5.05%, which was 0.7% higher than the lowest content of 5.01% in *Kharif* season residue (KR) (Table 4). Relative to CT, the CA treatments improved the oil content and oil yield by 1.3 and 3.2%, respectively.

3.3. Grain and straw micronutrient content and uptake

ZT treatments increased Fe content by 7.2–13.6%, Zn content by 4.9–12.2%, Mn content by 3.1–6.7%, and Cu content by 8.3–

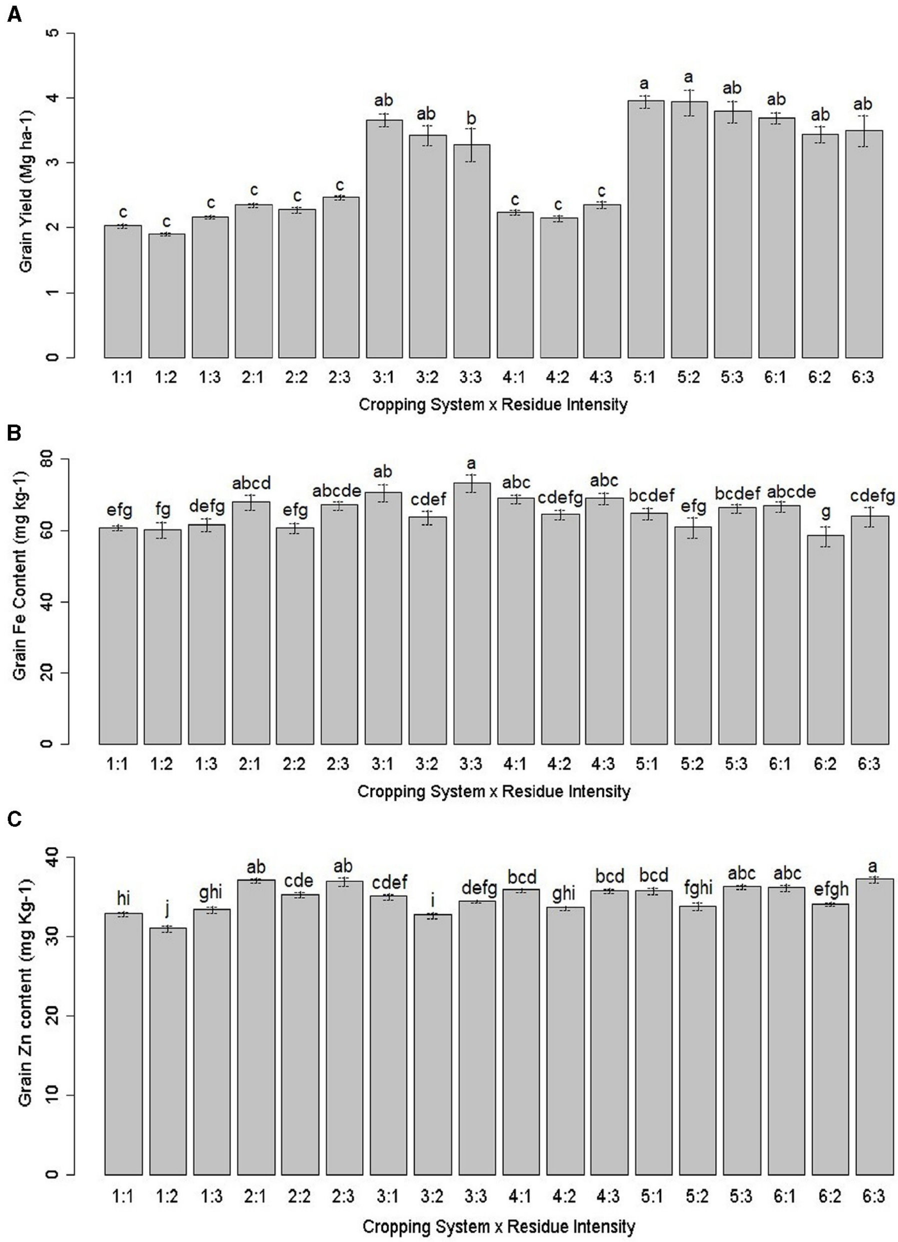

FIGURE 2
Interactions effect of cropping system and residue intensity on **(A)** pearl millet grain yield (MG ha^{-1}), **(B)** grain Fe (mg kg^{-1}), and **(C)** Zn content (mg kg^{-1}). Treatment details are given in Table 2. Different lowercase letters represent significant differences between different treatments.

16.7% relative to CT (Table 5). The order of grain Fe content was ZT (P + C – M+B) > ZT (P +CL – M) > KR > ZT (P – M + C) > ZT (P – M) > CT (P – M). The Zn content was greater with chickpea integration compared to cluster beans in the PMCS system. Mn content in ZT (P + C – M + B) remained 22.3% higher as compared with CT (P – M) (Table 5). The highest grain contents of Fe, Zn, and Mn were observed in the ZT (P + C – M + B) treatment with KR (Tables 3, 5). Regarding residue intensity, the highest grain contents of Fe, Zn, and Mn were observed in the both season crop residue retention (BR) and KR treatments, while the highest grain contents of Fe and Cu were observed in the ZT (P + C – M + B) and grain Zn and Mn contents in the ZT (P – M) treatment. The grain Fe, Zn, Mn, and Cu uptake was significantly higher in ZT regimes and followed the order of ZT (P + CL – M) > ZT (P + C – M + B) > ZT (P + C – M) > ZT (P – M + C) = ZT (P – M) (Table 5). The interaction effect showed a different trend for grain Fe and Zn content. Fe content was non-significant; however, it was the highest with ZT (P + C – M + B) × BR, followed by ZT (P + C – M) × RR (Figure 2B). As shown in Figure 2C, Zn content was highest in the ZT (P + C– M) × BR combination. Overall, the combination of ZT and KR can enhance the biofortification of pearl millet significantly.

TABLE 5 Effect of cropping system and residue management on micronutrient content (mg kg^{-1}) and uptake (g ha^{-1}) (2-year pooled data).

Treatments	Grain Fe content (mg kg^{-1})	Grain Zn content (mg kg^{-1})	Grain Mn content (mg kg^{-1})	Grain Cu content (mg kg^{-1})	Grain Fe uptake (g ha^{-1})	Grain Zn uptake (g ha^{-1})	Grain Mn uptake (g ha^{-1})	Grain Cu uptake (g ha^{-1})
Cropping system and tillage configuration (main plot)								
CT (P – M)	60.9^{d}	32.4^{e}	54.0^{d}	14.4^{d}	124.4^{d}	66.3^{d}	110.2^{d}	29.5^{d}
ZT (P – M)	65.3^{bc}	36.4^{a}	57.6^{a}	15.6^{c}	155.1^{c}	86.4^{c}	136.8^{c}	37.1^{c}
ZT (P + C – M + B)	69.2^{a}	34.0^{d}	55.7^{c}	16.8^{a}	239.3^{ab}	117.9^{b}	192.9^{b}	58.3^{ab}
ZT (P – M + C)	67.5^{ab}	35.0^{c}	56.5^{bc}	16.1^{b}	152.6^{c}	79.1^{c}	127.5^{c}	36.4^{c}
ZT (P + CL – M)	64.0^{c}	35.2^{c}	56.6^{b}	15.7^{c}	249.6^{a}	137.3^{a}	220.4^{a}	61.4^{a}
ZT (P + C – M)	63.1^{cd}	35.8^{b}	57.8^{a}	15.7^{c}	224.1^{b}	127.1^{b}	205.4^{ab}	55.8^{b}
Residue intensity (sub plot)								
KR	66.7^{a}	35.4^{a}	56.4^{a}	15.9^{a}	200.4^{a}	106.4^{a}	169.2^{a}	48.0^{a}
RR	61.5^{b}	33.4^{b}	56.2^{a}	15.4^{b}	175.6^{b}	95.8^{b}	161.5^{b}	44.3^{b}
BR	66.9^{a}	35.6^{a}	56.4^{a}	15.9^{a}	196.6^{a}	104.9^{a}	165.9^{ab}	46.9^{a}

Different letters within same columns are significantly different (Tukey's HSD). Treatment codes are given in Table 2.

3.4. GGE biplot and stability analysis

3.4.1. Genotype × trait biplot and genotype × yield × trait biplot

The "genotype (here treatment) × trait (GT)" biplot (Figure 3A) displays the association between various traits. The biplot exhibited high accuracy, having 88.8% goodness of fit. The angle among the trait vectors was <90°, indicating a positive correlation between all the traits. The biplot shows that treatments 18 and 13 (see Table 2 for treatment details) had high Fe and Cu content. The grain Fe and Cu content was the lowest with treatments 11 and 12. Grain yield contents of Zn and Mn were the highest with treatments 18 and 14 and the lowest with treatments 9 and 10. The genotype × yield (Y) × trait (GYT) biplot (Figure 3B) was used to select the treatments on the basis of their relative performance. The goodness-of-fit for the GYT biplot for grain yield and nutrient content was 98.1%. Treatment 18 and 14 combinations had the most significant values for Y × Mn and Y × Zn, indicating that these treatments were best combined for grain yield with Mn and Zn content. Similarly, treatment 18 had the highest levels of Y × Fe and Y × Cu, meaning that this treatment was the best combination for grain yield with Fe and Cu content.

3.4.2. Which-Won-Where/What polygon

The "Which-Won-Where/What" polygon (Figure 3C) was used to find out the best treatments with respect to grain yield and micronutrient content (NC). The first two principal components (PC1 and PC2) explained 76.8% variation between treatment and trait. The biplot had five different sectors, but all the traits were located only in two sectors. Fe and Cu were located in the same sector, and treatment 9 [ZT (P + C – M + B)*RR] was the best treatment (farthest treatment) for these nutrients, followed by treatment 7. Grain yield (GY), Zn, and Mn content were placed in another segment, and treatment 18 [ZT (P + C – M)*BR] was the vertex treatment in the segment, indicating that this treatment performed best for these three traits. Treatment 2 [ZT (P – M) × KR] was the poorest treatment for all the traits. The "Which-Won-Where/What" polygon of Figure 4A shows the best treatments for grain yield and micronutrient uptake (NU) in grain and stover. The first two principal components (PC1 and PC2) explained a 75.6% variation in treatment × trait. All the traits were located only in four segments. Fe, Cu, and Mn uptake in grain and the GY were consolidated, and treatments 13, 14, 15, and 16 were the best for these traits, followed by treatment 7. Fe, Mn, and Cu uptake in grain and stover was strongly correlated and situated in one sector, and treatments 18 and 6 were outperformers. The polygon in Figure 4C shows the pooled result of quality aspects of nutrient content and uptake, oil, and protein content with grain yield, eliminating stover yield and stover uptake traits. Out of the eight sectors, all traits were observed in four sectors only. Grain yield, protein content, and uptake of Fe, Zn, Mn, and Cu in the grain were correlated, and the best treatment for this pooled trait was treatment 16, followed by treatments 15 and 7. Oil content (%) was observed in a decent number of other traits, and the best treatment was treatment 13 [CT (P – M) × BR].

3.4.3. Mean vs. stability biplot

The "Mean vs. Stability" biplot (Figure 3D) was used to identify the highly stable treatment among all the traits under study. The treatment stability was inversely related to the magnitude of the projection on the typical environment coordinate. Across the traits, treatments 1, 2, and 3 were highly stable, but their performance was poor. Treatments 7, 9, and 18 had the highest values of the traits but were highly unstable in their performance, whereas treatment 10 had a higher content of Fe and Cu, which was also highly stable. Likewise, treatment 16 was relatively stable and had higher GY, Zn, and Mn values. The "Mean vs. Stability" biplot (Figure 4B) was used to determine the highly stable GY, NC, and NU treatments. Across the treatments, 1, 2, and 3 were highly stable but poor performers. Treatments 14, 15, 6, and 4 had the highest GY, NC, and NU pooled

