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Orphan plant species for food security and nutrition: Successes, challenges, and a way forward

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Enoch G. Achigan-Dako, Iago Lowe Hale, Katherine Steele
and E. O. Deedi Sogbohossou

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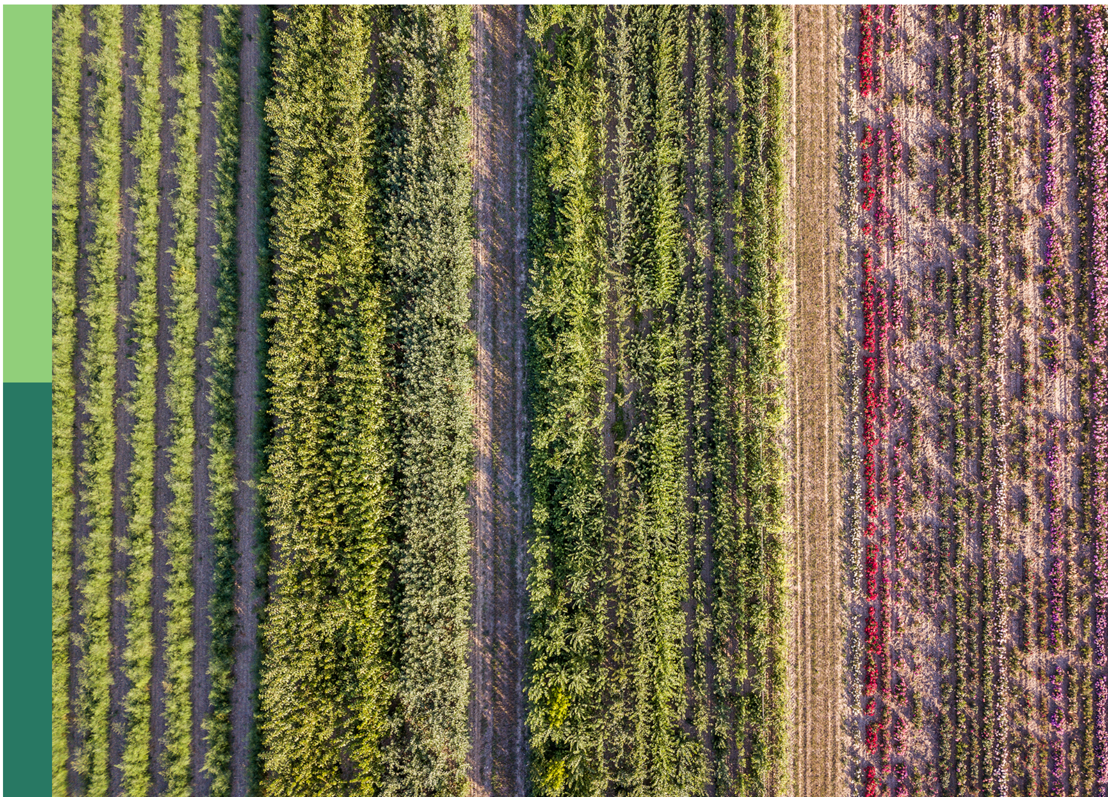

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Orphan plant species for food security and nutrition: Successes, challenges, and a way forward

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Enoch G. Achigan-Dako — University of Abomey-Calavi, Benin
Iago Lowe Hale — University of New Hampshire, United States
Katherine Steele — Bangor University, United Kingdom
E. O. Deedi Sogbohossou — Wageningen University and Research, Netherlands

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Finger Millet [*Eleusine coracana* (L.) Gaertn]: An Orphan Crop With a Potential to Alleviate the Calcium Deficiency in the Semi-arid Tropics of Asia and Africa

Theivanayagam Maharajan[1], Stanislaus Antony Ceasar[2]*, Thumadath Palayullaparambil Ajeesh Krishna[1] and Savrimuthu Ignacimuthu[3]

[1] Division of Plant Biotechnology, Entomology Research Institute, Loyola College, Chennai, India, [2] Department of Biosciences, Rajagiri College of Social Sciences, Cochin, India, [3] Xavier Research Foundation St. Xavier's College, Tirunelveli, India

Edited by:
E. O. Deedi Sogbohossou,
Wageningen University and
Research, Netherlands

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Salej Sood,
Indian Council of Agricultural Research
(ICAR), India

***Correspondence:**
Stanislaus Antony Ceasar
antony_sm2003@yahoo.co.in

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Finger millet plays a vital role in the food and nutritional security of many people in developing countries particularly in Asia and Africa. It is a staple food for poor people in many regions of Asian (India, China, Nepal, and Sri Lanka etc.) and African (South Africa, Ethiopia, Kenya, Uganda, and Nigeria etc.) countries. Finger millet contains nutrient rich components such as dietary fibers, minerals, vitamins, and phytochemicals that include phenolic compounds with several potential health benefits. Calcium (Ca) is an important macronutrient for healthy life of plants, humans and animals. It plays an indispensable role in structure and signaling and its deficiency causes low bone density, osteoporosis, colon cancer etc. Finger millet grains contain exceptionally higher amount of Ca (>300 mg/100 g) when compared to other major cereals. Ca transporter and sensor family genes are involved in the uptake, transport and accumulation of Ca. Understanding the molecular mechanisms of Ca transporter and sensor family genes is important for growth, development and seed fortification in finger millet. Expression analysis of Ca transporter and sensor family genes has been carried out in various tissues of finger millet. Only a very little research work has been done to understand the Ca accumulation in the grains of finger millet. In this review, we discuss the nutritional importance and health benefits of finger millet. We discuss the studies on Ca sensor, accumulation and transport genes that help to improve the grains of finger millet with special reference to Ca. Improved Ca content in finger millet may help to alleviate the Ca deficiency throughout the world particularly in the semi-arid tropics of Asia and Africa.

Keywords: calcium deficiency, calcium transporters, finger millet, genome-wide association studies, health benefits, quantitative trait loci

INTRODUCTION

Food security remains a major challenge especially in less developed countries. Orphan crops like cereals, legumes, vegetables and tubers play a vital role in the food security and livelihood of resource poor farmers and consumers in the developing countries of Asia and Africa (Tadele, 2019). Cereals including rice, wheat, maize, sorghum and millets are rich sources of nutrients for humans and animals in these countries. Finger millet (*Eleusine coracana*) is a nutrient rich crop. Finger millet is being used as food (grains) in developing countries and as animal feed (straw) in developed countries indicating that it is considered as a poor man's food (Ceasar et al., 2018; Wambi et al., 2020). It is a major crop in the arid and semiarid regions of developing countries of Asia and Africa (Ceasar et al., 2018; Krishna et al., 2018, 2020). Among the various millets, finger millet ranks fourth on a global scale of production next to sorghum (*Sorghum bicolor*), pearl millet [*Cenchrus americanus* (new name), *Pennisetum glaucum* (old name)] and foxtail millet (*Setaria italica*) (Upadhyaya et al., 2007; Maharajan et al., 2019). Millets are also nutritionally superior to rice and wheat since their grains contain high amounts of vitamins, iron, carbohydrate, calcium (Ca), potassium, zinc, phosphorus, magnesium, and essential amino acids (Saleh et al., 2013).

Ca is required for a number of basic regulatory functions such as transmission of nerve impulses, contraction and relaxation of muscles, blood coagulation cascade, activation of enzymes, stimulation of hormonal secretion and so on in human body and so on (Pravina et al., 2013). It also helps in the protection from cancers including colorectal, ovarian, breast, and prostate cancers (Goodman et al., 2002; Gao et al., 2005; Lin et al., 2007). Intake of Ca is important for development of fetal skeleton, increasing birth weight prenatal hypertension and avoidance of pre-clamsis in pregnant women (Chan et al., 2006; Puranik et al., 2017). Around the world, 3.5 billion people are affected by Ca deficiency, with ∼90% of people affected in Africa and Asia (Kumssa et al., 2015). Ca deficiency is a serious health problem both in the developing countries of Asia and Africa (Sharma et al., 2017). Human diets with low levels of Ca has been linked to diseases such as osteoporosis and indirectly to rickets (through Vitamin D) (Heaney, 1993; Chan et al., 2006; Pettifor, 2008). Such diets with low Ca levels is prevalent among 80% of elderly population and are consequently affected by osteoporosis worldwide (Bhatia, 2008; Pettifor, 2008). Elderly population is mostly affected by Ca deficiency predominantly in the form of osteoporosis and osteopenia (Puranik et al., 2017). The onset of bone decalcification and demineralization leads to reductions in bone mass causing the osteoporosis among above 50 years old men and menopausal women (Michaelsson et al., 2005). In particular, 80% of the women suffer from osteoporosis (Sharma et al., 2017). Therefore, osteoporosis is a major problem in developing countries (Sozen et al., 2017). The World Health Organization (WHO) considered that osteoporosis as the next main public healthcare concern globally, after cardiovascular diseases afflicting almost 75 million people in Europe, the United States of America and Japan. Hypocalcaemia disease occurs in human body when Ca levels are low in the blood (Fong and Khan, 2012). Milk as well as dairy products are one of the major sources of Ca in human diets. If people consume 1 L of milk per day they will get the recommended amount 1000–1300 mg of Ca (Thorning et al., 2016). Most poor people living in the developing countries of Asia and Africa cannot afford to buy and consume 1 L of milk per day (Puranik et al., 2017). These people consume <500 mL of milk per day. Unavailability of recommended amount of milk among poor population and lactose intolerance lead to Ca deficiency (Curry, 2013).

Plants are the cheap, convenient and alternative source of dietary Ca. People frequently consume cereal based food products which are low in Ca. Major cereal crops may not provide adequate amount of Ca for low income people. Finger millet contains higher amount of Ca compared to the other major cereals (**Figure 1**). For example, the Ca content in finger millet (344 mg/100 g) is almost 10-fold higher than wheat (*Triticum aestivum*) (41 mg/100 g), maize (*Zea mays*) (26 mg/100 g) and rice (*Oryza sativa*) (33 mg/100 g) and three times higher than milk (Kumar et al., 2016). So finger millet is an example of Ca rich crop in developing countries of tropical and subtropical regions. Finger millet grains also contain higher content of minerals such as phosphorus, iron and manganese compared to other cereals (Kumar et al., 2016). The seeds can be stored for more than 5 years without insect damage that makes it a valuable crop for famine hit areas (Latha et al., 2005). Crops such as rice and wheat provide food security, but finger millet provides nutritional security to the world (Devi et al., 2014; Ceasar et al., 2018).

Plants uptake Ca by root and translocate to the aerial parts of plants including fruits and seeds by symplastic and apoplastic pathways (Kumar et al., 2014; Mirza et al., 2014). In finger millet, Ca is present in aleurone layer followed by seed coat and embryo (Nath et al., 2013). The Ca transporters are actively involved in the uptake and transport of Ca in the cells, while Ca sensors are involved in the regulation of Ca transporters. Only a few Ca transporters have been identified before the releasing of draft genome sequence in finger millet. A few molecular marker-based studies have been conducted to identify the quantitative trait loci (QTL) for Ca traits in finger millet. In this review, we describe the nutritional importance and health benefits of finger millet. We have provided the detailed information on Ca sensor, accumulation and transport genes and identification of QTL related to Ca traits in finger millet.

NUTRITIONAL IMPORTANCE AND HEALTH BENEFITS OF FINGER MILLET

Finger millets serve as a major food in resource-poor countries of Asia and Africa by providing 75% of total calorie intake next to fine cereal grains (Singh et al., 2019) and gluten-free cereal products (Chandrasekara et al., 2012; Saleh et al., 2013). It is nutritionally superior to rice and wheat due to high amount of dietary fibers, iron, zinc, Ca, phosphorus, potassium, vitamin B, and essential amino acids (Parameswaran and Sadasivam, 1994; Ceasar and Ignacimuthu, 2011). Finger millet also possesses more lysine, threonine, and valine than

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Abbreviations: Ca, Calcium; *CaM, calmodulin*; *CaMK, CaM dependent protein kinase*; *CAX, Ca^{2+} cation exchanger/antiporter*; *CBL, Calciuneurin B-like protein*; CDPK, *CaM independent protein kinases*; *CIPK, Calciuneurin B-like protein (CBL)-interacting protein kinases*; DAS, days after sowing; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; TPC1, two pore channel.

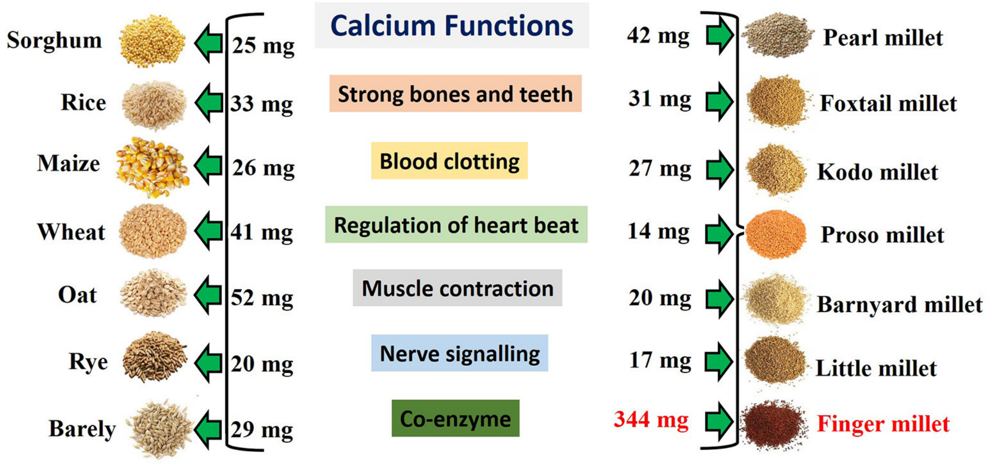

FIGURE 1 | Calcium (Ca) content of various cereals. The Ca content of finger millet and other major cereals and small millets are presented for strengthening the human health. The given Ca content is expressed per 100 g of seeds for all cereals including finger millet. Ca content data of all cereals were collected from Shobana et al. (2013), Saleh et al. (2013), and McKevith (2004).

other millets (Ravindran, 1991; Sripriya et al., 1997). It has anti-nutrients including phytates, polyphenols, tannins, trypsin inhibitory factors and dietary fibers. Finger millet's dietary fiber and polyphenols offer various health benefits like antidiabetic, antioxidant, hypocholesterolaemic, antimicrobial, delayed nutrient absorption, increased fecal bulk, lowering of blood lipids effects (Devi et al., 2014). Over 50 phenolic compounds belonging to several classes such as phenolic acids and their derivatives, dehydrodiferulates and dehydrotriferulates, flavan-3-ol monomers and dimers, flavonols, flavones, and flavanonols were identified in finger millet (Saleh et al., 2013).

Finger millet's phenolic compounds like gallic, protocatechuic, p-hydroxy benzoic, p-coumaric, vanillic, syringic, ferulic, trans-cinnamic acids, and quercetin exhibit major antidiabetic and antioxidant properties and also inhibit cataract effectively (Saleh et al., 2013). Finger millet yields less glucose than the polished rice which is good for diabetic patients (Wang et al., 2018). Consumption of finger millet based diets resulted in significantly lower plasma glucose levels due to its higher fiber content and antinutritional factors than rice and wheat (Kumari and Sumathi, 2002). It also has beneficial effect on nerve growth factor production and wound healing properties at early diabetic condition (Rajasekaran et al., 2004). The grains and various fractions of finger millet have the potentials to prevent and treat diabetics (Saleh et al., 2013; Devi et al., 2014; Kam et al., 2016). Globally, most countries face high and increasing rates of cardiovascular diseases due to obesity, smoking, unhealthy diet, and lack of physical activity. This reduce plasma triglycerides in hyperlipidemic rats and prevent cardiovascular disease (Lee et al., 2010). The antioxidants and phenolics rich finger millet grains can also contribute to improve health, to protect against aging and metabolic syndrome (Bravo, 1998). Seed coat extracts of finger millet showed antimicrobial activity against *Bacillus cereus* and *Aspergillus flavus* (Radhajeyalakshmi et al., 2003).

IDENTIFICATION OF QTL FOR CA TRAITS IN FINGER MILLET

Molecular markers are one of the most crucial diagnostic tools for plant breeding programmes. It is used to analyse genomes and enables the study of the genetic structure and traits of crop genomes. These markers are used to identify the specific genes responsible for better growth, development and resistance to abiotic and biotic stresses. The microsatellite markers have been used to identify the agronomically important traits such as grain yield, quality, disease resistance, drought resistance, nutritional quality, nutritional use-efficiency, etc (Maharajan et al., 2018). The availability of simple sequence repeats (SSR) markers in finger millet is limited compare to those for other cereal crops (Ceasar et al., 2018; Krishna et al., 2018). Only few QTL associated with Ca traits have been reported in finger millet. For example, 23 anchored SSR markers were used to identify nine QTL associated with Ca content in 113 genotypes of finger millet by association mapping (Kumar et al., 2015b). Recently, Yadav et al. (2020) identified two QTL (UGEP78 and UGEP60) for grain calcium content in 238 genotypes of finger millet through association mapping using 85 SSR markers. They also identified two genotypes with the highest grain calcium content [GPHCPB45 (452.8 mg) and (IE 2957 (447 mg)] among the 238 genotypes. This type of study is needed to detect high Ca content genotypes from more than 28,000 accessions of finger millet. The detection of genotypes with high Ca content may help to eradicate Ca deficiency around the world, particularly in Asian and African countries. Only association mapping populations were used for QTL studies so far in finger millet. There is a crucial need to develop the linkage maps for finger millet for the identification of QTL for the agronomically important traits including Ca. High-resolution research on the identification of QTL in finger millet for agronomically important trait is essential for molecular breeding and finger millet improvement. The advanced backcross QTL (AB-QTL) methods are also helpful for a variety development (Kumari et al., 2020). Methods of AB-QTL analysis have been further used to identify and transfer the valuable QTL from un-adapted to cultivated germplasm in a single process (Wang and Chee, 2010; Bhanu et al., 2017). Back cross population (BC_2 and BC_3) are used for detection of QTL in this method. If worked effectively, AB- QTL analysis can prove to be a potential method to exploit unadapted and exotic germplasm for the quantitative trait improvement of a number of crop plants including finger millet. The released finger millet draft genome provides excellent opportunities to identify expressed sequence tag derived SSR markers for crop improvement (Hittalmani et al., 2017; Ceasar et al., 2018; Pandian and Ramesh, 2019).

GENOME-WIDE ASSOCIATION STUDIES FOR THE ENRICHMENT OF NUTRITIVE VALUE IN FINGER MILLET

The introduction of next-genome sequencing (NGS) technology helps for rapid and accurate detection of the genetic basis of phenotypic variation in crops. Due to its significant contribution to genome analysis, it can aid in understanding the genome organization of crops. NGS technology is useful to identify the genetic variability across the genomes of individuals and identify their genotype-phenotype associations in the huge germplasm of the crops. Therefore, genome sequencing plays a vital role in GWAS for crop improvement through a genome-assisted breeding (GAB) program. A very little effort was paid on GWAS in finger millet and only one report is available till date. Six nutritional traits such as iron, zinc, Ca, magnesium, potassium, and sodium were evaluated through GWAS using 190 finger millet genotypes and 418 single nucleotide polymorphism (SNP) linked with nutritional traits were identified (Swati et al., 2020). Out of 418 SNP, 18 SNP markers showed homology with candidate genes having putative functions in binding, remobilization/transport of metal ions in finger millet (Swati et al., 2020). This study provides the insight to genotypic variation of the phenotypes for the selection of the best breeding materials to improve grain nutritional content and nutrient use-efficiency in finger millet. Researchers need to pay more attention to GWAS of finger millet for improving their nutritive traits which may contribute to human health in the future. Furthermore, genotyping by sequencing (GBS) technology generated SNP markers are used to analyze the agro-morphological traits of 113 finger millet accessions and identify consistent SNP markers linked to grain yield and its component traits (Sharma et al., 2018). The identified SNP was associated with the candidate genes of rice and foxtail millet, which were responsible for flowering, maturity, and grain yield (Sharma et al., 2018). In another recent report, Tiwari et al. (2020) identified QTL and genes for seed protein content, days to maturity and grain yield in finger millet using 2977 SNPs by GBS technology. Among these, five SNPs were associated with seed protein content and grain yield and three for days to maturity. They have identified four candidate genes responsible for seed protein content using SNPs through *in-silico* analysis (Tiwari et al., 2020). Notably, *aspartyl protease* has been found to be the most promising candidate gene for seed protein content of finger millet. The plant breeders and molecular biologists need to focus more on GWAS in finger millet since it would be a key tool of GAB for finger millet improvement in the future.

TRANSPORTERS AND SENSORS FOR CA ACCUMULATION IN FINGER MILLET

The Ca is taken up by roots from the soil solution in a divalent cation (Ca^{2+}) form (White and Broadley, 2003). The primary roles of the Ca are maintaining chemical balance in the soil, reducing soil salinity, activating the plant growth regulating enzymes and improving the water penetration and disease resistance in plants. Ca also improves the absorption of the other nutrients by roots and their translocation within the plant (Hepler, 2005). Ca transporters are classified into three types viz. *Ca^{2+} channel*, *Ca^{2+} ATPase*, and *Ca^{2+} cation exchanger/antiporter* (*CAX*) (Sze et al., 2000; Demidchik et al., 2018). These transporters export Ca^{2+} to the apoplast and into the cellular organelles specifically to the endoplasmic reticulum, golgi/endosome/pre-vacuolar compartments, and plastids/vacuoles (Karley and White, 2009). Plants absorb Ca by roots from the soil solution, which reaches the shoots through the xylem stream.

Two contrasting finger millet genotypes (GP-1; low Ca containing and GP-45; high Ca containing) were used to identify the expression pattern of *CAX1*, two *pore channel* (*TPC1*), *calmodulin* (*CaM*), *Ca^{2+}ATPase*, and *CaM dependent protein kinase* (*CaMK1* and *2*) genes in vegetative tissues and various stages of developing spike in finger millet (Mirza et al., 2014). This study laid the foundation for the identification of Ca transporter genes in finger millet and same genotypes were also utilized in the following studies. Same two genotypes were used to identify two *CaM independent protein kinases* (*CDPK3* and *13*), four *Calciuneurin B-like protein (CBL)-interacting protein kinases* (*CIPK2*, *4*, *10*, and *14*), one *CaM*, one *TPC1*, two *CAX*s (*CAX1* and *3*) and four *ATPases* (*CaATPase*, *CaATPase1*, *6* and *9*) transporters in developing spikes of finger millet (Kumar et al., 2015a). *CIPK10, CDPK13, CIPK4, CaATPase*, *TPC1* and *CAX3* and *CaM*, *CDPK3*, *CIPK2*, *CaATPase6*, *9*, *CAX1*, and *3* were expressed in developing spikes of GP-45 at S3 and S4 stages respectively (**Figure 2**). *CDPK3*, *CaM*, *CIPK14*, *CaATPase1*, *TPC1*, and *CAX1* were expressed in the developing spikes of GP-1 at S4 stage. Among these, expression level of *CIPK14* and *CaATPase1* were higher in GP-1 at S4 stage compared to other genes (Kumar et al., 2015a). This basic study revealed the spatial and temporal expression of these genes. Therefore, further study is required to characterize both the genes in GP-1 which may help to improve the low Ca containing genotype. The above results show that most of the Ca transport and accumulation genes were expressed in high Ca genotype compared to the low Ca genotype.

Likewise, 19 Ca transporter genes, which includes 11 *Ca^{2+}ATPases*, seven Ca exchangers and one Ca^{2+} channel genes were identified in GP-1 and GP-45 through RNA-seq

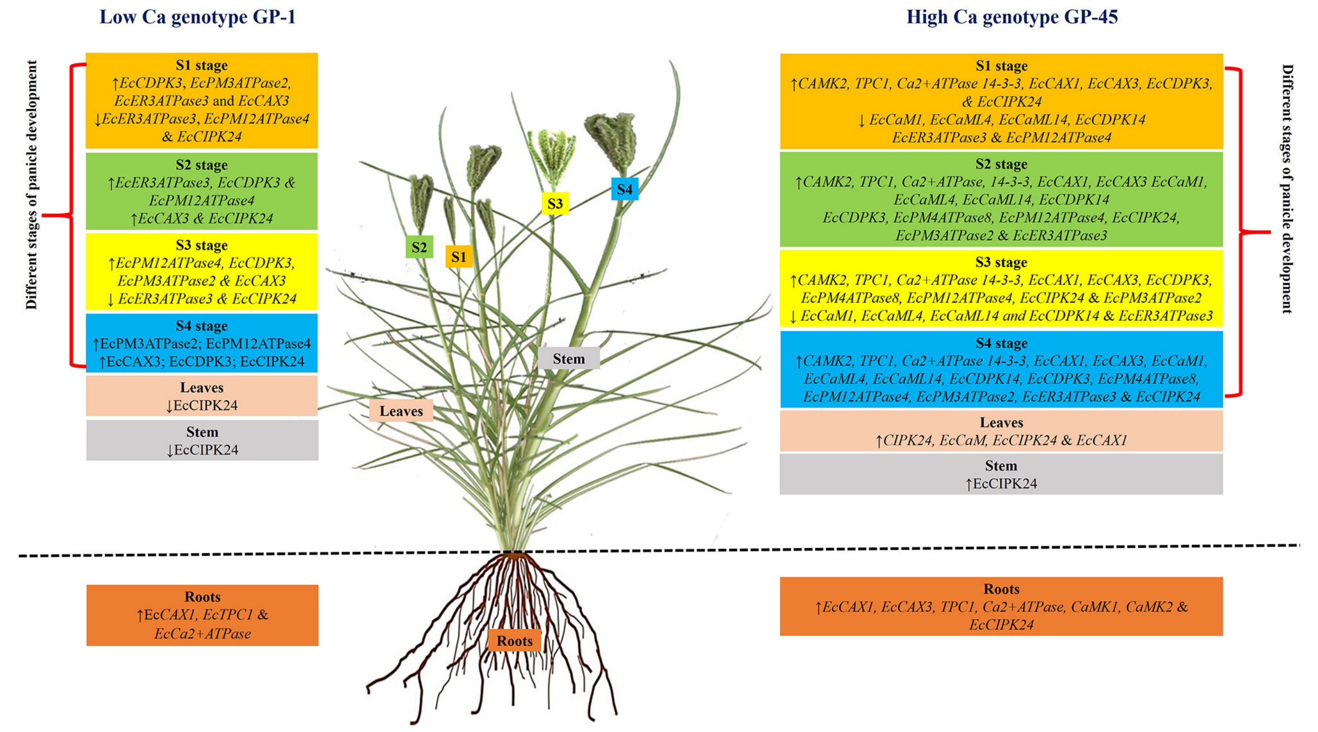

FIGURE 2 | Comparisons between genes related to calcium (Ca) uptake, transport, and accumulation identified in low Ca containing (GP-1) and high Ca (GP-45) containing genotypes. The Ca uptake, transport and accumulation related genes were identified in root, leaves, stems at vegetative stage and developing spikes at various stages of [S1 (booting), S2 (anthesis), S3 (grain ripening), and S4 (maturation)] of finger millet. Stages S1–S4 are marked with a rectangular shape and filled in orange, green, yellow, and blue, respectively. More number of Ca uptake, transport and accumulation genes were expressed in high Ca containing genotype as compared to the low Ca containing genotype.

(Singh et al., 2015). RNA-seq analysis revealed that nine (*EcVCax3*, *EcER3ATPase3*, *EcPM12ATPase4*, *EcPM8ATPase*, *EcPM5ATPase4*, *EcPM4ATPase8*, *EcPM3ATPaseB*, *EcPM3ATPase2*, and *EcPM2ATPase1*) and three (*EcCaX1b*, *EcCL1pore*, and *EcPM12ATPase4*) genes were highly expressed in GP-45 and GP-1 genotypes, respectively. The *EcPM4ATPase8* was highly expressed in developing spike of GP-45 at S2 and S3 stages under 20 mM and 5 mM Ca concentrations, respectively. Ca accumulation in seed is a polygenic trait and Ca^{2+} transporters (especially EcCAX3) might play very important role in seed Ca accumulation (Singh et al., 2015). This study reported first time an extensive list of Ca^{2+}sensing and transport related genes in finger millet. However, functions of these gene have not yet been validated by knock-out and knock-in studies. Another study shows that Ca sensor gene *CIPK* (*EcCIPK24*) was highly expressed in root, shoot, leaves and developing spike of GP-45 compared to GP-1 (**Figure 2**) (Chinchole et al., 2017). CIPK activates the CAX protein by interaction with CBL proteins. Docking analysis for EcCIPK24 protein of the both genotypes with CBL proteins (EcCBL4 and 10) were performed by Chinchole et al. (2017). The docking study proposed that EcCBL4 has a strong binding affinity with EcCIPK24 and might play a significant role in the accumulation of Ca in seeds (Chinchole et al., 2017). These predictions reveal the functions of the key Ca sensor gene in finger millet and further functional characterization of this gene may help to understand the specific role in finger millet Ca accumulation.

In another study, *CAX1*, *CAX3*, *CaM*, *CBL4*, *CBL10*, and *CIPK24* genes were identified in roots, stem, leaves and developing spike of GP-1 and GP-45 (**Figure 2**) (Kokane et al., 2018). Most of the genes were highly expressed in roots (*CAX1* and *CAX3*), leaves (*CIPK24*), and stems (*CIPK24*, *CaM*, and *CAX1*) of GP-45 genotype compared to GP-1. Much of these works were carried out before the availability of draft genome of finger millet. So the full length gene sequences were not used in designing the primers and other molecular studies. Draft genomes of finger millet were released in 2017 by two groups (Hittalmani et al., 2017; Hatakeyama et al., 2018). Many reviewers have suggested that draft genomes of finger millet will help to improve the finger millet growth and yield through various molecular studies under both biotic and abiotic stress (Ceasar et al., 2018; Vetriventhan et al., 2020; Wambi et al., 2020). Hittalmani et al. (2017) identified 330 Ca transporter and accumulation related genes (*28 CaM ATPase*, *145 CaMK1*, *125 CaMK2*, *29 CAX1*, and *3 TPC1* genes) through transcriptome analysis. The identified genes will help in exploring more finger millet genotypes for Ca uptake, translocation and accumulation studies in future.

Apart from preliminary studies mentioned above, Ca transport and accumulation related genes have not yet been studied extensively. Overexpression of Ca-signaling transporter genes is directly proportional to increased levels of Ca (Demidchik et al., 2018). For example, overexpression of *CAX1* transporter in *Arabidopsis thaliana* (Hirschi, 2001) and rice (Kim et al., 2005) enhanced the Ca levels in seeds of both the plants. Ca is acquired by root from the soil solution in free ionic form. Once Ca enters inside the root epidermal cells, it can move both apoplastically and symplastically to the cortex and then to the stele (Kumar et al., 2015c). Members of Ca sensors, exchangers and transporters are involved in Ca uptake, transport and accumulation at cellular levels in plants. Seed consists of maternal and filial tissues with no direct vascular connection between them (Wolswinkel et al., 1992). Ca is deposited in seed during its development which is mediated by specialized Ca transporters. It gets pumped toward the apoplast from seed coat, where it is absorbed by aleurone and then in endodermal cell (Sharma et al., 2017). Therefore, further studies to characterize the Ca sensors, exchangers and transporters may help to improve the finger millet growth and especially to accumulate Ca in seeds. When compared to research on Ca transporter family genes in finger millet to that of *A. thaliana* and rice, very little research has been carried out. Further study on Ca transporters' structure, function, affinity, and other molecular studies will throw more light on the complex function of Ca transporters in Ca uptake, transport, remobilization, and utilization in finger millet. Future high-resolution study on various aspects of Ca transporters in finger millet and the future development of new finger millet varieties with high Ca content will surely aid in enhancing nutritional security of the poor in the developing world.