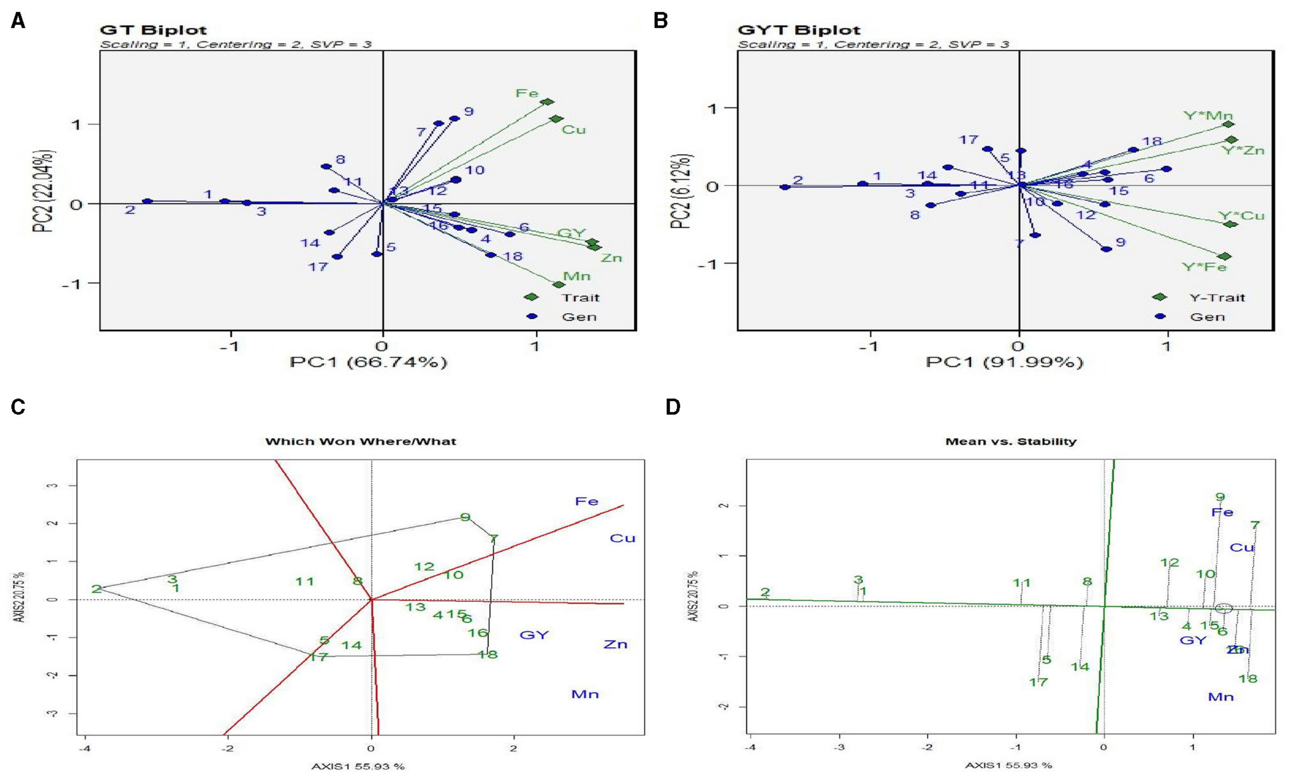

FIGURE 3
GGE analysis of interaction between treatments, micronutrients content, and grain yield. **(A)** GT plot: treatments × grain yield × micronutrient content (Fe, Zn, Cu, and Mn). **(B)** GYT biplot: treatments × (grain yield × micronutrient). **(C)** Which Won Where/What polygon. **(D)** Mean vs. stability biplot. Treatment details are given in Table 2.

grain and stover values. However, treatments 9, 18, and 16 were highly stable for higher GY and grain NC. The "Mean vs. Stability" biplot (Figure 4D) was also used to determine the stability between grain yield and uptake of Zn, Fe, Mn, and Cu. Treatments 8, 10, 13, and 16 were highly stable but performed poorly in terms of grain yield and micronutrient uptake, whereas treatments 7, 14, 1, and 18 showed better performance and stability.

3.5. Grain fatty acid composition

The fatty acid composition analysis of pearl millet showed significant differences between ZT with crop residue retention and CT with incorporated crop residue. The most notable difference was observed in the percentage content of (9Z, 12Z) 9,12-octadecadienoic acid (linolic acid), which increased from 55.9% in CT to 58.7% in ZT with crop residue retention (Table 6). This indicated that ZT with crop residue retention positively impacts quality composition by promoting the accumulation of this unsaturated fatty acid, which is known to play a crucial role in maintaining human health. Furthermore, the increase in 9,12-octadecadienoic acid was accompanied by a decrease in the percentage content of 10-octadecenoic acid, which decreased from 25.0% in CT to 16.8% in ZT (Figures 5A, B) with crop residue retention.

4. Discussion

4.1. Effect on crop productivity and grain quality

Crop production performance is influenced by various factors such as soil water, fertilizer availability, gas exchange, heat supply, and ultimately the economic yield of the crop (46–48). In rainfed conditions, enhancing crop productivity requires the conservation of soil moisture, increased nutrient availability, optimal soil health, and efficient utilization of solar radiation. The evaluation of long-term tillage and diverse cropping systems allows for a comprehensive assessment of the advantages and disadvantages of tillage practices and system intensification effects while also considering crop cycles and yield stability on a broader scale (48, 49). The findings of this study demonstrate that adopting ZT and residue retention techniques has a positive impact on pearl millet equivalent yield (PEY) and stover yield. These practices contribute to increased moisture and nutrient availability, as well as improved infiltration due to the retention of crop residues (7, 50). These results align with previous studies that have shown the stabilizing effect of ZT with crop residue retention on yield and grain quality of pearl millet crops (9, 13). Furthermore, the implementation of ZT and residue retention promotes higher microbial activity and diversity, leading to improved soil nutrient availability. Rational system intensification and appropriate tillage

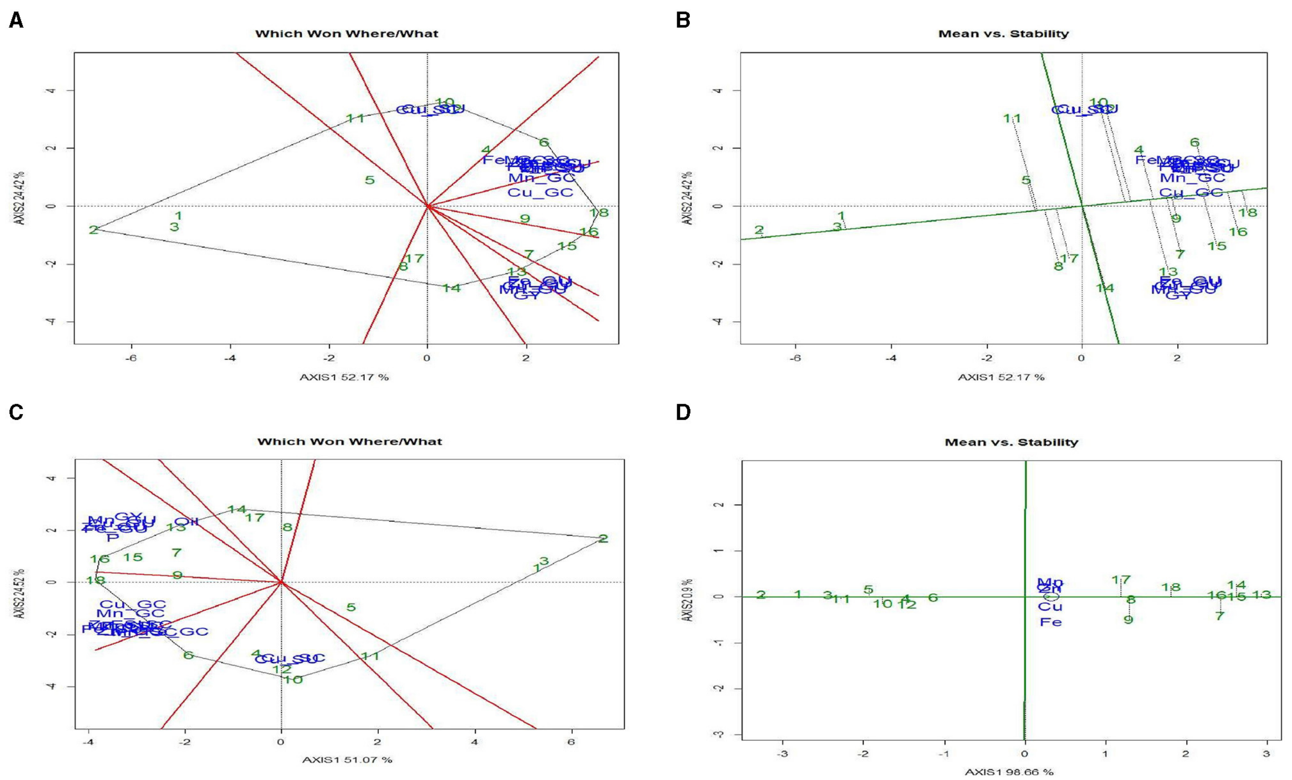

FIGURE 4
GGE analysis for grain yield (GY) and micronutrients content and uptake (Fe, Zn, Cu, and Mn). **(A)** Which Won Where/What polygon. **(B)** Mean vs. stability biplot; GGE analysis for grain, yield, straw yield, micronutrient content and uptake, oil and protein content. **(C)** Which Won Where/What polygon. **(D)** Mean vs. stability biplot for micronutrient grain uptake. Treatment details are given in Table 2.

practices also contribute to improved soil quality by fostering better soil structure and reducing soil bulk density (51, 52). Crop residue retention plays a crucial role in building soil organic matter (SOM) and reducing mineral-N losses (15, 53, 54). In comparison to CT, conservation tillage practices like ZT with residue mulching hold promise as they provide favorable conditions for moisture, nutrient availability, and soil structure, thereby ensuring high and stable crop yields (46).

The quality of pearl millet grain was assessed in terms of oil and protein content. The highest grain quality was observed in systems combining ZT with legume intensification, such as the incorporation of cowpea or cluster bean. Legumes are well-known for their ability to fix atmospheric nitrogen through root nodules and increase microbial activity in the rhizosphere. Legumes also contribute to soil organic matter through leaf fall, ultimately enhancing soil chemical and microbial health and thereby promoting increased yield and grain quality (45, 55). Higher protein content in grain is often associated with increased nitrogen availability to the plant, a correlation that was also observed in this experiment (56, 57). The ZT and residue retention systems exhibited higher levels of available sulfur (S) compared to the conventional tillage system. The build-up of soil organic carbon (SOC) and available sulfur contributed to a higher oil content (58). The improved oil concentrations, coupled with a higher grain yield, resulted in a higher oil yield. Overall, the combination of ZT, residue retention, and legume inclusion proved to be a beneficial nexus, leading to improved crop yield. The proposed study holds merit in addressing key knowledge gaps, providing practical insights for improving agricultural practices, and enhancing the nutritional value and resilience of pearl millet production in rainfed agro-ecosystems.

TABLE 6 Effect of tillage and cropping systems on management on fatty acid profile of pearl millet crop (2-year pooled data).

S.N.	Retention time	Compound	Relative percentage (%)
Zero tillage with soil surface retention of crop residues			
01	45.42	Hexadecanoic acid	21.0
02	49.71	9,12-Octadecadienoic acid	58.7
03	49.82	9-Octadecenoic acid	16.8
04	49.99	Octadecanoic acid	3.5
Conventional tillage with crop residue-incorporation			
01	45.29	Hexadecanoic acid	19.1
02	49.54	9,12-Octadecadienoic acid	56.0
03	49.66	10-Octadecenoic acid	25.0

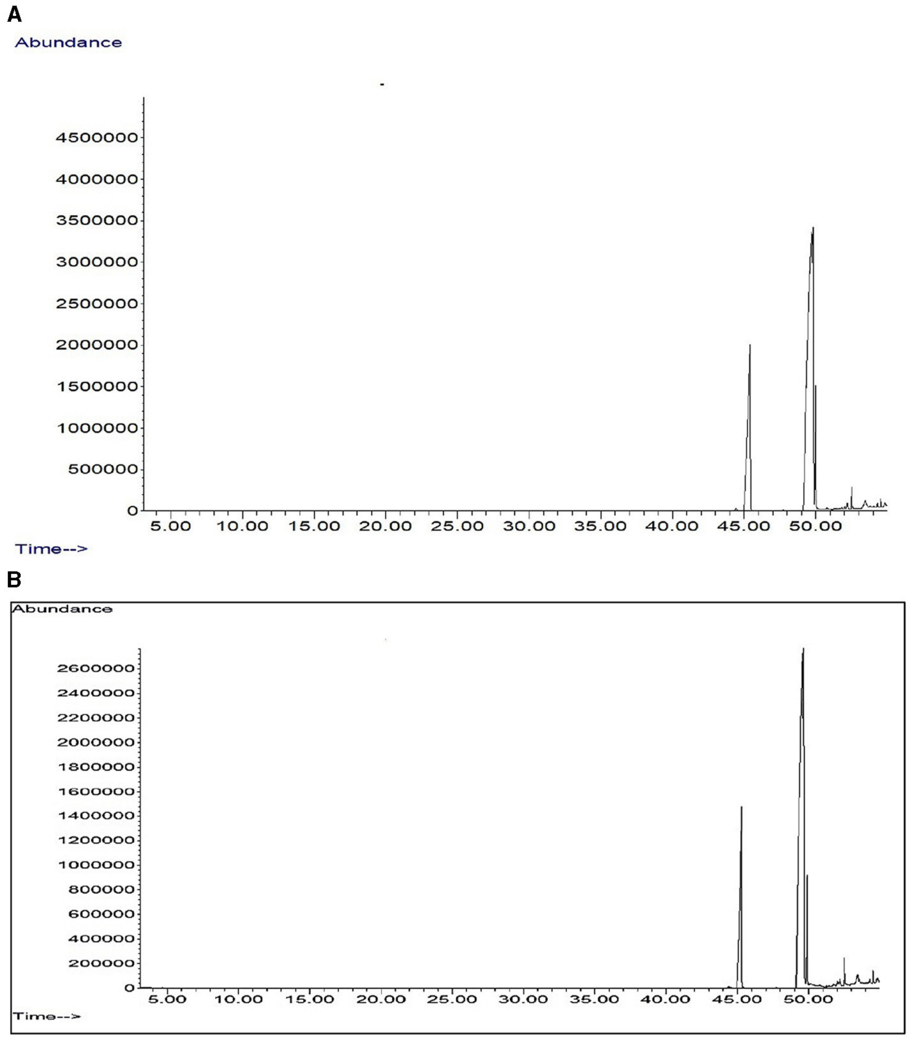

FIGURE 5
GC-MS total ion chromotogram of fatty acid profiling of pearlmillet oil in **(A)** zero tillage regimes and **(B)** conventional tillage system.