CONCLUSION AND FUTURE PROSPECTS

Crop improvement has been one of the major priority areas of research in agriculture. Ca deficiency is a widespread problem across the globe, however, very little effort has been made to understand the mechanisms Ca accumulation in finger millet seeds. It may be due the reason that finger millet is being grown and consumed by less developed regions in the world. Further study on the post-transcriptional and post-translational regulation of Ca transporters needs to be fine-tuned to exploit them in finger millet production to meet the ever increasing global population and food demand. Recently, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) gene-editing tool is projected to play a vital role in crop improvement. CRISPR/Cas9 tool could be applied to Ca transporter genes in future to dissect and improve the Ca accumulation in seeds of finger millet. Finger millet genotype with high Ca content will help to reduce Ca deficiency worldwide.

Bio-fortification is a food based approach to overcome the nutrient starvation by delivering nutrient dense crops at the door steps of poor populations. Bio-fortified crops, particularly finger millet contain high amount of proteins, essential amino acids, minerals, and vitamins which can help to reduce hidden hunger in the most vulnerable populations in the world. Finger millet is called as a super cereal because it is the richest source of Ca among all the cereals. Farmers who grow bio-fortified crops including finger millet can easily access the nutritious foods with minimal investments. Hence the incorporation of naturally Ca-rich cereals like finger millet into global bio-enrichment programs can be a good starting point to alleviate Ca malnutrition.

AUTHOR CONTRIBUTIONS

TM, TA, and SA conceptualized, wrote, edited, and updated the manuscript. SA and SI contributed critically in revising and improving the manuscript for publication. All authors contributed to the article and approved the submitted version.

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REVIEW
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Improvement of a Traditional Orphan Food Crop, *Portulaca oleracea* L. (Purslane) Using Genomics for Sustainable Food Security and Climate-Resilient Agriculture

Ajay Kumar[1*†], Sajana Sreedharan[1†], Pardeep Singh[2], Enoch G. Achigan-Dako[3*] and Nirala Ramchiary[4*]

[1] Department of Plant Science, Central University of Kerala, Kasaragod, Kerala, India, [2] Department of Environmental Science, PGDAV College, University of Delhi, New Delhi, India, [3] Laboratory of Genetics, Biotechnology and Seed Sciences, Faculty of Agronomic Sciences, University of Abomey-Calavi, Cotonou, Benin, [4] School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

Edited by:
Matteo Balderacchi,
Independent Researcher,
Piacenza, Italy

Reviewed by:
Maria Grazia Melilli,
National Research Council (CNR), Italy
Md. Amirul Alam,
Universiti Malaysia Sabah, Malaysia

***Correspondence:**
Ajay Kumar
botanistajay@gmail.com
Enoch G. Achigan-Dako
e.adako@gmail.com
Nirala Ramchiary
nrudsc@gmail.com

[†] These authors have contributed equally to this work

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Purslane (*Portulaca oleracea* L.) is a popular orphan crop used for its nutritional properties in various parts of the world. It is considered one of the richest terrestrial sources of omega-3 and omega-6-fatty acids (ω-3 and 6-FAs) suggesting its importance for human health. This ethnomedicinal plant is also an important part of traditional healing systems among the indigenous people. Many studies have indicated its tolerance against multiple stresses and found that it easily grows in a range of environmental gradients. It has also been considered one of the important biosaline crops for the future. Despite its huge nutritional, economic, and medicinal importance, it remains neglected to date. Most of the studies on purslane were focused on its ethnomedicinal, phytochemical, pharmacological, and stress-tolerance properties. Only a few studies have attempted genetic dissection of the traits governing these traits. Purslane being an important traditional food crop across the globe can be valorized for a sustainable food security in the future. Therefore, this review is an attempt to highlight the distribution, domestication, and cultivation of purslane and its importance as an important stress-tolerant food and a biosaline crop. Furthermore, identification of genes and their functions governing important traits and its potential for improvement using genomics tools for smart and biosaline agriculture has been discussed.

Keywords: purslane, sustainable food systems, traditional foods, food security, climate change, climate smart agriculture, biosaline agriculture, ethnic foods

INTRODUCTION

The *Portulaca* L. is the only genus in the family Portulacaceae as per APG III classification of angiosperms (The Angiosperm Phylogeny Group, 2009; Ocampo and Columbus, 2012) and comprises more than 100 species which are widely distributed and adapted to a range of environmental conditions (Nyffeler and Eggli, 2010). The genus *Portulaca* shows a great phenotypic plasticity in its various traits such as flower, fruit, leaves, stem, and growing habit

(Coelho and Giulietti, 2010). The members of *Portulaca* genus are either herbaceous perennials or annuals usually with tuberous roots, slightly succulent stem, and leaves with alternate or rarely opposite phyllotaxy and cymose terminal inflorescences (Nyffeler and Eggli, 2010; Walters et al., 2011; Ocampo and Columbus, 2012; Dalavi et al., 2018). The flowers are sessile or rarely pedicellate and are easily distinguishable by their inflorescence and fruits which are operculate capsules termed as pyxidia (Nyffeler and Eggli, 2010). The *Portulaca* is characterized by thick and dense homogenous populations in nature (Walters et al., 2011). Due to the presence of facultative C4/CAM photosynthetic pathways in purslane, it has become an important model crop for facultative C4/CAM photosynthesis evolution in plants and its future role for food security (D'Andrea et al., 2014; Ferrari et al., 2020). The species of *Portulaca* are adapted to the shady and moist environments, show extensive branching (Matthews and Levins, 1985), and are generally characterized by tuberous roots (Geesink, 1969; Carolin, 1987). The leaves are succulent in nature, and fruits are round or egg-shaped operculated capsules and produce tiny, oval-shaped reddish-, brown-, or black-colored seeds which are rich in phytochemicals (Matthews and Levins, 1985; Grubben, 2004). The leaves and inflorescences show the presence of trichomes (Matthews and Levins, 1985). It produces many seeds with very high dormancy (Alam et al., 2014e). Most of its species such as *P. oleracea*, *Portulaca grandiflora*, *Portulaca amilis*, *Portulaca molokiniensis*, *Portulaca pilosa*, and *Portulaca umbraticola* show C4 photosynthesis (Koch and Kennedy, 1982; Guralnick et al., 2002; Voznesenskaya et al., 2010). Interestingly, some C4 species such as *P. oleracea*, *P. grandiflora*, *P. australis*, *P. pilosa*, *Portulaca digyna*, and *Portulaca cyclophylla* also show Crassulacean acid metabolism (CAM) pathway, a special type of photosynthesis found in the succulent plants adapted to desert conditions (Sage, 2002). Most of the species except a few such as *Portulaca cryptopetala* show Kranz anatomy (Ocampo et al., 2013). However, the CAM pathway in the C4 *Portulaca* species operates only during drought conditions when water availability is reduced (Lara et al., 2004; D'Andrea et al., 2014). Besides a model plant for C4/CAM photosynthesis, it is also an important crop for understanding the salt-tolerance mechanisms in plants (Borsai et al., 2018, 2020). Of the more than 100 species of *Portulaca*, *P. oleracea* L. (hereafter purslane; **Figures 1a–d**) is the most important and well-studied species. Purslane also shows wide phenotypic plasticity in its flower colors that make it an important ornamental plant (**Figure 2**). Purslane is an important plant rich in important phytochemicals and nutritional components possessing medicinal, nutritional, medicinal, phytoremediation, stress-tolerance, and pharmacological properties (Alam et al., 2014c, 2015b; Uddin et al., 2014; Zhou et al., 2015). Purslane is also traditionally used as an ethnomedicinal plant (Sultana and Raheman, 2013; Iranshahy et al., 2017). Despite its multiple benefits, such as its nutritional and phytochemical richness, purslane still remains a neglected food crop of the indigenous communities, and studies on the genetic regulation of important traits and their improvement strategies is limited. Except for the C4-CAM switch, very limited information on distribution, domestication, and cultivation of purslane is available. Information on application of genetics and genomics for the improvement of nutritional and stress-resilient traits of purslane, and hence to be adopted for the extensive cultivation and industrial applications is not available. Furthermore, since the purslane grows profusely across a range of environmental

FIGURE 1 | Natural habitat and parts of purslane plant. **(a)** Purslane plant in natural habitat; **(b)** young stem with leaves; **(c)** harvested plant showing roots; and **(d)** young fruits, known as pyxidia with black-colored seeds.

FIGURE 2 | (a–i) Morphological diversity of flowers observed in purslane (scale bar, 1 cm).

gradients, and shows salinity tolerance, it is not surprising that the species holds a potential as a biosaline crop for urban agriculture. Taken together with this background, this review summarizes current knowledge on various aspects of purslane with an emphasis on its distribution, cultivation, domestication, and its importance as a nutritional, medicinal, and biosaline crop. It further discusses a futuristic approach combining genomics and gene-editing tools for the editing of genes governing important traits for urban and biosaline agriculture.

DISTRIBUTION AND DIVERSITY OF PURSLANE

Purslane is the eighth most commonly distributed plant in the world (Anastácio and Carvalho, 2013). The molecular phylogenetic study of the genus *Portulaca* shows that it is a monophyletic genus with two major clades, namely, opposite leaves (OL) and alternate leaves (AL; Ocampo and Columbus, 2012). The study further showed that OL clade includes species that possess opposite leaves and are distributed in Africa, Asia, and Australia, whereas AL clade represents species that possess alternate to subopposite leaves and are distributed in the New World. There was a debate around its origin in the Old World and its further introduction to the New World countries during the Columbus period. However, the presence of Pre-Columbian archaeological evidence in the New World suggests that purslane is distributed in the Old World as well as New World countries thereby ending the debate whether it was introduced by Columbus (Byrne and McAndrews, 1975). Recovery of the carbonized fossils of purslane from prehistoric human settlement sites suggests its importance as an important food plant for the indigenous communities since prehistoric times (Chapman et al., 1973; Byrne and McAndrews, 1975; Simopoulos et al., 1995). Simopoulos et al. (1995) suggested Mexico as the center of origin of purslane because the maximum number of species including its three ploidy types exists there. Although germplasm collections and their characterization for various important traits such as stress tolerance, superior metabolic and nutritional traits, and biomass accumulation are important for improvement of purslane; however, no such study reports are available in plenty.

DOMESTICATION AND CULTIVATION OF PURSLANE AND MEASURES TO UPSCALE ITS PRODUCTION AND CONSUMPTION

A very few studies have focused on the cultivation and domestication of purslane. Cros et al. (2007) carried out comparative study of its cultivation using peat, vermiculite, coir, perlite, and mixtures of peat and perlite and found that plants grown on peat showed the highest total fatty acid, alpha-linolenic acid, and linoleic acid. Application of *Rhizophagus irregularis*, an arbuscular mycorrhizal fungi (AMF) increased the phosphorus uptake, grain yield, biomass weight, total antioxidant activity, and unsaturated fatty acid, leaf essential oil, and leaf ω3 and improved the soil cation exchange capacity (CEC) of plants (Hosseinzadeh et al., 2020, 2021). Application of nitrogen fertilizers also improved phosphorus and nitrogen uptake, biomass weight, flavonoid content, total antioxidant capacity, and fatty acid content (Hosseinzadeh et al., 2020). The best season for its sowing is spring of fall, and it completes its life cycle in 60–75 days (Alam et al., 2014e). In Malaysia, nearly seven cultivars are cultivated for ornamental purposes and are propagated through cuttings (Alam et al., 2014e). However, the wild purslane can germinate through the seeds (Alam et al., 2014e). Although it is nutritionally and medicinally important across different cultures, it is considered a weed in several parts of the world (Banerjee and Mukherjee, 2002; Rapoport and Drausal, 2013; CABI, 2021).

Safdari and Kazemitabar (2009) have attempted tissue culture in wild and cultivated races of purslane using different explants and hormone concentrations, and the results show that $10\,\mu M$ IBA combined with 5 or $10\,\mu M$ BAP sufficiently induce callus formation in the wild race whereas $2.5\,\mu M$ IBA was enough for root generation in both the races. A study on the development of *in vitro* plant regeneration and *in vitro* flowering using various concentrations of PGRS showed that $0.5\,mg\ L^{-1}$ of kinetin is optimum for maximum shooting and $0.2\,mg\ L^{-1}$ of GA_3 induced bud formation in purslane (Sharma et al., 2011). Shekhawat et al. (2015) observed maximum shooting when MS medium was supplemented with $2.0\,mg\ L^{-1}$ BAP. These studies provide a basis for the cultivation of purslane. However, to better understand the best performance conditions, further field trial experiments are needed. Moreover, the standardization of tissue culture protocols in purslane would be helpful for future breeding programs aimed at upscaling cultivation of purslane. The future studies should be aimed at the methods for improved productivity, phytochemical composition, and its suitability for urban agriculture. Dewanti et al. (2021) found the highest amount ($9.73\,mg\ kg^{-1}$) of vitamin C in purslane obtained from lowland areas.

IMPORTANCE OF PURSLANE

The purslane is cultivated in various parts of the world mostly for ornamental purposes because of its phenotypic diversity in its flower color and for nutritional and medicinal purposes because of its richness in important phytochemicals (Simopoulos et al., 1992; Coelho and Giulietti, 2010; Melilli et al., 2020). **Figure 3** provides an overview of the importance of purslane for sustainable food security and human benefits.

Nutritional Value

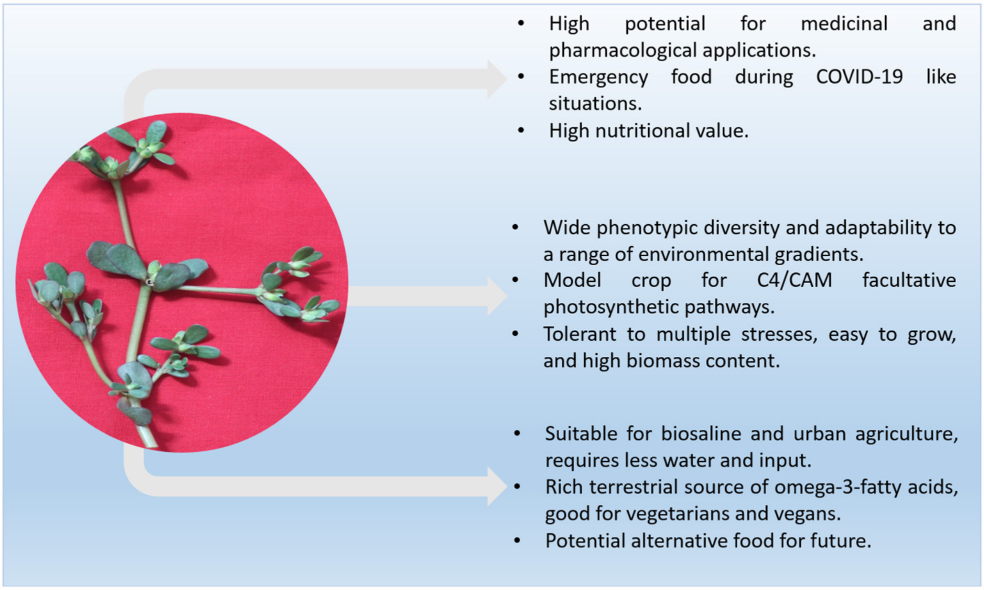
A number of studies have shown that purslane is very rich in important nutritional components such as vitamins, proteins, carbohydrates, ω-3-FAs, carotenoids, and minerals (Simopoulos et al., 1995, 2005; Aberoumand, 2009; Uddin et al., 2014; Petropoulos et al., 2016; Alam et al., 2021). It shows a very high amount of ω-3-fatty acids which is not generally found in vegetarian diets suggesting its important role as a functional food (Palaniswamy et al., 2001; Alam et al., 2014e; Petropoulos et al., 2016). Several studies have done analysis of its various nutritional constituents. The purslane contain high quantity of protein, ash, fiber content, and minerals (Aberoumand, 2009; Almasoud and Salem, 2014; Uddin et al., 2014; Chugh et al., 2019). It is also a very good source of various types of vitamins including vitamins A and C (Guil-Guerrero and Rodríguez-García, 1999; Chugh et al., 2019). The high nutritional composition of purslane indicates its potential as an important nutraceutical food for the future.

Medicinal Potential

Purslane is an important traditional ethnomedicinal plant as its leaves, roots, and stems contains rich medicinally important phytochemicals such as alkaloids, flavonoids, phenolic acids, homoisoflavonoids, lignans, polysaccharides, and catecholamines (Banerjee and Mukherjee, 2002; Xiang et al., 2005; Lim and Quah, 2007; Alam et al., 2014b; Zhou et al., 2015). Several authors have reviewed the phytochemical composition of purslane. It has been a part of indigenous healthcare systems across the continents (Xiang et al., 2005; Bosi et al., 2009; Sultana and Raheman, 2013; Hwess et al., 2018; Sdouga et al., 2020; Zaman et al., 2020). Many ethnobotanical studies have reported its use against multiple diseases and ailments (Ahmad and Beg, 2001; Bosi et al., 2009; Nedelcheva, 2013; Sultana and Raheman, 2013; Hwess et al., 2018; Chaachouay et al., 2019; Manzanero-Medina et al., 2020; Nanagulyan et al., 2020). Recent pharmacological studies also suggest its important medicinal benefits against a number of diseases such as cancer, diabetes, and viral, bacterial, and fungal infections (Dong et al., 2010; Ye et al., 2015; Jin et al., 2017; Park and Han, 2018; Zhao et al., 2018; da Silva et al., 2019; Li et al., 2019; El-Desouky, 2021; Park et al., 2021; Tleubayeva et al., 2021). These results of pharmacological studies provided important evidence toward the potential of purslane in drug development against diseases.

Potential for Biosaline Agriculture

Salinity is also an important stressor that limits crop productivity. There is plenty of saline water, and if salinity-tolerant crops are



- High potential for medicinal and pharmacological applications.
- Emergency food during COVID-19 like situations.
- High nutritional value.

- Wide phenotypic diversity and adaptability to a range of environmental gradients.
- Model crop for C4/CAM facultative photosynthetic pathways.
- Tolerant to multiple stresses, easy to grow, and high biomass content.

- Suitable for biosaline and urban agriculture, requires less water and input.
- Rich terrestrial source of omega-3-fatty acids, good for vegetarians and vegans.
- Potential alternative food for future.

FIGURE 3 | Potential of purslane for sustainable food security and health benefits.

available, we can use the saline water for agriculture and can reduce the usage of freshwater in agriculture (Flowers, 2004; Yamaguchi and Blumwald, 2005; Roy et al., 2014; Fita et al., 2015). Moreover, the current agricultural practices which involve the use of chemicals have increased the salinity of agricultural soils (Shrivastava and Kumar, 2015). Therefore, soils have become less fertile and the normal varieties cannot survive in the saline environments. Plants respond to salinity stresses in a variety of ways including expression of important genes related to salinity tolerance (Chandna et al., 2014; Borsai et al., 2018). Therefore, looking for crop plants which can withstand salinity condition should be one strategy so that saline environment can be utilized for agriculture.

Purslane is considered salinity tolerant and can perform even under high salinity conditions (Yazici et al., 2007; Kafi and Rahimi, 2011; Alam et al., 2014b,e; Hniličková et al., 2019). Experimental evidences by several studies showed that purslane could grow in saline soils, therefore, it could be popularized as an important biosaline crop (Hasanuzzaman et al., 2014; Panta et al., 2014; Dagar, 2018; Elouafi et al., 2020). The plant responds to salinity stress by the production of proline which is an important osmolyte and helps in salt tolerance (Yazici et al., 2007; Rahdari et al., 2012). Similar response is observed in another species, *P. grandiflora* suggesting its adaptability to stresses (Kichenaradjou et al., 2018). Studies have shown reduced accumulation of proteins and lipids following salinity stress and increased accumulation of carbohydrates (Morgan, 1992; Dhanapackiam and Ilyas, 2010). Unlike salt-sensitive crops, purslane is known to keep its photosynthetic activity on even under higher salinity conditions through increased chloroplast biosynthesis and chlorophyll accumulation (Rahdari et al., 2012), although, the application of salinity decreases its germination potential. Another study found that intermediate levels of salinity are not detrimental to purslane as they continue to perform well under moderate salinities (Teixeira and Carvalho, 2009). Several metabolites in response to salinity have been identified in *P. oleracea* (Zaman et al., 2020). Increased accumulation of proline in the salt-tolerant purslane genotypes correlated with higher expression of *pyrroline-5-carboxylate synthetase* (*PC5S*) gene which is a key enzyme for the biosynthesis of proline (Sdouga et al., 2019). Increased accumulation of proline and its correlation with *PC5S* show important transcriptional control of salt tolerance in purslane. Due to its salt-tolerance potential, it is suggested to be an appropriate crop, with greater pharmacological and nutritional potential that can be grown in areas where the irrigation water is saline and solar radiation levels are high (Franco et al., 2011).

Potential for Urban Agriculture

Another issue that we face today is the nonavailability of land resources in urban areas (Fischel, 1982; Fazal, 2000; Satterthwaite et al., 2010; Kapil, 2019). The urban populations are dependent on market foods that are not fresh and sometimes, it takes many days to reach the kitchens of urban areas (Satterthwaite et al., 2010). However, if crops are devised that takes very less space and gives high biomass, it can be adopted in the future especially by urban people/consumers to have quick chemical-free fresh vegetables and foods. As purslane takes very less space and produces huge biomass rich in very important nutrients and health-promoting phytochemicals, this crop has tremendous potential for urban agriculture (Ren and White, 2019).

Rich Terrestrial Source of Omega-3-Fatty Acids

Several quantitative studies from various parts of the world have reported very high amounts of ω-3-fatty acids in its various plant parts of purslane (Davis and Kris-Etherton, 2003; Simopoulos et al., 2005; Uddin et al., 2014). It is considered one of the richest sources of ω-3-fatty acids. Marine algae are also rich in ω-3-fatty acids; however, they are not available beyond the coastal areas. Moreover, cultivation of algae needs skill and expertise. In contrast, cultivation of purslane is very easy and does not require special care or expertise. Vegetarian and vegan diets are relatively low in alpha-linolenic acid (ALA), an important ω-3-fatty acid (Davis and Kris-Etherton, 2003). ALA is further broken down to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the latter are essential for cardiovascular health. However, plasma, tissue, and blood of vegetarians and vegans are also low EPA as well as DHA as these (Davis and Kris-Etherton, 2003; Rosell et al., 2005; Saunders et al., 2013). Lower EPA and DHA in vegetarians and vegans may lead to cardiovascular-related health issues. Dieticians and health experts suggest increased intake of ALA to vegetarians and vegan people since their diets are deficient in ALA (Davis and Kris-Etherton, 2003). The presence of a high amount of ALA in purslane suggests its suitability for vegetarians and vegans as an important source of ALA (Uddin et al., 2014). Therefore, the promotion of consumption of purslane among vegetarians and vegans is an important step toward enriching their diets with ALA, EPA, and DHA.

Wide Phenotypic Diversity and Adaptability to a Range of Environmental Gradients

The *P. oleracea* is a cosmopolitan species, and huge phenotypic plasticity is observed for various traits such as flower color, phytochemical constitution, leaf size and number, growth habit, and overall plant size (Danin et al., 1978; Ezekwe et al., 1999; Ren et al., 2011; Alam et al., 2014d). Ren et al. (2011) reported genetic variation study in purslane using amplified fragment length polymorphic markers in 10 drought-tolerant accessions from different geographical locations, namely, POS, PO, and Turkey (Turkey), Keren and Tokombiya (Eritrea), Golden E (UK), Golden G and T (Netherlands),Wild Greece (Greece), and Egyptium (Egypt). They reported Tokombiya and Egyptium as the most tolerant accessions to drought stress at the adult stage (Ren et al., 2011). Commercial cultivars, Glystrida 0425 and Purslane Dark Green, and local populations, Sari, Gorgan, and Aliabad originating from Iran had relatively higher biomass yield (Karkanis and Petropoulos, 2017). Ezekwe et al. (1999) have characterized eight accessions namely *P. oleracea*, *P. sativa*, Golden Gerber (GG Dutch), Garden (Gn Dutch), Golden (G England), and wild (W) accessions (Beltsville, Egyptian, and Greece) of purslane from different geographical regions for chemical composition, crude protein, total lipids, and carbohydrate content. They reported the highest crude protein content (27.1%) in Wild Greek accession and the highest omega fatty acid content in Dutch Garden accession (Ezekwe et al., 1999). Alam et al. (2014d) studied 45 accessions of purslane from the Western Peninsula of Malaysia and grouped them into seven types based on their characters such as leaf type and color, stem color, and flower type and color. Molecular analysis of these 45 accessions of purslane using expressed sequence tag (EST)-derived simple sequence repeat markers (ES-SSR) showed high genetic diversity among the accessions (Alam et al., 2015a). Evaluation of antioxidant compounds, antioxidant activities, and mineral composition of 13 collected purslane accessions showed wide phenotypic diversity (Alam et al., 2014c). Several accessions of purslane were screened for high salt tolerance (Alam et al., 2014b), and it was found that salinity affects phenolic compounds and antioxidant activities of 13 collected purslane germplasms (Alam et al., 2015b). Characterization of 45 accessions of West Peninsular, Malaysia for various morphological and physiological parameters and mineral composition identified potential accessions that can be used for supplementing the nutritional and mineral requirements (Alam et al., 2014a,e). Liu et al. (2000) have evaluated 12 varieties of purslane (nine Australian wild, two North American origins and one local), and the results of the study showed a wide variation in fatty acids and β-carotene composition. Lim and Quah (2007) characterized six cultivars (PO1–PO6) of purslane for total phenol content (TPC) and found that the ornamental cultivars have higher total phenolic (TPC) content and antioxidant activities than the common variety (PO1). Among the six varieties, PO6 showed the highest TPC and antioxidant activity. Tiwari et al. (2008) found that two species of *Portulaca*, i.e., *P. tuberosa* and *P. oleracea* are good heavy metal accumulators. Zhu et al. (2010) used 11 *Portulaca* accessions from five provinces of China and obtained consistent GC-MS and IR spectral fingerprints that are important for quality assurance. A comparative study of two subspecies of *Portulaca*, i.e., *P. oleracea* subs. *granulatostellulata* and *P. oleracea* subs. *edulis* for their growth and nutritional quality showed that both the subspecies were rich in minerals. However, K content was higher in *P. oleracea* subs. *edulis*, whereas the Ca content was higher in *P. oleracea* subs. *granulatostellulata* (Yun et al., 2016). Analysis of physicochemical properties of oil from seed, leaf, and stem of purslane from the Dire Dawa region of Ethiopia showed the highest oil content in seeds (11.25%) compared with leaf and stem (Desta and Cherie, 2018). Furthermore, the analysis of 13 common Malaysian-cultivated accessions of purslane showed differential morphological responses to salinity stress (Alam et al., 2016). The results showed that except plant height in Ac 1 (accession 1), all morphological parameters showed negative correlation with salinity and Ac 13 was the most affected accession among all. Egea-Gilabert et al. (2014) characterized 12 accessions of purslane using morphological, molecular, agronomical, and biochemical analyses and identified CM 13-00809 as an important accession with superior traits such as yield, dry matter, and potassium content. Kaşkar et al. (2009) analyzed agronomic parameters of several accessions of purslane such as Golden purslane, "C," one local Turkish accession, and three Spanish accessions and found variation in various parameters such as nitrate and oxalate content, plant height, number of leaves, leaf area, and yield component. They further found that accession "C" which was obtained from Pasa Seeds company showed better agronomic traits which includes plant height, number of leaves, leaf area, and yield component. The Turkish accession showed minimum amounts of oxalate and nitrates (undesirable/antinutrients) suggesting their suitability for human consumption. Analysis of 19 characters in four populations of purslane showed wide interpopulation heterogeneity in Tunisia (Salah and Chemli, 2004). Sdouga et al. (2020) found wide diversity in morphological and biochemical characters between wild populations and cultivated varieties. Karkanis and Petropoulos (2017) studied comparative physiological responses under Mediterranean semiarid conditions between two commercial cultivars, three local populations of Iran, and one local population of Greece and found that the Aliabad, a local population of Iran showed the highest yields. Balabanova et al. (2020) analyzed 33 Bulgarian and five Greek accessions of purslane and reported 50 metabolite markers. Metabolic profiling of leaves and roots of two purslane genotypes, Tall Green (TG) and Shandong Wild (SD), under the control and saline exposures was performed by Zaman et al. (2020) who found a huge variation in the concentration of 132 metabolites under control and treated conditions. Furthermore, salinity-induced reduction in the number of leaves and roots, diameter of the stem, and length of roots was reported.

Besides its tolerance to a range of environmental conditions (Miller et al., 1984; Simopoulos, 2011), it produces a very high number of seeds with very good germination rate even under stress conditions (Fernández et al., 2008; Chauhan and Johnson, 2009; Alam et al., 2014e; Ren and White, 2019). Purslane grows profusely even on the less fertile and waste lands (Nyffeler and Eggli, 2010; Subramanyan, 2017). Its adaptability to various climatic conditions makes it a suitable crop for extensive agriculture. Various varieties are regionally important, and people collect it from the natural habitats (Turner et al., 2011; Uddin et al., 2014; Borelli et al., 2020). In some cases, people also cultivate it at a small scale especially for ornamental purposes (Osma et al., 2014). Efforts must be taken to exploit its genetic diversity for regional agricultural programs based on the availability and suitability of *Portulaca* species/cultivar. Moreover, except for *P. oleracea*, only a few other species of *Portulaca* are studied to date. This suggests that further studies on other species of *Portulaca* will help us to identify potential genotypes including higher content of ω-3-fatty acids and other nutritional and phytochemical components.

Important Model Crop for C4/CAM Facultative Photosynthetic Pathways

Portulaca oleracea is an important plant that uses both C4 and CAM photosynthetic pathways depending upon the prevailing situations (Lara et al., 2004; D'Andrea et al., 2014). The C4-CAM switching depending upon the prevailing environmental conditions makes it an important and desirable crop for the twenty-first century. The scientists are working toward development of climate-smart crops using the gene and genome engineering/editing tools (DeLisi et al., 2020; Zaidi et al., 2020). However, purslane is a naturally occurring smart crop that operates its machinery smartly (D'Andrea et al., 2014). Due to the presence of C4 and CAM enzymes in purslane, it has become an important model crop for C4-CAM facultative photosynthesis studies. It behaves as a C4 plant when grown under normal conditions whereas it switches to CAM pathway under drought conditions. Since CAM pathway is a highly water-efficient system of photosynthesis, switching into CAM by purslane surely helps it to survive under drought conditions (D'Andrea et al., 2014). Further investigation on the mechanisms governing C4-CAM switching in purslane and the identification of the genes would provide important insights into this important facility used by purslane (Winter and Holtum, 2014). The enzymes that are activated during these situations have been studied (Lara et al., 2003, 2004; D'Andrea et al., 2014, 2015).

Emergency Food During COVID-19-Like Situations

Various indigenous communities rely on traditional food plants including purslane for their nutritional requirements (Kuhnlein and Receveur, 1996; Pieroni et al., 2005; Muthoni and Nyamongo, 2010; Uddin et al., 2014; Pawera et al., 2020). Traditional plants are also used for medicinal purposes in various parts of the world (Mahomoodally et al., 2012; Shikov et al., 2017; Kumar et al., 2019). As discussed, purslane is also one of the most important ethnomedicinal plants among various countries (Ross, 2003; Bosi et al., 2009; Iranshahy et al., 2017). The traditional food plants are regionally very important and local communities rely on them for their nutritional needs. During the coronavirus disease 2019 (COVID-19)-like situations, purslane can be one of the important emergency foods for far-flung areas where food aid is disrupted due to COVID-19-like situations in the future. It is not surprising that COVID-19 has disturbed the global supply chains of the foods, and in the future, such crops can be important for sustaining the communities that lack basic food distribution.

Stress Tolerance in Purslane

The depletion of water resources is one of the biggest challenges to modern agriculture in the world (FAO, 2011). It directly translates to reduced productivity and yields, and if arising in a large-scale agricultural land, would lead to global food insecurity (Falkenmark, 2013; Misra, 2014). Scientists across the globe are putting efforts to devise and innovate strategies to engineer new varieties of crops that can perform well even under extreme conditions such as drought condition (Zhang et al., 2000, 2018; Jewell et al., 2010; Vandenbroucke and Metzlaff, 2013; Jaganathan et al., 2018; Zaidi et al., 2020). Considering the global water shortages, and the susceptibility of the modern crop cultivars to drought conditions, it is highly desirable to discover new crops/varieties that are naturally drought tolerant.

Researchers are putting efforts to use genetic engineering tools to modify currently available crops for increased stress tolerance. Some studies have shown encouraging results, although, not up to the mark. Therefore, the alternative option is to discover naturally occurring stress-tolerant plants that can perform well under stressful conditions. Among all, drought, salinity, heat, and temperature stresses are some of the important abiotic stresses concerning agriculture (Yang et al., 2012; Jin et al., 2015). Drought is also an important environmental stress which limits plant growth and development and affects yields (Ren et al., 2011). Several studies have indicated that purslane is drought tolerant and can easily grow in extreme drought conditions (Ren et al., 2011; Rahdari et al., 2012). Jin et al. (2015) studied drought and heat stress tolerance of purslane and observed significant increase in the malondialdehyde (MDA), proline, electrolyte leakage, reactive oxygen species (ROS), and antioxidant activities and decrease in leaf water content (LWC) and chlorophyll content during the progressive drought treatment. The rehydration following drought stress reversed the negative parameters such as LWC and chlorophyll content. Activation and regulation of various physiological mechanisms makes purslane more efficient to cope up with stress conditions and recover during rehydration (Jin et al., 2015). Earlier study has also shown rapid recovery of purslane plants following dehydration (D'Andrea et al., 2014). Purslane responds to stresses at multiple levels using various mechanisms. One study suggests that switching of CAM pathways enhances the water use efficiency and drought tolerance in purslane (Liu et al., 2018). Differences in the metabolites and the enzymes required during their synthesis in the well-irrigated and drought conditions are also observed, suggesting a biochemical basis of salt tolerance in purslane (Lara et al., 2003, 2004). It also improves its tolerance by enhancing the production of antioxidants, proline content, and heat-shock proteins which are all important for survival under high stress conditions (Yang et al., 2012).

TRADITIONAL FOOD SYSTEMS THAT ARE BASED ON PURSLANE

Traditional food plants are an important part of indigenous diets, and they are produced within the restricted local areas (FAO, 1988). Culturally accepted food patterns evolved from available local resources are referred to as traditional food systems (Kuhnlein and Receveur, 1996). Traditional food systems reflect the culture indigenous communities and their dietary patterns (Trichopoulou et al., 2007). Traditional food plants are healthy, nutritious, and serve as the sustainable food resources for indigenous rural communities (Legwaila et al., 2011). A wide variety of food plants form a part of traditional food systems across the world (Turner et al., 2011). Various studies have reported the use of purslane as an important part of traditional food systems (Welcome and Van Wyk, 2019). It is consumed as a vegetable in a variety of ways across the globe, and the mode of preparation and consumption of purslane varies from region to region. It is used as a raw salad in Turkey, Kufla ka saag in India, and as traditionally processed papads in India (Kapoor et al., 2010; Renu and Waghray, 2016; Borelli et al., 2020). It is also consumed in many other countries and forms a basis for novel traditional foods. **Table 1** presents a summary of traditional food systems that are based on purslane across 18 countries. This table provides information related to its local names, mode of preparation, and consumption.

GENETICS OF PHYTOCHEMICAL, NUTRITIONAL, AND STRESS-RESILIENT TRAITS

Since purslane is known for its medicinal and nutritional properties due to the synthesis of important nutrients and phytochemicals such as alkaloids, flavonoids, catecholamines, lignans, terpenoids, betalains, carotenoids, vitamins, and ω-3-fatty acids (Oliveira et al., 2009; Mulla and Swamy, 2010; Singh et al., 2011; Patil et al., 2012; Petropoulos et al., 2016; Verma et al., 2016; Fernández-Poyatos et al., 2021) and tolerance toward several stresses such as drought, temperature, salinity, moisture, and heat (Ichimura and Suto, 1998; Yazici et al., 2007; Alam et al., 2014b; Jin et al., 2016; Borsai et al., 2020; Xing et al., 2020), it is attaining greater attention (Sultana and Raheman, 2013; Uddin et al., 2014). Many plant-derived secondary metabolites have been used as drugs in modern times as these compounds have specific biological activities (Kumar et al., 2019). It is important to isolate and identify the genes that govern stress-resilient traits and biosynthesis of metabolites. Identification of genes involved in biosynthetic pathways of important metabolites and stress-resilient traits is useful for the trait improvement programs and for the development of superior varieties. Only a few studies have reported the genes regulating important traits in purslane. Among the various phytochemicals synthesized in purslane, dopamine is an important catecholamine with a neuroprotective potential (Chen et al., 2003; Chugh et al., 2019). It is important to identify and characterize the genes that synthesize dopamine. Recently, genes for two enzymes tyrosine decarboxylase and tyrosinase have been identified from hair roots using *A. rhizogenes* (Babashpour et al., 2018). Two genes encoding ω-3 fatty acid desaturases *viz. FAD7* and *FAD8* which are involved in the conversion of linoleic to ω-3-linolenic acid have been identified from purslane (Teixeira et al., 2010). In addition to synthesizing ω-3 fatty acids, purslane also produces ω-6-fatty acids. Du et al. (2021) identified 94 genes involved in the biosynthetic pathway of unsaturated fatty acids. Three genes encoding ω-6 fatty acid desaturases *viz. FAD2-1*, *FAD2-2*, and *FAD-6* have been isolated (Teixeira et al., 2009). *FAD2* genes are plasticidal whereas FAD6 is microsomal. Differential expressions of several genes in response to drought stress and after rewatering have been reported in purslane (D'Andrea et al., 2015). The study showed that three important genes, namely, *Ribosomal protein S15A* (*RPS15A*), *Ribosomal protein L2* (*RPL2*), and *ω-6-fatty acid desaturase 2* (*FAD2*) were induced at higher levels following rewatering/recovery of purslane, suggesting their important roles in imparting stress tolerance. FAD2 and stearoyl-acyl-carrier-protein desaturase (SAD) are also important genes involved in ω-3 fatty acid biosynthesis, and there differential expression is reported in stem and leaves of *P. oleracea* (Du et al., 2021). C4/CAM switching is one of the most important mechanisms reported from purslane in response to the differences in environmental conditions. It uses C4 machinery at normal irrigated conditions whereas it switches to CAM when water scarcity is sensed. The enzymes that are activated during C4 and CAM environmental conditions have been studied (Lara et al., 2003, 2004; D'Andrea et al., 2014, 2015). However, the identification of genes governing C4/CAM switch which enables plants to survive under different environmental conditions would be immensely helpful to manipulate this mechanism in purslanes by genetic engineering.

TABLE 1 | Some traditional food systems based on purslane from different countries.

Sl. No.	Country	Traditional food	Preparation/uniqueness	References
1	Turkey	Salad	Used as salad or side dish.	Borelli et al., 2020
2	Spain	Special soup "sop salem korkat"	Sop salem korkat is made by boiling it with local celery, leek, and citrus and sweet soy sauce.	Tarkergari et al., 2013
3	India	"Kulfa ka saag" and papads	Leaves are cut and boiled by adding salt till it becomes paste. Coriander seeds and red chillies fried in mustard oil mixed with this paste. Experience a bit of sour taste.	Kapoor et al., 2010; Renu and Waghray, 2016
4	Indonesia	Oseng-oseng	Leaves cooked with tamarind, *Oseng-oseng* are prepared by stirring and frying young leaves and tender stems along with shallot, garlic, red chilies, palm sugar, salt, salem leaves, and galangal.	Mishra et al., 2020
5	Sri Lanka	Cooked vegetables	Entire plant of purslane is cooked and used as a vegetable.	Ediriweera, 2010
6	Italy	Salad	Plants are eaten as salads and cooked like spinach.	Bosi et al., 2009
7	Mexico	Snacks and sauces	Rolled in omelets and tortillas and added in soups or stews or flowers, leaves, and stems are steamed and consumed.	Irawan et al., 2003; Manzanero-Medina et al., 2020
8	China	Stir fry, pancakes, soups, and sauces	In South China, tender leaves and stems are eaten as stir fry and in egg pancakes, soup, and sauces.	Xu et al., 2020
9	Bulgaria	Salad and soups	Above-ground parts are used for preparing salad and soups.	Nedelcheva, 2013
10	Australia	Roasted roots, raw leaves, and seed floor.	Roots are eaten after roasting. Leaves and stems are consumed raw or in slightly heated form. Seed flour is used in cakes.	Smith, 1991
11	Armenia	Fries, salads, lacto-fermented foods	Fried aerial parts are eaten or pickled in brine. Young leaves and stems are used in salads. Aerial parts are lacto-fermented	Nanagulyan et al., 2020; Pieroni et al., 2021
12	Albania	Raw fresh parts consumed as such. Leaf used to make drinks.	Drink made from leaf juice mixed with milk and sugar.	Pieroni et al., 2005
13	Georgia	Food (Phkhali)	Leaves are used as delicious food.	Bussmann et al., 2016
14	Tunisia	Flours, sauce, and salads	Leaves and stems are used in sauce and salads and seeds crushed into flours.	Ismail, 2013
15	South Africa	Snacks and vegetables	Stem is used as a vegetable, leaves for snacks and vegetables.	Magwede et al., 2019; Welcome and Van Wyk, 2019
16	Madagascar	Soup/salad	Locals use it as salad or soup.	Beidokhti et al., 2018
17	Benin	Cooked vegetables	Locally used as a green vegetable. In Benin, it is used against rheumatism, gynecological diseases, dysentery, fever, and other infections.	Achigan-Dako et al., 2010
18	Greece	Purslane salad	Raw purslane is mixed with potato slices, onions, tomatoes, green chillies, and oil.	Irawan et al., 2003

IMPROVEMENT OF PURSLANE USING GENOMICS AND GENE-EDITING TOOLS FOR SUPERIOR TRAITS AND URBAN AGRICULTURE

Crop improvement involves strategies for the trait enhancement of the plants for superior characters (Singh et al., 2020). Various strategies including conventional and modern approaches have been used for crop improvement programs (Breseghello and Coelho, 2013). The characterization and identification of diverse germplasm is an important step toward further crop-improvement programs (Ezekwe et al., 1999; Alam et al., 2014b; Egea-Gilabert et al., 2014). Few researchers have attempted and successfully standardized tissue culture protocols and *Agrobacterium*-mediated transformation (Rossi-Hassani et al., 1995; Sedaghati et al., 2019, Sedaghati et al., 2021). Although the advancement of molecular techniques including genomics and other omics technologies enabled the analysis of genomes, proteomes, metabolomes, and ionomes of the crop plants (Salt, 2004; Kumari et al., 2015; Ramalingam et al., 2015), however, such studies are lacking in purslane. The genomics studies in combination with other omics studies such as metabolomics and proteomics will provide useful information about the regulatory networks that contribute to the stress tolerance, diversity of phytochemicals, and other nutritional traits in purslane besides shortening the time required for breeding superior cultivars (Singh et al., 2020). Furthermore, the gene-editing tools can also be exploited for editing the important genes and customization of the purslane cultivars for urban and biosaline agriculture (Abdallah et al., 2015). The purslane improvement studies must focus on traits such as biomass, yield, maturity, flowering, seeds, and stress tolerance (Alam et al., 2014e). Since purslane is highly nutritious orphan crops with a number of superior traits, attempts should be made to decipher its genetic makeup and other regulators such as noncoding RNAs and epigenetic marks using genomics tools. The identification of genes will enable to manipulate and improve desirable traits including enhancement of ω-3-FAs, its CAM pathway, nutritional content, stress tolerance/resistance,

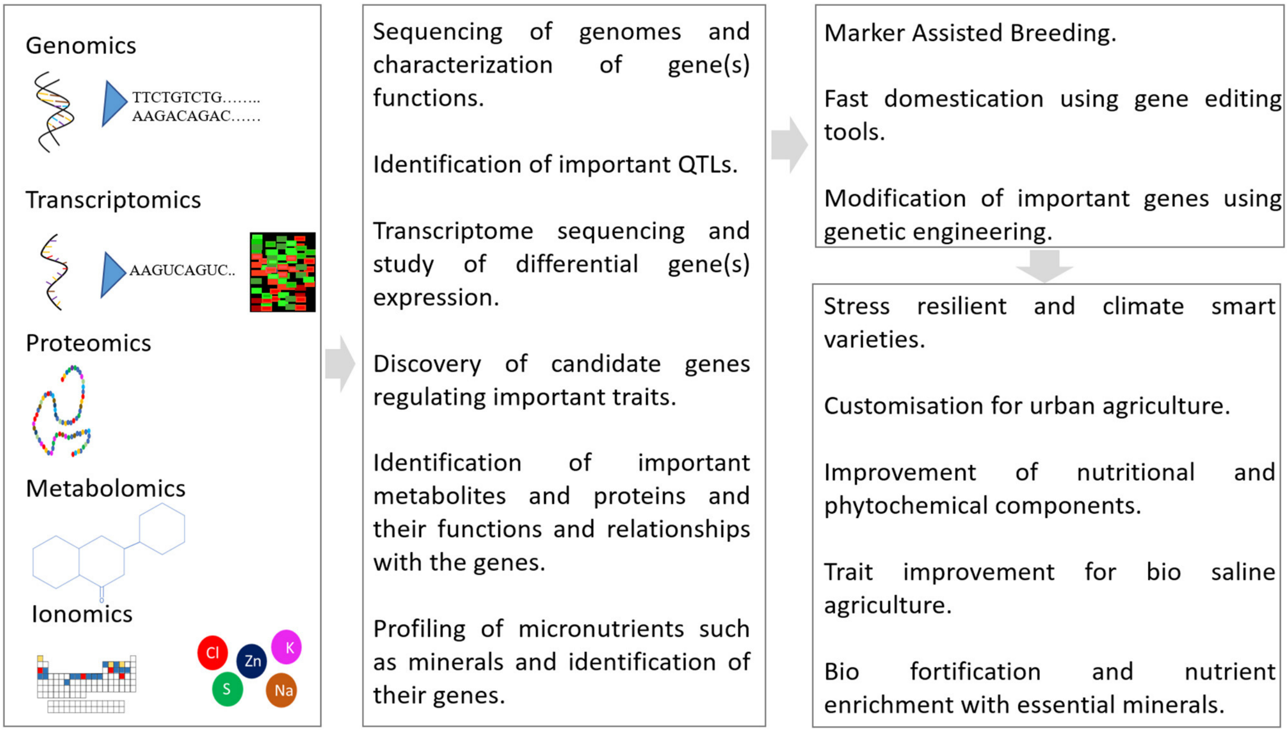


FIGURE 4 | Integrated strategies to improve purslanes using genomics and gene-editing technology.

and other important traits using conventional and modern genome-editing tools. Furthermore, a large-scale profiling of nutrients and essential mineral elements using metabolomics and ionomics and establishing links with their genes would help us to decipher the importance of genetics of these traits. **Figure 4** represents an integrative strategy involving the use of genomics and gene editing for the improvement of purslane.

CHALLENGES TO CULTIVATION AND IMPROVEMENT OF PURSLANE

Purslane is a nutritionally rich traditionally important orphan crop which is not either cultivated or consumed on a large scale. Although it has multiple desirable traits, it has unpalatable taste due to which it is not widely consumed by people. To increase its production and consumption, awareness programs about its health benefits must be conducted. Although wide variation of purslane is reported, detail collection and characterization to identify potential germplasm to be used in crop improvement has not been reported (Ramanatha Rao and Hodgkin, 2002; Alam et al., 2014a, 2015a). The lack of germplasm collections in different gene/germplasm banks across the globe is also one of the reasons why it remained neglected to date. Furthermore, there are very limited genetic and genomics resources of purslane to date. Future attempts should be made to increase germplasm collections; their characterization, phenotyping, and genotyping using various omics techniques should be attempted for purslane to be used in improvement programs.

CONCLUSION AND FUTURE DIRECTIONS

Purslane, being an important traditional crop with multiple health benefits and inherent stress-tolerant mechanism has tremendous potential to be adopted for cultivation during this time of global climate change, salinity, drought, and urbanization-related problems. The climate-smart crops should be able to grow and adapt in stressful environments such as drought, high temperature, and submergence machinery, and these properties are inherently present in purslane. Purslane uses water-efficient CAM pathway for photosynthesis during drought conditions and switches to C4 machinery when the temperature is very high (Simopoulos et al., 1995; Kamil et al., 2000; Ferrari et al., 2020), which is a potential trait for designing climate-smart crop (D'Andrea et al., 2014). Facultative CAM pathway of purslane and C4 metabolism makes it an important crop for water-scarce regions of the world (Welkie and Caldwell, 1970; Koch and Kennedy, 1980; Ocampo et al., 2013; Winter and Holtum, 2014; Liu et al., 2018). Therefore, its cultivation can also be attempted in the drought-prone regions of the world. Furthermore, its salinity-tolerance character makes it fit for biosaline agriculture where freshwater (normal) is not in plenty (Borsai et al., 2018, 2020). However, the mechanisms of salinity tolerance still need to be investigated in detail. The development of transformation protocols is important for gene editing or genetic modification of crops. A research group has standardized and developed an efficient protocol of *Agrobacterium*-mediated transformation in *P. oleracea* (Sedaghati et al., 2019). The induction of hairy root is a key step toward *in vit*ro production of secondary metabolites, and it has been successfully achieved in *P. oleracea* (Moghadam et al., 2014). Therefore, the availability of *Agrobacterium*-mediated transformation protocol may ensure genetic manipulation experiments once the genes governing important traits are identified. Furthermore, other gene editing technologies may also be attempted in purslane for manipulating genes governing important traits. The application of multi-omics high-throughput technologies such as genomics, transcriptomics, metabolomics, proteomics, and ionomics and their integrated analysis would help to identify genes, understand the regulatory mechanism, and help in manipulation of important traits. Lastly, we suggest and recommend for popularizing the cultivation and consumption of traditional orphan food crop purslane in a large scale owing to its high nutrition and health beneficial compound content and easy to cultivate in less space and multiple stress environments.

AUTHOR CONTRIBUTIONS

All authors have directly and significantly contributed to this work and approved the final draft for publication.

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Genome-Wide Scanning Enabled SNP Discovery, Linkage Disequilibrium Patterns and Population Structure in a Panel of Fonio (*Digitaria exilis* [Kippist] Stapf) Germplasm

Abdou R. Ibrahim Bio Yerima [1,2]*, ***Kassari Ango Issoufou*** [2], ***Charlotte A. Adje*** [1], ***Aissata Mamadou*** [2], ***Happiness Oselebe*** [3], ***Mame Codou Gueye*** [4], ***Claire Billot*** [5] **and** ***Enoch G. Achigan-Dako*** [1]

[1] Laboratory of Genetics, Biotechnology and Seed Sciences (GBioS), Faculty of Agronomic Sciences (FSA), University of Abomey-Calavi (UAC), Cotonou, Benin, [2] Department of Rainfed Crop Production (DCP), National Institute of Agronomic Research of Niger (INRAN), Niamey, Niger, [3] Department of Crop Production & Landscape Management, Faculty of Agriculture & Natural Resources Management, Ebonyi State University, Abakaliki, Nigeria, [4] Centre d'Étude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (CERAAS), Institut Sénégalais de Recherches Agricoles (ISRA), Thiès, Senegal, [5] UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France

Edited by:
Matteo Balderacchi,
Independent researcher,
Piacenza, Italy

Reviewed by:
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Flinders University, Australia
Celine Blanc-Jolivet,
Thünen Institute of Forest
Genetics, Germany

****Correspondence:***
Abdou R. Ibrahim Bio Yerima
abdourazakoui01@gmail.com

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White fonio (*Digitaria exilis)* is a staple food for millions of people in arid and semi-arid areas of West Africa. Knowledge about nutritional and health benefits, insights into morphological diversity, and the recent development of genomic resources call for a better understanding of the genetic structure of the extant germplasm gathered throughout the region in order to set up a robust breeding program. We assessed the genetic diversity and population structure of 259 fonio individuals collected from six countries from West Africa (Nigeria, Benin, Guinea, Mali, Burkina Faso and Niger) in this study using 688 putative out of 21,324 DArTseq-derived SNP markers. Due to the inbreeding and small population size, the results revealed a substantial level of genetic variability. Furthermore, two clusters were found irrespective of the geographic origins of accessions. Moreover, the high level of linkage disequilibrium (LD) between loci observed resulted from the mating system of the crop, which is often associated with a low recombination rate. These findings fill the gaps about the molecular diversity and genetic structure of the white fonio germplasm in West Africa. This was required for the application of genomic tools that can potentially speed up the genetic gain in fonio millet breeding for complex traits such as yield, and other nutrient contents.

Keywords: *Digitaria exilis*, staple food, genotyping by sequencing, genetic diversity, population structure, linkage disequilibrium patterns

INTRODUCTION

Plant breeders often use the diversity of plant genetic resources to create new and improved cultivars with desirable features such as production potential, nutritional quality, large seed size, and resistance to biotic and abiotic stresses (Govindaraj et al., 2015). Modern crop varieties are typically inappropriate for low-input agriculture in marginal environments, despite the fact that they were created largely for high yielding potential under well-defined conditions (Evenson and Gollin, 2003), compared to traditional varieties or landraces that are well-adapted to various farming systems under different ecological regions, thus contributing to food and nutrition security of subsistent small-scale farmers (Ceccarelli and Grando, 2002). Among traditional cereals, fonio is one of West Africa's oldest native cereal crops, with social, economic, and nutritional significance (Dansi et al., 2010). Fonio grain is used to make salads, couscous, stews, candies, and alcoholic and non-alcoholic beverages, as well as traditional porridges, fonio Jollof, and flour creams (Abdul and Jideani, 2019). Furthermore, the ecological versatility of fonio, makes the crop an important component to be considered for tackling issues related to food insecurity and malnutrition in the future (Raneri et al., 2019). However, in spite of its inherent advantages in playing a critical role for food and nutrition security, and income generation, no improved varieties have been developed so far. With the current advances in molecular breeding, additional efforts are therefore needed to bridge the gaps and generate knowledge that can contribute significantly to increase precision and accuracy in fonio improvement using molecular data.

During the last decades, the efficiency and precision of conventional breeding have been remarkedly improved through the application of molecular tools in both basic and applied research (e.g., diversity studies, phylogenetic and evolutionary analyses, linkage QTL mapping, association mapping studies, marker-assisted selection, genomic-assisted selection) (Gross-German and Viruel, 2013). Various molecular markers have been reported earlier in fonio millet (*Digitaria exilis* [Kippist] Stapf) for diversity, phylogenetic and evolutionary studies. These markers included isozyme (Adoukonou-Sagbadja et al., 2010), Random Amplified Polymorphic DNA (RAPD) (Hilu et al., 1997; Kuta et al., 2005), Inter-Simple Sequence Repeat (ISSR) (Animasaun et al., 2018), and Simple Sequence Repeat (SSR) (Barnaud et al., 2012; Ngom et al., 2018; Olodo et al., 2019). Amplified Fragment Length Polymorphism (AFLP) markers were found to be effective in detecting genetic diversity and determining population differentiation, and moderate levels of genetic diversity were detected in *Digitaria exilis* (Adoukonou-Sagbadja et al., 2007). Furthermore, SSR markers were frequently used for diversity, phylogenetic, and evolutionary investigations (Ellegren, 2002) due to their co-dominance, high polymorphism, high global mutation rates, and repeatability, even if designing primers requires a thorough understanding of DNA sequences (Xu and Crouch, 2008; Mondini et al., 2009). With the recent advances in genotyping by sequencing (GBS) technologies, single nucleotide polymorphisms (SNPs) are becoming the markers of choice in crop genetic studies because of their bi-allelic nature, which allows for accurate variant calling, high reproducibility, and the fact that they provide a rapid, high-throughput, and cost-effective tool for exploring plant genetic diversity on a genome-wide scale. Ching et al. (2002), Chagné et al. (2008),and Studer and Kölliker (2013) without prior knowledge of the species of interest's genome. Most importantly, these markers enabled dissection of the genetic basis underlying complex quantitative traits through biparental linkage and mapping approaches (Sehgal et al., 2020) as well as comparative genomics (Abrouk et al., 2020; Wang et al., 2021). Unlike linkage mapping, the association mapping approach, also known as linkage disequilibrium (LD) mapping, makes use of the existing genetic diversity in natural germplasm populations and exploits their historic recombination events to map or fine map genes with greater precision (Buckler IV and Thornsberry, 2002). Thus, for a successful association mapping and genomic prediction, a better understanding of LD's patterns and decay within a population is very critical to figure out the number of markers needed (Porto-Neto et al., 2014). Khatkar et al. (2008) reported that when low LD levels exist in a population, to capture the genetic variation across the genome, a higher marker density is required. To date, the most commonly used measure of LD is the r^2 being the squared value of the correlation coefficient between two polymorphic loci's allelic states (Pritchard and Przeworski, 2001). LD patterns based on SNP markers have been widely studied in different crop species including cereals (Chen et al., 2017; Serba et al., 2019) and legumes (Otyama et al., 2019; Sodedji et al., 2020). However, assessment of genetic diversity, population structure analysis, and Linkage Disequilibrium patterns of fonio millet at genome-wide scale were not performed. Therefore, to better characterize the diversity of the current collection of fonio at genomic level and understand the population structure at regional level, advanced high throughput genotyping platforms is needed. This is the first time, as far as we know that genome-wide diversity is explored on a regional basis in fonio millet including collections from Guinea, Mali, Niger, Benin, Nigeria and Burkina Faso. The paper intends to respond to the following questions: (a) What is the genetic basis of the population structure? (b) how are fonio individuals from various ecological regions related? (c) Does population differentiation link with geographical patterns? and What are the LD patterns? The aims of this research are to investigate the genetic diversity in fonio millet accessions, to determine the structure and level of population differentiation, and to measure LD. We assume that investigating the genetic diversity on a genome wide-scale using genotyping by sequencing analysis will reveal association that is useful in fonio molecular breeding.

MATERIALS AND METHODS

Description of Plant Materials

The plant material included 259 fonio millet accessions collected across six West African countries (Benin, Burkina Faso, Guinea, Mali, Niger, and Nigeria) and kept at the genebank of the Laboratory of Genetics, Biotechnology and Seed Science (GBioS) of the University of Abomey-Calavi (UAC) in Benin. The diversity panel has also been collected from a range of ecological regions including arid, semi-arid and sub-humid. The most accessions in the collection were from semi-arid ecology (183), followed by arid (50) and sub-humid (26) ecologies. According to the country of provenance, 42 fonio accessions were collected from Benin, four from Burkina Faso, 29 from Guinea, 28 from Mali, 28 from Niger, and 128 from Nigeria. The distribution map of fonio accessions collected from six West African countries is presented below (**Figure 1**).

DNA Extraction and Genotyping

Fonio plants were grown in plastic bags in the GBioS laboratory at the University of Abomey-Calavi (Benin) and three weeks old leaves were sampled from a single plant and collected into 96 deep well samples collection plates and sent to the Integrated Genotyping Service and Support (IGSS) platform (https://ordering.igssafrica.org/cgibin/order/login.pl) now SEQART AFRICA located at Biosciences Eastern and Central Africa (BecA-ILRI) Hub in Nairobi for Genotyping. The Nucleomag Plant Genomic DNA extracting kit was used to extract genomic DNA from 50–100 ng/ul, with the quality and quantity of the DNA extracted being checked on 0.8% agarose. DArT-Seq has been optimized for *D. exilis* by choosing the best method to reduce complexity (*PstI-MseI* restriction enzymes). The DNA samples were processed using Kilian et al. (2012) digestion and ligation reactions method, but they replace a single *PstI*-compatible adapter with two different adapters that match two different restriction enzymes. The Illumina flow cell attachment sequence, sequencing primer sequence, and staggered, varying length barcode have been built into *PstI*-compatible adapter while the reverse adapter contained the flow cell fitting region and the overhang sequence that was *MseI*-compatible. PCR was used to amplify only mixed fragments (*Psti-MseI*) following these reactions conditions: initial denaturation stage (1 min at 94°C); denaturation stage (30 rounds each consisting of 20 s at 94°C); annealing stage (30 s at 58°C); extension stage (45 s at 72°C); and finally, an extension step of 7 min at 72°C. Following PCR, equimolar amounts of amplification products were bulked and applied to c-Bot bridge PCR for each sample of the 96-well microtiter plate and sequenced on Illumina Hiseq2500. Single read sequencing took place over 77 cycles. Sequences produced by every lane were processed using proprietary DArT analytical pipelines. In order to filter poor-quality sequences, the FASTQ files were first processed in the primary pipeline with more stringent selection criteria compared to the remaining sequence in the barcode region. The sequences of specific samples contained in the barcode split-step were therefore assigned more consistently. The barcode/sample sequences were used in the marker calling with ∼2,500,000 (±7%). At the end, the same sequences were collapsed into "fastqcall files." and these files were used in the secondary pipeline for DArT P/L's proprietary SNP and SilicoDArT (present = 1 and absent = 0) calling algorithms (DArTsoft14). The sequence data were processed using analytical pipeline. SNP markers were aligned to the reference genome of Fonio CM05836 (*D. exilis*) (Abrouk et al., 2020) to identify chromosome positions.

Data Cleaning, Imputation and Filtering

TASSEL (Trait Analysis by aSSociation, Evolution and linkage) software version 5.2.56 (Bradbury et al., 2007) was used for data cleaning or quality control. Prior to the analysis of genetic parameters, tree filters were generated before missing data imputation, whereby loci with minor alleles frequency (MAF) <0.05 along with loci departing from Hardy–Weinberg equilibrium at 5% and loci with a call rate <70 % were pruned using the PCCA algorithm in KDCompute (https://kdcompute.igss-africa.org/kdcompute/login). The imputed and filtered data were used for structure and genetic relatedness analyses, the analysis of molecular variance (AMOVA), and as well as the discriminant analysis of principal components (DAPC).

Genetic Diversity Analysis

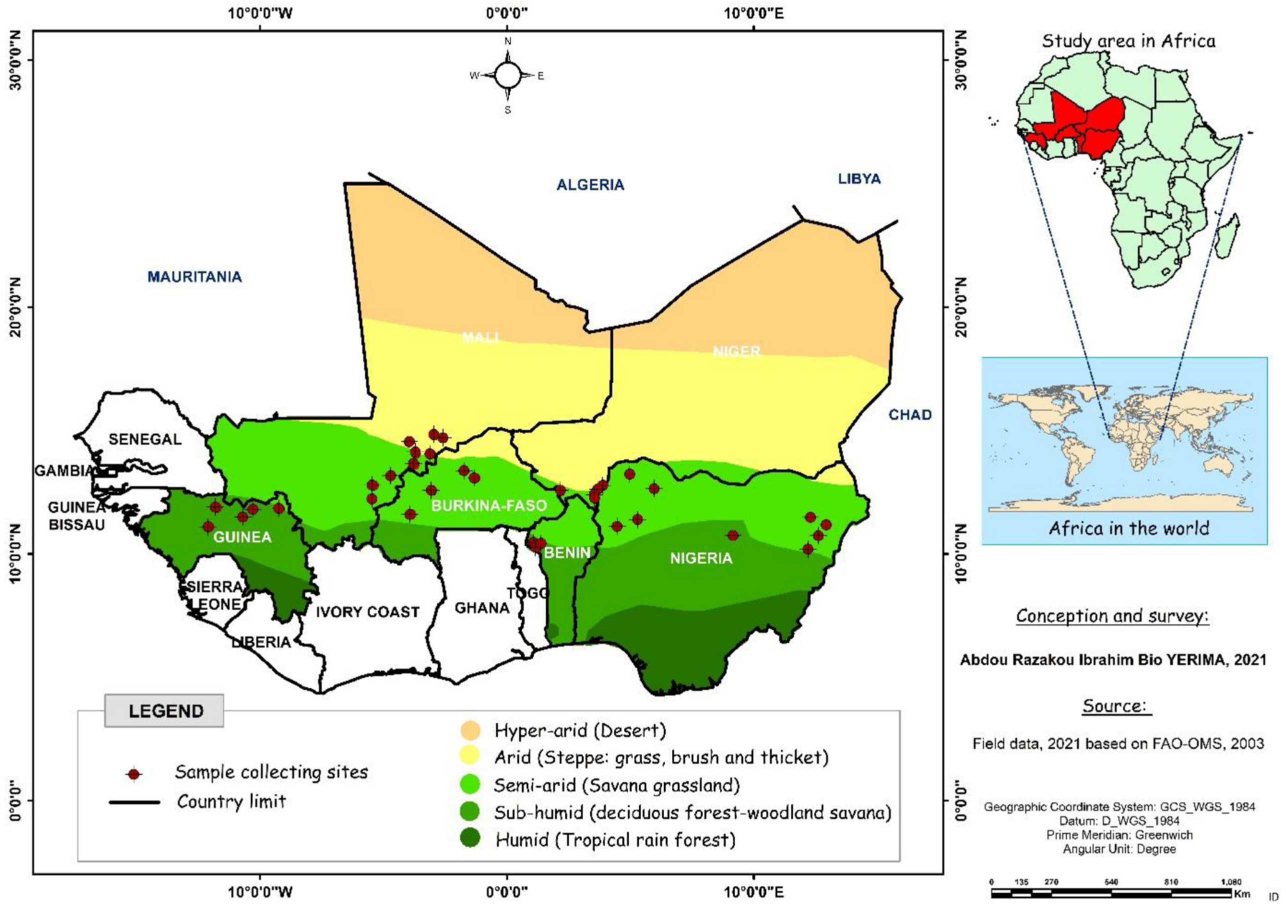
Parameters of genetic diversity such as observed and expected heterozygosities and inbreeding coefficient were estimated at different levels including the entire panel, ecological regions and as well as country of provenance using the package "adegenet" (Jombart, 2008) implemented in R software version 3.6.1 (Core Team, R, 2019). The analysis of molecular variance (AMOVA) was estimated using the package "poppr" (Kamvar et al., 2014) implemented also in R software.