4.2. Micronutrient content and uptake

This study investigated the effect of ZT, residue retention, and system intensification on micronutrient content and uptake in the soil-plant system. Micronutrients in the soil are often present in forms that are not readily available to plants, particularly in their native cationic form. However, the combination of ZT and crop residue retention (CRR) practices in this study facilitated improved availability and uptake of certain micronutrients, such as iron (Fe) and manganese (Mn), which exist in reduced forms (Fe^{2+} and Mn^{2+}). The increased soil moisture under CRR on the soil surface favored the reduced forms of these nutrients and facilitated their movement and diffusion from the soil to plant roots (59). Moreover, the better moisture conservation achieved under ZT and CRR promoted the uptake of micronutrients that move via mass flow into the plant system and subsequently translocate to the grain. In contrast, CT practices led to the mineralization of soil organic carbon (SOC) and limited the complexation of micronutrients with SOC, resulting in lower micronutrient concentrations.

Several previous studies have reported higher concentrations of Zn, Cu, Fe, and Mn under the ZT system with residue retention (14, 60, 61). Consistent with these findings, our study also observed higher grain nutrient content and grain yield, leading to significantly higher micronutrient uptake. A similar pattern was reported in a study focused on a no-till-based lentil cropping system (53). Additionally, micronutrient build-up in the soil under ZT and CRR on the soil surface has been reported, indicating replenishment of the micronutrient pool in real time (62, 63). The combination of ZT, CRR, and intercropping practices increases the availability of micronutrients, including Zn, Fe, Mn, and Cu, in both extractable and organic forms (14, 61). The improved microbial activity observed in CRR plots with legume intercropping likely contributed to the increased solubilization of micronutrient cations (53). The formation of organic complexes between micronutrient cations and organic acids generated during the decomposition of plant residues is believed to be responsible for the increased micronutrient content, particularly Zn and Cu, in plots with residue retention (9, 64, 65). In CT practices, crop residues are often not recycled, resulting in reduced carbon (C) input and the loss and depletion of nutrients in the soil. In contrast, CA practices disturb the soil less, and the associated crop residue retention in this system leads to nutrient recycling and increased C input. These practices also stimulate microbial and enzymatic activity in the soil.

Legume-based systems, due to nitrogen fixation and faster biomass decomposition with a narrow carbon-to-nitrogen (C:N) ratio, increase carbon sequestration and enhance micronutrient acquisition (49). The resulting soil organic matter (SOM) may also have facilitated the synthesis of organic acids in the rhizosphere, which acted as micronutrient chelates, influencing the translocation and remobilization of micronutrients (66).

4.3. Fatty acid composition

Generally, fatty acids give a unique flavor to food. Pearl millet is known to contain higher levels of fatty acids compared to other cereal grains. Linoleic and oleic acids are two of the essential fatty acids present in pearl millet. The balanced compositions of these fatty acids are essential for the oil's stability and the quality of the grain (67). The impact of farm management practices on fatty acid levels in grain needs to be better understood. In this study, we observed differences in fatty acid content between different tillage practices. Drought stress has been reported to increase the oleic acid and decrease the linolic acid content (68, 69). Hexadecanoic acid possesses antioxidants, anti-inflammatory, and hypocholesterolemic properties. Di-linoleic acid (9,12-octadecadienoic acid) has been reported to have anti-arrhythmic properties (67, 68). These findings suggest that ZT with crop residue retention can help improve soil quality and health impacts in diets by altering the fatty acid composition, which can significantly impact the nutritional value and productivity of pearl millet crops. This aligns with our findings because ZT coupled with CRR increased the moisture availability and thus decreased the oleic acid and linolic acid content in pearl millet grain. However, further studies are required to understand the role of farm practices on fatty acid composition.

5. Conclusion

The combination of residue retention in the zero-tillage system along with system intensification using leguminous crops shows great potential for enhancing micronutrient biofortification and achieving stable yields in pearl millet-based cropping systems. The success of this sustainable approach relies on effective soil moisture conservation, improved soil chemical and biological health, and increased system productivity. The interactive effects of tillage and residue recycling play a significant role in micronutrient biofortification, while yield stability is primarily influenced by tillage and system intensification practices. This sustainable approach offers a promising solution to address the challenges of micronutrient malnutrition and low crop yields in rainfed dryland areas. For future research, it is essential to gain a deeper understanding of the hydro-thermal dynamics under zero tillage and the impact of residue management in diverse intercropping systems on nutrient distribution in different plant parts. Additionally, investigating the effects of residue mulching and legume intercropping on greenhouse gas emissions from all crops within each system will provide valuable insights for advancing sustainable agricultural practices.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

AYo: conceptualization, methodology, investigation, monitoring, data curation, and writing of the original and final draft. RB: conceptualization, investigation, review, writing, and editing. SG: review and editing and data analysis. RN: draft finalization and editing. SS: data curation. AC: methodology conceptualization, investigation, review, writing, and editing. YS: review and methodology. DS: data analysis and data curation. SB: writing and review. TS: writing and editing. AYa: draft initiation and supervision. SN: methodology and analysis. NS: investigation, review, and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1205926/full#supplementary-material

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Farmers' and millers' experiences and attitudes towards the production and processing of zinc biofortified wheat in Pakistan: a mixed methods study

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Background: Zinc biofortified wheat may be a sustainable strategy to increase zinc intake in areas where fortification and dietary diversification are not feasible or are limited by household purchasing power. This convergent mixed methods study aimed to explore the farmers' and millers' experiences and attitudes towards the production and processing of zinc biofortified wheat in Pakistan.

Methods: A telephone survey was conducted with farmers ($n = 418$) who were provided with Zincol-2016 biofortified wheat seed for the 2019–2020 growing season, as part of a wheat grain micronutrient mapping study across Punjab Province. The survey explored the farmers' experiences of growing Zincol-2016 and whether they opted to grow it again in the subsequent season. Semi-structured focus group discussions were undertaken in a separate group of farmers in Khyber Pakhtunkhwa (KP) province ($n = 12$) who grew Zincol-2016 for the BiZiFED2 RCT. Millers were also interviewed in KP, both those who had processed Zincol-2016 for the trial ($n = 12$) and those who had no experience of processing biofortified wheat ($n = 12$). Survey data were analyzed using descriptive statistics and transcripts of focus groups were analyzed using thematic analysis.

Results: Nearly half of farmers who responded to the survey (47%) re-cultivated Zincol-2016 in the following season. The drivers for Zincol-2016 re-cultivation were seed availability (100%), grain yield and growth resistance (98%), quality of the flour from the previous harvest (97%) and nutritional benefit (94.5%). Discussions with farmers suggested that the main motivators for potential scale-up of biofortified wheat were the perceived quality of the grain, wheat, and flour. Millers saw it as an opportunity to expand their business. Farmers and millers valued the health benefits of the wheat. Challenges for scale-up include the need of additional support to produce it, unfamiliarity with the biofortification process, production costs, and external threats to the supply chain.

Conclusion: Farmers and millers showed a strong implicit preference for Zincol-2016 over alternative varieties. Crop performance and product yield were the most cited motivators for growing Zincol-2016. Farmers and millers are willing to produce and process biofortified wheat if financial and educational support is provided.

KEYWORDS

biofortification, farmers, millers, staple crops, producers, acceptability, wheat value chain

Introduction

Mild-to-moderate zinc deficiency may lead to growth faltering in children, impaired immune function, and altered integrity and function of the gastrointestinal tract (1). Zinc deficiency remains a serious public health problem, particularly in low-middle income countries. In Pakistan, 22.1% of women of reproductive age and 18.6% of children under 5 years of age are zinc-deficient (2). In rural areas of Pakistan, where diet diversity is low, we recently reported that 68.8% of adolescent girls were zinc deficient (3).

Leading public health strategies to address zinc deficiency include supplementation, fortification and increasing dietary diversity. However, the sustainability of these strategies can be challenging in remote impoverished rural areas where access to affordable diverse diets, supplementation interventions and centrally fortified food products are limited (4). Biofortification is a process by which the density of vitamins and minerals in the edible component of a crop are increased through conventional plant breeding, transgenic techniques, agronomic practices, or a combination of these (5). Agronomic biofortification refers to the addition of nutrient rich fertilizer which can be applied through foliar (fertilization to plant leaves and steams) or basal (pre-plating fertilization) methods. Recent evidence has demonstrated that that foliar application of zinc can increase the zinc concentration and bioavailability in wheat grain and flour (6).

There is increasing evidence that consumption of biofortified foods improve micronutrient status (7, 8). Biofortification strategies consider the specific nutrient needs of the population and the staple foods of the region so that reach, and affordability are maximized (9). If these criteria are met, biofortification of staple crops presents a potential long-term cost-effective and self-sustaining strategy for increasing dietary micronutrient intake (10), in contrast to supplementation and commercial fortification programs which incur higher ongoing costs to sustain them (5).

The success of biofortification strategies to improve micronutrient status on a population scale depends not only on the evidence of a positive impact on relevant health outcomes, but also on high rates of adoption and consumption by the producers and intended beneficiaries (11, 12). Systematic reviews have provided evidence that there is sensory acceptability (11) and a willingness to pay (13) for biofortified crops among consumers. However few studies have explored the views of wheat farmers (14–17), and none to our knowledge have sought the opinions of the millers who process the resulting grain. Studies in Nigeria and Uganda found that farmers have limited knowledge of biofortification (14) or the benefits of fertilizer application (17) and that an increased awareness of the benefits of and positive perceptions towards the biofortified crop were strong determinants of its adoption (14). Two studies investigated farmers' opinions on the hypothetical introduction of genetically modified maize in Mexico (15) and biofortified pearl millet in India (15). Both studies showed significant heterogeneity among farmers' views, dependent on their location (related to soil quality, yield, their involvement in local labor markets), age group, and whether they produce mainly for household consumption or market sale. This heterogeneity among farmers' views towards biofortified crops demonstrates the importance of tailoring biofortification strategies to local needs by considering the views and expectations of local producers.

In 2016 a new variety of zinc biofortified wheat (Zincol-2016) was released by HarvestPlus in partnership with the Research & Development Institutions of Pakistan. As wheat is the main staple food and cultivated on the largest acreages in Pakistan (18), this biofortified crop is a promising approach to improve zinc intake on a population level, especially when combined with zinc fertilizers (6, 19). The BiZiFED2 (Biofortification with Zinc and Iron for Eliminating Deficiency, BBSRC Global Challenges Research Fund, Grant Number BB/S013989/1) trial was established to investigate the potential of biofortification as a strategy to reduce zinc and iron deficiencies in Pakistan. At the start of the study, Zincol-2016 was the only variety of selectively bred zinc biofortified wheat available in Pakistan. The primary objective was to examine the effects of consuming zinc-biofortified wheat flour on the zinc status of adolescent girls aged 10–16 years. We have found that consumption of zinc-biofortified wheat grown with zinc fertilizers has a positive impact on total dietary zinc intake (3, 20) and is perceived positively among consumers (21). Here we present the findings of a convergent mixed-methods study, to explore the views and experiences of the famers and millers of zinc biofortified wheat and flour to inform future programs seeking to scale-up zinc biofortified wheat in Pakistan.

Materials and methods

This study is part of the BiZiFED2 project (Biofortification with Zinc and Iron for Eliminating Deficiency) (3). One of the main objectives of this research was to improve understanding of the socio-cultural factors and market systems that affect the sustainable uptake of biofortified wheat in Pakistan. To achieve this objective, a mixed method study was undertaken to explore the views and perspectives of farmers, millers and community members to identify what factors influence decisions around their acceptance of biofortified wheat.

Study procedures

Study design

The study used a mixed methods convergent parallel design in which two independent strands of complementary quantitative and qualitative data were collected and analyzed independently and merged in the integration phase (22). This approach was chosen because it would allow us to triangulate our results and obtain a multidimensional understanding that would have not been available

Abbreviations: KP, Khyber Pakhtunkhwa; BiZiFED2, Biofortified zinc flour to eliminate deficiency in Pakistan; WP, Work packages.

through separate qualitative or quantitative approaches. Data were collected using a survey of farmers recruited to cultivate Zincol-2016 the prior growing season as part of the larger BiZiFED2 effectiveness trial (23). Focus group discussions (FGDs) were carried out with farmers and millers who produced zinc biofortified wheat and flour for the BiZiFED2 trial. One additional FGD was conducted with millers who had no experience of milling biofortified wheat.

Survey: recruitment and implementation

In 2019, 686 farmers across the Punjab region were recruited to participate in the BiZiFED trial. They were provided (free of charge) with 25 kg Zincol-2016 wheat seed sufficient for 0.5 acre (~0.2 hectare) using standard broadcast sowing practices. Farmers who had granted their permission to be followed up during the initial recruitment period were contacted to take part in a survey. Up to three attempts were made to reach each farmer by telephone. Upon contact, the nature of the study was explained to each potential participant and consent was reconfirmed. Farmers were provided with the contact details of the researchers and encouraged to ask any questions and seek clarification from the research time if required. The survey was conducted by an experienced agronomy extension worker (SA) who was fluent in the local language and trained in the skills required to collect data for this project by telephone, using KoboCollect software (24). The survey was conducted by telephone to maximize response rate and minimize risks of exposure to COVID-19 for all concerned.

The survey was designed to capture the extent of biofortified wheat cultivation, the farmers' experiences of growing Zincol-2016 in the growing season 2019–2020, and whether they had continued to grow Zincol-2016 in the 2020–2021 growing season. The full suite of questions is provided in Supplementary file 1. Survey data were collected between 10th February and 1st July 2021. Participant responses were captured in KoboCollect software (24) using a handheld tablet and the survey took between 4 and 15 min to complete depending on the number of questions responded to (i.e., farmers who had sown Zincol-2016 in the second growing season were asked more questions than farmers who did not). The survey responses were checked by the research team after the first 10 surveys were conducted to ensure that the survey was working as intended for the participants and the researchers. This revealed that more farmers than expected were growing a second biofortified wheat variety "Akbar-19", that was released in Pakistan in 2019. Therefore, one additional question was added to the survey to establish the acreage given to growing Akbar-19 on farms cultivating this variety. This enabled us to more accurately capture the production acreage given to biofortified wheat on each farm, as reported by the farmer in the remaining interviews.

Focus groups: recruitment and implementation

Four FGDs were planned of which two were with farmers and two with millers. The farmers were recruited from a total pool of 59 tenant farmers cultivating land belonging to two landlords in the Peshawar area. Farmers were small scale farmers, relying mostly on manual techniques for sowing and harvesting and who were given resources (Zincol-2016 seed and zinc fertilizer) to grow Zincol-2016 which was then purchased for use in the BiZiFED2 effectiveness trial. The millers were recruited from two mills that were initially chosen to process the wheat for the trial flour. These mills were selected according to the affordability, accessibility, and condition of the machinery. Of these two mills only one (Mill 1) was chosen to grind the Zincol-2016 wheat grain for the trial. The second mill (Mill 2) acted as a potential substitute in case of any mechanical fault or power shortages in the area. Only non-biofortified wheat was processed in Mill 2, which was used at the start of the trial as a source of control flour. For the FGDs, potential participants were eligible if: they were over the age of 18, were employed at the selected farms or mills, and could willingly give informed consent. The selection of the participants for the FGDs was conducted by the BiZiFED2 RCT trial management team who identified individuals who were willing to speak openly.

A total of four FGDs with a duration of between 30 and 60 min were conducted between November and December 2020. The location of the FGDs was selected based on ease of access for the participants and where COVID-19 safety measures could be ensured. Topic guides were used to lead the FGDs (See Supplementary file 1) and were designed to gather information about local farming/milling practices, their views on biofortified wheat and their willingness to continue to use it, and any challenges farmers and millers may have faced during the COVID-19 pandemic. Topic guides were reviewed by all members of the research team and translated into the local language (Pashto). Each FGD was facilitated by two research assistants who were fluent in the local language and received training from experienced qualitative researchers prior to conducting the FDGs. FGDs were audio recorded and transcribed and translated into English by an independent third-party provider in the UK.

Data analysis

Simple descriptive statistics, such as frequencies and percentages, were used to analyze the survey data, using Microsoft Excel for Microsoft 365 MSO (Version 2,208).

FGD transcripts were imported into NVivo® 12 (QSR International) for analysis. An inductive thematic analysis was conducted to generate themes following the approach by Braun and Clarke (25). This approach involves an iterative process of six phases: familiarization with the data, generating initial codes, searching for themes, reviewing themes, defining, and naming themes, and producing the report. One researcher in the UK (MCR) and one researcher in Pakistan (UM) independently read and re-read the transcripts to familiarize themselves with the data and undertook an initial coding of the dataset. Similarities and dissimilarities in the coding were discussed between the two researchers until a consensus regarding the overarching codes was achieved. One researcher (MCR) collapsed the codes into themes and sub-themes and a coding tree was generated. This coding tree was reviewed by a second (UM) and third researcher (VHM) and checked against the transcripts. Adjustments were made until consensus was achieved. Further coding and reviewing were undertaken by a researcher (MCR) using the final coding tree. Examples of quotations that best exemplified each theme and sub-theme were chosen by one researcher (MCR) and reviewed by a second researcher (VHM).

Following the analysis of the qualitative and quantitative data, results were carefully examined, and parallels and contradictions were identified, interpreted, and integrated into the discussion.

Results

Punjab farmers' survey

The participation rate for the survey was 61%, with 418 of 686 farmers who were initially contacted agreeing to take part. The findings related to: biofortified crop cultivation; drivers of Zincol-2016 second season cultivation; use of the 2020 harvested Zincol-2016 grain; agronomy and training priorities; and pandemic effects.

Biofortified crop cultivation

Of all farmers surveyed, 278 (67%) kept some or all their harvested Zincol-2016 grain separated from their usual variety post-harvest, while the remaining 33% mixed it with any other variety. Of the farmers who kept some separate, some 227 (82%) reported storing this seed for growing in the 2020–2021 season. Almost half of the farmers (47%, $n=197$) reported that they chose to grow Zincol-2016 again in the following season (2020–21). Nearly two thirds (61%, $n=253$) of all farmers were aware of Akbar-19, a recently released zinc biofortified wheat variety.

Agronomy and training priorities

The majority of farmers did not use foliar zinc fertilization on their crops to increase grain zinc (83%, $n=191/230$).When asked what factors would influence their decision to apply basal or foliar zinc fertilizer, which would typically incur additional expense for the farmer, the most frequent responses were cost of zinc fertilizer ($n=138$), whether there was sufficient market demand/buyers interest in the biofortified product ($n=130$), and lack of knowledge on how to apply foliar zinc fertilizer ($n=102$), while discoloration of leaves ($n=25$), preference of organic matter ($n=26$) and access to credit ($n=32$) were less commonly identified as influencing factors. Most farmers (82%, $n=189/230$) indicated they would be more likely to use foliar zinc on wheat intended for their own consumption. Most participants (84% $n=341/407$) indicated that they would appreciate some training on this approach.

Drivers of Zincol-2016 second season cultivation

The 197 participants who reported growing Zincol-2016 again in the following season were asked to rate how important certain factors were in their decision to grow this variety again. The farmers indicated that the availability of Zincol-2016 seed ($n=166/185$), growth and disease resistance ($n=117/190$), grain yield (120/188) and the quality of flour from the previous harvest (107/180) were very important motivators to growing Zincol-2016 again. Nutritional benefit was identified as "important" ($n=86/166$) or "very important" ($n=71/166$) by most participants. The cost of seed was rated as "not important" by 28% of those who responded to this option ($n=50/177$) Figure 1.

Use of the 2020 harvested Zincol-2016 grain

Farmers who had indicated that they had kept some Zincol-2016 grain separate from their other variety post-harvest were asked how they used the retained grain. Of the 278 farmers who responded, the majority (79%) reported having more than one use for the grain. As shown in Figure 2 the most frequently given responses were that the grain had been used for consumption within their own household ($n=221$), gifted or shared with neighbors ($n=144$), or was stored for multiplication sowing in the following season ($n=227$). Of those who consumed the Zincol-2016 grain within their own household, 29% had consumed it for less than 3 months ($n=64$), 57% consumed it for 3–6 months ($n=127$), and 14% consumed it for more than 6 months ($n=30$). Of those who had consumed bread made with Zincol-2016 grain, most felt it had a better taste ($n=198$, 90%), better texture ($n=174$, 79%) and lighter color ($n=140$, 63%) than bread made with their usual variety (Figure 3). Only a minority of farmers used the

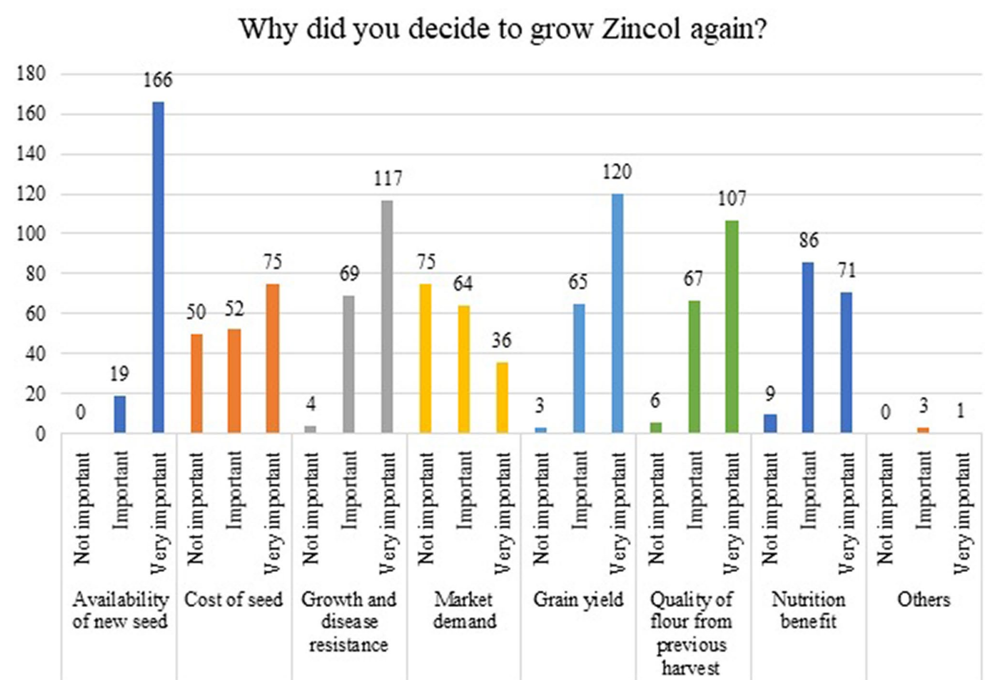

FIGURE 1
Participant farmers importance attached to selected attributes influencing their decision to grow Zincol-2016 in the follow-on growing season.

grain as payment to a landowner ($n = 6$) or as payment to laborers ($n = 31$) and 19% ($n = 52$) reported that they had sold the Zincol-2016 grain.

Pandemic effects

Farmers were asked if the COVID-19 pandemic impacted on the amount of grain they sold in 2020 to which 49% responded that they had sold the same amount as usual, 23% had sold less than usual, and 28% had sold more than usual.

Focus group discussions with Peshawar farmers and millers

A total of 12 farmers and 12 millers agreed to take part on the FGDs. Information derived from the thematic analysis enabled the identification of two main themes: (1) Enablers for scaling up of biofortified wheat and (2) Challenges and considerations for scaling up.

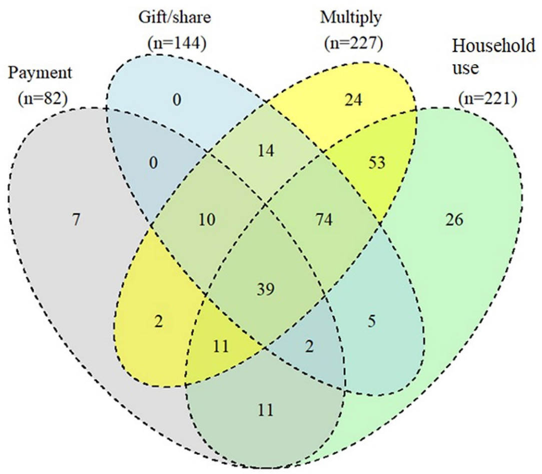

FIGURE 2
The use of 2020 harvested Zincol-2016 grain reported by participants.

Enablers for scaling up of biofortified wheat

Among the enablers for scaling up we identified four subthemes: (1.1) Perceived health benefits; (1.2) Improved grain quality and production; (1.3) Willingness to produce and process biofortified wheat if provided with support; and (1.4) An opportunity for millers to expand their business.

Perceived health benefits

The farmers and the millers who had produced and processed the biofortified wheat, recognized that adding minerals to the wheat or flour through biofortification or fortification was beneficial to alleviate mineral deficiencies among the population. The health benefits were seen as an incentive to produce and/or process biofortified wheat.

> "Sir, the reason we consented (to grow biofortified wheat) was that there was deficiency in our country, children weakness, their mothers' weakness, vitamin deficiency, when we read your description we found good things in it, and that wheat was beneficial for us. We consented for the purpose of having a healthy society" – A farmer.

Improved grain quality and production

The farmers acknowledged that the process of growing biofortified wheat differed from their usual ways of farming (i.e., the way in which the land is prepared) and required additional care. However, farmers did not verbalize any concerns about the process of growing biofortified

FIGURE 3
The participant responses to questions about Zincol-2016 bread quality compared to their usual variety.

wheat or about the wheat grain provided to them during the trial. On the contrary, farmers expressed satisfaction with the quality of seeds and believed that the zinc sprays enabled the grain to grow stronger and be of better quality. Farmers also believed that the sprays were beneficial to the soil and felt that the chemicals they received increased their yield.

> "It came up very good and its production was beautiful, we were very much happy with it. Every grain of wheat was very big and beautiful, every grain of it was like a red berry, it was the mercy of Allah and it happened due to the grace and mercy of Allah" – A farmer.

> "It was very good because when we grew it the production was more than it used to be and additionally zinc and everything were given to it, so it brought a very good result" – A farmer.

The farmers expressed that they did not have any issues while growing Zincol-2016 for the BiZiFED trial, other than those that they would normally face while growing a non-biofortified variety. These common challenges included disease and weather issues.

> "In the name of Allah, the most merciful, the most compassionate Mr. Rashid, when we would do farming previously, it would be on our (inaudible) but when you came, the method you did the wheat farming it was in a very good way, and we were to get its products but unfortunately some storms and rain occurred which caused the wheat's grain to remain incomplete" – A farmer.

Willingness to produce and process biofortified wheat if provided with support

The farmers and millers who produced and processed the Zincol-2016 wheat grain expressed that, given the benefits of biofortified wheat for the population, their production and business, they would be willing to produce and process it if given support (i.e., technical help, machinery, soil, fertilizers, seeds, wheat, and training) from the government or other organizations.