Population Structure Analysis

In order to cluster fonio accessions onto different groups, population structure was investigated using DArTseq-derived SNP markers distributed across the fonio genome using the Bayesian clustering approach in STRUCTURE version 2.3.4 (Pritchard et al., 2000). The analysis included three ecological regions (e.g., arid, semi-arid and sub-humid zones) as putative phytogeographic origins of accessions. A burn-in period of 10,000 Markov Chain Monte Carlo (MCMC) iterations and 10,000-run length, with an admixture model following Hardy-Weinberg equilibrium and correlated allele frequencies were considered in the structure analysis (Evanno et al., 2005). For each value of K (number of subpopulations), 10 independent runs were performed ranging from 1 to 7. The Structure Harvester (Earl, 2012) was used to analyze the results from the Structure, which enabled the identification of the best K-value as the distinct peak in the change of likelihood (ΔK) (Evanno et al., 2005). The extent of genetic differentiation (Fst) between the three ecological regions was estimated with Structure harvester simulation results (**Supplementary File 1**). DArTseq SNP data were numerically coded as follows: $A = 1$, $T = 2$, $C = 3$, $G = 4$ as suggested in Structure V2.3.4 user manual.

Genetic Relatedness and Population Differentiation

A Neighbor-Joining (NJ) dendrogram was drawn using Darwin software V6.0.21 (Perrier and Jacquemoud-Collet, 2016), in order to investigate the genetic relatedness among accessions. Prior to the analysis, the 259 accessions were grouped according to their country of provenance to describe the distribution in the identified clusters. Furthermore, discriminant analysis of principal components (DAPC) was performed to infer and describe clusters in population of genetically related individuals



Conception and survey:

Abdou Razakou Ibrahim Bio YERIMA, 2021

Source:

Field data, 2021 based on FAO-OMS, 2003

Geographic Coordinate System: GCS_WGS_1984
Datum: D_WGS_1984
Prime Meridian: Greenwich
Angular Unit: Degree

FIGURE 1 | Distribution map of fonio accessions collected from six West African countries (Benin, Burkina Faso, Guinea, Mali, Niger and Nigeria).

using ≪ adegenet ≫ (Jombart, 2008) and ≪ hierfstat≫ (Goudet, 2005) packages implemented in R V.3.6.1 (Core Team, R, 2019). In DAPC, data were set first for principal components analysis (PCA) and subsequently clustered using discriminant analysis (DA) with respective eigenvalues. Moreover, in order to estimate genetic differentiation among fonio clusters, Weir and Cockerham's Fst-values (Weir and Cockerham, 1984) were calculated using R software.

Linkage Disequilibrium (LD)

Genome-wide LD patterns of known position of SNPs on the chromosomes (Huang et al., 2012) out of the complete set of 21,324 polymorphic markers were estimated using TASSEL (Trait Analysis by aSSociation, Evolution and Linkage) V5.3.1 software (Bradbury et al., 2007). LD patterns between markers was estimated using r^2, which is the correlation between alleles at two loci, as well as an indicator for evaluating the resolution of association approaches. Farnir et al. (2000) stated that for all possible combination of alleles and then weighting them according to the allele's frequency, a weighted mean of r^2 was calculated between the two loci. In order to test the LD's significance, we also obtained *P-values* determined by permutational test to calculate the ratio of permuted gamete distributions that was less likely than the gamete distribution observed under the null independence hypothesis (Weir, 1996). Furthermore, the rate of LD decay was drawn as the variation r^2 and the physical genetic distance (Mb) between pairs of SNP markers using "dartR" (Gruber et al., 2018) and "Sommer" (Covarrubias-Pazaran, 2016) packages implemented in R.

RESULTS

Genetic Diversity

A total of 21,324 codominant single nucleotide polymorphisms (SNPs) were obtained from the DArTseq analysis with 46.12% of missing data of the overall fonio accessions. The call rate ranged from 37.83 to 100% with a mean of 53.87% ± 0.080. These markers showed high reproducibility varying from 0.90 to 1.00 with an average of 0.99 ± 0.011. The minor allele frequency (MAF) ranged from 0.002 to 0.5 with a mean of 0.153 ± 0.08 while the major allele frequency (MaF) varied from 0.5 to 0.99 with an average of 0.84 ± 0.08 (**Table 1**). Furthermore, 17,821 markers (83.57%) had minor allele frequency >0.05 and

TABLE 1 | Genetic properties of markers.

Parameters	CR	MAF	MaF	GD	PIC	RP
Minimum	0.3783	0.002	0.5	0.09	0.00	0.90
Maximum	1.00	0.5	0.99	0.50	0.5	1.00
Mean	0.5387	0.153	0.84	0.30	0.25	0.99
SD	0.080	0.08	0.08	0.12	0.11	0.011

CR, call rate; MAF, minor allele frequency; MaF, major allele frequency; GD, total genetic diversity; PIC, polymorphic information content; RP, reproductibility; SD, standard deviation.

the majority (83.83%) had a polymorphic information content (PIC) value >0.1 with a mean value of 0.25 ± 0.11. About 80% (17,045) of SNPs were aligned to the reference genome of fonio CM05836. These markers were almost uniformly distributed across the genome with the most SNPs observed on chromosome 09B (2,004 markers) followed by chromosome 05B (1,590 markers). The lowest numbers of SNP markers were observed on chromosomes 06A and 08A with 406 and 386 markers, respectively (**Supplementary Figure 1**).

The discovered SNPs were also categorized according to nucleotide substitutions as either transitions (A↔G or C↔T) or transversions (A↔C, C↔G, A↔T, G↔T). Our results of nucleotide changes showed that guanine (G) and cytosine (C) nucleotides mutate at 1.2-fold higher rates than adenine (A) and thymine (T) nucleotides (**Table 2**). The results also indicated that at a higher proportion of missing data, both minor allele frequency (MAF) and proportion of heterozygous reached lower values (**Figure 2**). Six hundred and eighty-eight (688) putative out of 21,324 SNP markers obtained after filtering and imputation were used for downstream analyses. The gene diversity (GD) analysis revealed that the entire panel varied from 0.09 to 0.50 with an average of 0.30 ± 0.12. Moreover, among the three ecological regions, the highest level of genetic diversity was observed in accessions collected from sub-humid ecology (0.201), and the lowest in the arid ecology (0.004). Also, based on country of provenance, accessions from Guinea and Nigeria showed the highest level of genetic diversity with respectively 0.209 and 0.200 while the least level was observed in accessions from Niger (0.003) (**Table 3**). The AMOVA results indicated that a large proportion of total genetic variation (67%) segregates among fonio accessions rather than between ecological zones, as well as the country of provenance (**Table 4**).

Population Structure and Genetic Relatedness

The outputs of the structure analysis indicated that the likelihood of Delta K (ΔK) peaked at $K = 2$ (**Figure 3A**) revealing that two sub-populations (population I and population II) showed the highest probability for population clustering (**Figure 3B**). The distribution of fonio accessions among the two sub-populations revealed that population II had more fonio accessions (61.39%) than population I (38.61%). The accessions from population I were mainly composed of accessions from arid and semi-arid ecologies (Nigeria, Mali and Niger) whereas most accessions from population II were from semi-arid and sub humid ecologies (Benin, Guinea, Burkina Faso). In addition, both of the two sub-populations diverged significantly as explained by the mean value of fixation index (Fst) for each of the sub-populations with 0.355 and 0.935 for population I and population II respectively (**Table 5**).

Furthermore, the Neighbor-Joining dendrogram results based on dissimilarity among 259 fonio accessions revealed also two heterogenetic clusters confirming the results of the Structure analysis. Cluster I (45.18%) was mainly composed of accessions originating from Benin, Niger and Mali while Cluster II (54.82%) was essentially composed of accessions from Nigeria, Guinea, Mali, and Burkina Faso (**Figure 4**).

Population Differentiation

The degree of differentiation among fonio genotypes from different ecological regions and countries of provenance are shown in **Table 6**. Significant genetic differentiation was found among fonio accessions from different ecologies as indicated by the overall Weir and Cockerham's Fst-value of 0.24. Pairwise Fst-values varies from 0.19 to 0.71. The lowest Fst-value (0.19) was observed between arid and sub humid ecologies while the highest Fst-value (0.71) was observed between arid and sub-humid ecologies. However, the overall Weir and Cockerman's Fst-value based on country of provenance of fonio accessions was merely higher (0.26) compared to that of different ecological regions (0.24). Furthermore, pairwise Fst-values varied from 0.10 to 0.59. The highest Fst-value was observed between Nigeria and Benin (0.59) while the lowest value was observed between Burkina Faso and Guinea (0.10) (**Table 6**).

Discriminant analysis of principal components (DAPC) was estimated using the detected number of clusters. The 10 first PCs (60% of variance) of PCA and two discriminant eigenvalues were retained (**Figure 5**). The results showed that fonio accessions can be clustered in three groups and discriminant function 1 clearly differentiated cluster 2 from cluster 3. Cluster 1 consisted of 66.02% (171 accessions), while cluster 3 and cluster 1 had 20.46% (53 accessions) and 13.52% (35 accessions), respectively. Accessions from Benin, Burkina Faso, Mali, and Niger were more dominant in cluster 1 whereas the majority of accessions from clusters 2 and 3 were originated from Nigeria and Guinea. Results of pairwise Fst revealed high genetic differentiation among clusters with overall Weir and Cockerham's Fst-value of 0.68. Pairwise Fst-values ranged from 0.31 to 0.72 (**Supplementary Table 1**). The lowest Fst-value (0.31) was found

TABLE 2 | Proportion of transition and transversion SNPs discovered.

Allelic information	Transition		Transversion			
	A/G	C/T	A/C	A/T	G/C	G/T
Number of allelic sites	5,861	5,790	2,231	1,196	4,044	2,202
% of allelic sites	27.485	27.153	10.462	5.608	18.965	10.327
Total	11,651		9,673			
Percentage	54.638		45.362			

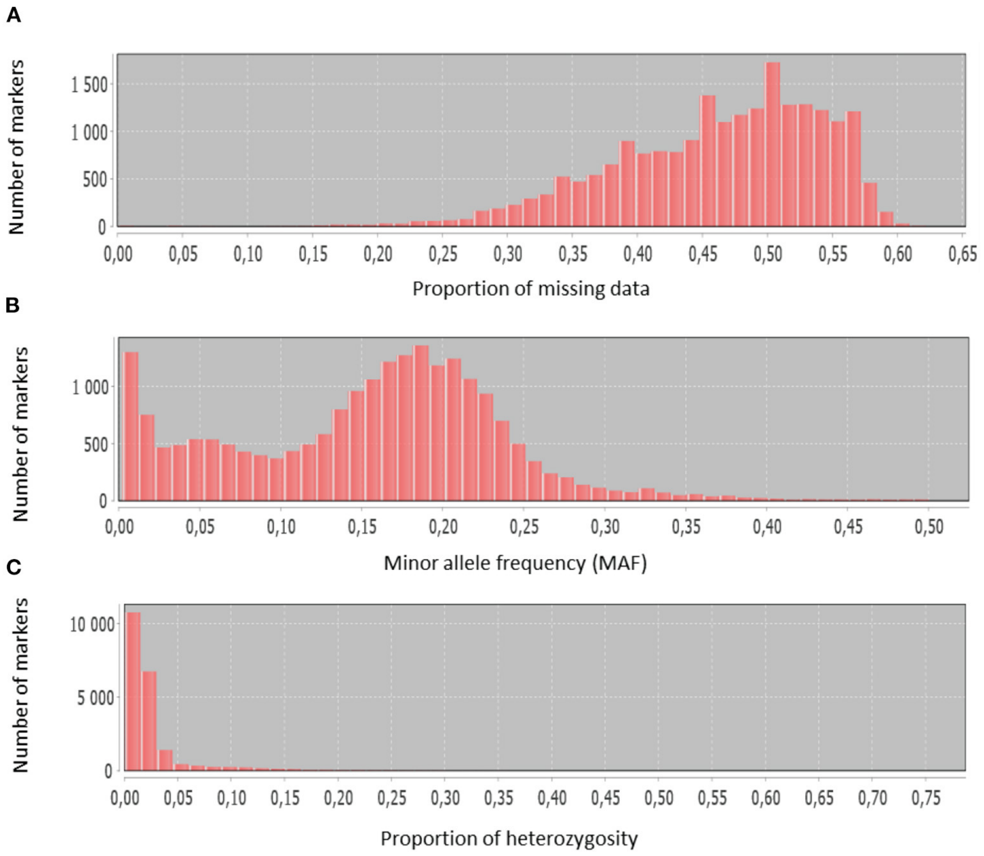


FIGURE 2 | Histograms showing basic SNP characteristics: **(A)** proportion of missing data, **(B)** minor allele frequency, **(C)** proportion of heterozygosity.

between cluster 2 and cluster 3. An intermediate Fst-value (0.69) was observed between cluster 1 and cluster 2. Moreover, the highest Fst-value (0.72) was detected between cluster 1 and cluster 3.

Linkage Disequilibrium (LD)

LD was analyzed across the fonio genome. Of the 21,324 SNPs, 17,045 (80%) covering the entire genome were in true LD based on the Comparison between pairs of markers. The LD patterns analysis revealed that the large red blocks of haplotypes along the diagonal of the triangle plot indicate the high level of LD between the loci in the blocks (**Figure 6**).

The LD decay plot showed that few LD values were found over a short physical genetic distance which decreased rapidly, with very low decline at longer distances between markers across the genome. In the present study, LD decayed rapidly below $r^2 = 0.64$, reaching 0.56 at a distance of 5 Mb (**Figure 7**).

DISCUSSION

SNP Markers Discovery and Genetic Diversity

Genotype-by-sequencing (GBS) provides new technologies for breeders with cost-effective, high-depth coverage, and high throughput sequencing platforms (Elshire et al., 2011; Fu and

TABLE 3 | Genetic diversity estimations of 259 fonio accessions based on ecological regions and country of provenance.

Population	Number of individuals	Observed heterozygosity	Expected heterozygosity	Inbreeding coefficient
Entire panel	259	0.026	0.274	0.87
Ecology				
Arid	50	0.004	0.005	0.22
Semi-arid	183	0.018	0.164	0.86
Sub-humid	26	0.042	0.201	0.79
Country of provenance				
Benin	42	0.004	0.057	0.92
Burkina Faso	4	0.005	0.006	0.19
Guinea	29	0.036	0.209	0.83
Mali	28	0.006	0.005	0.17
Niger	28	0.004	0.003	0.32
Nigeria	128	0.028	0.200	0.86

TABLE 4 | Analysis of molecular variance (AMOVA) results.

	Df	Sum of Squared	Mean Squared	Variance	% Est.variance	*P-value*
Between Population (ecological regions)	2	4512.53	2256.26	29.91	28.66	< 0.05
Between Subpopulation (country of provenance)	5	820.04	164.008	4.72	4.52	< 0.001
Within samples (accessions)	251	17503.34	69.73	69.73	66.82	< 0.001
Total	258	22835.91	88.51	104.36	100	

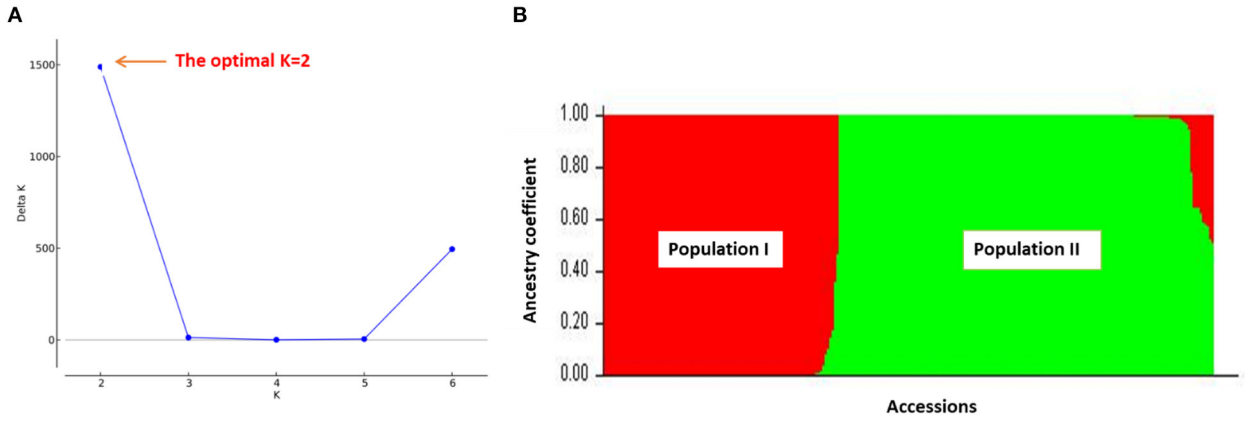


FIGURE 3 | The STRUCTURE results of 259 fonio accessions using 688 SNP markers: (**A**) the optimal value of K ($K = 2$); (**B**) Bar plot of accessions showing two subpopulations (Population I = red color and Population II = green color).

Peterson, 2011; Egan et al., 2012; Poland and Rife, 2012; Mir et al., 2013; Kim et al., 2016; Voss-Fels and Snowdon, 2016). The high throughput multiplexing genotyping platforms used in this study for assessing genetic diversity and population structure in a panel of 259 fonio accessions yielded 21,324 quality SNP markers. The relatively high proportion of missing data observed in this study might be due to the low read coverage in this dataset during genotyping because SNPs with low read coverage are more prone to errors than SNPs with high read coverage (Furuta et al., 2017). This shows the importance of sequencing to higher read depths to minimize missing data. On the other hand, this could also be explained by DNA contamination during leaf sampling which affects the quality of DNA. This result is in accordance with that of Taberlet et al. (1996) who reported that both DNA quality and or low quantity cause genotyping errors by favoring allelic dropouts and false alleles. Furthermore, in this study, the observed nucleotide changes may be due to inherent characteristics of DNA (e.g., cytosine deamination) that often cause a mutational bias in plant genomes (Gaut et al., 2011). However, the mutation rate obtained in this study was

TABLE 5 | Proportion of membership, estimate of genetic diversity parameters obtained from Structure results.

Population	Inferred clusters	Mean Fst	Expected heterozygosity	No. of genotypes
Population I	38.61	0.355	0.356	100
Population II	61.39	0.935	0.013	159

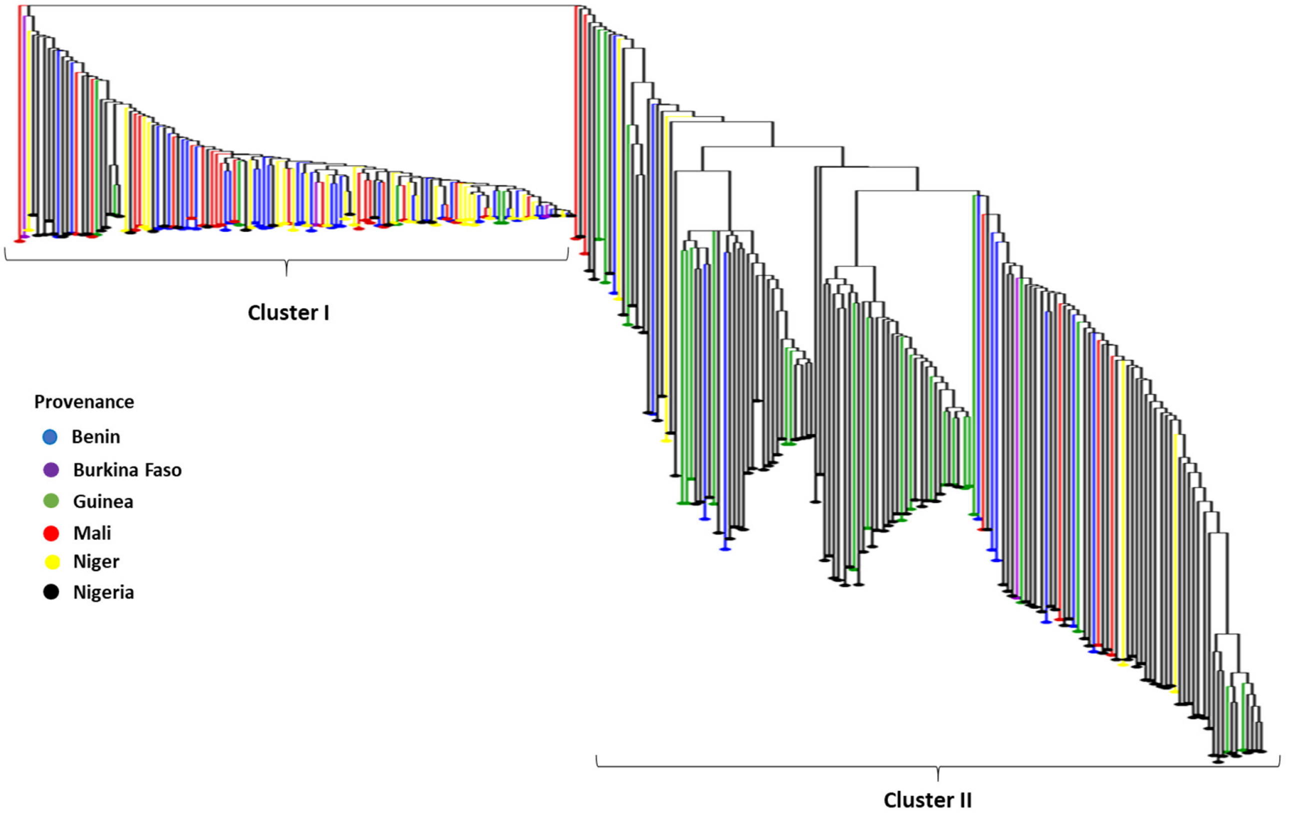


FIGURE 4 | The Neighbor-Joining dendrogram based on dissimilarity among 259 fonio accessions using 688 SNP markers. NB: Depending on the provenance accessions were colored as follows: Benin=blue, Burkina Faso = purple, Guinea = green, Mali = red, Niger = yellow and Nigeria = black.

lower compared to that reported earlier in maize (Morton et al., 2006).

Previously, genetic diversity of fonio millet was investigated with a different type of markers and based on small data sets limiting the chance of getting high polymorphic and informative markers (Hilu et al., 1997; Adoukonou-Sagbadja et al., 2007; Animasaun et al., 2018). The results revealed that the markers developed in this study were highly reproducible, with a high call rate and high polymorphism information content suggesting that these markers are very important for future fonio genetic improvement. This shows the extreme importance of genotyping by sequencing analysis for SNPs discovery, harnessing genetic diversity, and genotyping in most orphan small cereals where reference genome is not yet available. The high polymorphic markers obtained in this study are within the range of values reported in earlier SNP-based genetic diversity studies in other small millets (Kumar et al., 2016; Johnson et al., 2019). However, since genetic diversity is a true indicator of the degree of genetic variance within individuals in a population, in this study a moderate level of genetic diversity was observed among fonio accessions. This was further supported by the result of AMOVA, with a large proportion of total genetic variance found within fonio accessions reflecting that to some extent our collection has captured the diversity in the crop. This result is also consistent with that reported earlier by Adoukonou-Sagbadja et al. (2007) who assessed genetic diversity of 122 fonio accessions using AFLP markers. Furthermore, compared to these results a lower level of genetic diversity was reported in foxtail millet (Wang et al., 2010). This could be attributed to factors such as inbreeding due to the self-pollinated nature and in other hand the small population size (Barnaud et al., 2012; Abrouk et al., 2020). These factors have been reported to contribute to a reduction in genetic diversity in crop plants (Bhandari et al., 2017). The results revealed also that the genetic diversity in *D. exilis* is concentrated in sub humid and semi-arid ecologies of West Africa comprising the Upper Niger basin (Guinea and Mali), Northwestern Benin Republic,

TABLE 6 | Pairwise Fst analysis results among ecological regions and countries of provenance of 259 fonio millet accessions.

Ecology

	Arid	Semi-arid	Sub-humid
Arid	-		
Semi-arid	0.19*	-	
Sub-humid	0.71**	0.19*	-

Overall Weir and Cockerham's Fst-value = 0.24*

Country of provenance

	Mali	Niger	Benin	Guinea	Burkina Faso	Nigeria
Mali	-					
Niger	0.29*	-				
Benin	0.28*	0.32**	-			
Guinea	0.30*	0.52**	0.28*	-		
Burkina Faso	0.07*	0.27*	0.13*	0.10*	-	
Nigeria	0.29**	0.53***	0.59***	0.27**	0.17*	-

Overall Weir and Cockerham's Fst-value = 0.26**

= significant at 5%, **= significant at 1%, *= significant at 0.1%.*

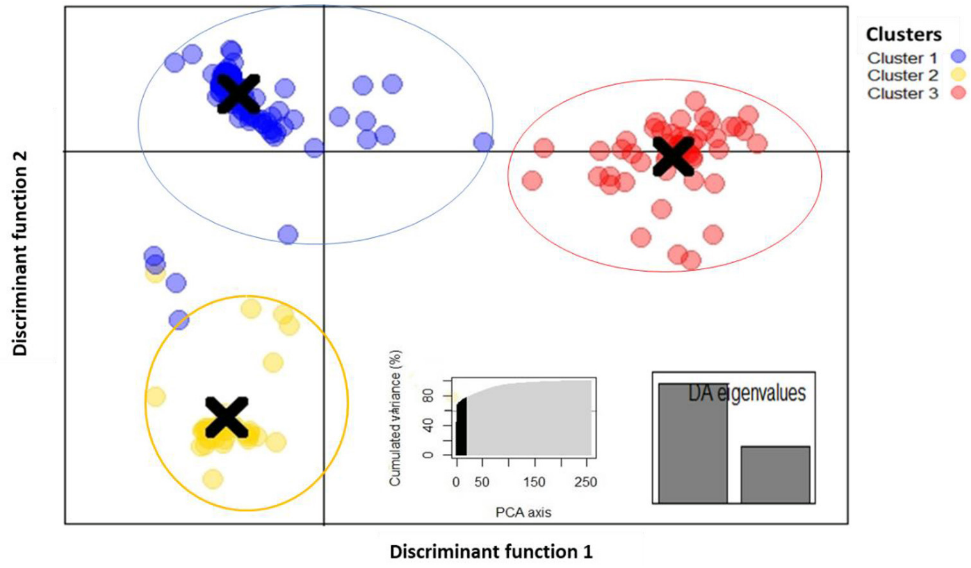


FIGURE 5 | Discriminant analysis of principal components plot of the 259 fonio genotypes. using 688 SNP markers grouped into 3 clusters using 2 discriminant functions. Within the graph PCA axis (left) and eigenvalues (right).

Southwestern Burkina Faso and Northern Nigeria. This result confirms the Upper Niger basin as center of diversity of *D. exilis* as reported earlier by Portères and Harlan (1976). However, the level of genetic diversity observed in Nigeria suggests the proposition of Northern Nigeria as the second center of diversity of white fonio. The low genetic diversity found in the arid ecology of Niger and part of Mali may therefore be related to the founder effect that occurs when a small group of individuals that is not genetically representative of the population from which they came establish in a new area. Similar results were reported by Adoukonou-Sagbadja et al. (2007) who stated that founder effect was the main cause of low genetic variation observed in white fonio from the Atacora mountain zone (Benin and Togo areas).

Clustering and Genetic Relatedness of Fonio Accessions

Structure analyses revealed two divergent subpopulations while the Neighbor-Joining dendrogram clustered fonio genotypes in two distinct groups based on ecological regions and country of provenance, respectively. On the contrary, previous studies revealed that fonio accessions clustered in three groups and six subpopulations based on principal component analysis (PCA) and structure analysis, respectively (Abrouk et al., 2020). This difference in clustering fonio accessions could be attributed to the sample size and geographical sources where fonio accessions were collected, as well as the number of SNP markers used. For instance, in our study, we used 21,324 SNP markers and

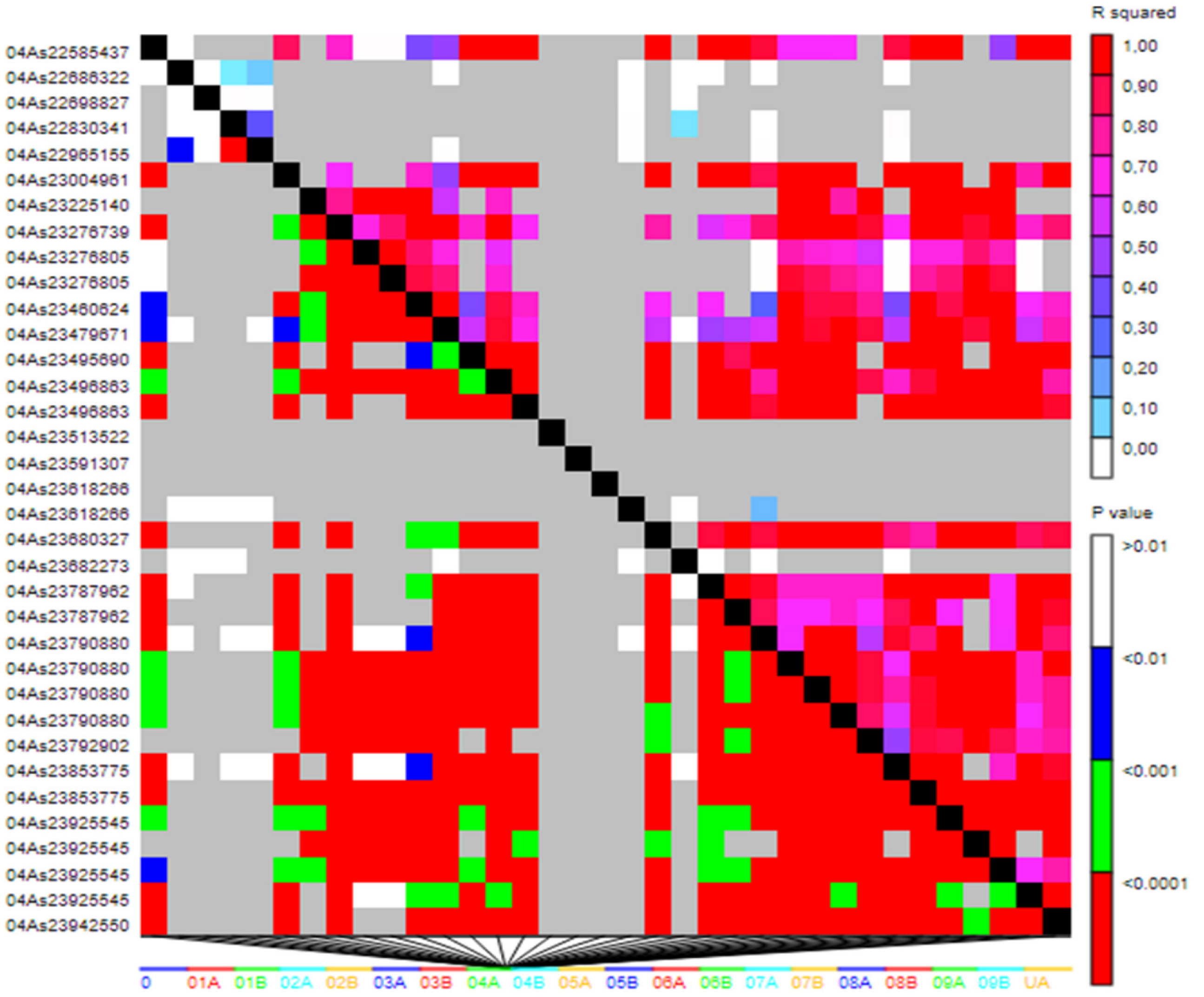


FIGURE 6 | LD patterns plot based on 17,045 SNP markers. The relationship between two markers is represented by each cell with the color codes for the presence of significant LD. In both diagonals, colorful bar codes are shown for the significant threshold levels.

259 fonio accessions collected from six West African countries while in previous studies, Abrouk et al. (2020) used 11,046,501 SNP markers and 166 *D. exilis* accessions collected from seven West African countries (Guinea, Mali, Benin, Togo, Burkina Faso, Ghana, and Niger). Nonetheless, Wang et al. (2021) used 13,546 SNP markers and identified three clusters while assessing the genetic diversity of 130 fonio accessions collected from Mali and Niger based on principal coordinates and dendrogram relationship analyses. This confirms our results of discriminant analysis of principal components, although the numbers of countries where fonio germplasm was collected, sample size, and SNP markers were lower compared to in our study. This shows the importance of diversifying sources of germplasm collection and using dense markers in order to capture the maximum diversity. Furthermore, the discrepancies between the results of our study and those of Adoukonou-Sagbadja et al. (2007) may be due to different type of markers (SNP vs. AFLPs markers) and the small data sets used, although fonio accessions were collected from nearly the same countries of provenance.

Varshney et al. (2007) reported that AFLP and SSR markers were more appropriate for fingerprinting and diversity analysis while SNP markers were considered as the best class for characterizing and conserving the genebank materials. Importantly, SNP markers can be digitalized or represented as graphic formats (Garafutdinov et al., 2020) across different genotypes making them the markers of choice for genetic diversity studies with recent advances in next generation sequencing technologies (NGS). The results of clustering fonio accessions in different groups demonstrate the influence of environmental factors and human activities on genetic variability as reported earlier (Abrouk et al., 2020). According to Wondimu et al. (2021), populations were structured based on geographical

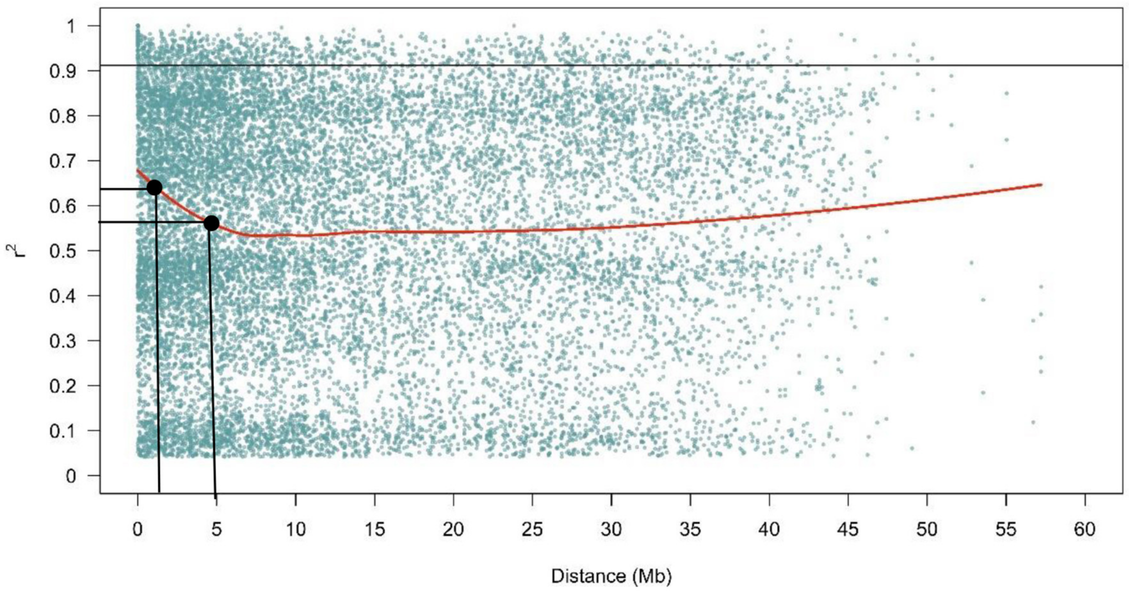


FIGURE 7 | The LD decay plot in the fonio diversity panel. The values on the Y-axis represent r^2 while the X- axis constitute the physical distance in Mb.

patterns and botanical races in sorghum. This is supported by the finding of Deu et al. (2010) who stipulated that seed exchange and food preferences were other factors that affected population structure in sorghum. However, in this study fonio landraces clustered irrespective of their ecological regions and country of provenance backgrounds implying that alleles from different sources co-occur within individuals. This shows the importance of admixture, a phenomenon that potentially increases genetic variation by decreasing inbreeding depression (Shi et al., 2018). This phenomenon could likely be due to the existence of gene flow through germplasm exchanges that frequently occur between different regions as reported earlier (Sidibé et al., 2020). This result is consistent with that of Kabbaj et al. (2017) who reported among wheat genotypes including landraces, varieties, and elite lines collected from diverse origins higher admixtures. The difference observed between the two clustering approaches Structure model and DAPC analysis could be attributed to the sensitivity of the Bayesian clustering algorithm to sample size, number of populations, number of loci scored and the type of markers (Evanno et al., 2005). On the other hand, with DAPC a considerable inaccuracy in terms of both the suggested number of clusters and the placement of individuals in those clusters was reported when the migration rates were high (Miller et al., 2020).

Regional Differentiation

A large genetic differentiation was found among fonio accessions based on country of provenance compared to that of ecological regions suggesting that the regional differentiation of fonio accessions is a country based rather than ecological regions. This may be likely due to farmers' management practices that are specific to ethnolinguistic groups because fonio accessions used in this study were provided by different ethnic groups of farmers from different countries. This is supported by the findings of Abrouk et al. (2020) who cited farmers' ethnolinguistic groups as among the factors that shaped genetic diversity in fonio. A possible explanation can be related to the self-pollination nature of the crop (Barnaud et al., 2012) that limits gene flow among fonio genotypes. This result corroborates the findings of Adoukonou-Sagbadja et al. (2007) who reported a high degree of differentiation within fonio accessions. Conversely, a low level of regional differentiation was reported by Wondimu et al. (2021) for 338 cultivated sorghum in Ethiopia. However, the low degree of differentiation observed between semi-arid and sub-humid zones can be explained by seed exchanges among fonio farmers due to the proximity of these zones. This finding is in agreement with that reported previously by Akohoue et al. (2020) in Kersting's groundnut and confirms the high genetic distance observed between arid and sub-humid ecologies probably due to isolation by distance (Wright, 1946). Furthermore, the great divergence observed between fonio accessions from Nigeria and the remaining countries could be also explained by geographic isolation leading to speciation which is the formation of distinct species in the course of evolution. Surprisingly, fonio accessions from Nigeria and Benin Republic for instance were collected from the same ecology (semi-arid region), but the highest divergence among fonio was observed between these two countries confirming the hypothesis that regional differentiation was country-based. Since the two groups of population (Nigeria and Benin) are thriving in the same ecology, the divergence may likely arise from management practices firstly because farmers are from different ethnolinguistic groups (e.g., Hausa ethnic group from Northern Nigeria and Ditamari from the North-western Benin Republic). This is further supported by Abrouk et al. (2020) who highlighted the strong impact of anthropogenic factors on shaping the genetic diversity of fonio. Thus, future research should investigate drivers that influence management practices of fonio millet (*D. exilis*) across different ethnolinguistic groups of West Africa. Secondly, the two populations might have experienced different pressures of biotic and abiotic stresses and thirdly, the informal seed systems that limit the seed exchange of improved fonio varieties (Adoukonou-Sagbadja et al., 2006; Sekloka et al., 2015) may probably cause this genetic divergence.

Linkage Disequilibrium (LD) Patterns

Even though any quantitative traits loci (QTLs) and/or markers associated with different traits on the chromosomes have been reported, yet in fonio the results show potential markers distributed on different chromosomes in the genome that were in true LD, paving ways for genome- wide association studies and genomic selection. Hence, further investigation should be made in order to map genomic regions associated with important well-preferred traits for both farmers and consumers. Furthermore, the results indicated a high level of non-random association between the loci in the blocks suggesting that there has been a low or no recombination rate since LD block formations. Moreover, our finding revealed that on average fonio germplasm was characterized by a high linkage disequilibrium with slow LD decay over long physical distances between markers. This could be attributed to the mating system of the crop because selfing limits heterozygosity, while increasing homozygosity with the consequence of reducing the effectiveness of recombination rate. As a result LD is expected to be maintained over long physical distances (Gaut and Long, 2003). Similar results were reported in wild barley (Morrell et al., 2005). The application of artificial hybridization techniques between distant genetic parents, mutagenesis and transgenesis could potentially increase recombination rate, thereby increase genetic diversity among fonio germplasm. In addition, the results of LD patterns provide relevant information on the recombination history under natural selection since no breeding efforts through artificial hybridization have been reported yet. Therefore, future LD patterns analysis should be investigated in-depth in order to shed light on other factors that alter certain genomic regions leading to the differentiation in the LD patterns among fonio accessions.

CONCLUSION

The present study demonstrated the potential of genotyping by sequencing (GBS) to discover SNPs, to assess genetic diversity and population structure of fonio millet accessions. The results indicated also that grouping of fonio accessions was irrespective of geographical origin backgrounds while the regional differentiation was based on the country of provenance rather than the ecological regions. Furthermore, the high genetic divergence observed between accessions from Nigeria to the other countries particularly the Benin Republic suggests geographic isolation. Moreover, the relatively high non-random association between alleles at two loci observed in this study could be attributed to the selfing nature of the crop, which reduces the recombination rate. Finally, our findings highlighted the importance of population structure, and Linkage Disequilibrium patterns in genetic diversity study and provide relevant information for association mapping and genomic selection in fonio millet.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Dryad and https://doi.org/10.5061/dryad.vmcvdncsr.

AUTHOR CONTRIBUTIONS

AI and EA-D conceptualized the study, wrote the manuscript, and edited the manuscript. AI, KI, and AM carried out data analysis. EA-D, CA, KI, AM, HO, MG, and CB critically revised the draft and updated the manuscript for publication. All authors contributed to the article and approved the submitted version.

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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.2021.699549/full#supplementary-material

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Seed Systems of Traditional African Vegetables in Eastern Africa: A Systematic Review

Mathieu A. T. Ayenan[1,2]*, **Lys Amavi Aglinglo**[1], **Herbaud P. F. Zohoungbogbo**[1], **Sognigbe N'Danikou**[3], **Judith Honfoga**[1], **Fekadu F. Dinssa**[3], **Peter Hanson**[1] and **Victor Afari-Sefa**[1]

[1] World Vegetable Center, West and Central Africa—Coastal and Humid Regions, Cotonou, Benin, [2] West African Centre for Crop Improvement, College of Basic and Applied Sciences, University of Ghana, Legon, Ghana, [3] World Vegetable Center, Eastern and Southern Africa, Arusha, Tanzania

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South Africa

Correspondence:
Mathieu A. T. Ayenan
mathieu.ayenan@worldveg.org

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Traditional African Vegetables (TAV) play an important role in the livelihoods, food and nutritional security of local populations. Access to high-quality seeds of improved varieties is a foundation for increasing crop productivity. TAV seed systems have received little attention. We systematically reviewed the literature to assess the sustainability (seed quality, availability, accessibility, affordability, and profitability of seed businesses) of TAV seed systems in Eastern Africa. The review revealed that the private sector mediated seed system (i.e., formal) offered higher potential for seed quality, and profitability for seed growers. The community-based seed system showed higher potential in ensuring a better access to seeds. The quality of TAV seeds was partially addressed in the studies with a focus on germination percentage and purity, which varied across systems, crops, and geography. While there was a trend of poorer seed quality in the informal system, seed quality in the formal and community-based systems was not necessarily better. The affordability of seed from the various systems (private sector mediated, community based and informal) needs further investigation. Because TAV seed systems are localized and differ significantly among and within countries, tailored interventions are required when promoting a given TAV seed system. We identified inter-regional gaps in TAV seed systems studies, with all the studies concentrated in Eastern Africa, mainly in Tanzania and Kenya. Filling these gaps will require more investment in other regions in sub-Saharan Africa to document existing initiatives or initiate interventions seeking to promote access to high-quality TAV seeds. This review sheds light on existing gaps in research on TAV seed systems to guide future interventions.

Keywords: traditional vegetables, seed quality, orphan crops, neglected and underutilized species, seed system

INTRODUCTION

Food and nutritional insecurity are a significant concern in developing countries, especially in sub-Saharan Africa, where 57% of the population cannot afford a healthy diet (FAO, 2020). Food insecurity is aggravated by micronutrient deficiencies, also known as "hidden hunger," a major impediment to social and economic development (Nugent et al., 2020). High malnutrition rates leading to non-communicable diseases such as hypertension, cancer, and obesity are public health challenges (World Health Organization, 2020). In addition, the consumption of fruits and vegetables is far below the recommended amount of 400 g/capita/day (FAO, 2020).

Traditional African Vegetables (TAV) are "plant species that are indigenous or naturalized to Africa, well adapted to or selected for local conditions, whose plant parts are used as a vegetable, and whose modes of cultivation, collection, preparation, and consumption are deeply embedded in local cuisine, culture, folklore, and language" (Towns and Shackleton, 2018). TAV [(e.g., amaranth (*Amaranthus* spp.), African eggplant (*Solanum* spp.)] are micronutrient-dense and provide nutritional balance to diets among populations whose main staple food is carbohydrate-based (FAO, 2020). The promotion of sustainable production and consumption of TAV is widely recognized as an effective strategy to contribute to diet diversification and to improve nutrition and food security in developing countries (Grubben et al., 2014; Keatinge et al., 2014, 2015; Ojiewo et al., 2015; Ochieng et al., 2018). Many TAV are rich in vitamins and phytochemicals with antibiotic, antioxidants, and anticancer properties and have the potential to reduce the occurrence and severity of non-communicable diseases like hypertension, cancer, and obesity (Yang et al., 2013; Keatinge et al., 2015; Ojiewo et al., 2015). Some TAV are adapted to marginal environments and require little external farm inputs (Ambrose-Oji, 2009; Ebert, 2014); hence, they are an asset for resource-poor farmers and especially women who often lack access to fertile land (Doss et al., 2015; Burke et al., 2018). TAV can contribute to adaptation to climate change by increasing the resilience of local farming systems. Leafy TAV have relatively shorter production cycles (about 30 days for some species) and can be less risk-prone than staple crops, which tend to be more vulnerable to environmental stressors (Ojiewo et al., 2015).

In most African countries, TAV production uses local landraces in low input systems and is intended for household consumption or sale in nearby towns and villages. In some countries, however, the production of certain TAV is shifting into more input-intensive and commercial systems targeting urban areas with high consumer demand (Cernansky, 2015; Ojiewo et al., 2015). Consequently, farmer demand for high-quality seeds of improved TAV varieties that meet the quality requirements of urban markets is also increasing. Like other TAV research areas, breeding improved varieties has received little attention. However, a few improved TAV varieties are available that can be promoted and produced by seed producers, including amaranth, African eggplant, African nightshade, jute mallow, kale/Ethiopian mustard, cowpea leaf, spider plant (Dinssa et al., 2016). The available varieties are predominantly open-pollinated (Dinssa et al., 2016). Only one private seed company has developed hybrid African eggplant (Dinssa et al., 2016).

Despite the potential of TAV as a source of income and in ensuring food and nutritional security (Schreinemachers et al., 2018), their production faces several challenges. Lack of a sustainable supply of quality seed is a primary constraint limiting TAV production (Adebooye et al., 2005; Afari-Sefa et al., 2013; Keatinge et al., 2015). Demand for TAV has increased in recent years but, limited availability and accessibility of quality seed of preferred varieties has constrained the ability of farmers to deliver quality products to consumers (Afari-Sefa et al., 2013). The primary seed source of TAV is the informal sector (Adebooye et al., 2005; Onyango, 2007; Coomes et al., 2015; Croft et al., 2018; Pincus et al., 2018) in which seed quality (genetic purity, germination, phytosanitary status) is highly inconsistent (Keatinge et al., 2015; Pincus et al., 2018). Seed companies are reluctant to invest in orphan crops such as TAV for several reasons, including non-supportive seed legislation, perceived low profit margin, low seed replacement rate, and fragmented seed markets (Minot, 2008), as well as uncertain prospects for development of hybrids of these crops and their demand on the seed market. The need to invest in the TAV seed system is equally underplayed by certification and quality control agencies (Keatinge et al., 2015; Ojiewo et al., 2015). These challenges have prompted increasing interest in developing alternative, farmer participatory TAV seed production and delivery systems. These include private sector mediated seed system (contracted seed production) and community-based seed production systems to make high-quality seed available to farmers (Karanja et al., 2011; Afari-Sefa et al., 2013; Kimenye, 2014; Kansiime and Mastenbroek, 2016). The different smallholder participatory seed production systems raise a question: Do private sector mediated and community-based seed systems ensure better seed quality, availability, accessibility, affordability, and higher profitability than informal seed systems in sub-Saharan Africa? A comparative analysis of the various studies in which TAV seed production systems were tested is necessary to assess their sustainability. Schreinemachers et al. (2021) provide an excellent analysis of the vegetable seed industry and strategies to strengthen the industry as a whole. Kuhlmann et al. (2021) depict how seed laws and regulations affect the private vegetable seed business in SSA. The present systematic review adds to the literature on vegetable seed systems, focusing on TAV and the sustainability of seed production systems in sub-Saharan Africa.

CONCEPT CLARIFICATION

Seed Systems

A seed system is a set of activities contributing to variety development and seed production and delivery to farmers. Seed systems are often categorized into three types: formal, semi-formal, and informal. A formal seed system is characterized by a well-regulated and organized set of activities, from breeding to delivering certified seeds of known and registered varieties to farmers (Louwaars, 1995). The key distinctive features between formal seed systems and other systems are the registration of seed producers or seed companies and the certification process, which is usually controlled by a public regulatory body. The formal seed system entails various types of arrangements among actors. This review considers contracted seed production or a private sector mediated seed system as part of the formal seed system. A private sector mediated seed system involves individual smallholder seed producers or their cooperatives producing certified seeds under a contract with a private seed company (e.g., Simlaw Seed Limited, East African Seeds, Alpha Seeds Plc) (Karanja et al., 2011; Afari-Sefa et al., 2013; Kimenye, 2014; Kansiime et al., 2021). The informal seed system is outside the control of government agencies, with no external seed quality control. This system includes farmer-saved seed, gifts, barter, and seed purchasing from local markets. The semi-formal seed system (termed community-based) is at the interface of formal and informal seed systems. A community-based seed production system involves individual farmers or farmer cooperatives producing Quality Declared Seed (QDS).

Quality Declared Seed

Quality Declared Seed is a category of seed produced by a registered seed producer subject to quality control and complying with the minimum standards for the crop species concerned (FAO, 2006). The requirements for QDS are less stringent than those of certified seeds while guaranteeing satisfactory seed quality. QDS is an alternative seed quality assurance adapted to contexts where official seed regulatory bodies have limited resources to implement a complete certification scheme (FAO, 2006).

Sustainability of Seed Production

A sustainable seed production system ensures that "high-quality seeds of a wide range of varieties and crops are produced and fully available in time and affordable to farmers and other stakeholders" (FAO, 2021). A sustainable seed production system encompasses several components: seed quality, availability, accessibility, and profitability of seed businesses. Seed quality refers to genetic (adaptation, varietal purity), physiological (germination, vigor), sanitary (absence of diseases), and physical integrity (percentage of good seeds, free of stones and weed seed) (Almekinders and Louwaars, 1999). Seed availability is the ability to supply sufficient quantities of quality seed to meet the needs of farmers. Seed availability is critical to ensure seed security. Seed accessibility refers to the ability to deliver quality seed in locations within reasonable proximity to farmers when the seed is needed. Seed is affordable when it meets farmers' purchasing power. Profitability is the extent to which a business yields financial gain. Profitability is at the heart of the seed business. In the absence of government subsidies, it determines the viability of the business entity since it influences the decision to participate in seed production.

MATERIALS AND METHODS

Literature Search and Eligibility Criteria

We performed a literature search using a combination of the following terms: formal seed system, farmer-led seed enterprises, quality declared seed, community-based seed system, informal seed system, seed quality, seed accessibility, seed affordability, vegetable seed business, traditional vegetable, traditional African vegetable, traditional leafy vegetable, indigenous vegetable, neglected vegetable, underutilized vegetable, vegetable landrace (**Supplementary Table 1**). We searched in English and French (with translated search strings) without limiting the publication date in various databases, including Web of Science, Scopus, CAB abstract, Google Scholar, and AGRIS. The search in Web of Science, Google Scholar, and Scopus was conducted using Publish or Perish Software with functions "Title words" and "Keywords" (Harzing, 2007). The eligibility criteria are presented in **Table 1**. Our initial intent in this systematic review was to cover sub-Saharan Africa, but the relevant studies were only carried out in Eastern Africa, hence the focus on this region in the subsequent sections.

Validity Assessment

Traditional risk of bias tools developed for randomized control trials or observational studies in clinical settings or evaluations of interventions (Moher et al., 2009) cannot directly be applied to the studies on seed production systems. Instead, we used the following four internal and external validity checklist elements identified from West et al. (2002): Description of the study population, sample size justification, application of appropriate statistical, factual outcomes (conclusions supported by data). We also assessed the strength of each paper following Koutsos et al. (2019) ranking conference (papers) as "low"; case and observational studies as "medium"; and experimental studies as "strong."

Data Extraction and Analysis

We carefully read the 13 papers included in the review and extracted information relevant to our objective using a prepared template. Besides the metadata (authors, title, source of title, keywords, abstract, document type, authors' affiliation), the extracted information was related to the country of studies, type of seed system, seed production system (i.e., private sector mediated, community-based, informal), seed categories (certified, QDS, non-certified), effects of the seed systems on seed quality, availability, accessibility, affordability and profitability, and production and marketing challenges. A data matrix was prepared using these themes as column names and the studies as row names. The extracted data were coded (one when a paper addressed a theme or zero otherwise) and subjected to the computation of frequencies and visualization. Two researchers extracted data from the full texts, and a third researcher cross-checked the extracted data for consistency.

RESULTS

Literature Search Results

We exported the search results as CSV files, and after removing duplicates, we obtained 1,742 publications. These publications underwent a screening using the inclusion and exclusion criteria (**Table 1**). We first screened the titles of the papers and excluded 1,511 papers. The majority of the papers dealt exclusively with the nutritional and economic importance of TAV, ethnobotany and diversity studies, and utilization of TAV. After the first screening, we retained 198 for abstract screening and excluded 165 papers for geographical coverage, publication type (review article), and focus. We read the full texts of 33 papers, and 20 were excluded (**Figure 1**). Finally, 13 papers met the inclusion criteria and were deemed relevant for the review (**Figure 1**).

Overall, the studies included in the review had good quality reports, and the population was described. However, eight studies (Onim and Mwaniki, 2008; Karanja et al., 2011; Afari-Sefa et al., 2013; Kimenye, 2014; Kansiime et al., 2016; Rajendran et al., 2016; Mvungi et al., 2020; Ndinya et al., 2020) failed to present the justification of the sample size. Five studies

TABLE 1 | Inclusion and exclusion criteria of the papers in the review.

Criteria	Inclusion	Exclusion
Language	English or French	Other languages
Type of research	Primary research	Secondary data
Type of document	Peer-reviewed articles, book chapters, conference papers, proceedings, working papers, Project reports, online theses	Book review, note, erratum, opinion papers, review articles
Focus and content	Main focus of the paper is seed systems of TAV Paper explicitly addresses relationship between seed system and seed quality, availability, accessibility, affordability and/or profitability	Seed systems of vegetables in general without explicit references to TAV Paper does not address relationship between seed system and seed quality, availability or accessibility or affordability (e.g., Paper on topics such as mode of pollination/reproduction, varietal preferences, diversity studies, ethnobotany, economic importance, nutrient contents of TAV)
Geographical coverage	Areas within sub-Saharan Africa (SSA)	Studies carried out in other regions than SSA

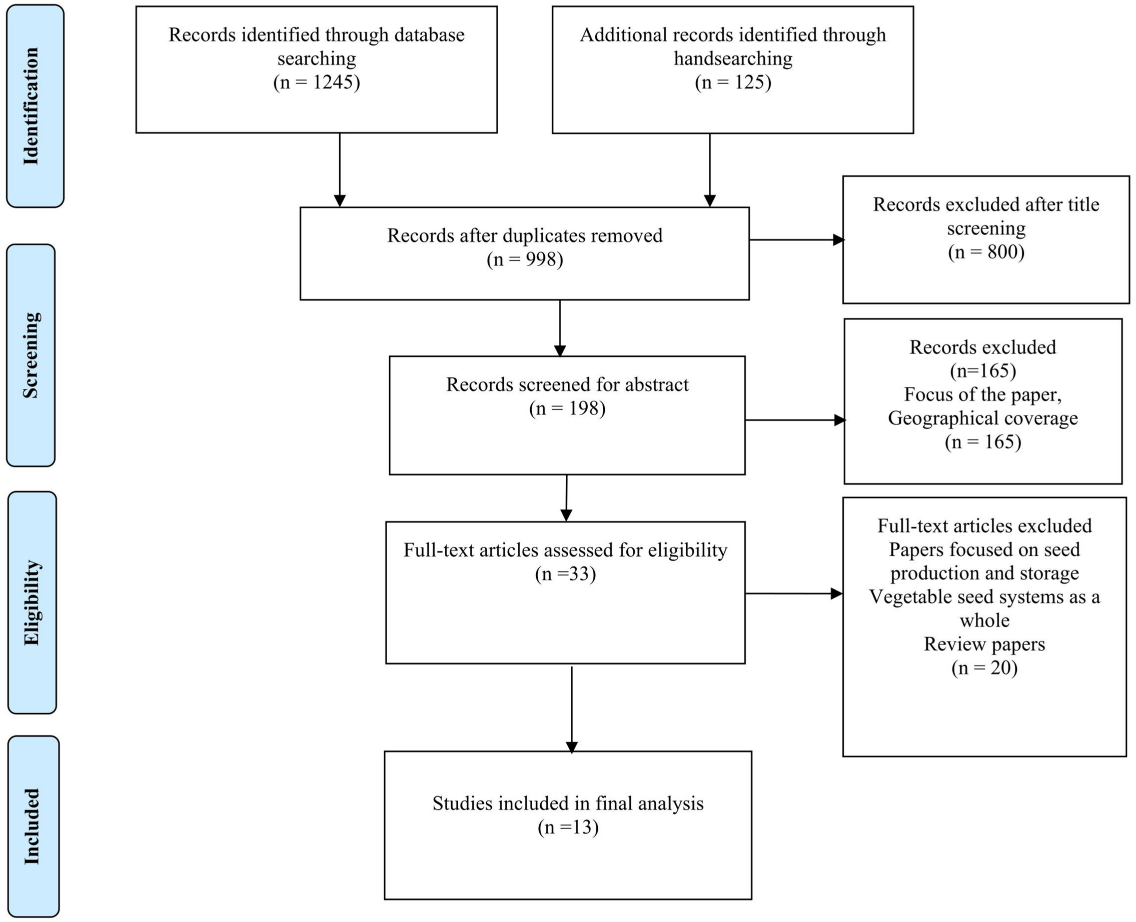

FIGURE 1 | Modified preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram. Adapted from Moher et al. (2009).

(Onim and Mwaniki, 2008; Karanja et al., 2011; Afari-Sefa et al., 2013; Kimenye, 2014; Kansiime et al., 2016) were narrative and did not apply satisfactory analytic techniques. The strength of evidence ranged from low (Onim and Mwaniki, 2008; Karanja et al., 2011) to strong (Croft et al., 2018) (**Supplementary Table 2**). The remaining studies (Afari-Sefa et al., 2013; Kimenye, 2014; Kansiime et al., 2016, 2018, 2021; Rajendran et al., 2016; Pincus et al., 2018; Kimaru et al., 2019; Mvungi et al., 2020; Ndinya et al., 2020) had moderate strength of evidence.

The studies on TAV seed systems were all carried out in Eastern Africa, with seven of the studies conducted in Kenya followed by Tanzania (6). The studies were primarily carried out by researchers from the World Vegetable Center Eastern

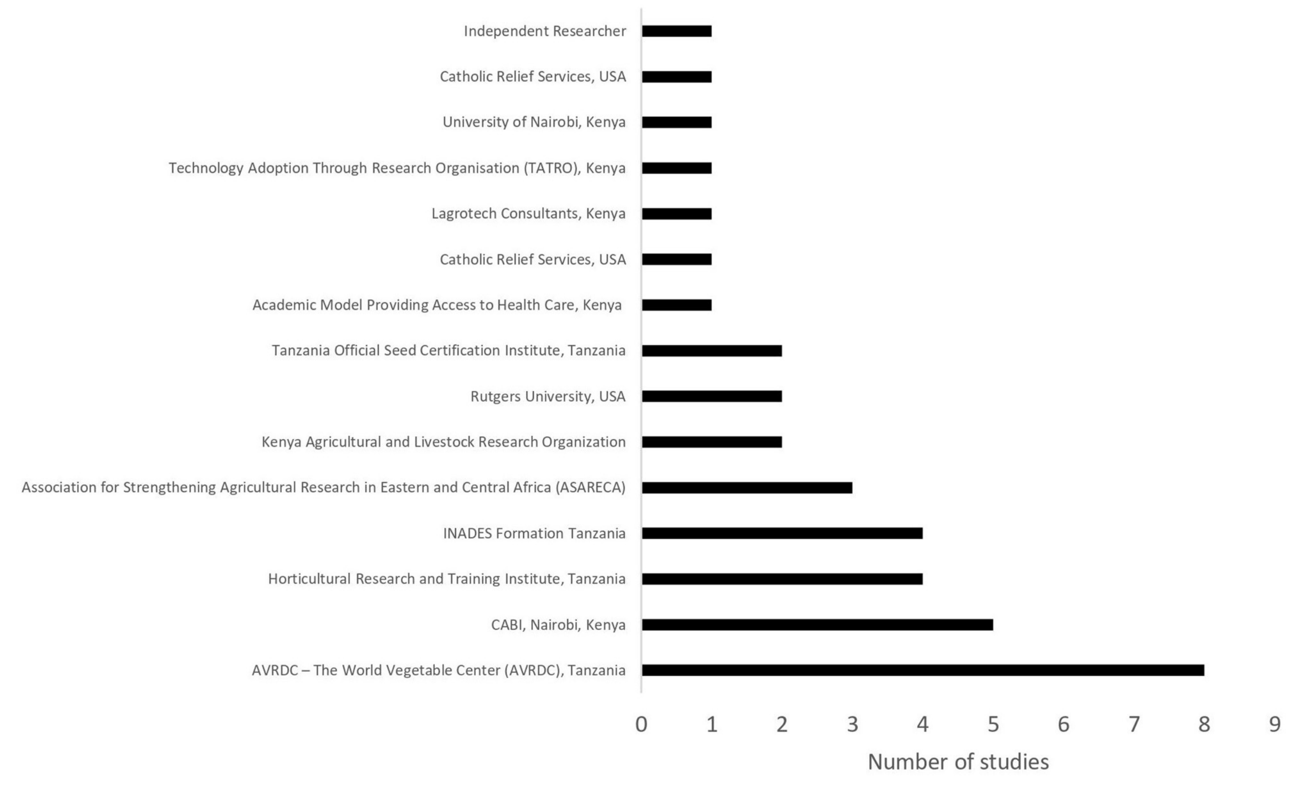

FIGURE 2 | Affiliations of the researchers working on TAV seed systems.

and Southern Africa regional office in Tanzania and the Center for Agriculture and Bioscience International (CABI) in Kenya (**Figure 2**).