> "Absolutely. We will be growing yeah (multiple voices). This is beneficial for us, this thing is good for our coming future, we like it very much, yes. We want the government to help us like that then God willing we will keep growing this. We request the government to help us the way you came last year and helped us in terms of chemicals, soils, wheat seeds" – A farmer.

> "If the government provides us with biofortified wheat, then we are ready by all means to grind it. If we get this sort of wheat we will grind and sell in the bazaar" – A miller, Mill 1.

Some participants indicated the need for government initiatives to promote the scale up of biofortified wheat. A miller expressed his belief that improved community benefit would arise from a government supported program of biofortification.

> "If the government supports this program as this work is done for the welfare and good of our people. Therefore, we should participate in it, and we should become part of it. If this done by the government then it would be carried out with enforcing people and it would develop easily. Instead of doing it privately if it is carried out through the government then it would bring up better results" – A miller, Mill 1.

The farmers expressed that receiving support during the BiZiFED2 trial in the form of soil, seeds, fertilizers and knowledge enabled farmers to increase their production and allowed unused lands to be re-used. Farmers expressed their interest for continuing to receive the support provided during the trial and expressed their concerns of a decline in production when the trial came to an end.

> "The chemicals and the seeds increased our products and as Mr. (Name) told you that before when we would plough a field then we would plant a mound (37.324 kg) or two in it, but this time, Praise be to Allah, they showed us a proper limit and we used Urea as well in its proper limit, God willing. We were expecting after that as well, but it did not happen. God willing, we will fully cooperate with you if we receive all these things next time again" – A farmer.

An opportunity for millers to expand their business

Discussions with millers also revealed that milling biofortified wheat was beneficial, not only for the potential health benefits that this could confer to the population, but as an opportunity to expand their business and possibly gain interest from the consumers, as had occurred when millers fortified wheat with support of the World Food Program (WFP).

> "If we get it (biofortified wheat) then we would accept it with happiness, because we try to expand our business so it would help us to expand our business. And we would be able to provide people with such standard flour which would be good for their health, and it would meet their nutritional requirements as well" – A miller, Mill 1.

Participants from both mills believed that improving the nutritional value of the wheat through the WFP fortification initiative had boosted consumer demand, and one participant suggested that an identifier label or stamp may help consumers differentiate fortified products from standard varieties.

> "Our production has increased with it (fortification) because the item that is put into it, which is iron, that is in fact for some requirement such as some illness and so. Therefore, people consume it with good interest" – A miller, Mill 2.

> "Whichever market this flour goes, we have told all of our customers regularly that you should first look at its own fortified monogram and then buy the flour so that you make sure this thing is available in it" – A miller, Mill 1.

Possible challenges and other considerations for scaling up biofortified wheat

Four sub-themes were identified as possible challenges and considerations for scaling up. These were: (1) Unfamiliarity with the biofortification process and crop; (2) Production costs; (3) Need of support from the landlord; (4) Millers' beliefs about local wheat; and (5) External threats to supply chain of wheat and resources (i.e., COVID-19).

Unfamiliarity with the biofortification process and crop

Prior to the BiZiFED2 trial, it appeared that neither farmers nor millers had previously produced or processed biofortified wheat, nor did they know what biofortification was. The farmers that produced biofortified wheat for the first time during the BiZiFED2 trial suggested to the research team that they use a simple local name to refer to biofortified wheat as they would find easier to understand.

> "My suggestion is that you give us some advice, for this wheat which you call it biofortified, choose a name which a farmer finds that name sweet and dignified, yeah. Just call it 'Sona' [Sona is a fertilizer name used in this conversation but is an Urdu language term as well which is widely used in Pakistani Pashto, and it means 'gold' so in my judgement the famers here use it in this sense – Transcriber] wheat as a lot of hard work is done on it. As 'Sona' has a high quality in the soil so is this wheat" – A farmer.

Some millers described not being aware of what biofortified wheat was despite previously having ground it for the BiZiFED2 trial, but they were open to receiving more information about it. A miller believed that the farmers should be given training on the process since they would be the ones that grew the wheat.

> "Those who need to be told first are the farmers as they are the one who grow the wheat and that wheat comes to us after that. We have not been briefed about this, so when they come then we will receive briefing about it. When they teach us, then good results will be achieved" – A miller, Mill 1.

The millers that had not ground Zincol-2016 before, expressed their beliefs that the quality of the local wheat was poor, hence they would not be interested in milling biofortified wheat if it was grown locally unless they had evidence that the quality of the flour produced by the biofortified grain was good.

> "We will not be paying attention to this wheat firstly because its bread is weak, and secondly, we will see next year, if the wheat is good then we will be buying it. Although we do not buy the local wheat because even if we use it then it gets returned to us from every side, the wheat that particularly the wheat that is native to Peshawar" – A miller, Mill 2.

Production costs

For the farmers, the costs of the chemicals required to support biofortified wheat crops was the main barrier to its adoption. Farmers expressed not being able to afford the chemicals as they needed to prioritize other expenditures due to severe resource limitations.

> "Yeah, white fertilizer black fertilizer, chemicals, if these things are expensive then we will not be able to afford it, because we would be wondering whether to spend what we earn through labor work on the field or on ourselves. So, if we cannot do the crops, it would be due to the poverty and helplessness" – A farmer.
>
> "Yes, it does require expenditure and we cannot afford it; it needs fertilizers, garbage [a particular garbage, mostly consisting of ashes which people use as natural fertilizer – Transcriber], and we cannot afford all of these. We can only put soil and so into it" – A farmer.

The price of the biofortified wheat was not directly mentioned by millers as a barrier to processing it, but they did state that when wheat prices increased, for example as they did during the COVID-19 pandemic due to supply chain issues, the price of their product also increased, and consumers may choose not to buy their flour. This suggests that millers may not be willing to mill biofortified wheat if the cost to consumers exceeded the cost of government-produced wheat.

> "No, no, there is shortage of wheat. It is not available at all. When it is available then its price is 5,000 Rupees, yeah, when we flour it then people do not buy it because there is a big difference between the 5,000 and the price of the government wheat, the difference is almost 1,200 Rupees at this time" – A farmer.

The need for support

Farmers and millers both described a requirement for third party support if they were to produce or process biofortified wheat. Decisions concerning the type of crops cultivated by the farmers was not only dependent on the farmers themselves but also on their landlords, which would ultimately impact on whether they would be able to grow biofortified wheat crops.

> "We have (Name of landlord) land, we cultivate it. Whatever he orders us we cultivate the land in accordance to that" – A farmer.

Typically, farmers gave a proportion of their crop to their landlord and the rest was retained by the famers for self-consumption or for selling, either to millers or in the markets. Some of the money earned from the farmers' sales was used to buy farming resources, but they also expressed a need for more support from their landlords for such expenses.

> "There are a lot of expenses, the farmers spend money on tractor. The landlord just halves it [the harvest] and they do not

> care about anything else. There are expenses, at least half of those expenses should be accepted by the landlords" – A farmer.

Millers also believed that if there was any special equipment or training to process biofortified wheat they would need support from a third party.

> "Whatever machinery is necessary for this, the government should provide us with, and we will go along with it" – A miller, Mill 1.

External threats to the supply chain of wheat and resources

Market and road closures and transportation disruption were a threat to the wheat and resource supply chain (e.g., fertilizer and transport) and economy of the farmers and millers during the COVID-19 pandemic. Both groups described how the disruption in transportation would cause delays or an inability to deliver their products to the market or to their customers, making it difficult to sell their products.

> "The disease that Corona [COVID-19] has brought has had a big impact. As my friend said when we take a crop to the bazaar, it is a challenge. Firstly, vehicles are not available and so going to the bazaar is difficult but when we manage to take it to the bazaar then there is the issue of selling; when we sell it, it goes for cheaper but when we buy something it cost expensively for us" – A farmer.
>
> "Due to the Corona [COVID-19] the transportation has been affected very much. A work that would have been done for ten rupees before, has turned to be done for 100 rupees. Therefore, for a poor man wheat in the open market became very expensive. The wheat that the government would subsidize, and we would grind, that flour was available to the public with subsidy. But the private wheat's transport cost doubled for the flour mills because of the closing of vehicles stations, and lack of transportation. So, this was a loss for the mills" – A Miller, Mill 1.

Farmers and millers also noted the impact of reduced opening times at the markets where they usually sold their products. To ensure that products were sold, farmers would directly sell their products to the mills or sell them at a lower price which represented losses for them. Both farmers and millers expressed that the market closures would cause shortages and therefore an increase in the price of the resources (e.g., fertilizers and wheat) required for producing their products.

> "It would certainly have been affected because it is about the bazaar, when the bazaar is closed then it definitely gets affected. When a product does not reach the bazaar on time then the product that was supposed to be sold for ten, now it gets sold for eight. This issue is there yeah" – A Farmer.

However, these adverse circumstances may have been short-lived as participants in subsequent focus groups (conducted in February 2021) did not describe market closures and attributed this to support from the government. The millers acknowledged the work of the government in reducing the shortage of wheat during the pandemic and reducing its price.

> "The price was high at that time because the wheat was short and wasn't available. But at the present time the wheat is gradually increasing from every side and the government provides a lot of facilities, so a month ago our wheat was 1,250 Rupees and now it is 1,100 Rupees, and due to the government wheat, the flour is getting cheaper" – A miller, Mill 2.

The farmers expressed that the COVID-19 pandemic led to an unstable labor force as they were not able to leave their homes to go to work. This would affect their farming activities as those who had other jobs in addition to farming would not be able to afford the resources required to grow the wheat. The farmers explained that this instability in the labor force would cause economic difficulties among their consumers and hence their products would not get sold.

> "It affects the work of a laborer as he cannot find work, so it impacts it in a big way. The farmers' products do not get sold" – A farmer.

Millers, however, believed that people would continue to consume the same amount of flour during the pandemic therefore they would still be able to sell their products.

> "Flour is something which is for daily consumption, therefore there has been no impact on it" – A miller, Mill 2.

Discussion

Zinc biofortified wheat may be a sustainable strategy to increase the dietary zinc intake among populations with low access to nutritious diets (3). Given the importance of the producers' acceptability of the biofortified crop to enable successful scaling up of biofortified wheat in Pakistan, the aim of this mixed methods study was to explore Pakistani farmers' and millers' experiences and attitudes towards the production and processing of zinc biofortified wheat.

The survey and focus group data provided evidence that the farmers were satisfied with the Zincol-2016 wheat variety. Almost half of farmers (47%) who received Zincol-2016 seed to grow in the 2019–2020 season, stored a portion of the grain harvest and expanded its production in the following season (2020–2021), which suggests a preference of Zincol-2016 over their existing varieties. In addition, in the focus groups the farmers expressed their willingness to grow the biofortified crop given the perceived health benefits of the grain. Our findings are similar to previous studies in Uganda and Nigeria that investigated stakeholders' perceptions towards the adoption of biofortified crops, where farmers expressed a positive response to agronomic biofortification (14, 17), particularly if awareness of the benefits of biofortification was high (14).

In our study we were able to identify some potential motivators that could increase the likelihood of biofortified wheat adoption among producers and processors. These included perceptions related to the superiority of the grain yield and flour quality, perceived health benefits of the biofortified flour, acceptability of the flour among consumers, and potential for increased marketing opportunities. It was also noted that adoption is likely to be improved if support, in the form of training and resources, is provided alongside the biofortified seed.

Crop performance was the most cited motivators for growing Zincol-2016 among farmers, who observed that the quality of the Zincol-2016 seeds, together with the fertilizers, increased their yield. Two thirds of farmers who cultivated the Zincol-2016 grain in the following season (2020–2021) cited good growth, disease resistance and yield as reasons for why they chose to do so. Farmers who took part in the focus groups had no concerns about the health of their Zincol-2016 crops above and beyond the common challenges they regularly faced (i.e., common fungal disease and weather issues). These findings provide promising indicators for the success of scaling up of biofortified wheat, since maximizing yield and reducing production threats due to disease positively impact the farm income potential (26). Crop resilience to adverse climate conditions is a major factor affecting farming decisions. The frequency of extreme climate events is increasing in Pakistan as a consequence of climate change, and include extreme heat wave occurrences, a shortfall in irrigation water and drought conditions at sowing (18). This highlights the critical importance of developing new crop varieties that consider grain resistance, crop resilience and yield, alongside improved nutritional quality.

Production costs are a key factor that influence producers' intention to produce biofortified crops, particularly in relation to the cost of foliar zinc fertilizer application, which may be in addition to soil enrichment with zinc and organic fertilizers. Foliar zinc fertilizers increase the zinc content and quality of the wheat crops and have been used with Zincol-2016 to enhance the grain's performance (6, 19, 27). However, data suggests that the use of fertilizers in Pakistan is low (19). The survey with the farmers showed that the majority (83%) of participants reported that they did not apply foliar zinc fertilizer to their wheat crops, with more than half stating that their decision to apply foliar fertilizer would depend on the costs incurred. This was supported by views expressed in the focus groups, with farmers explaining that they would not be able to afford the fertilizers due to severe resource limitations. Wheat (input and output) prices in Pakistan are regulated by the federal government (28), and farmers are not incentivized for the commercial production of foods with higher nutritional qualities. Therefore, as it has been previously discussed (29), higher production costs may demotivate farmers to choose zinc biofortified wheat varieties or use zinc fertilizers if these are perceived as necessary to achieve the desired enhanced quality for market advantage.