We found nine TAV species (**Figure 3**) covered by the seed systems. African nightshade ranked first in terms of the number of occurrences in the studies (nine studies), followed by amaranth (eight studies), spider plant, *Crotalaria*, and jute mallow (five studies) (**Figure 3**). Pictorial representation of TAV reported in this study is given in **Figure 3**.

TAV Seed Categories Reported in the Studies

In Tanzania, Quality Declared Seed and certified seed were the predominant seed categories identified across the studies (Onim and Mwaniki, 2008; Karanja et al., 2011; Afari-Sefa et al., 2013; Kimenye, 2014; Kansiime et al., 2016, 2021; Rajendran et al., 2016). Non-certified TAV seed was the most frequent TAV seed category in Kenya (Onim and Mwaniki, 2008; Croft et al., 2018; Pincus et al., 2018; Kimaru et al., 2019).

Seed production and distribution channels were context-dependent and varied widely between and within countries. For instance, Rajendran et al. (2016) reported an exclusive Quality Declared Seed in the Dodoma region of Tanzania, while in Tanga and Morogoro regions, non-certified seed was the only seed category available to farmers.

Sustainability of Seed Production Systems

We evaluated the sustainability of the different systems using five criteria: quality of seed produced and delivered, seed availability, accessibility, affordability, and the profitability of the seed systems (Reyes and Maredia, 2014; FAO, 2021).

Seed Quality

Seed quality is of utmost importance for the viability of seed enterprises and food security as a whole. Good quality seed strengthens the trust of farmers in seed producers and encourages them to buy seed. Eleven studies addressed the effects of different systems on components of seed quality. Germination and genetic purity were the most common dimensions addressed (**Supplementary Table 3**). None of the studies assessed seed sanitary conditions.

Seed quality differs significantly within the same species, depending on the seed systems (private sector mediated, informal, community-based) (**Supplementary Table 3**). Seed of TAV produced and marketed in informal seed systems was generally of lower quality. In Kenya, Kimaru et al. (2019) found that only certified seed met the International Seed Testing Association (ISTA) standards, while non-certified and farmer-saved seed of African nightshade and other seed sold in the markets were of extremely low quality, with purity below 74%. Similarly, germination rates ranging from 33% for spider plant to 74% for slender leaf (*Crotalaria brevidens*) were reported in

A- African nightshade (9)
B- Amaranth (*Amaranthus* spp) (8)
C- Spider plant (5)
D- Slenderleaf (5)
E- Jute mallow (5)
F- African eggplant (3)
G- Cowpea (2)
H- African kale (Ethiopian mustard) (2)
I - Pumpkin (2)
Photo Credit: World Vegetable Center

FIGURE 3 | Overview of the TAV species reported in the studies. The values in brackets denote the number of occurrences of the TAV species in the studies included in the review **(A)** African nightshade (9), **(B)** Amaranth (*Amaranthus* spp) (8), **(C)** Spider plant (5), **(D)** Slender leaf (5), **(E)** Jute mallow (5), **(F)** African eggplant (3), **(G)** Cowpea (2), **(H)** African kale (Ethiopian mustard) (2), **(I)** Pumpkin (2).

Western Kenya in the informal system (Pincus et al., 2018). Poor quality of TAV seeds was also reported from seed companies (Onim and Mwaniki, 2008). Likewise, in Kenya, the germination percentage of amaranth and African nightshade seeds produced by farmers (informal seed system) was about two times higher than seed sourced from seed companies (Croft et al., 2018).

In Tanzania and Kenya, farmers involved in private sector mediated and community-based seed systems improved their seed quality (Karanja et al., 2011; Afari-Sefa et al., 2013; Kimenye, 2014; Kansiime et al., 2016, 2021). This improvement was linked to training received by seed producers, which improved their knowledge and technical know-how (Afari-Sefa et al., 2013; Kimenye, 2014). In Western Kenya, TAV seed producers were trained and received seed for multiplication from the Kenya Agricultural and Livestock Research Organization and its partners (Ndinya et al., 2020). However, the seed produced by those farmers was below the minimum required standards; it was not of better quality in germination percentage and purity than that produced by their non-trained counterparts (Ndinya et al., 2020). The seed produced by the trained farmers was not subjected to any quality assurance. In addition to the training provided to seed producers, a close follow-up during production, seed processing, and the certification process either through certified seed or QDS would be critical to ensure seed quality.

Seed Availability for TAV Production

Eight studies addressed the effect of the various systems on seed availability. In Tanzania, private sector mediated and community-based seed systems improved the availability of TAV seeds (Karanja et al., 2011; Afari-Sefa et al., 2013; Kimenye, 2014; Kansiime et al., 2016, 2021; Rajendran et al., 2016) (**Datasheet 1**). For example, from 2009 to 2010 in Kenya, through private sector mediated seed production, there was an increase of the quantity of amaranth seed from 2,134 kg to 5,918 kg; African nightshade from 3,832 kg to 27,997 kg; jute mallow from 1,770 kg to 17,706 kg; and *Crotalaria* from 6,669 kg to 24,253 kg (Kimenye, 2014). In Western Kenya, the informal seed system failed to ensure year-round seed availability for TAV, including amaranth, spider plant, and slender leaf (Pincus et al., 2018). These findings showed that the availability of TAV seed is context-dependent, and private sector mediated and community-based seed systems tend to contribute to improved seed availability (**Datasheet 1**).

Seed Accessibility for TAV Production

Seed accessibility is an essential component of the sustainability of seed systems and was investigated in eight studies. Private sector mediated and community-based seed systems improved seed accessibility across the countries (Afari-Sefa et al., 2013; Kimenye, 2014; Kansiime et al., 2016) (**Datasheet 1**). In Uganda, TAV seed dealers in private mediated seed system failed to meet farmers' seed demand (Kansiime et al., 2018). Similarly, farmers experienced challenges accessing TAV seed in the informal system in Kenya (Pincus et al., 2018). In Tanzania, the community-based seed system ensured higher accessibility of TAV seed to farmers because the produced seed was sold within the community, while in the private sector mediated system, the seed was not readily available within the community (Kansiime et al., 2021).

Access to TAV seed was found to be gender-biased in Tanzania, where female-headed households and older farmers were reported to have less access to certified TAV seed compared to their male and younger counterparts, respectively (Rajendran et al., 2016). Identifying the underlying causes for the disparity between male and female and young and adult farmers for access to good quality TAV seed is critical to designing gender-and age-appropriate seed interventions.

Seed Affordability for TAV Production

Seed affordability has not been addressed widely in the studies included in this review. Only three studies (Onim and Mwaniki, 2008; Kansiime et al., 2016; Croft et al., 2018) assessed the effect of seed systems on seed affordability. Seed from the informal system was reported to be more affordable than certified seed in Kenya (Onim and Mwaniki, 2008; Croft et al., 2018). In Tanzania, Kansiime et al. (2016) reported that seed from the private sector mediated seed and community-based seed systems was affordable. It is worth noting that none of these studies reported figures assessing the extent of seed affordability.

Profitability of TAV Seed Production

Providing evidence for the profitability of TAV seed could attract investments in the sector. Interestingly, seed system profitability was addressed in six studies (Afari-Sefa et al., 2013; Kimenye, 2014; Rajendran et al., 2016; Kansiime et al., 2018, 2021; Mvungi et al., 2020). In Tanzania, a comparative study between contract seed growers and community-based seed growers revealed that farmers engaged in the latter system had lower input costs and higher returns than contract seed growers (Afari-Sefa et al., 2013). Similarly, Kansiime et al. (2021) reported that farmers in community-based seed production systems earned about US$ 857 per ha while farmers in contract-based production systems (private sector mediated) earned US$ 192 from TAV seed. However, these findings were not in line with the report of Mvungi et al. (2020) in Kenya, where contracted TAV seed growers profitably produced seeds of a range of TAV species in contrast with their counterparts in the community-based model, who were only profitable in the production of spider plant seed. Seed growers in private sector mediated systems had higher yield and selling prices (Mvungi et al., 2020). More specifically, farmers involved in private sector mediated seed system had a higher profit margin ratio (>50%) than non-contracted farmers who had a negative profit margin for African nightshade and amaranth. An in-depth analysis of the profitability of the seed production system through cost-benefit ratio (CBR) revealed that one dollar invested by contracted seed growers in the production of African nightshade and amaranth seed would yield about US $ 7.92 and US $ 6.27, respectively (Mvungi et al., 2020). In the same way, Kimenye (2014) found that farmers in the private sector mediated seed system (Kenya) had higher revenue than community-based (QDS) seed growers (Tanzania). Per cropping season, seed growers involved in private sector mediated seed system earned on average a gross income of US $ 4,500 producing amaranth, African nightshade, jute mallow, and *Crotalaria* seed in Kenya. Community-based (QDS) seed growers earned US $ 300 producing African eggplant, amaranth, and African nightshade seed in Tanzania per cropping season (Kimenye, 2014). Similarly, in Tanzania, income generated per hectare per season from TAV seed was US $ 907.1, US $ 892.6, and US $ 576.5 for the contract seed growers, community-based seed growers, and seed growers in the informal system, respectively (Rajendran et al., 2016). Seed producers in a private sector mediated system earned a gross margin of US $ 1325 and US $ 4500 per ha for *Solanum aethiopicum* and *Amaranthus lividus*, respectively (Kansiime et al., 2018). A low profit margin was reported for TAV seed producers in the informal seed system in Kenya (Pincus et al., 2018). For instance, the average annual income from selling TAV seed from the informal seed system ranged from US $ 15 in Bungoma County to US $ 28 in Kisumu County in Kenya (Pincus et al., 2018), which was quite low compared to the values reported in Tanzania and Kenya for private sector mediated and community-based seed systems (Afari-Sefa et al., 2013; Kimenye, 2014; Mvungi et al., 2020).

Factors Limiting TAV Seed Production and Marketing

TAV seed production and marketing were constrained by several biophysical, technical, organizational, and institutional factors. On the production side, pest attacks, low accessibility to improved TAV germplasm, access to land, availability of irrigation water, and lack of technical know-how of seed producers as well as efficient seed extraction methods were major factors limiting TAV seed production (Onim and Mwaniki, 2008; Afari-Sefa et al., 2013; Kansiime et al., 2021). On the market side, difficulty in exploring viable markets and poor differentiation between informal seeds sold in the open market and QDS were major constraints (**Table 2**). As a result, there was competition between QDS and non-certified farmer-produced seed (Afari-Sefa et al., 2013). Building seed producers' capacity concerning market research, branding, and packaging would improve their market access. Lack of resources (human and technical), understaffing in seed certification agencies, poor or no collaboration between seed sector stakeholders, and non-conducive seed legislation for TAV seed business in many

TABLE 2 | Factors limiting TAV seed production and marketing.

Constraints of TAV seed production	Number of studies reporting the constraint	Authors
Pest attacks	3	Onim and Mwaniki, 2008; Afari-Sefa et al., 2013; Kansiime et al., 2021
Lack of technical know-how on seed production	3	Onim and Mwaniki, 2008; Afari-Sefa et al., 2013; Kansiime et al., 2021
Poor quality of farm saved seed	2	Onim and Mwaniki, 2008; Kimaru et al., 2019
Low accessibility to improved TAV germplasm	2	Onim and Mwaniki, 2008; Afari-Sefa et al., 2013
Difficulty exploring viable markets in QDS	2	Onim and Mwaniki, 2008; Afari-Sefa et al., 2013
Rudimentary seed processing mechanisms	1	Kansiime et al., 2018
Poor processing and packaging of seeds from farmers	1	Onim and Mwaniki, 2008
Lack of access to foundation seed and quality assurance for QDS growers	1	Kansiime et al., 2021
Climatic and other abiotic challenges		Kansiime et al., 2021
Access to water	1	Afari-Sefa et al., 2013
Unavailability of adequate land for seed production	1	Onim and Mwaniki, 2008
Delay in receiving complementary inputs (Contracted farmers)	1	Afari-Sefa et al., 2013
Institutional bottleneck (understaffing in seed certification agencies)	1	Afari-Sefa et al., 2013
Challenges in negotiating and adhering to contracts for farmers under contract farming	1	Kansiime et al., 2021
Lack of strong collaborative links between seed sector stakeholders	1	Afari-Sefa et al., 2013
Fragmented markets for QDS	1	Kansiime et al., 2021
Competition between QDS and non-certified farmer-produced seeds	1	Afari-Sefa et al., 2013
Insufficient attention given to TAV by private mediated seed sector	1	Afari-Sefa et al., 2013

countries are institutional factors to be tackled to foster the production and delivery of high-quality TAV seed to farmers.

DISCUSSION

Regional Gaps in the Implementation of TAV Seed Systems

Research on TAV and especially on the development of viable seed systems has received little attention (Afari-Sefa et al., 2013; Dinssa et al., 2015), exemplified by the limited number of studies relevant to be included in this review. Within Eastern Africa, Kenya and Tanzania are predominant in the development of TAV seed systems, for two main reasons:

Project interventions like the Good Seed Initiative funded by Irish Aid through CABI (Kansiime et al., 2016, 2018; Rajendran et al., 2016), and the Multi-Donor Trust Fund (MDTF) to ASARECA (Kimenye, 2014). The majority of the studies stemmed from these projects. This finding shows the vital role of funding in disseminating improved varieties and promoting access to good quality TAV seed. Implementing interventions to improve farmers' access to improved varieties and good quality TAV seed in other regions of the continent is imperative.

There is scarce or no reported information on promoting access to good quality TAV seed in other countries, especially in Western, Central, and Southern Africa where TAV contribute to income generation and nutritional security. More attention is needed to assess, develop, test, and scale promising TAV seed systems, especially in the regions where no study was identified for inclusion in this review.

Sustainability of TAV Seed Systems

As one can expect for a developing seed system, there is still a pre-dominance of the informal seed system in TAV—an indication of its critical role in providing farmers with TAV seed (Adebooye et al., 2005; Onyango, 2007; Coomes et al., 2015; Croft et al., 2018; Pincus et al., 2018). The TAV seed systems in Eastern Africa are at stage one as per the Douglas scale (1980), a range that describes seed systems. Stage one is characterized by a predominance of farmer-saved seed with low availability of improved varieties. In contrast, stage four depicts a well-developed seed sector driven by commercial seed production and marketing. Significant investment in varietal development and creating a conducive policy environment is needed to enhance the availability, accessibility, and affordability of high-quality TAV seed.

The three TAV seed systems have some merits in delivering seed to farmers. The studies we reviewed focused primarily on seed quality (germination and purity), seed availability, and accessibility. However, seed affordability to farmers has received little attention. Seed affordability is a major driver of seed purchase by farmers (Simtowe et al., 2019). We recommend that future studies to assess the viability and sustainability of seed production and marketing systems also address the demand side by evaluating seed affordability for farmers. Such studies should investigate the decision-making process of farmers regarding their willingness to invest more into seed purchases.

The informal seed system seems to be more limited in producing and delivering high-quality seed. However, there were instances where seed quality in the formal or community-based system was either poorer or of the same quality as seed from the informal sector (Coomes et al., 2015; Croft et al., 2018). This system deserves special attention (Croft et al., 2018), especially where the formal and the semi-formal seed systems are not operational. Besides potential shortcomings in seed production, processing, and storage from some seed companies, poor seed quality reported from the private sector mediated seed system could be due to the presence of "fake seed" injected into the seed market—a challenge to farmers and seed companies alike. Poor-quality seed from a private sector mediated seed system could create distrust and encourage sourcing seed from an informal system. Insufficient technical information is available on methods to optimize seed production (ideal temperatures/rainfall, soil types, isolation distances, crop management practices, avoidance of seed-borne pathogens, seed extraction, seed drying) of many TAV. Such knowledge is often specific for each TAV and should be developed and publicized. The conditions under which TAV are stored are critical for long-term seed health. Actors in TAV seed systems may need to revisit their cultural and postharvest practices, including seed harvesting, threshing or extracting, drying, and storage.

The private sector mediated system with an outgrowing scheme stands out in terms of profitability for seed growers (Kimenye, 2014; Rajendran et al., 2016; Mvungi et al., 2020). In the private sector mediated system, seed growers had access to services, including credit, and improved technologies from seed companies enabled them to realize high profit margins (Mvungi et al., 2020). Those services would likely allow them to improve their productivity, seed quality, and profitability. As long as the producer complies with the contractual arrangement, there is a guaranteed buyer (seed companies), which is not often the case in the other systems (Afari-Sefa et al., 2013). For instance, seed producers engaged in community-based seed systems had difficulty exploring viable markets and selling their seed due to competition from the informal seed sector (Afari-Sefa et al., 2013), and lacked access to foundation seed (Kansiime et al., 2021). It is worth noting that the private sector-mediated system may be limited in facilitating farmers' access to seed (Kansiime et al., 2021). Private seed companies purchase seed from out-growers but do not necessarily sell it within the community where the seed is produced. As a result, TAV growers in the community may have low access to high-quality seed produced by their fellow farmers. Nevertheless, where a private company is willing to invest in TAV seed production, it may be more sustainable to promote contract-based seed production. However, seed producers must carefully consider the challenges involved in negotiating and complying with the terms of their contracts (Kansiime et al., 2021). Seed quality requirements such as varietal purity, germination, moisture content, etc. must be clear to the seed producers, and seed companies must honor the prices given in contracts so that both parties feel confident, trustful, and secure.

Women play an important role in the production and utilization of TAV. However, current TAV seed systems are not sufficiently gender-responsive. In one example of the informal system, male seed producers earned more than female producers (Pincus et al., 2018). Females experience difficulty in getting contracts with seed companies because of decision-making at the household level and land ownership (Kansiime et al., 2021). Development-oriented interventions in the TAV seed sector should consider gender differences and promote the inclusion of females and youth. Failing to do so would worsen gender inequalities (Pincus et al., 2018).

TAV seed systems are diversified and the predominant systems depend on the crop species and the location. Therefore, one should observe caution in generalizing the information on TAV seed systems. Before developing and implementing seed interventions, a thorough situational analysis should be conducted to achieve the desired impact and avoid disruption in access to high-quality seed (McGuire and Sperling, 2016; Almekinders et al., 2019). Where there is no private seed company interested in TAV seed, promoting a community-based seed system with Quality Declared Seed as quality assurance, coupled with capacity building for seed growers in production skills, management, and marketing would help ensure sustainable TAV seed production. The choice of a system to be promoted also depends on the objective of the intervention. Interventions seeking to ensure seed availability at the national level could promote the private sector mediated system; when the emphasis is on a given community, it would be preferable to support a community-based seed system with a QDS option if regulations allow.

Diversifying Quality Assurance Options for TAV

In COMESA, the regional harmonized seed regulations (COMESA, 2014) have not made room for other seed systems except for the formal seed system. This system is characterized by centralized laboratory testing and requires complex logistics and full-time inspectors for field inspections and laboratory testing. These seed quality control services are often understaffed with limited resources for timely field inspection, laboratory testing, and delivery of certificates or tags (Afari-Sefa et al., 2013; Ayenan et al., 2017). Considering the low amounts of seed produced by TAV seed producers, payment for the certification process is almost prohibitive for many local seed companies or seed producer cooperatives. The COVID-19 pandemic has further exposed the low resilience of the system. During the pandemic, lockdowns and other restrictions on movement in many countries have undermined the ability of certification officers to carry out their duties (field inspection, seed sampling, and testing), which could compromise seed quality (Sperling et al., 2020). In addition, there has been a limitation on seed importation from other countries or seed transport within a country, stressing the dire need to foster local seed production. Sperling et al. (2020) rightfully pointed out the need for policy change to redesign the seed system to serve farmers better, reach farmers in remote areas (last mile), and function better under normal and stressful conditions. QDS as quality assurance is gaining recognition, and grassroots stakeholders are embracing it, even in challenging seed policy environments. For instance, in Mali, seed regulations do not officially recognize QDS, but initiatives for community-based seed production involve improved and non-improved varieties with unofficial QDS (Diallo and de Boef, 2012; Louwaars et al., 2013). National seed legislation should create enabling conditions for farmers to access quality seed for all crops, especially TAV, because of their importance for improving nutrition and livelihoods.

In addition to QDS, Participatory Guarantee Systems (PGS) used in organic farming for quality assurance could be tested in TAV seed production. "Participatory Guarantee Systems (PGS) are locally focused quality assurance systems. They certify producers based on the active participation of stakeholders and are built on a foundation of trust, social networks, and knowledge exchange." (IFOAM-Organic International, 2008, 2019). This system integrates the simplification of the seed certification process promoted by the QDS scheme and the principles of participation, trust, and empowerment of stakeholders involved in the system.

CONCLUSION

Studies on TAV seed systems in sub-Saharan Africa are recent and mainly carried out in Kenya and Tanzania. Despite the small number of studies reviewed, we identified interesting trends that could guide future studies and interventions on TAV seed systems. Even though seed quality varied widely within a system, private sector mediated and community-based seed production tends to provide better seed quality. Farmers involved in private sector mediated seed and community-based seed systems had higher profitability than their counterparts in informal systems. The private sector mediated seed system was more profitable to seed growers compared to community-based seed system. Private sector mediated and community-based seed systems improved seed availability. Community-based seed production systems had a better effect on seed accessibility compared to private sector mediated systems. The advantage of the private sector mediated seed system over the community-based seed system lies in the guaranteed market that the former offers to seed growers. The available information from the studies could not discriminate among the three systems based on the affordability of the seed they delivered to farmers. We identified the private sector mediated system as the most sustainable model. However, the promotion of the private sector mediated system requires the existence and willingness of a private seed company to venture into TAV seed production and to contract seed growers. In the light of the regulatory and status of seed sector development in a given intervention area, community-based and informal seed systems could be promoted to improve access to high-quality TAV seeds.

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

MA: conceptualization and literature search. MA, HZ, LA, and SN'D: methodology. MA, HZ, LA, and JH: full text review and writing—original draft preparation. MA, HZ, LA, SN'D, FD, PH, and VA-S: writing—review and editing. PH and VA-S: supervision. All authors have read and approved the version to be published.

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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.2021.689909/full#supplementary-material

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Dietary Phytochemical Screening of Spider Plant (*Gynandropsis gynandra* (L.) Briq.) Accessions From Africa and Asia to Identify Genotypes for Use in Nutraceutical Breeding

Barthlomew Yonas Chataika [1]*, ***Levi Shadeya-Mudogo Akundabweni*** [1], ***Aristide Carlos Houdegbe*** [2,3], ***Enoch G. Achigan-Dako*** [2], ***Julia Sibiya*** [3] ***and Kingsley Masamba*** [4]

[1] Crop Science Department, Faculty of Agriculture and Natural Resources, University of Namibia, Oshakati, Namibia,
[2] Laboratory of Genetics, Biotechnology, and Seed Science, Faculty of Agronomic Sciences, University of Abomey-Calavi, Cotonou, Benin, *[3] School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa,* *[4] Department of Food Science and Technology, Lilongwe University of Agriculture and Natural Resources, Lilongwe, Malawi*

Edited by:
Giuseppina Rea,
National Research Council (CNR), Italy

Reviewed by:
Umakanta Sarker,
Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh
Shyamal Nandi,
Govind Ballabh Pant National Institute of Himalayan Environment and Sustainable Development, India

***Correspondence:**
Barthlomew Yonas Chataika
barthchataika@gmail.com

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Increased public awareness of healthy foods and healthy living, coupled with escalating medicinal costs and recent advances in research and technology, has sparked a paradigm shift to nutraceuticals, which guarantee human health and disease prevention. Spider plant (Gynandropsis gynandra) contains dietary phytochemicals with high nutritional and medicinal properties that can contribute to healthy living. A study was conducted to identify spider plant (*Gynandropsis gynandra* (L.) Briq.) accessions with superior levels of dietary phytochemicals and anti-oxidative activity for use in nutraceutical breeding. Thirty-three accessions of spider plant, representing a wide genetic diversity based on geographic areas of origin (Asia, East Africa, Southern Africa, and West Africa), were used. Total phenolic acids, tannins, and anthocyanins were extracted and quantified using the Folin-Ciocalteau colorimetric, spectrophotometric, and pH differential methods, respectively. Antioxidant activity was determined using phosphomolybdenum method. Results showed significant variation in levels of total phenolic compounds, tannins, anthocyanins, and antioxidant activity ($P < 0.05$) amongst the spider plant accessions and regions of origin; ODS-15-037 (464 mg TAE/g DW), ODS-15-053 (270 mg GAE/g DW), and BC-02A (127 mg cyanidin-3-glucoside/g DW) had the highest levels of total tannins, phenolic compounds, and anthocyanins, respectively. Antioxidant activity was high in ODS-15-053 (492.2 mg AAE/100 g DW), NAM 2232 (445.3 mg AAE/100 g DW), and NAM-6 (432.5 mg AAE/100 g DW). On average, West African accessions had significantly high tannin concentrations (239 mg TAE/g DW), while Southern Africa accessions contained significantly high anthocyanin content (58.9 mg cyanidin-3-glucoside/g DW). The superior accessions are potential candidates for use in nutraceutical breeding, while the regions of origin could be used as gene pools for specific phytochemicals for improving dietary supplements of nutraceuticals. The strong antioxidant activity exhibited by spider plant accessions suggests the presence of compounds responsible for scavenging free oxygen or nitrogen radicals. Further studies are recommended to identify the chromosomal regions that contain genes controlling the dietary nutraceuticals in the genetic materials and to determine their association with foliage yield and other phenotypes, which can be utilized in spider plant improvement.

Keywords: dietary phytochemicals, nutraceutical breeding, indirect selection, free radicals, accessions, spider plant, antioxidant

INTRODUCTION

Global economic development continues to influence the changes in disposable incomes, spending levels, and quality of lifestyle associated with major diseases related to nutritional deficiencies (Pandey et al., 2010). Increased public awareness regarding healthy eating and living, escalating medical costs, and recent advances in research and technology have ignited a paradigm shift to nutraceuticals (Das et al., 2012), promising good health and disease prevention.

The nutraceuticals, such as vitamins, minerals, and phenolics, reduce the risk of chronic diseases by scavenging reactive oxygen and nitrogen species in the human body (Zhang et al., 2015; Sarker and Oba, 2018d) and are thus referred to as antioxidants (Sarker et al., 2018b). Over a couple of decades, there has been an interest in utilizing natural antioxidants from leaves of plant species, particularly for different types of pigments (Sarker and Oba, 2019a), phenolic acids (Sarker et al., 2018a), flavonoids (Sarker and Oba, 2018a), and vitamins C, E, and A (Sarker and Oba, 2020e). The leaves are also the essential sources of minerals, such as microelements (Sarker et al., 2017) and macroelements (Chakrabarty et al., 2018), protein (Sarker and Oba, 2019d), dietary fiber (Keleş et al., 2016), vitamins (Sarker et al., 2015a) and carbohydrates (Sarker et al., 2015b) for human food supplements. The indigenous vegetables, commonly consumed by the local population, are reported to contain higher levels of dietary phytochemicals and antioxidant activity than commercial and exotic vegetables (Moyo et al., 2013; Chepkwony et al., 2020), suggesting their importance and inclusion in healthy diets. Among different orphan crops, the spider plant (*Gynandropsis gynandra* (L.) Briq.; family—Cleomaceae), a semi-wild indigenous vegetable species in sub-Saharan Africa (Shilla et al., 2019), has gained recent attention due to the presence of high levels of secondary metabolites which contribute to strong antioxidant properties (Sowinmi and Afolayan, 2015), and therefore, chosen for this investigation.

Studies on nutritional value and medicinal use of *G. gynandra* have suggested the high potential of the vegetable in the nutraceutical industry (van der Walt et al., 2009). The important nutraceutical phytochemicals in spider plant include phenolic compounds (Adhikari and Paul, 2018), tannins (Kutsututsa et al., 2014), and anthocyanins. These phytochemicals are reported to have antioxidant and therapeutic characteristics, including anti-inflammatory (Narendhirakannan et al., 2005), anticancerous (Bala et al., 2010), anti-diabetic (Trilochana et al., 2017), immunomodulator, and free radical scavenging (Adhikari and Paul, 2018) properties and other age-related disorders (Anbazhagi et al., 2009).

Although the above studies report the medicinal and nutraceutical role of *G. gynandra*, the levels of the important phytochemicals in spider plant accessions have not been conclusively investigated. For example, Chweya and Mnzava (1997) observed that phytochemical research in *G. gynandra* mainly focused on individual compounds, with very little done on quantification and assessment of genetic variability. From the available literature, it is evident that studies have concentrated on quantifying the nutritional levels while ignoring the nutraceutical potential. Some phytochemicals are believed to be associated with the species' phenotype that can allow selection if the relationships are adequately investigated. For example, the purple color is believed to be linked to bitter taste due to high anthocyanin content, which also affects the nutraceutical potential of the plant and influences the willingness of consumers to utilize the plant as food (Mueller-Harvey, 2001) or medicine (Sogbohossou et al., 2020).

Tannins produce pronounced astringent properties associated with binding and gelatin precipitation from solution, consequently affecting the nutritive value of foods and feedstuffs eaten by humans and animals, respectively (Mueller-Harvey, 2001). In addition, researchers in Zimbabwe (Kutsututsa et al., 2014) observed the need for variability in tannin levels to employ crop improvement techniques to reduce the bitterness in *G. gynandra* genotypes without compromising the nutraceutical properties. Their observation pointed toward the need for adequate genetic characterization of spider plant accessions to quantify tannin levels. Although they screened and identified a superior genotype, their research was only limited to five genotypes. Thus, determining genetic diversity from a wider gene pool remains key to identifying superior candidates for use in demand-driven crop improvement programs.

Phenolic compounds and tannins are amongst the most important phytochemicals responsible for antioxidant and therapeutic properties in spider plant. The antioxidant activity of phenolic compounds is attributed to their ability to break the strong chains and scavenge the free radicals to provide protection against reactive oxygen species (Uusiku et al., 2010). These compounds account for the spider plant's potential to treat a number of non-communicable diseases, including hypertension, diabetes, cancer, and other cardiovascular diseases (Kumari and Jain, 2012; Kutsututsa et al., 2014). In addition, tannins are reported to enhance glucose uptake, insulin functions and regeneration of beta cells (Anderson and Polansky, 2002), protection of kidneys, and the relief of sore throats and diarrhea (Tuli et al., 2016).

The null hypothesis was that no difference exists in the dietary phytochemical content (total phenolic compounds, anthocyanins, and tannins) and in antioxidant activity amongst 33 accessions of spider plant (*G. gynandra*), representing a wide genetic diversity based on geographic areas of origin (Asia, East Africa, Southern Africa, and West Africa). The study's objectives were to screen and identify spider plant accessions, with high levels of dietary phytochemicals and antioxidant activity, for use in nutraceutical breeding.

MATERIALS AND METHODS

Analytical Chemicals

The analytical chemicals used in the study were Folin—Ciocalteu phenol reagent, gallic acid (3,4,5-trihydroxy benzoic acid), sodium hydrogen carbonate ($NaHCO_3$), methanolic solution, Folin-Denis solution, Na_2CO_3, gallic acid solution, sulphuric acid, sodium phosphate, ammonium molybdate, ascorbic acid, hydrochloric acid, potassium chloride, and Natrium/sodium acetate. All the chemicals used were of analytical grade and were obtained from SAAR Chem-Trade Pvt. Ltd, Mumbai, India.