A particularly striking finding of the farmer's survey is that, with a starting provision of sufficient grain for half an acre of cultivation, seed saving, and multiplication saw this extend to nearly one third (31%) of the cultivated wheat area just 1 year later, for the 47% of initial farmers who continued to grow Zincol-2016. It also showed a strong preference of home consumption and gifting of seed to neighbors, rather than more commercial transfers to landowners, workers, or sale. Data from both focus groups and survey shows that seeds were retained to sow for the next season, providing evidence that an initial investment for the provision of seed could be a sustainable strategy to support the reduction of micronutrient deficiencies if the crop maintains its high zinc trait over successive growing season.

Compared to control flour, the additional zinc content of the Zincol-2016 flour produced in this study was 3.69 mg Zinc/kg (3). Farmers and millers who produced or processed Zincol-2016 recognized the importance of additional micronutrient content of the biofortified wheat to their community. Likewise, 40% of survey participants considered the nutritional qualities of the crop an important motivator when deciding whether to sow Zincol-2016 in the following season. A study conducted in Nigeria suggested that adoption of biofortified cassava among farmers may be improved by increasing their awareness of the health benefits of the crop (14). These findings, however, are in contrast to a Ugandan study which reported that farmers growing biofortified banana did not value the improved mineral content of the product since they believed that consumers were unconcerned about nutritional value and only cared about buying enough food to feed their families (30). Our earlier study on consumers perceptions of biofortified wheat challenges this view, as consumers of zinc biofortified flour valued the health benefits that they perceived it gave, which may suggest a preference over standard varieties (21).

The results of our study suggest that decisions to grow biofortified wheat does not only rely on the farmers, but also on the landowners who may have ultimate control of what the farmers grow. Moreover, it is possible that farmers may not want to invest in zinc fertilizers or improvements in the soil if their tenure is uncertain. A study in Pakistan (31) suggested that tenure influenced farmers' decisions regarding soil and yield improvement measures, such as fertilizer application. Therefore, stakeholders wishing to promote adoption may need to engage landowners and landlords in discussion to actively influence their farm management decisions.

The present study was conducted during a critical period of the COVID-19 pandemic, when intermittent lockdowns were in place across Pakistan. In the FGDs farmers described their experiences during lockdown, including difficulties with access to markets affecting the price of fertilizers and pesticides and their ability to sell their products, and increased prices of basic food items. This concurs with similar reports from Pakistan (32) and India (33). A survey by Hussain et al., showed that farmers in Pakistan faced many difficulties with crop cultivation during the COVID-19 lockdown, including a perceived increase in price of fertilizers and pesticides and difficulties accessing markets affecting the price of foods (32). Similarly, a survey in India reported that farmers faced delays on their ability to sell their products due to travel restrictions, representing a financial loss for farmers who were unable to safely store their goods, and difficulties accessing a varied diet due to the lack of availability of certain foods or increased prices (33). These studies highlight the fragility of the food system and a need for strategies to support farmers in their ability to sustainably produce nutrient rich foods even under challenging circumstances. Although the COVID-19 pandemic is currently controlled, other external threats such as climate change and international conflict pose a potential risk to global wheat production and resulting food security and dietary diversity of vulnerable populations (18).

Evidence suggests that a key driver to the adoption of Zincol-2016 and other biofortified wheat varieties in rural areas in

Pakistan is the provision of adequate support for both producers and processors of the grain. Our survey and focus group discussions suggested that most farmers were willing to grow biofortified wheat if given affordable access to foliar zinc fertilizers and training on their application. A previous study in Pakistan exploring the factors influencing the adoption of improved wheat varieties in rural areas revealed that access to extension services and micro credit schemes was found to be a key factor (34). In this context, the farm advisory/extension services visited the farmer, inspected crop health, and took soil samples (for onward lab recommendations) to issue advice. These services were provided free of cost to the farmer by the provincial agriculture department as well as by private fertilizer companies. These services provided an opportunity for creating awareness about the biofortified crops, promoting the adoption of biofortified varieties and provision of training for the farmers.

Finally, our study revealed that the millers considered consumer acceptability and the quality of the food staple when deciding whether to adopt new technologies. The millers who did not process Zincol-2016 grain in our study expressed that they would not adopt it until they had evidence that the flour and bread it produced were of good quality. Our survey data indicated that the majority of farmers who had consumed the Zincol-2016 flour believed that the bread it produced had a better taste and texture compared to their usual varieties. Similarly, our earlier study of consumers' perceptions of Zincol-2016 (21) revealed that participants felt that the resulting flour had good sensory and cooking attributes (21), which suggests that good quality produce may be a facilitator for the adoption of Zincol-2016 among both consumers and producers. The importance of consumer acceptability was also highlighted in one study (35) where findings showed that millers were reluctant to invest in fortification spraying technologies to increase the nutritional quality of food staples as they perceived that the resulting produce (in this case micronutrient fortified rice) was not well received by consumers (31).

To our knowledge, this is the first study that has been conducted exploring producers' experiences of growing biofortified wheat in Pakistan. The quantitative survey 418 of farmers, together with the qualitative exploration of experiences and attitudes allowed the triangulation of data and added richness and depth to the findings. This study also has some limitations. The farmers in the focus group discussions received support from the BiZiFED2 project, which included the provision of the seed, fertilizers, and the purchase of their crop at a favorable rate. The farmers in the survey received free Zincol-2016 seed the previous growing season and soil fertility data at the time of their selection, which could have introduced bias to their responses: they do not represent an unbiased sample of Punjab Province wheat growers in relation to biofortified wheat cultivation. Moreover, our survey did not collect the reasons for why some farmers did not grow Zincol-2016 in the following season. Additionally, the views of farmers not involved in the BiZiFED2 trial were not sought, and it is possible that their views may differ from those of farmers involved in the study.

In conclusion, our study suggests that biofortified wheat was well received among the producers in KP and Punjab provinces. Although both farmers and millers valued the nutritional qualities of the crop, farmers felt that crop performance and yield were among the most valued characteristics of the grain, while millers saw it as a marketing strategy and would be willing to adopt it due to the high quality of the product. Further awareness and training about the benefits of biofortification is required among both farmers and millers. Farmers require support to acquire and utilize zinc foliar fertilizers to optimize the zinc content of the Zincol-2016 grain. The results of this study strongly suggest that farmers and millers are willing to produce biofortified wheat providing support is given in the form of resources and training. These finding can inform the scaling up of biofortification for the provision of more nutritious foods to populations, particularly in areas outside the reach of centralized fortification interventions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethical approval granted by the University of Central Lancashire (REFTEMH1014), Khyber Medical College Peshawar (reference BZ/000628) and University of Nottingham (BIO-1819-001A) ethics committees. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

NML, VHM, ELA, EJMJ, HO, MZa, MZi, and SA contributed to the design and development of the study. UM, HO, SA, ELA, MZi, and MZa managed data collection. MC-R and UM completed initial coding and analysis of the qualitative data, which were double checked by VHM and EJMJ. SA and ELA cleaned and analyzed the survey data. M-CR integrated the qualitative and quantitative data and wrote the manuscript. VHM, NML, and EJMJ revised the paper and finalized the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MZi was employed by Fauji Fertilizer Company.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1158156/full#supplementary-material

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Ultrasound-assisted fortification of yellow sweet potato (*Ipomoea batatas*) with iron and ascorbic acid

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The objective of this study was to evaluate the effect of ultrasound on the incorporation of iron and ascorbic acid (AA) in sweet potato *(Ipomoea batatas)* and to optimize the process parameters to obtain a fortified food. The incorporation was carried out using cubes of sweet potato submerged in 0.1% m/v ferrous sulfate and 1% m/v AA solutions, treated at different times and sonication frequencies (37 and 80 kHz), at 100 watts of power and 30 ± 5°C. ANOVA and Tukey's test at 5% significance were applied to establish significant differences and the process was evaluated using a factorial design. The results revealed that the application of ultrasound influences the content of iron and AA, incorporating greater amount of iron and AA compared to samples not treated with ultrasound. Similarly, longer times led to higher incorporation of iron and AA content in sweet potatoes; the frequency was not statistically significant. The highest iron content was 105.91 ± 0.03 mg/100 g and for AA, it was 392.65 ± 4.84 mg AAE/100 g. The defined ultrasonic process conditions produced an increase of 4928.99 and 610.65%, respectively, in iron and AA content in sweet potato.

KEYWORDS

ultrasound, iron, ascorbic acid, fortified sweet potato, food processing

1. Introduction

The need and consumption of fortified foods have increased significantly in recent years, there is great concern about the nutritional aspects and processed foods products consumed in the daily diet, this is due to the high nutritional deficiencies in the world population. Iron deficiency anemia is one of the most prevalent nutritional deficiencies worldwide, and is considered as a public health problem (Ministerio de Salud [MINSA], 2017). Worldwide, anemia affects 500 million women of reproductive age (15–49 years), 40% of pregnant women, and 42% of children under five years of age (Meilianti et al., 2023). In Peru, 40% of the population suffers from anemia (Instituto Nacional de Salud [INS], 2020), 33.6% of children under 5 years old, 20.6% of women between 15 and 49 years old by 2022 (Instituto Nacional de Estadística e Informática [INEI], 2023).

This issue can be approach by adhering to a strict diet, containing iron-rich foods, and consuming supplements or fortified and/or enriched foods. Iron fortification is considered the most effective option to prevent malnutrition on a large scale, and this strategy has been applied in different governmental programs (Joshi et al., 2019). Iron is a micronutrient necessary for the human body; it participates in the production of hemoglobin and myoglobin, which are responsible for oxygen transport. Likewise, contributes to the metabolism of certain enzymes and in the synthesis and catabolism of neurotransmitters, therefore this deficiency has an impact on behavioral, mental and motor development, and also induce a slower speed of conduction of the sensory, auditory and visual systems (Rivera et al., 2012; Ministerio de Salud [MINSA], 2017).

Fortification is the addition of a micronutrient to food to increase the content of one or more essential micronutrients, correct or prevent a demonstrated deficiency (dietary, biochemical, functional, and/or clinical) of a nutrient in the population (Latham and FAO, 2002). Iron fortification is one of the most complex, there are different iron compounds concerning their solubility and chemical state; ferrous sulfate is the most widely used source of iron in the industry, due to its high bioavailability of the micronutrient, and its low cost (Shubham et al., 2020). To enhance absorption, iron is combined with vitamin C, which acts as a potentiator. Vitamin C in molar relations with iron greater than 1:1 can double the absorption of non-heme iron, despite the presence of dietary inhibitory factors (Tostado-Madrid et al., 2015). However, when iron incorporated directly into the food matrix, it can oxidize and cause undesirable changes in organoleptic properties (odor, taste, color) (Genevois et al., 2014). For this reason, the optimal fortification of foods with complex structures such as fruits and vegetables, requires a great deal of research, most of the traditional technologies used cause instability in the added nutrients; this is why new technologies such as ultrasound (US) are being used (Carvalho et al., 2021).

Ultrasonic technology has been the subject of numerous studies aimed at improving mass transfer in foods. These studies utilized different media, such as water, osmotic solutions and ethanol, to transmit acoustic waves into the food matrix. This method enables the efficient inclusion of compounds, such as iron, more effectively, improving their homogeneous dispersion of micronutrients (Rojas et al., 2019). Furthermore, the use of this technology aids in decreasing nutrient loss and processing times, favoring the quality of the sensory properties of the product (Bhargava et al., 2021).

Carvalho et al. (2021) demonstrated the efficacy of the combination of ultrasonic technology, microencapsulation, and convection drying in the production of fortified pineapple chips. The authors showed that optimal ultrasonic pre-treatment of ethanol (25 kHz - 30 min), significantly increases the iron content (up to 1,000% more than the control sample) in the final product. Similarly, Bonto et al. (2020) incorporated iron in rice with ultrasonic technology (40 kHz- 5 min). The absorption of iron in the rice sample was 321 ± 13.43 mg/kg of rice, achieving a 28-fold increase when compared to the untreated rice sample. Furthermore, the experiment revealed efficient iron diffusion, and achieved 82.9% retention rate after washing and cooking. Similarly, Rojas et al. (2019) incorporated iron and carotenoid microencapsulates in pumpkin and apple, respectively. The application of ultrasound resulted in a more homogeneous distribution of iron increased iron content by over than 1,000% compared to the control samples.

Mashkour et al. (2018) incorporated iron into whole potatoes by vacuum impregnation in combination with ultrasonic technology (37 kHz - 45 min) as a pre-treatment. Their study showed that the combination of IV (vacuum impregnation) with the US (ultrasonic waves), resulted in higher iron incorporation of 210%. In addition, no significant effects on color and texture parameters were observed. Miano and Augusto (2018) incorporated iron during the bean hydration process with ultrasound application (91 W/L; 25 kHz), after 510 min, the incorporation obtained with ultrasonic application was 60.1 mg Fe/100 g, compared to 34.4 mg Fe/100 g without ultrasonic application. Mason et al. (2015) mentioned that ultrasound generates positive effects on processed food, this effect occurs due to the collapse of cavitation bubbles that are caused by pressure fluctuations, exerted by the passage of acoustic waves from the US bath. This cell disruption can produce a higher mass transfer (solids gain and water loss), allowing better impregnation of the solvent and thus facilitating the incorporation of micronutrients in foods (Bonto et al., 2018; Yılmaz and Ersus Bilek, 2018).

The incorporation of iron in different foods has been investigated with positive results, reflected in the increase of the content of this micronutrient. To this extent, it is interesting to investigate the incorporation in food matrices, being sweet potato is a food of great interest and has great potential to become a food vehicle, since it ranks seventh in most produced food. Sweet potatoes are one of the most significant crops worldwide, producing over 104 million tons in 2014 (FAOSTAT data, 2017). In addition, production cost is relatively low (Grozo and INEI, 2021). Likewise, this food has valuable nutritional content and is highly rich in nutrients, such as vitamin C, Fe, K, and *Ca*. It is a member of the *Convolvulaceae* family, genus *Ipomoea,* and the type species *Ipomea batatas L*. The orange-fleshed sweet potato has antioxidant properties, anti-inflammatory effects, high iron and zinc content and a low glycemic index being a great ally in countries suffering food shortages (Oladejo and Ma, 2016; Renee et al., 2018; Nyathi et al., 2019). Although heme iron contained in animals is more bioavailable than non-heme iron (found in vegetables), many countries have low meat consumption and high prevalence of iron deficiency anemia, therefore, non-heme iron is an alternative option to increase iron intake and combat iron deficiency. Furthermore, vegetable sources may contain a significant content of AA, which favors a higher bioavailability of iron (Andre et al., 2018). Although studies on ultrasound-assisted food fortification are reviewed in the literature, there are no studies that propose the incorporation of iron and AA in sweet potato. Consequently, considering the great importance of iron-fortified foods for the world population, the objective of this work was to study the effect of ultrasound on the incorporation of iron and AA in sweet potato *(Ipomoea batatas)* and to evaluate the ultrasound parameters for obtaining an fortified food.

2. Materials and methods

2.1. Reagents

All the reagents were of analytical grade, for the determination of ascorbic acid (AA) we used 2,4-dinitrophenylhydrazine (Lobachemie, India), glacial acetic acid (Scharlau, Spain), bromine water, thiourea, sodium acetate, metaphosphoric acid, sulfuric acid (98%, m/v), the reagents were acquired from Movilab (Lima, Peru). For iron determination, 1, 10-phenanthroline (Biolab, Argentina), ammonium iron (II) sulfate ($Fe(SO_4)(NH_4)_2(SO_4)6H_2O$) (Movilab), hydroxylamine hydrochloride (Scharlau, Spain), hydrochloric acid (37% m/v) was purchased from (J.T.Baker, Mexico), nitric acid (65% m/v) was purchased from (Merck, Germany). AA $C_6H_8O_6$ and ferrous sulfate ($FeSO_4$) were purchased from Movilab.

2.2. Raw material and sample preparation

Yellow sweet potatoes *(Ipomoea batatas)* were acquired from a local supermarket in Sullana (Peru) and kept in storage at 27 ± 3°C for 2 to 3 days prior to utilization. The sample had a soluble solids content of 10 ± 1 °Brix and a moisture content of 79.8 ± 1.5%. The sweet potato samples were sliced into pieces measuring11 mm x 11 mm x 5 mm for further processing.

2.3. Ultrasound-assisted incorporation of iron and acorbic acid

Iron and AA were incorporated following the methodologies reported by Carvalho et al. (2021) and Rojas et al. (2019). First, the solution was prepared containing ferrous sulfate at 0.1% w/v and AA at 1% w/v (FA). Next, sweet potato slices were added to a beaker containing FA solution in a 1:5 w/v ratio. The above samples were placed in an the ultrasonic bath (Elmasonic P 30H, Germany) and treated at different frequencies (37 and 80 kHz) for 10, 20, 30, 40, 50, and 60 min., and ultrasound power of 100 watts and temperature inside the ultrasonic bath was 30°C. A control sample (without ultrasonic treatment) was also prepared in parallel. Subsequently, both the ultrasound-treated and control samples were drained, and dehydrated in a tray dryer (Dehydrator ST-04, 30–90°C, 0 − 15 h), at 45 ± 2°C for 8 h at air circulation speed (2.5 m/s). The Schematic diagram of the ultrasound-assisted incorporation of iron and AA in sweet potatoes is shown in Figure 1.

2.4. Determination of AA

Total AA (ascorbic acid + dehydroascorbic acid) was quantified using the UV spectrophotometric method described by Rahman et al. (2007). Ascorbic acid is oxidized to dehydroascorbic acid by the action of bromine water in an acidic medium, generated by the presence of acetic acid. The AA was extracted from the samples; for this, 1 g of the sample was mixed with 20 mL of 5% w/v metaphosphoric acid solution and 10% v/v acetic acid centrifuged at 2500 rpm for 4 min. Next, the obtained mixture was filtered with Whatman No. 1 filter. Next, 3 mL of filtered solution (SF) was reacted with 0.15 mL of bromine water, and then gently stirred. Additionally, 0.80 mL of 10% m/v thiourea was added to remove excess bromine, then 1 mL of 2,4- dinitrophenylhydrazine solution was added and gently stirred. To finish the reaction, the samples were maintained in an incubator (Usamed, DNP-9052A) at 37°C temperature for 3 h; subsequently, the samples solutions were immediately cooled in an ice bath, added 1 mL of 85% m/v H_2SO_4 with constant stirring and made up to 5 mL with distilled water. The absorbance of the colored solution obtained was measured at 521 nm in a UV–visible spectrophotometer (Thermo Scientific, model Genesys 150). The calibration curves were obtained with standard solutions of AA ranging from 2.5 to 20 mg/L, following the same procedure used for the samples, and the concentrations were expressed in mg of AA (AAE) /100 g. All determinations were performed in triplicate and all steps were repeated for the blank sample.

2.5. Determination of total iron

The determination of total iron was performed according to the AOAC (1994) method (944.02) with adaptations. In this method, Fe^{2+} reacts with 1,10-phenanthroline to form a characteristic red-orange complex that absorbs strongly in the region around 510 nm of the visible spectrum. Hydroxylamine hydrochloride solution was applied as a reducing agent to reduce Fe^{3+} to Fe^{2+}.

To perform the analysis, a crucible was used to weigh 1 g of the sample and which was subsequently placed in a muffle furnace (Sel-Horn "R- 8 L) at 550 ± 15°C for a period of 5 h. Next, 0.1 mL of nitric acid was added to the ashes obtained and evaporated on a hot plate. Next, 1 mL of hydrochloric acid was added and again evaporated on a hot plate. Finally, 0.2 mL of hydrochloric acid was added, and the volume was filled up to 10 mL with distilled water. For quantification, an aliquot of 2 mL of the above solution was reacted with 0.6 mL of hydroxylamine hydrochloride, then allowed to stand in a dark place for 5 min, followed by the addition of 1.5 mL of acetate buffer and 0.6 mL of 1,10-phenanthroline, stirred gently and distilled water was added to make up the 5 mL. Finally, the solution was agitated in a vortex (Cole-Parmer, model SA8) at 1000 rpm × 10s and kept at rest for 10 min. The absorbance was measured at 510 nm in a UV–visible spectrophotometer. The calibration curves were obtained with standard solutions of Fe^{2+} ranging from 0.5 to 5.0 mg/L, following the same procedure applied to the samples, and the concentrations were expressed in mg /100 g. All determinations were performed in triplicate and all steps were repeated for the blank sample.

2.6. Experimental design and statistical analysis

For screening purposes, a 2^2 full factorial design, with two levels (−1 and + 1) was applied to the two independent factors frequency (37–80 kHz) and ultrasound exposure time (10 and 60 min) in the responses of iron (mg/100 g) content and AA (mg AAE/100 g), in a total of 4 runs (Table 1). All the experiments were performed in triplicate.

Apart from the factorial design, aliquots were evaluated every 10 min (20, 30, 40, and 50 min) in 37 and 80 kHz to process control, in a total of 8 runs. For the analysis of results, the Analysis of Variance method (ANOVA) and Tukey's test were used to determine differences between different treatments. The process was evaluated using factorial design with Statgraphics Centurion XVI software and the analysis was performed with IBM-SPSS software.

3. Results and discussion

3.1. Incorporation of iron and AA

Table 2 presents the iron and AA content in the dehydrated sweet potato after the different treatments with and without ultrasound. The control dehydrated sweet potato (CT) presented an iron content of 2.15 ± 0.01 mg/100 g and an AA content of 63.61 ± 0.50 mg/100 g, values higher than those found by Amagloh et al. (2022), who reported an AA content in fresh sweet potato ranging from 3.040 to 16.698 mg AAE/100 g. Peruvian food composition tables indicated an even lower

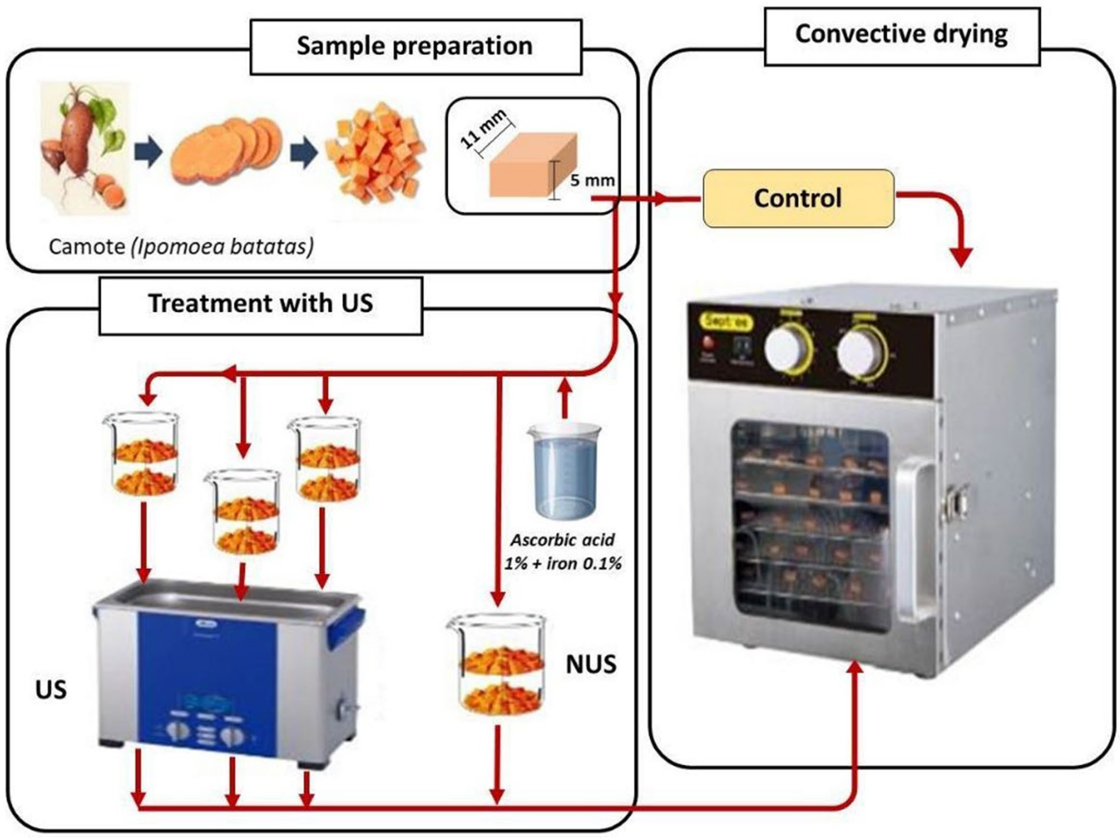

FIGURE 1
Schematic diagram of ultrasound-assisted incorporation of iron and AA in sweet potato (*Ipomoea batatas*). US, Ultrasonic treatment; NUS, No ultrasonic treatment.

TABLE 1 Coded (−1 and + 1) and real parameters of the 2^2 full factorial design.

Run	US Frequency (kHz)		US time (min)	
1	−1	37	−1	10
2	+1	80	−1	10
3	−1	37	1	60
4	+1	80	1	60

AA content of 0.80 mg/100 g was reported for dehydrated sweet potato; however, the iron values were 2.90 mg/100 g (María et al., 2017); which is in agreement with the findings of the present study. Both ultrasonic (US) and non-ultrasonic (NUS) treatments were effective in incorporating iron and AA into the sweet potato. Longer treatments resulted increased the content of both iron and AA content; however, ultrasonic frequency, as well as the interaction (frequency versus time), did not influence AA content; on the other hand, in the case of iron content, time and interaction were significant, as observed in Table 2.

According to Mashkour et al. (2018), the use of a 37 kHz frequency generated higher micronutrient incorporation compared to an 80 kHz frequency, due to the cavitation phenomenon induced by the US, the cavitational collapse produces the cellular rupture in the plant tissue, which allows the increased of cell permeability, resulting in better mass transfer. Lower US frequencies generate longer wavelengths, and longer compression cycles and produce a violent cavitational implosion, resulting in greater cell disruption. In contrast, Yu et al. (2016) testing three different frequencies (25, 45, and 100 kHz) to enrich peanuts with resveratrol, used US as a pretreatment, the optimal frequency was found to be 100 kHz. The difference in these results may be attributed in the different in the food matrix, pretreatment methods, and frequencies utilized.