Trial Establishment and Collection of Phenotypic Data

The trial was established in Lilongwe, Malawi (1138 m asl; 13° 50′ 58″ S & 33°46′ 07″ E) in June 2020 under irrigation. The area comprises of fertile dark loam soils belonging to the class of luvisols. It is characterized by a medium amount of rainfall of around 900 mm per annum, and temperatures range from 17 to 27°C. The 33 accessions of spider plant (**Table 1**), which originated from Asia, East Africa, Southern Africa, and West Africa, were grown in an 11 × 3 alpha lattice design, replicated three times. Accessions were planted in single rows of three meters long, 0.3 m inter-row spacing. Seeds were soaked in warm water (40°C) for 30 min before planting to enhance germination. The field was kept weed-free by manual weeding and moist through regular watering. Watering was done every day during the first 14 days and then reduced to once every three days. Basal NPK fertilizer (23:21:0 + 4S) was applied at planting at the rate of 40 kg N ha^{-1}.

Phenotypic data, including quantitative and qualitative traits, were collected when the accessions reached 50% flowering, which was six weeks after planting. The quantitative phenotypic data was the number of leaves per plant. Qualitative data collected included stem trichome density, stem color, petiole trichome density, petiole color, growth tip trichome density, leaf color, leaf trichome density, and flower color. All the qualitative data were collected as described by Sogbohossou et al. (2018, 2019).

Sample Preparation and Extraction

Mature leaf samples were harvested six weeks after planting when the plants were at the 50% flowering stage. Leaf samples were cleaned with distilled water and freeze-dried using liquid nitrogen before being transported to the Lilongwe University of Agriculture and Natural Resources (LUANAR) nutrition laboratory for analysis of dietary phytochemicals. The phytochemical extraction from the leaf samples was done following the method described by Chaves et al. (2020) with minor modifications. Freeze-dried leaf samples were ground in a mechanical grinder to obtain a homogenous powder. The phytochemicals were extracted using 80% methanol in a 1:4 w/v ratio for 24 h with continuous shaking. The extracts were filtered using a Whatman no. 1 filter paper (Whatman International Ltd., Maidstone, England), and the filtrate/extracts were concentrated by evaporating excess solvent using a Buchi Rotavapor-R-205, at 40°C. The extracts were diluted in a 1:4 w/v ratio in 80% methanolic solution to obtain absorbance readings that were within the standard curve concentration range of 0.0–200.0 mg gallic acid equivalent (GAE)/mL for total phenolic content determination.

Determination of Total Phenolic Content (TPC)

The TPC was determined by using the Folin-Ciocalteau colorimetric method presented by Singletone and Rossi (1965). One milliliter (1 mL) of the sample extracts was mixed with 2.5 mL of Folin-Ciocalteau reagent (1:10 v/v) and left to stand for 5 min followed by the addition of 2.5 mL of 7.5% $NaHCO_3$ in test tubes. Standard gallic acid samples of 0, 1, 2, 3, 4, 5, 6 mg were prepared from a stock solution of 1 mg/mL by pipetting 0–6 mL into six different test tubes. Similarly, 2.5 mL of Folin-Ciocalteau reagent and 2.5 mL of $NaHCO_3$ were added in the six standard test tubes. The samples were incubated for 20 min at room temperature for color development. The absorbance was measured at 765 nm using a spectrophotometer (Thomas-Willy Laboratory mill model 4). TPC expressed as mg of GAE per g of DW sample was calculated from the standard gallic acid curve using linear equation $Y = 0.739x$ (**Figure 1A**). TPC was finally calculated as follows:

$$TPC\ \left(mg\ GAE\ g^{-1}\right) = \left(\frac{C\ \times\ V}{m}\right)\ \times\ 100 \tag{1}$$

Where C = concentration of the standard solution of gallic acid; V = total dilution volume, and m = mass of sample in grams.

Determination of Total Tannins Composition

Total tannin content was determined by the spectrophotometric method described by Shrin and Prakash (2010) with minor modifications. One milliliter (1 mL) of the 80% methanolic extracts was mixed with 0.5 mL of Folin-Denis solution (1:10 v/v) and concentrated Na_2CO_3, and the solution was diluted to 10 mL. A stock solution of 1 mg/mL tannic acid was prepared, and standard solutions of 0–8 mg were prepared. The standard solutions were similarly treated as the samples, and absorbance was measured at 760 nm after 30 min of color development using a UV spectrophotometer (model: UVA 094615, England, UK). The total tannic acid content, as mg TAE g^{-1}, was calculated from the standard tannic acid curve using the linear equation $Y = 0.1780x$ (**Figure 1B**).

TABLE 1 | List of spider plant accessions used in the study.

No	Accession name	Institution	Country of Origin	Region
1	TOT1048	World Vegetable Center	Thailand	Asia
2	TOT7198	World Vegetable Center	Malaysia	Asia
3	TOT7197	World vegetable center	Malaysia	Asia
4	TOT7486	World Vegetable Center	Lao People's Democratic Republic	Asia
5	TOT3536	World Vegetable Center	Lao People's Democratic Republic	Asia
6	TOT3527	World Vegetable Center	Lao People's Democratic Republic	Asia
7	TOT7196	World Vegetable Center	Malaysia	Asia
8	TOT4976	World Vegetable Center	Thailand	Asia
9	TOT5799	World Vegetable Center	Thailand	Asia
10	ELG 19/07A	KENRIK	Kenya	East Africa
11	BAR 1807B	KENRIK	Kenya	East Africa
12	TOT8926	World Vegetable Center	Kenya	East Africa
13	TOT6420	World Vegetable Center	Tanzania	East Africa
14	KF-07	KENRIK	Kenya	East Africa
15	KSI 2407A	KENRIK	Kenya	East Africa
16	NAM2232	Vergenoeg	Namibia	Southern Africa
17	LA 1	Ogongo campus	Namibia	Southern Africa
18	BC-01B	LUANAR	Malawi	Southern Africa
19	BC-03A	LUANAR	Malawi	Southern Africa
20	BC-03B	LUANAR	Malawi	Southern Africa
21	BC-01A	LUANAR	Malawi	Southern Africa
22	CZ-01	Chitedze Research Station	Malawi	Southern Africa
23	NAM-6	Ogongo	Namibia	Southern Africa
24	TOT6439	World Vegetable Center	Zambia	Southern Africa
25	BC-02A	LUANAR	Malawi	Southern Africa
26	ODS-15-038	GBioS	Benin	West Africa
27	ODS-15-053	GBioS	Togo	West Africa
28	ODS-15-121	GBioS	Ghana	West Africa
29	ODS-15-045	GBioS	Togo	west Africa
30	ODS-15-013	GBioS	Benin	west Africa
31	ODS-15-020	GBioS	Benin	west Africa
32	ODS-15-037	GBioS	Benin	west Africa
33	ODS-15-044	GBioS	Benin	west Africa

KENRIK, Kenya Resource Centre for Indigenous Knowledge; LUANAR, Lilongwe University of Agriculture and Natural Resources; GBioS, Laboratory of Genetics, Biotechnology and Seed Science.

Determination of Total Anthocyanins Content by the pH Differential Method

Anthocyanins Extraction

Anthocyanins in the samples were extracted by using 80% methanol acidified with 1.5 M hydrochloric acid (85:15 HCl, v/v) as described by Tonutare et al. (2014) with slight modification. One gram (1 g) of dry samples was placed in 100 mL beakers, and 10 mL of the solvent was added. The mixture was macerated and stirred overnight at room temperature before filtering through a Whatman no. 1 filter paper. The residues were filtered three times; then, the filtrates were combined and made up to 20 mL volume.

Determination of Total Anthocyanins

Total anthocyanins were determined by the pH differential method as outlined by Giusti and Wroltad (2001) with minor modifications. Four milliliters (4 mL) of the extracts were pipetted into test tubes, and solutions of pH 1.0 (prepared from potassium chloride, 0.025 M) and pH 4.5 (0.4 M Natrium/sodium acetate) were added into the different test tubes. The test tubes were let to stand for 30 min for color development, and absorbance was measured at 520 and 700 nm, respectively, using a Helios spectrophotometer (Thomas-Willy Laboratory). The total anthocyanins composition was presented as cyanidin-3-glucoside per gram using the following equation:

$$TA\ (mg\ g^{-1}) = \frac{A \times M \times DF \times 1000}{[\varepsilon] \times l \times m} \tag{2}$$

where A = $(A_{520}\text{-}A_{700})_{\text{pH } 1.0} - (A_{520}\text{-}A_{700})_{\text{pH } 4.5}$, M = molar mass of cyanidin-3-glucosides (449.2 g/mole), DF = dilution factor, 1,000 = converting grams to mg, ε = molar extinction coefficient /absorptivity of cyanidin-3-glucosides (26

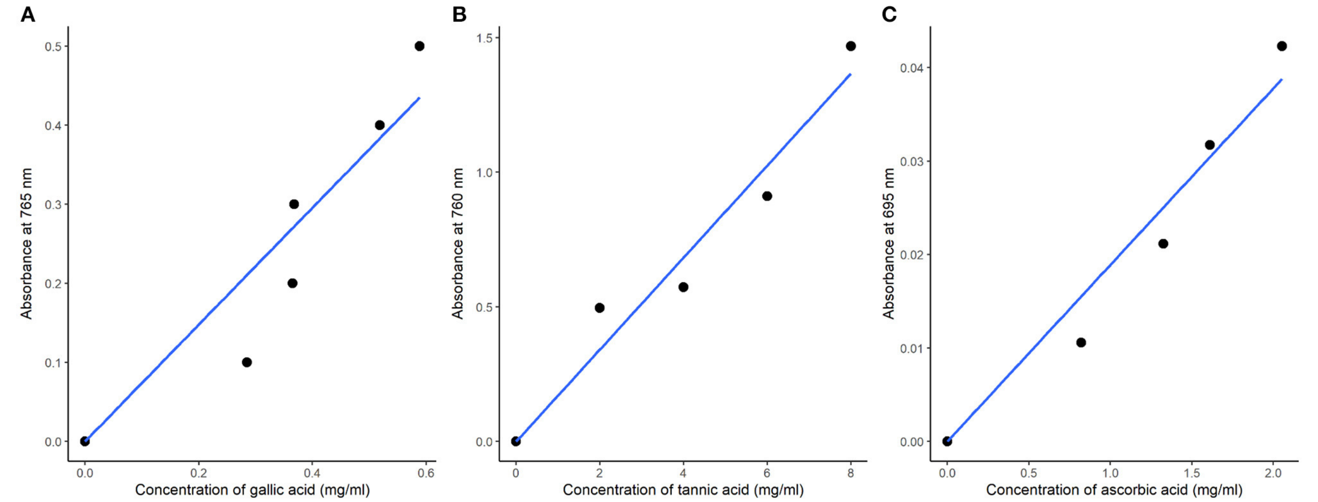

FIGURE 1 | Standard calibration curves of total phenolic compounds **(A)**, total tannins **(B)**, and total antioxidant activity **(C)** of leaves of spider plant.

900 l/mol.cm), l = width of cuvette (1 cm), and m = mass of sample in grams.

Determination of Total Antioxidant Activity

Total antioxidant capacity in the plant extracts is believed to depict the combined effects of phenolics, flavonoids, and other reducing compounds and is expressed in terms of ascorbic acid equivalent (AAE) (Kumar et al., 2014). The total antioxidant activity was determined by using the phosphomolybdenum method with minor modifications (Prieto et al., 1999). The basic principle of the assay was based on the reduction of Mo (VI) to Mo (V) by the sample extracts with a subsequent formation of a green phosphate complex in an acidic condition. One milliliter (1 mL) of the extract was added to test tubes, with 1 mL of the reagents (0.6 M sulphuric acid, 28 mM sodium phosphate, four mM ammonium molybdate), and the tubes were incubated while capped in a water bath at 95°C for 90 min. Standard ascorbic acid samples of 0–6 mg concentrations, prepared from a 1 mg/mL stock solution, were similarly treated. After cooling, the absorbance of the samples and standards was measured at 695 nm using a UV spectrophotometer against the blank. A standard calibration curve of ascorbic acid was plotted (**Figure 1C**), and total antioxidant activity was calculated as ascorbic acid equivalent (AAE) in mg AAE/g dry weight (DW).

Data Analysis

All the statistical analyses were performed using R software version 4.0.5 (R Core Team, 2021). Descriptive statistics (minimum, maximum, range, mean, standard deviation, and coefficient of variation) were used to describe the quantitative traits, including the number of leaves and the phytochemical compounds (total phenolic acids, total tannins, total anthocyanins, and antioxidant activity). Shapiro Wilk test was used to verify the normality of the data, and none of the quantitative data was normally distributed. Consequently, Kruskal–Wallis test was used to test the significant difference amongst the accessions and the geographical origins of the accessions. Due to the non-normality of the quantitative traits, the Spearman Rho's coefficient of correlation and their significance were performed using the function rcorr of the R package Hmisc (Harrell, 2021). Factorial analysis of mixed data (FAMD) was run on combined quantitative and qualitative traits to identify the most important components using the function *FAMD()* of the R package FactoMineR (Lê et al., 2008). A hierarchical clustering on principal components (HCPC) based on the selected principal components was performed to cluster the accessions according to their similarities using the function *HCPC()* from the R package FactoMineR (Lê et al., 2008). Factormap of both quantitative and qualitative variables and the dendrogram were represented using the function *fviz()* of the factoextra package (Kassambara and Mundt, 2021).

RESULTS

Dietary Phytochemical Content in Different Accessions of Spider Plant

The dietary phytochemical content in leaves of vegetables is an indicator of its potential to provide nutritional and health benefits when utilized. The total phenolic content (TPC), expressed as gallic acid equivalent per gram of dry weight (GAE/g DW) in spider plant extracts, ranged from 64.7 mg GAE/g DW in TOT8926 to 270.7 mg GAE/g DW in ODS-15-053 (**Table 2**; **Figure 2A**). The total tannin content ranged from 41.1 to 466.3 mg TAE/g DW, while the total anthocyanin contents were in the ranges between 10.8 and 126.9 mg cyanidin-3-glucoside/g DW. The spider plant extracts exhibited the mean antioxidant activity of 315.3 mg AAE/100 g DW and were within the range of 189.9 to 492.3 mg AAE/g DW.

There were significant differences in TPC, total tannins, and total anthocyanins amongst spider plant accessions ($p < 0.001$) (**Figures 2A,C,E**). ODS-15-053 had the highest TPC (270.7 mg

TABLE 2 | Descriptive statistics for dietary phytochemicals and morphological traits of the 33 accessions of *Gynandropsis gynandra*.

Traits	Min	Max	Range	Mean	SD	CV (%)
Total phenolic compounds (mg GAE/g DW)	64.7	270.7	206.0	123.3	41.6	33.8
Total tannins (mg TAE/g DW)	41.1	466.3	425.2	190.1	105.5	55.5
Total anthocyanins (mg cyanidin-3-glucoside/g DW)	10.8	126.9	116.1	46.0	28.2	61.4
Antioxidant activity (mg AAE/100 g DW)	189.2	492.3	303.2	315.3	70.3	22.3
Number of leaves	12.3	142.0	129.7	46.3	28.8	62.2

GAE, gallic acid equivalent; TAE, tannic acid equivalent; AAE, ascorbic acid equivalent; DW, dry weight; SD, standard deviation; CV, coefficient of variation.

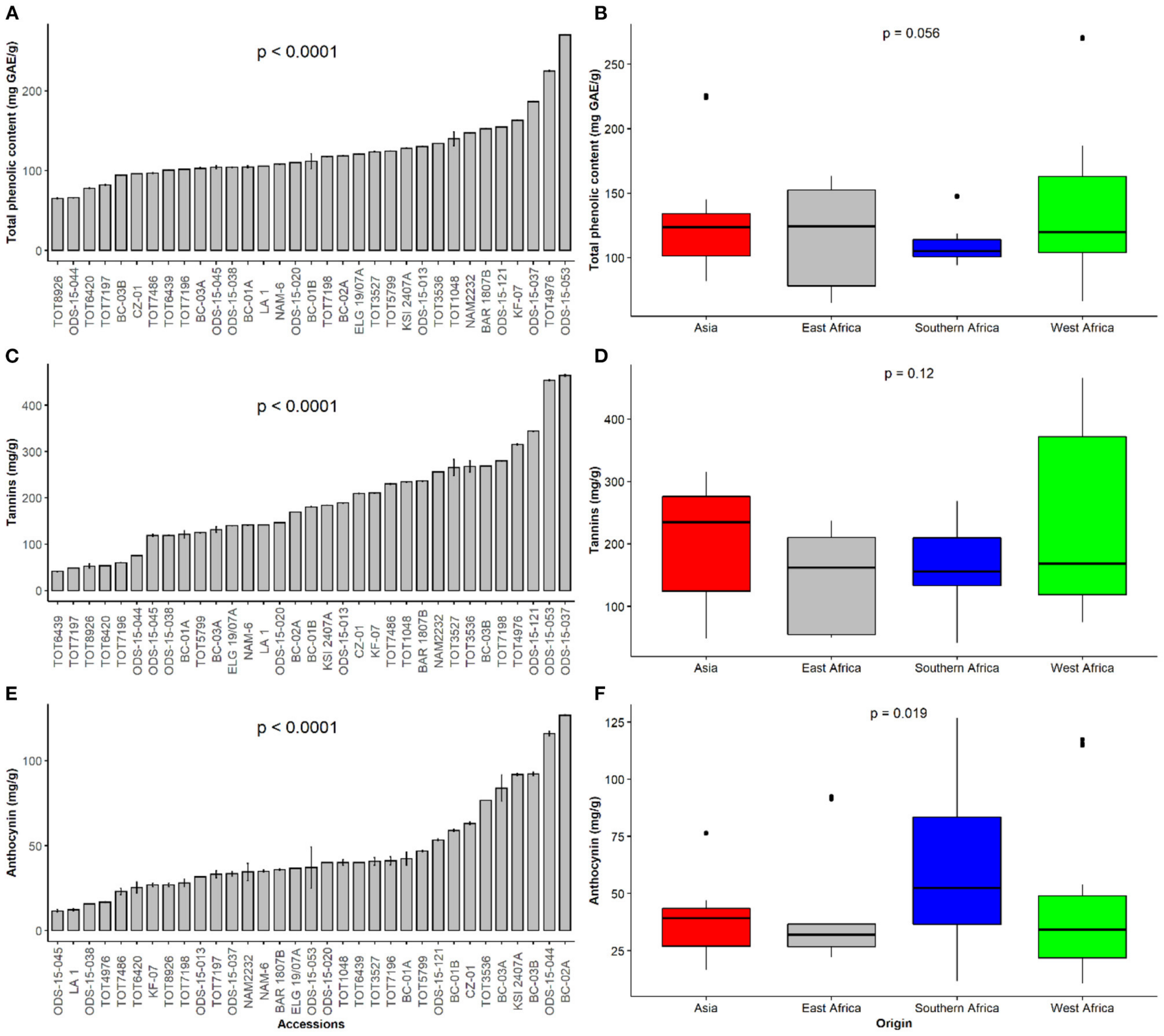

FIGURE 2 | Dietary phytochemical content of a collection of 33 *Gynandropsis gynandra* accessions and based on the regions of origin. **(A)** Total phenolic content among 33 accessions of spider plant. **(B)** Total phenolic content among accessions of spider plant based on their regions of origin. **(C)** Tannin content among 33 accessions of spider plant. **(D)** Tannin content among accessions of spider plant based on their regions of origin. **(E)** Anthocyanin content among 33 accessions of spider plant. **(F)** Anthocyanin content among accessions of spider plant based on their regions of origin.

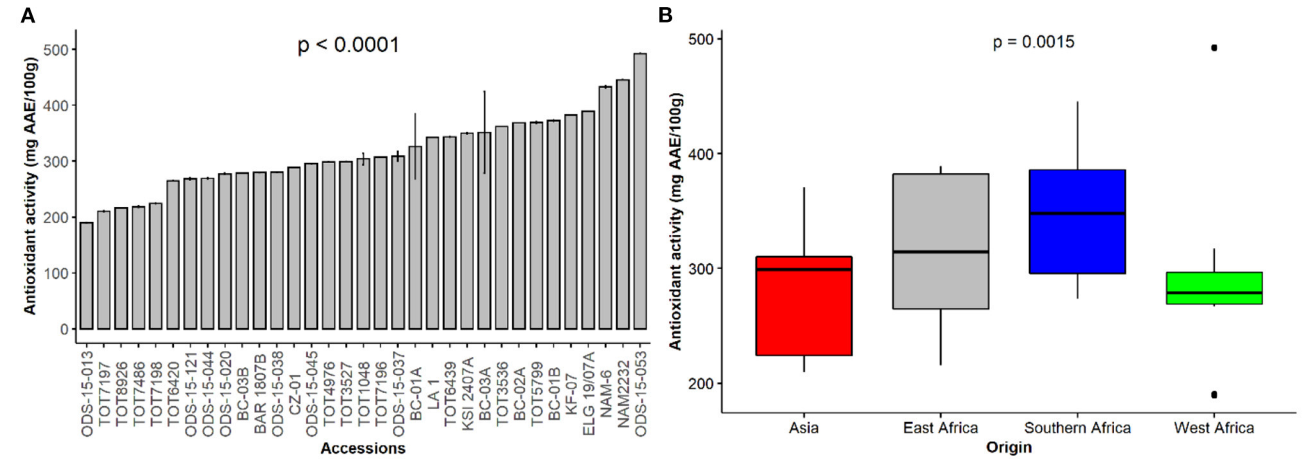

FIGURE 3 | Antioxidant activity of individual accessions **(A)** and based on their regions of origin **(B)** of a collection of 33 *Gynandropsis gynandra* accessions.

GAE/g DW), while TOT8926 had the least TPC (64.7 mg GAE/g DW). Fourteen spider plant accessions, representing 42.4%, had TPC below the overall mean of 190.1 mg GAE/g DW. The other accessions which contained high quantities of TPC were TOT4976 (225 mg GAE/g DW) and ODS-15-037 (187 mg GAE/g DW). The differences were not significant across the regions. However, the results showed wide variation in East Africa and West Africa, while southern Africa had low levels with narrow variability (**Figure 2B**).

Total tannin content was the highest in ODS-15-037 (464 mg TAE/g) and the lowest in TOT6439 (41 mg TAE/g DW) (**Figure 2C**). In addition to ODS-15-037, the other superior spider plant accessions (ODS-15-053, ODS-15-121, and TOT4976) showed high amounts of total tannins. Although a comparison of total tannin content across regions did not show significant differences, accessions from Asia had a higher median while those from West Africa contained some individuals with the highest content of total tannins (**Figure 2D**).

Total anthocyanin content significantly differed across the accessions ($p < 0.001$) (**Figure 2E**). Total anthocyanin content was the highest in BC-02A, followed by ODS-15-044, while ODS-15-045 had the lowest level. Accessions originating from southern Africa were characterized by significantly high levels of anthocyanins ($p < 0.05$), while the rest of the regions did not show statistical differences (**Figure 2F**). East African accessions had the lowest variation of anthocyanin content seconded by accessions from Asia.

Antioxidant Activity of Spider Plant Accessions

The antioxidant activity in vegetable plants indicates the availability of specific elements that scavenge free oxygen and nitrogen radicals. **Figure 3A** depicts the antioxidant activity, expressed as ascorbic acid equivalent (AAE) per 100 g dry weight (DW) of the spider plant accessions used in this study and the antioxidant activity across the regions of origin (**Figure 3B**). ODS015-053 (492.2 mg AAE/100 g DW), NAM2232 (445.3 mg AAE/100 g DW) and NAM6 (432.5 mg AAE/100 g DW) exhibited the highest levels of antioxidant activity, while ODS-15-013 had the lowest antioxidant activity ($p < 0.001$). Regional comparisons suggested accessions originating from southern Africa had high antioxidant activity (177.5 mg AAE/100 g DW) seconded by accessions from east Africa. In contrast, accessions originating from Asia had the lowest antioxidant activity (**Figure 3B**).

Relationships Amongst Dietary Phytochemicals and Morphological Attributes of Spider Plant Accessions

The number of leaves significantly varied amongst spider plant accessions and across regions of origin ($p < 0.001$) (**Figures 4A,B**). ODS-15-045 produced the highest number of leaves, while TOT7197 had the least. Comparison based on regions showed that accessions from West Africa produced the highest mean number of leaves while the Asian accessions had the least.

There was no relationship, however, between the level of the dietary phytochemicals and the number of leaves (**Table 3**). A significant and positive association was observed between TPC and total tannins ($r = 0.73$) and between TPC and antioxidant activity ($r = 0.44$) (**Table 3**).

A two-dimensional factor map of both qualitative and quantitative traits positioned tannins and TPC close to stem color, anthocyanins close to leaf color, while antioxidant activity was associated with leaf trichomes and number of leaves (**Figure 5**).

On the other hand, the hierarchical clustering on the results of the factorial analysis of mixed data (both quantitative and qualitative traits) grouped the 33 accessions of spider plant into five groups (**Figure 6**) with their characteristics presented in **Table 4**.

Cluster C1 was characterized by low TPC and tannin content and high antioxidant activity. The cluster C2 had only one

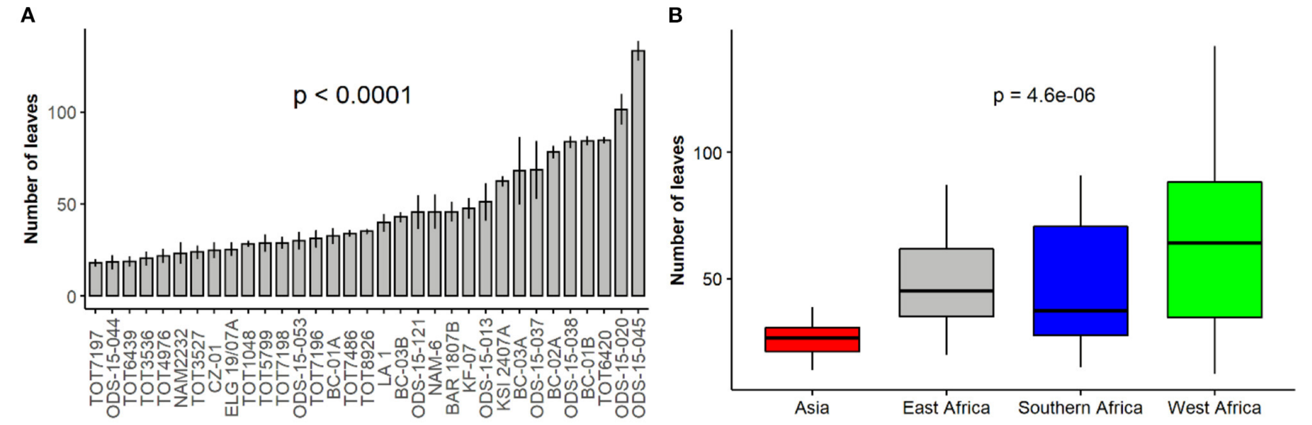

FIGURE 4 | Variation in the number of leaves among 33 accessions **(A)** and based on their regions of origin **(B)** of a collection of *Gynandropsis gynandra*.

TABLE 3 | Spearman rank phenotypic correlation between phytochemicals and number of leaves of 33 accessions of *Gynandropsis gynandra*.

	TPC	Tannins	Anthocyanin	Antioxidant activity
Tannins	0.73***			
Anthocynin	−0.03	0.08		
Antioxidant activity	0.44*	0.12	0.26	
Number of leaves	0.01	−0.05	−0.15	−0.04

*, ****significant at probability levels of 0.05 and 0.001, respectively.*

accession from West Africa with a low antioxidant activity and a high number of leaves. Clusters C3 and C4 had moderate TPC, tannins, antioxidant activity, and number of leaves, but they were distinct for anthocyanin content (cluster C3 and C4 had high and low anthocyanin content, respectively). Lastly, cluster C5 was characterized by high TPC, tannin content, and antioxidant activity with low number of leaves.

DISCUSSION

Phytochemical Content in Different Accessions of Spider Plant

Dietary phytochemicals are bioactive nutrient plant chemicals that play a role in providing desirable health benefits beyond basic nutrition, thereby reducing the risk of major chronic diseases (Liu, 2004). Vitamins, such as vitamins A, C, and E (Sarker and Oba, 2020b), some enzymes (Sarker and Oba, 2018b,c), and phytochemicals, such as pigments, phenolic, and flavonoid compounds, act as antioxidants (Sarker et al., 2018b, 2020a,b; Sarker and Oba, 2020a,d). These antioxidants neutralize/scavenge free oxygen and nitrogen radicals and prevent many diseases in the human body (Sarker and Oba, 2018e). Dietary phytochemicals of leafy vegetables, i.e., phenolics, are versatile compounds, such as phenol, coumarins, different groups of phenolic acids, and different groups of flavonoids, including flavonols, flavones, flavanols, flavanones, anthocyanins, chalcones, and different groups of non-flavonoids, including tannins, lignans and stilbenes that have been reported to have important antioxidant properties (Meda et al., 2013; Sarker and Oba, 2020e,f). The phytochemicals are distributed widely and occur in different concentrations within and across plant species. This study identified accessions of spider plant that contain high levels of TPC, total tannins, and anthocyanins. Based on our findings, as evidenced by the high levels obtained, these accessions are potential candidates for use in breeding programs aimed at enhancing the nutraceutical value of spider plant. For example, ODS-15-053, which had high levels of TPC, can be used as a parent in breeding programs that are aimed at enhancing the potential of spider plant in treating non-communicable diseases, including hypertension, diabetes, cancer, and other cardiovascular diseases, as previously reported by different researchers (Kumari and Jain, 2012; Kutsututsa et al., 2014). In addition, phenolic compounds are reported to play essential roles in defense mechanisms against pathogens, parasites, and predators of plants, suggesting that the accessions that have a high content of phenolic compounds could be possible sources of genes for insect pests resistance. In this study, the mean of TPC of the spider plant accessions (123.3 mg GAE/g) was higher than the values reported by Jiménez-Aguilar and Grusak (2017), but lower than the findings of Meda et al. (2013), who reported 133.0 mg GAE/g. In addition, other authors found lower levels of TPC (Somers et al., 2020), which were comparable to some African vegetables (Jiménez-Aguilar and Grusak, 2015). These authors used a small sample size and different extraction methodologies, which might account for the differences.

This study showed the diversity of TPC across accessions (66.0 to 270.0 mg GAE/g) and regions (108.9 to 140.8 mg GAE/g), suggesting both genetic and environmental effects on TPC. The gallic acid equivalent diversity in TPC across the genotypes of leaf vegetable amaranth species, such as *Amaranthus blitum* (Sarker and Oba, 2020c), weedy species (Sarker and Oba, 2019b), green morph amaranth (Sarker et al., 2020a,b), and red morph amaranth (Sarker and Oba, 2019c) were reported, which conform with the present findings. The differences in TPC and diversity

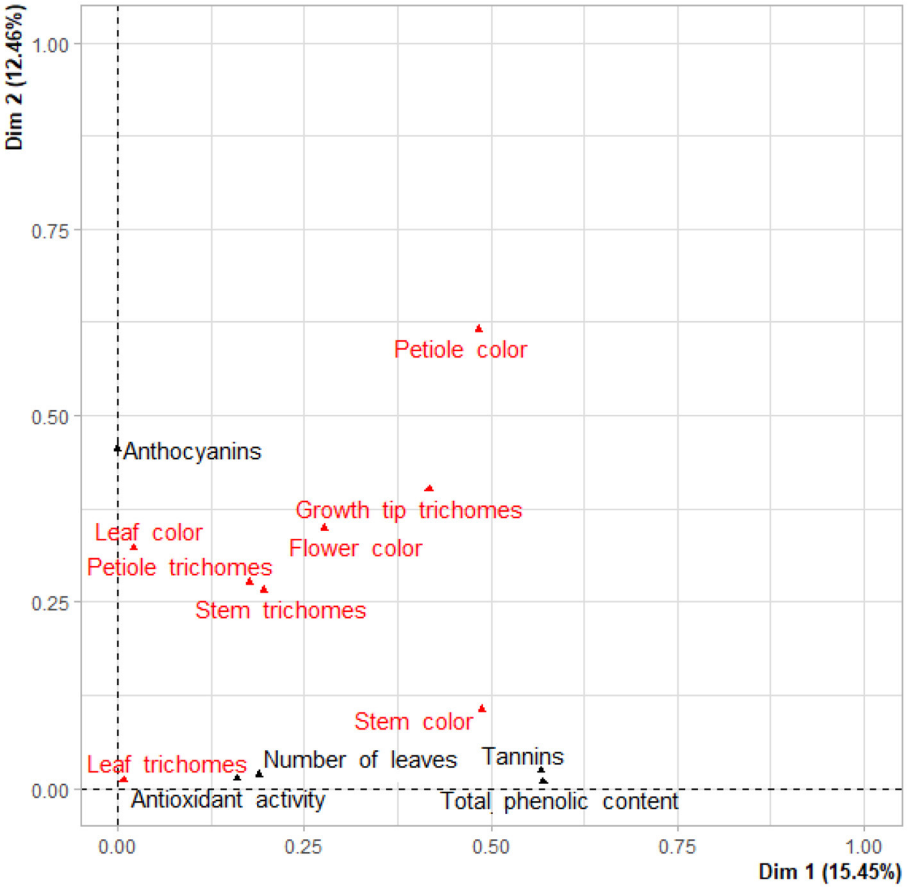

FIGURE 5 | Factor map positioning qualitative (in red) and quantitative (in black) variables on first and second components.

in the functions of dietary phytochemicals both in human health and in plant defense offer opportunities for using the identified superior accessions as candidates for pyramiding genes of multiple traits in breeding programs. However, there is a need to identify specific phenolic compounds responsible for specific functions and the location and mode of inheritance of these potential genes to enable effective and efficient breeding.