The iron content was incorporated in greater quantity in the treatments with longer exposure times (Figure 2B), for instance (Table 2) the treatment at 37 kHz for 50 and 60 min, showed an iron content of 72.96 and 105.91 mg/100 g. This represents an increase of 3,293 and 4,826% with respect to the control, and at 80 kHz the values were 60.52 and 77.98 mg/100 g in both times showing an increase in iron content of up to 2,715 and 3,427%, respectively. These results were higher than those observed at the same times in NUS. Incorporating iron in sweet potato with US at 30 min allows to obtain values equivalent to those demonstrated in the twice the duration in NUS, this demonstrates the effectiveness of the application of US. The iron content shown in each treatment studied, supplements the recommended daily intake of iron for pregnant women, which recommendation requires the consumption of 23 to 27 mg of iron/day. Additionally, the dietary iron requirement for adolescents is also covered, which ranges varies from 8 to 15 mg/day, depending on age and sex (Ministerio de Salud [MINSA], 2016).

TABLE 2 Total iron and ascorbic acid content in sweet potato, treated with and without ultrasound.

Treatments		Factors		Ascorbic acid	Total iron
		Frequency US	Time US	mg AAE/100 g	mg/100 g
CT				63.61 ± 0.50^{m}	2.15 ± 0.01^{p}
NUS	C1		10 min	99.58 ± 1.49^{jkl}	26.83 ± 0.18^{k}
	C2		20 min	93.32 ± 2.16^{kl}	17.18 ± 0.00^{o}
	C3		30 min	107.38 ± 3.13^{ij}	32.75 ± 0.00^{i}
	C4		40 min	106.24 ± 1.38^{ij}	26.82 ± 0.00^{k}
	C5		50 min	121.87 ± 0.00^{gh}	24.30 ± 0.05^{l}
	C6		60 min	152.73 ± 0.46^{f}	30.43 ± 0.04^{j}
US	T1	37 kHz	10 min	103.71 ± 0.82^{jk}	23.08 ± 0.04^{lm}
	T2	37 kHz	20 min	184.50 ± 4.54^{e}	22.63 ± 0.17^{m}
	T3	37 kHz	30 min	190.99 ± 4.60^{e}	43.58 ± 1.09^{f}
	T4	37 kHz	40 min	204.29 ± 4.37^{d}	50.72 ± 0.03^{e}
	T5	37 kHz	50 min	363.26 ± 3.16^{b}	72.96 ± 0.05^{c}
	T6	37 kHz	60 min	392.65 ± 4.84^{a}	105.91 ± 0.03^{a}
US	T7	80 kHz	10 min	91.82 ± 0.23^{l}	42.70 ± 0.75^{f}
	T8	80 kHz	20 min	127.91 ± 0.86^{g}	20.56 ± 0.76^{n}
	T9	80 kHz	30 min	115.63 ± 1.11^{hi}	34.91 ± 0.59^{h}
	T10	80 kHz	40 min	191.98 ± 8.30^{e}	40.59 ± 0.45^{g}
	T11	80 kHz	50 min	226.39 ± 8.78^{c}	60.52 ± 0.06^{d}
	T12	80 kHz	60 min	235.70 ± 3.30^{c}	77.98 ± 0.05^{b}
ANOVA				*p-value*	*p-Value*
Frequency (kHz)				>0.050	>0.050
Time (min)				<0.050	<0.050
Interaction				>0.050	<0.050

Values are mean ± standard deviation, each treatment with three replicates. Means in the same column with different superscripts are significantly different ($p < 0.05$). AAE, AA equivalent; CT, control treatment.

Currently, there are no precedents for incorporating iron into sweet potatoes; however, in other foods, it has been possible to incorporate iron and other micronutrients into other foods using the same technology. Carvalho et al. (2021) propose the iron fortification of pineapple chips by using microencapsulation, ethanol, ultrasound, and convective drying. The authors mentioned that the longer time (30 min) of pre-treatment in ethanol and US increased the iron content up to 1157.5%; however, compared to the pre-treatment in ethanol, the US treatment did not significantly improve the iron incorporation. Rojas et al. (2019) showed a similar result for the incorporation of iron and microencapsulated carotenoids in pumpkin and apple. The authors found that by applying US treatment for 30 min, an increase of more than 1,000% of iron incorporation was found and its application helped to improve the dispersion of ethanol microparticles, despite this, the US did not significantly affect the results compared to the treatment without US. This is consistent with Miano and Augusto (2018) found that the iron content in beans increases significantly at longer exposure times, they obtained an increase of 1418.6 mg/kg of iron during 510 min of processing.

The amount of AA incorporation in sweet potato depends significantly on the time factor, as shown in Table 2, which ranges from 93.32 to 152.73 mg AAE/100 g in NUS; US at 37 kHz from 103.71 to 392.65 mg AAE/100 g and US at 80 kHz from 91.82 to 235.70 mg AAE/100 g is observed; despite the frequency, factor did not evidence a significant statistical influence, at 37 kHz the highest AA ranges were observed (Figure 2A). Showing effective incorporation of AA from 63 to 517%, with respect to the control. In fact, 20 min of treatment was sufficient to exceed 60 min in NUS, achieving 50% higher AA. In similar studies, under the vitamin fortification approach in other food matrices, similar behavior was observed; for instance, Tiozon et al. (2021) incorporated folic acid (vitamin B) into rice using ultrasound as a pretreatment for 1, 3, and 5 min, found that longer sonication times resulted in greater incorporation and increased folic acid levels by 1,982% in brown rice and by 4,054 times in milled rice.; in addition, US improved the micronutrient retention capacity after cooking, by 93.53% (brown) and 86.52% (milled); however, the sonication time to be used should be evaluated according to the nature of the micronutrient, food matrix, among other factors. The incorporation of vitamin B5 in rice was study for different durations of 5, 15, 25, and 35 min; 25 min of sonication produced the greatest mass transfer by the rupturing of the cell walls (changes in cell microstructure), which provided an absorption of up to 140% more pantothenic acid than in

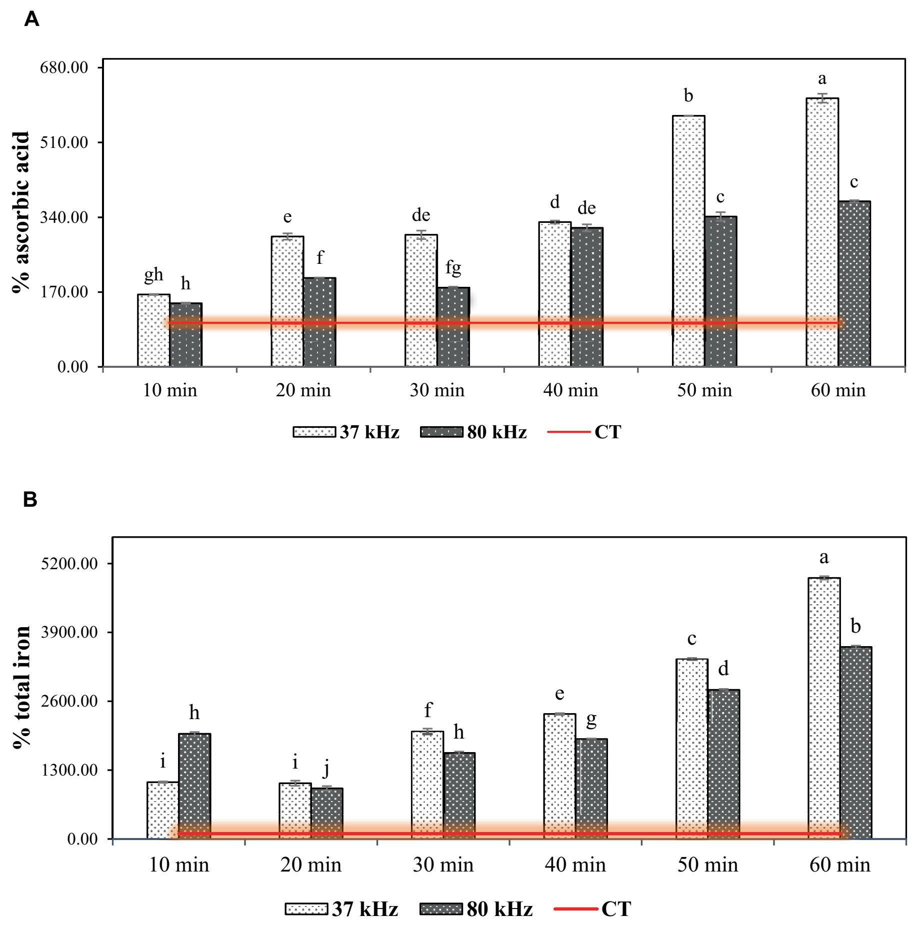

FIGURE 2
Ascorbic acid **(A)** and total iron **(B)** content expressed in percentage, using different times and frequencies of ultrasound treatment. CT, control sample. Values (mean ± SD) within each treatment with different letters differ significantly ($p \leq 0.05$).

the untreated sample; because a long time produced the gelatinization due to temperature variation (42–45°C) (Bonto et al., 2018).

3.2. Ultrasound-assisted fortification

The aim of the optimization of the process was to obtain the parameters that allow a greater increase in the iron and AA content, as shown in the surface response plot in Figure 3. As observed, the lowest frequency (37 kHz) and the longest exposure time (60 min) were the optimal parameters for the incorporation of iron and AA, obtaining an iron content of 105.91 ± 0.03, an increase of 4928.99% and an AA content of 392.65 ± 4.84, an increase of 610.65% compared to its initial content. The basis for these results, as mentioned above, is due to the increased cell permeability of the feed tissue as a result of the cavitation collapse caused by the ultrasonic treatment. Similar results were obtained by Oladejo and Ma (2016), in their study optimized the US-assisted osmotic dehydration process in sweet potato (*Ipomoea batatas*) using methodology response surface (MRS); the optimized values were the following, frequency of 33.93 kHz, sucrose concentration 35.69% and the exposure time was 30 min, this time was the maximum value, concluding that the use of US allowed more mass transfer and that the lower frequency favors the results.

Similarly, Azarpazhooh et al. (2020) investigated the impregnation of grape pomace phenols in *aloe vera* using ultrasound-assisted osmotic dehydration (25 kHz, 200–400 W), and optimized the process using a MRS. The optimum values were 50% sucrose, 50°C, 59% amplitude, 20% grape pomace extract and 173 min exposure, with the maximum time value being 210 min. They study concluded that that a low frequency (25 kHz) with an exposure time of 173 min achieves a higher gain of solids, leading to increase the content and retention of phenolic compounds.

Sucheta et al. (2020), optimized the process of spice impregnation in black carrots assisted with US (37 kHz), they used a MRS design, whose optimum values were 8.18% NaCl, 4.30% spice mixture, and 14.47 min of ultrasound exposure. It should be noted that the maximum exposure time was 15 min, and the US improved mass transfer.

Mashkour et al. (2018) obtained different results, they optimized the process of iron fortification of potatoes by vacuum impregnation and US pre-treatment, the optimized values were; 37 kHz frequency and 24 min exposure time. The authors conclude that longer exposure time affect the nutritional content as they observed leakage of

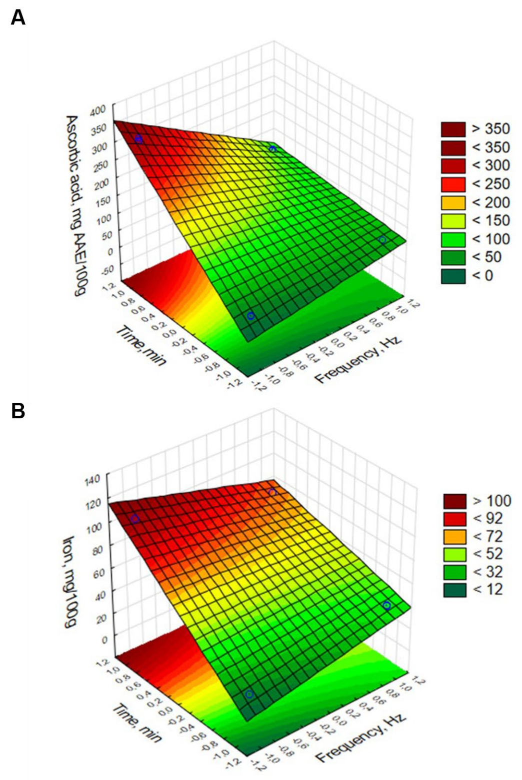

FIGURE 3
Surface response plot of frequency and time on AA **(A)** and iron **(B)** content in the ultrasound-treated sweet potato sample.

compounds from the potato; however, this is a side effect of the vacuum impregnation technique which was enhanced by the US. Similar results were reported by Maleki et al. (2020), implying that prolonged ultrasound exposure time damages carrot 303 microtissues by reducing mass transfer, the optimum values were 25 kHz frequency and 10 min.

4. Conclusion

The study introduces a novel approach to AA and iron fortification, achieved satisfactory results, showed that both US and NUS applications were effective for the incorporation of AA and iron in sweet potato (*Ipomoea batatas*). However, US treatments with longer exposure significantly influenced the content of AA and iron content. The frequency factor and the interaction (frequency vs. time) did not have a significantly influence on AA content. For iron content, the time factor and the interaction (time vs. frequency) had a significant effect. The optimized conditions of incorporation favored an increase of 4928.99% in iron content and 610.65% in AA; in comparison with the NUS treatment during the same time. It is concluded that, on a laboratory scale, the US application significantly increased the iron content from the minimum time of treatment.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

KP-S: conceptualization, experimentation, literature review, and writing the original draft. LR-F: experimentation, writing original draft, literature review, and data analysis. ZS: conceptualization, data analysis, supervision, data curation, and review and editing. EC: conceptualization, supervision, review and editing. ME-D: conceptualization, supervision, formal analysis, and review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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