Tannins are another category of dietary phytochemicals that are reported to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses, amongst other functions (Chung et al., 1998). Negative health effects have also been reported and are dependent on the intake levels (Chung et al., 1998). This study identified spider plant accessions with high levels of tannins (ODS-15-037) as well as accessions with low levels of tannins such as TOT6439. This implies that accessions in both extremes could be useful in breeding programs with different objectives. For example, Chung et al. (1998) reported some incidences of esophageal cancer emanating from eating tannin-rich foods such as betel nuts and herbal teas. However, the same authors were quick to indicate that the carcinogenic activity observed might be related to components associated with the tannins rather than the tannins themselves. Further studies would, therefore, be required to determine the levels of tannins that could be used to satisfy or accomplish specific objectives. Tannins have the potential of being anticarcinogenic and antimutagenic due to their anti-oxidative properties, which protect against cellular oxidative damage (Chung et al., 1998). However, a weak relationship of tannin levels with antioxidant activity was detected in this study. This could be due to different levels of the different types of tannins that might have been present in the extracts. Tannins are classified as hydrolyzable (Ashok and Upadhyaya, 2012); however, condensation of phenolic compounds results in tannins (Ghosh, 2015). The strong and significant correlation between total phenolic compounds and total tannins observed in this study, therefore, might imply the presence of condensed tannins in the total tannin extracts. These findings agree with other researchers who reported that spider plant contain high levels of condensed tannins (Kutsututsa et al., 2014). Furthermore, condensed tannins are reported to have strong antioxidant activity with high therapeutic potential (Haslam, 1966; Ghosh, 2015). Nevertheless, this study detected a relatively weak correlation between total tannins and antioxidant activity, thus suggesting the need for further studies to determine the levels and antioxidant activities of the different types of tannins in spider plant. Additionally, the presence of tannins was

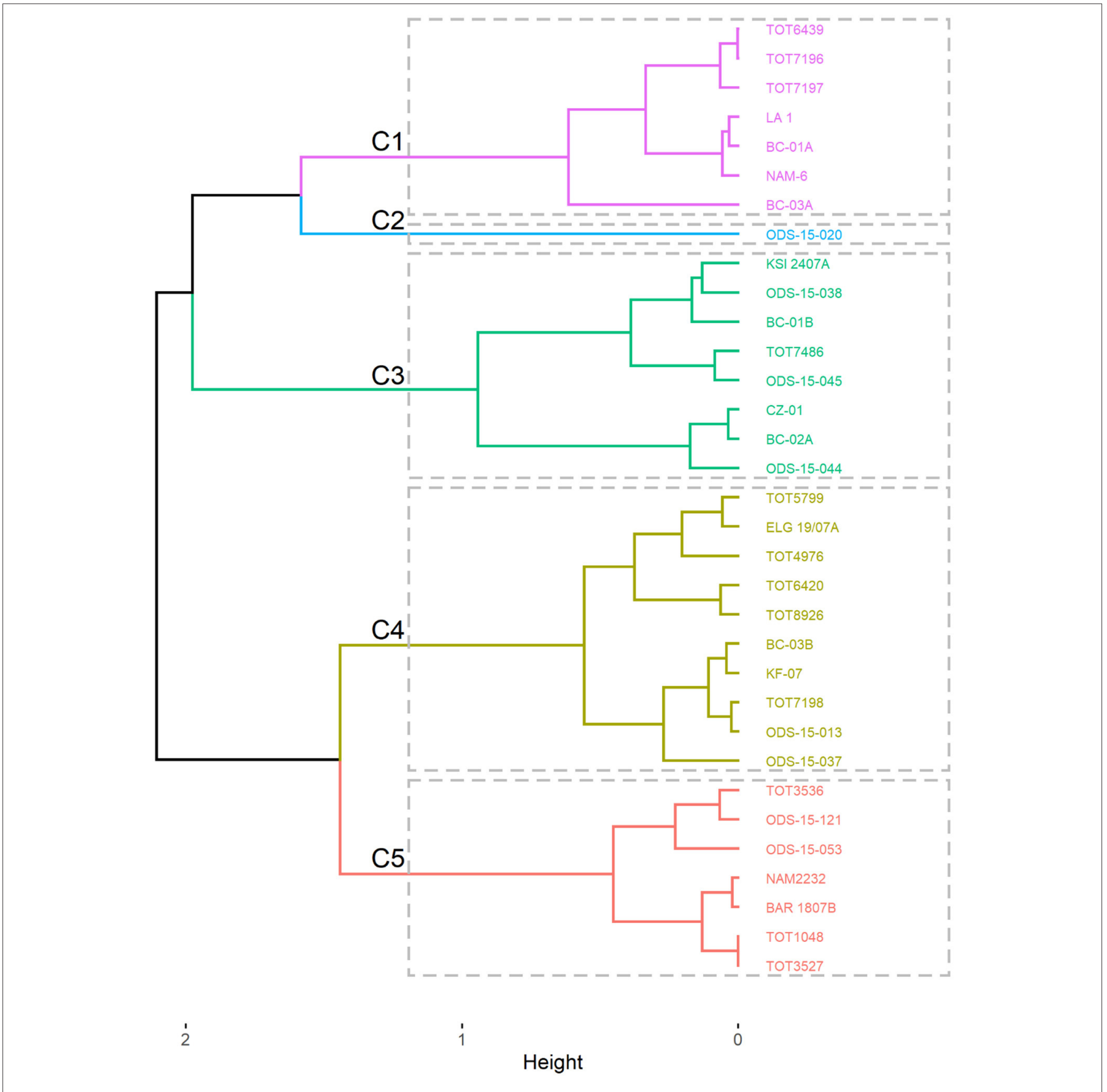

FIGURE 6 | Dendrogram showing the grouping of the 33 accessions of *Gynandropsis gynandra* into clusters.

reported to be responsible for the astringent taste (Kutsututsa et al., 2014) and influenced the palatability and utilization of the vegetable (Chataika et al., 2020). Identifying specific types of tannins and their antioxidant potential in different accessions of spider plant would therefore aid in targeted nutraceutical breeding for addressing specific human health and nutrition disorders and genetic improvements in the species.

Another dietary phytochemical that has been reported to affect palatability in spider plant is anthocyanin, which is believed to be responsible for the purple color of the different plants (Mueller-Harvey, 2001; Dasgupta and De, 2007; Gonzali and Perata, 2020). Furthermore, anthocyanins have been reported as having the capacity of lowering the risk of cardiovascular diseases, cancer, neurodegenerative disorders, and bone loss associated with aging (Chen et al., 2021; Hair et al., 2021), as well as lowering blood pressure, improving visual acuity, and preventing diabetes (Rozita et al., 2018). The accessions with the highest levels of anthocyanins were in cluster C3

TABLE 4 | Dietary phytochemicals and number of leaves differentiation among spider plant accession clusters.

Clusters	TPC	Tannins	Anthocyanin	Antioxidant activity	Number of leaves
C1	100.7	97.8	41.1	330.5	36.3
C2	109.9	146.7	40.1	277.3	**101.5**
C3	103.2	160.8	**63.3**	305.3	64.9
C4	130.5	209.8	36.5	292.1	43.5
C5	**160.3**	**294.0**	45.4	**350.1**	31.1

Values in bold are for high values characterizing the cluster.

and included BC-02A, ODS-15-044, BC-03B, and KSI-2407. This could be because of the diversity of the levels of these phytochemicals in different accession and across the regions of origin. The identification of superior accessions with high levels of both anthocyanins and tannins does not suggest any linkage of the genes controlling these traits, but another factor might be playing a role. One factor that could have been responsible for this observation is the agro-ecological factors associated with the regions of origin. This was evidenced by the significantly high anthocyanin content in Southern African accessions, while accessions from West Africa had high TPC and tannins. In addition, Adeka et al. (2019) reported significant changes in the levels of the bitterness of spider plant from different agro-ecologies, suggesting changes in anthocyanin levels due to agro-ecological factors. The observations suggest an opportunity for managing the levels of anthocyanin, including palatability, by changing specific environmental factors. This calls for further investigation to determine the specific environmental factors that affect the levels of anthocyanin and the extent of environmental influence.

Antioxidant Activity of Different Accessions of Spider Plant

Antioxidant activity, expressed as ascorbic acid equivalent (AAE), is believed to be a better way of depicting the combined effect of free radical scavenging phytochemicals in the plant extracts. The antioxidant activity of the spider plant accessions (189.2 to 492.3 mg of AAE/100 g) was slightly lower than what other authors found (Stangeland et al., 2009; Jiménez-Aguilar and Grusak, 2015; Somers et al., 2020). The variations in antioxidant activity across different leafy vegetable amaranth species (Sarker et al., 2018b,c; Sarker and Oba, 2019a) were reported, which conform with the present findings. Stangeland et al. (2009) reported antioxidant activity of 390 mg TE/100 g in spider plant, which was higher than the 35 Ugandan fruits and vegetables that were included in their study. Although the average antioxidant activity was lower (315.3 mg AAE/100 g) than what was reported by other authors, individual accessions with higher antioxidant activities, such as ODS-15-053 (492.2 mg AAE/100 g) and NAM2232 (445.3 mg AAE/100 g), were identified as candidates for breeding programs aimed at improving antioxidant activity of spider plant. These findings suggest the potential use of the spider plant accessions as a vegetable to provide the health benefits associated with antioxidant activity as well as the use of the superior accessions as donor parents in improving antioxidant activity.

Antioxidants are reported to protect cells from oxidative damage caused by free radicals, which can accumulate in the body due to diseases and exposure to chemicals, amongst other causes. Several studies suggest a close link between oxidative stress and the development of diseases such as cancer, AIDS, cardiovascular disease, diabetes, hypertension, inflammatory conditions, and neurodegenerative disorders (Stangeland et al., 2009; Kamble and Gacche, 2019). Furthermore, the antioxidants are reported to reduce DNA damage (Sevgi et al., 2015) and are believed to have antiaging and vitality strengthening properties (Giri et al., 2017). The results from this study suggest that the spider plant is a source of antioxidants, and this supports its use in traditional medicine (Chataika et al., 2020). Sowinmi and Afolayan (2015) also observed that *G. gynandra* possessed high secondary metabolites, which accounted for its strong antioxidant ability, thus justifying its use as naturally occurring antioxidants in folkloric medicine. The identification of *G. gynandra* with strong antioxidant ability availed an opportunity for targeted seed multiplication for conservation and sustainable use as a relish, dietary supplements, and for commercial purposes. Commercial production of the identified *G. gynandra* species, including the development of nutraceutical products, would potentially pave the way for deriving monetary benefits and economic betterment of the involved communities.

Accessions in cluster C5 are good candidates for the breeding program and cultivar release. They combine high levels of total phenolic, tannins, and antioxidant activity. This was further illustrated by the significant positive correlation among total phenolic, tannins, and antioxidant activity. This finding might suggest the possible control of these phytochemicals by a group of genes. Further investigations should focus on determining environmental factors influencing these phytochemicals and genes controlling them through the development of mapping populations. The development of the mapping population should consider the individuals from clusters C2 and C3, as they are characterized by high anthocyanin content and the number of leaves, respectively.

CONCLUSION

Results from this study have revealed that *G. gynandra* accessions contain high levels of dietary phytochemicals, which account for their strong antioxidant ability. The key phytochemicals in spider plant were the TPC, tannins, and anthocyanins. These phytochemicals vary in amounts in different accessions and are associated with the geographical origin of the spider plant accessions. It might be possible to identify candidate accessions for use in breeding programs aimed at improving the nutraceutical quality of the species. Plant breeders implementing breeding initiatives for managing lifestyle diseases associated with oxidative stress, including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders, can use these superior spider plant accessions as parental genotypes for crosses and for large-scale cultivation. The findings from this study provide science-based solid evidence in advocating for regular consumption of spider plant in order to minimize oxidative stress-related diseases. However, more studies are strongly recommended to dissect the genetic and environmental components affecting the inheritance of the phytochemicals to be able to develop a robust breeding program that would optimize the transfer and fixing of specific nutraceutical traits.

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

BC: conceptualization of the design of the research and the methodology, investigation, data curation, analysis, and drafted the original manuscript. AH: data analysis, reviewing the results, and editing the manuscript. LA, EA-D, and JS: review of the methodology and results, supervision of the research, and editing of the manuscript. KM: supervised the laboratory work, reviewed, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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REVIEW
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Nutritional Significance and Antioxidant-Mediated Antiaging Effects of Finger Millet: Molecular Insights and Prospects

Anil Kumar[1*†], Madhu Rani[2†], Shalini Mani[3*], Pallavi Shah[2], Dev Bukhsh Singh[4], Himabindu Kudapa[5] and Rajeev K. Varshney[5,6]

[1] Rani Lakshmi Bai Central Agricultural University, Jhansi, India, [2] Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, India, [3] Department of Biotechnology, Centre for Emerging Diseases, Jaypee Institute of Information Technology, Noida, India, [4] Department of Biotechnology, Siddharth University, Kapilvastu, India, [5] Centre of Excellence in Genomics & Systems Biology, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, [6] State Agricultural Biotechnology Centre, Crop Research Innovation Centre, Murdoch University, Murdoch, WA, Australia

Edited by:
Enoch G. Achigan-Dako,
Université d'Abomey-Calavi, Benin

Reviewed by:
Tahira Fatima,
Purdue University, United States
Nadia Fanou Fogny,
University of Abomey-Calavi, Benin

***Correspondence:**
Anil Kumar
anilkumar.mbge@gmail.com
Shalini Mani
shalini.mani@jiit.ac.in

† These authors have contributed equally to this work

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Aging is a multifaceted process that is associated with progressive, lethal, and unalterable changes like damage to different molecules (DNA, proteins, and lipids), cells, tissues, and organs. It is an inevitable process but can be delayed by both genetic and dietary interventions. Besides aging, premature death and age-associated diseases can be dealt with diet regulation and the use of compounds that inhibit the stress responsiveness or promote the damage repair signaling pathways. Natural compounds offer a repertoire of highly diverse structural scaffolds that can offer hopeful candidate chemical entities with antiaging potential. One such source of natural compounds is millets, which are minor cereals with an abundance of high fiber, methionine, calcium, iron, polyphenols, and secondary metabolites, responsible for numerous potential health benefits. The present review article elucidates the nature and significance of different phytochemicals derived from millets with a major focus on finger millet and highlights all the important studies supporting their health benefits with special emphasis on the antiaging effect of these compounds. The present article also proposes the possible mechanisms through which millets can play a significant role in the suppression of aging processes and aging-related diseases by influencing genetic repair, protein glycation, and stress-responsive pathways. We further discuss well-established natural compounds for their use as antiaging drugs and recommend raising awareness for designing novel formulations/combinations from them so that their maximum antiaging potential can be harnessed for the benefit of mankind.

Keywords: aging, antioxidant, bioactive compounds, millets, natural products, phytochemicals

INTRODUCTION

In the past few decades, there is a tremendous growth in research work connoting that certain major component in diet influence aging and associated age-related diseases. These dietary components include a myriad of constituents like fiber for the glycemic index in diabetes, vegetables, and fruits for cardiovascular disease, certain fats (saturated, polyunsaturated, and trans fatty acids) for cardiovascular disease, and vitamin D and calcium for osteoporosis and bone fracture (Everitt et al., 2006) and many more. With increasing public awareness for the wellness of health considering nutrition as a major role-playing factor, phytochemicals such as polyphenols and dietary fiber have gained popularity for their innumerable health beneficial properties. Several crops like millets are known for their numerous health benefits attributed to their dietary fiber and polyphenol content (Taylor, 2017).

Aging is often a major risk factor associated with non-communicable chronic diseases (NCDs) like cardiovascular disease, type II diabetes, certain cancers, obstructive pulmonary disease, osteoporosis, dementia, etc. (Shlisky et al., 2017). It is a complex multifaceted process, and many theories have been proposed to explain it. These theories include the free radical theory of aging which was later modified to the mitochondrial theory of aging by Harman (1972), the telomere shortening theory as proposed by Lu and Liu (2010), and the protein translational modification theory as proposed by Santos and Lindner (2017). Dietary components such as antioxidants and protein-modifying compounds influence different aspects as proposed in these theories and help delay and in cases overcome aging. Ribarič (2012) have described the epigenetic modifications by dietary factors which include direct effect on gene expression by influencing DNA methylation, histone modification, activation of nuclear receptors by ligands, and through modification of membrane receptor signaling cascades. Furthermore, the influence of caloric restriction on aging was reviewed in the above article with increasing clarity on molecular pathways like the mammalian target of rapamycin (mTOR), sirtuin pathway, and insulin/insulin-like growth factor signaling (IIS) cascades which are all involved in aging (Altintas et al., 2016; Chen et al., 2020; Yu et al., 2021). How these pathways are influenced by dietary components have helped us in establishing the major impact of dietary components on the aging process.

Millets are considered to have immense significance because of their nutritional and nutraceutical potential. Millets are an abundant source of minerals, dietary fibers, and phenolic compounds and offer health benefits such as antimicrobial, antidiabetic, anticancerous, antiartherosclerogenic effects, antioxidant, and antiaging properties (Yang et al., 2012; Si and Liu, 2014; Kumar et al., 2016). Among different types of millets, finger millet is known to possess almost the highest nutritional value (Taylor, 2017). In the recent past, various studies have also reported the preliminary antiaging effect of millet grains, mainly the finger millet (Hegde et al., 2002; Dykes and Rooney, 2006; Shobana et al., 2010; Zhang et al., 2011; Kakkar and Bais, 2014; Khan et al., 2015; Pei et al., 2016).

The present review covers the nutritional and phytochemical composition of finger millet and how they influence aging and age-related disorders. We further discuss well-established natural compounds for their use as antiaging drugs and recommend raising awareness for designing novel formulations/combinations from them so that their maximum antiaging potential can be harnessed for the benefit of mankind.

MILLET: A WONDER GRAIN

Millet includes the grains from the diverse group of forage grasses of the family Poaceae. They are considered "coarse cereals" similar to sorghum mostly due to their grain texture, which makes their processing as well as cooking inconvenient when compared with rice and wheat (Rao et al., 2017; Hassan et al., 2021). They are mainly grown in Asia and Africa and are in cultivation in East Asia for the last 10,000 years (Bhat et al., 2018). Millets produce small seed grains and are often cultivated as cereals. Millets are characterized by their remarkable abilities to survive in stress conditions, fairly resistant to pests and diseases, and are widely adaptable. Millet grains can be stored for a long duration during natural disasters such as famine (Obilana and Manyasa, 2002; Adekunle, 2012; Yang et al., 2012; Kumar et al., 2018).

India, several countries of Africa, and China produce the bulk of the world's millet crops. Several important millets grown in the Asian and African countries include barnyard millet (*Echinochloa* spp.), pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*), little millet (*Panicum sumatrense*), kodo millet (*Paspalum scrobiculatum*), and proso millet (*Panicum miliaceum*) (Rao et al., 2017). Whereas, Tef (*Eragrostic tef*) and Fonio (*Digitaria exilis*) are grown in Ethiopia, Nigeria, Niger, Togo, Senegal, and Mali (Bhat et al., 2018). Most millets are grown in different regions of the world from east to west, which is evident from their colloquial names, foxtail millet as Italian millet, proso as French millet, barnyard as Japanese barnyard millet, indicating their growing region. Proso millet is cultivated in the USA as well as in Russia and is a popular bird feed in both countries, whereas foxtail millet is largely cultivated in China and India. Millets are predominantly used for food purposes in India as well as in other developing countries but are mainly used as feed ingredients in developed countries.

Pearl millet is the most commonly grown species with large grains and accounts for 40% of the world's total millet production (ICRISAT, 2007; Mariac et al., 2007). Except for sorghum and pearl millet, all other millets are known as small or minor millets (Obilana and Manyasa, 2002; ICRISAT, 2007; FAO, 2012) (**Figure 1**). Finger millet is mainly grown in Eastern and Southern African countries (Uganda, Kenya, the Democratic Republic of the Congo, Zimbabwe, Zambia, Sudan, Tanzania, Nigeria, and Mozambique) and Southern Asia (mainly India and Nepal).

Nutritional Composition

The nutritional content of food is an important aspect for the overall health and well-being of humans especially for better health and development to maximize the human genetic potential. Millets as food are a rich source of micronutrients and phytochemicals (Hassan et al., 2021). In comparison with main cereals like wheat, rice, sorghum, and maize, millets possess a high nutritional value (Parameswaran and Sadasivam, 1994). Being an abundant source of nutrients especially minerals, vitamins, dietary fiber, and phytochemicals, millets provide numerous health benefits. They are nutritionally important as they possess high calcium content (0.38%), roughage or dietary fiber (18%), and phenolic compounds (0.3–3%) (Rao et al., 2017).

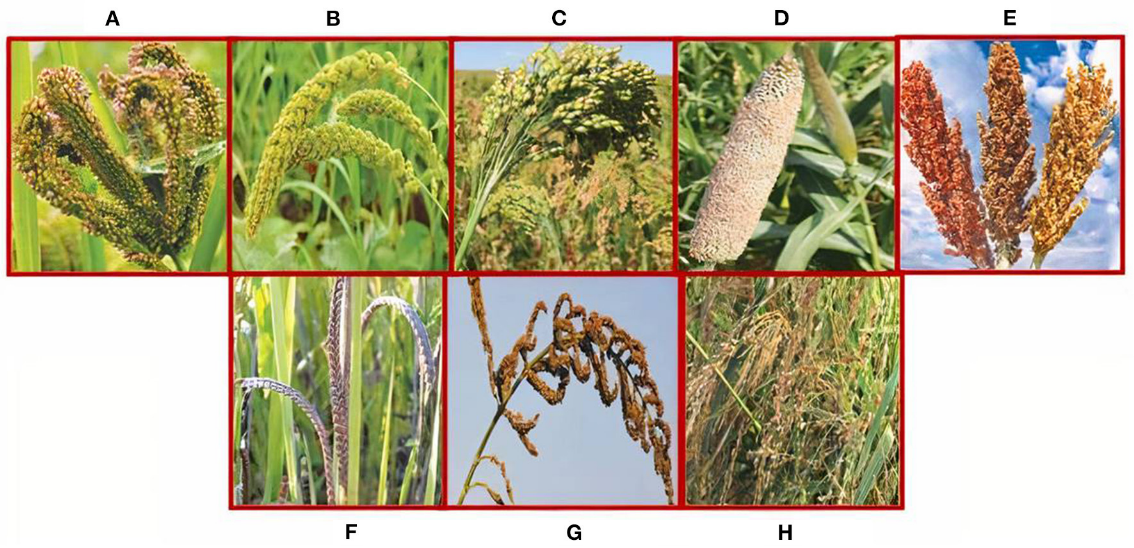

FIGURE 1 | Major millets: **(A)** finger millet (*Eleusine coracana*), **(B)** foxtail millet (*Setaria italica*), **(C)** proso millet (*Panicum miliaceum*), **(D)** pearl millet (*Pennisetum glaucum*), and **(E)** sorghum (*Sorghum bicolor*). Minor millets: **(F)** kodomillet (*Paspalum scrobiculatum*), **(G)** barnyard millet (*Echinochola* spp.), and **(H)** little millet (*Panicum sumatrense*).

Apart from these, proteins found in millet are rich in essential amino acids such as tryptophan, threonine, and sulfur-containing amino acids excluding lysine and threonine. They have a high-fat content in comparison with other cereals especially unsaturated fatty acids (Nithiyanantham et al., 2019). The abundance of phytochemicals and micronutrients of immense therapeutic potential signifies the importance of all varieties of millets (Mal et al., 2010; Singh et al., 2012). The average of the nutrient composition of different millets is shown in **Table 1**.

Millets possess proteins ranging from 7 to 12%, carbohydrates in the range of 65–75%, fat varying from 2 to 5%, and dietary fiber in the range of 15–20%. Of the different millet varieties, pearl millet is the richest in both protein (12–16%) and lipids (4–6%). Millet proteins contain greater amounts of healthy rich essential amino acids (Bhat et al., 2018; Shah et al., 2021). Finger millet, for example, contains a distinctive amount of sulfur-containing amino acids and is a better source of calcium and several micronutrients and pyridoxine. Furthermore, millets are rich in vitamin B complex. Due to the ample quantity of these key nutrients, millets or millet-based products are being used as food products (Subramanian and Viswanathan, 2007; Liu et al., 2012). On the other hand, millet proteins are poor in lysine similar to other cereals, and hence need to be complemented with lysine-enriched vegetables and animal proteins. The major portion of dietary fiber is made up of non-starch polysaccharides (95%), and they are present in seed bran as well as endosperm. Traditionally prepared food items from millets have been a major part of the food consumed in Central America, Africa, and the Indian subcontinent. Most traditional products are flour based, and these include pancakes made from either fermented or unfermented dough, porridge or *mudde*, snacks, deep-fried products, sweet or sour local liquor, some non-alcoholic beverages, and decorticated grains, prepared similar to rice by boiling (Taylor, 2017; Bhat et al., 2018). Based on their nutritional components and henceforth nutraceutical aspect, different types of millets are consumed in different seasons of the year, conventionally. Almost all of these millets are a good source of both soluble and insoluble dietary fibers, which is contributed by both seed coat as well as endosperm cell walls, unlike in rice, wherein mostly the bran layers account for its dietary fiber content. Since the dietary fiber component of the food offers several physiological benefits including the health of the gastrointestinal tract, millets could be gainfully utilized in the preparation of functional and healthy foods for the target population. Along with nutrition, bioactive compounds found in millets are also recognized for their health beneficial effects, such as antioxidant, antidiabetic, antiaging properties, etc. (Sripriya et al., 1997; Kumari and Sumathi, 2002; Shobana et al., 2007; Banerjee et al., 2012; Shahidi and Chandrasekara, 2013; Kumar et al., 2016).

Natural Compounds

Phytochemicals obtained from millets are mostly secondary metabolites with molecular weights ranging from 150 to 30,000 Da in size. Phenolic acids, flavonoids, and tannins are the major polyphenols reported in different parts of the millet grain (Rao and Muralikrishna, 2002; Sreeramulu et al., 2009). Furthermore, it also consists of terpenes, betalians, organosulfides, indoles/glucosinolates/sulfur compounds, protein inhibitors, and other organic acids, along with dietary fiber and phytates. Most millets possess colored coats and some possess colored endosperm. Carotene constitutes the major coloring pigment and hence several millet varieties are known

TABLE 1 | Proximate nutrient composition and nutritive value of various millets (g/100 g db and mg/100 g db).

Variety	Carbohydrate (g)	Protein (g)	Fat (g)	Ash (g)	Fiber (g)	Ca (mg)	Fe (mg)	Zn (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Energy (kcal)
Sorghum	71	10.4	3.1	1.8	2.0	25	5.4	3.1	0.38	0.15	4.3	329
Finger millet	59–75	6.9–10.9	1.5	2.6	15.2	350	3.9	3.13	0.42	0.19	1.1	336
Kodo millet	72–76	6.2–13.1	3.2–4.9	3.3	5.2	35	1.7	1.9–2.4	0.15	0.09	2.0	353
Foxtail millet	55–69	11.2	4.0	3.3	9.4	31	2.8	2.92	0.59	0.11	3.2	351
Fonio millet	68–75	8.4	3.3	3.4	18.2	20	2.1	1.5	0.17	0.22	1.15	379
Little millet	76	15	4.5	5.4	2.5	17	9.3	5.25	0.30	0.09	3.2	329
Barnyard millet	74	11.0	5.2	4.5	13.6	22	18.6	3	0.33	0.10	4.2	300
Pearl millet	67–72	11.8	5.1	2.2	13.8	42	11.0	3.29	0.38	0.21	2.8	363
Proso millet	64–76	12.6	2.9–11.6	2.7	13.1	15	2.2	2.36	0.41	0.28	4.54	316

All values represent mean/range of reliable published data.

Source: Adapted from Taylor (2017), Serna-Saldivar and Espinosa-Ramírez (2018), Kumar et al. (2018), Selvi et al. (2015), Shankaramurthy and Somannavar (2019), and Renganathan et al. (2020).

to have beta-carotene. Rich polyphenol content is responsible for the antioxidant potential of millets. The gelatinization temperature of the millet starch is slightly higher compared with rice and wheat. It also differs from other cereal starches concerning the crystallinity and the amylose and amylopectin organization. Hence, millet-based diet/foods may digest slowly compared with other cereal foods.

Polyphenols

Polyphenols are naturally occurring secondary metabolites which are generally characterized by the presence of multiple phenol rings (Pandey and Rizvi, 2009; Manuja et al., 2013). Several polyphenols have been detected from different plant species, and a majority of them are derivatives of phenylalanine or another precursor shikimic acid. Polyphenols are categorized on the basis of the number of phenol rings and how these rings are bound to each other. The major classes consist of phenolic acids (derivatives of benzoic acid and cinnamic acid), flavonoids, lignans, and stilbenes (**Supplementary Figures S1, S2**). Polyphenols are abundant in millets and are one of the most marketed dietary supplements (Ferguson, 2001). The finger millet seed coat is abundant in polyphenols when compared with many other kinds of cereal such as wheat, rice, barley, and maize (Viswanath et al., 2009).

Phenolic Compounds

Grain phenolics can exist both as free, soluble forms as well as insoluble bound forms. The majority of the phenolics found in millet exist as glycosides, whereas ferulic acid is the major bound phenolic acid (18.60 mg/100 g) and protocatechuic acid is the major free phenolic acid (45.0 mg/100 g) (Rao and Muralikrishna, 2002). The seed coat of finger millet is known to be rich in many potent antioxidant phenolics mostly benzoic acid derivatives (85%) such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and syringic acid (Hegde et al., 2002; Rao and Muralikrishna, 2003; Chandrasekara and Shahidi, 2010), whereas the rest consists of either flavonoid like quercetin or cinnamic acid derivatives such as *trans*-cinnamic acid, *p*-coumaric acid, caffeic acid, and sinapic acid followed by condensed tannins (Chethan and Malleshi, 2007; Shah et al., 2021). Apart from these bound phenolic acids such as ferulic and *p*-coumaric acid are also found in abundance in finger millet (**Table 2**).

Apart from these phenolics, direct infusion electrospray ionization mass spectrometry (ESI MS) of the extract from seed coat elucidated the presence of luteolin, naringenin, kaempferol, phloroglucinol, apigenin, catechins, malic acid, epigallocatechins, diadzein, and catechingallates (Shobana et al., 2009). Among various millets studied, finger millet had the highest content of ≤300 μmol catechin equivalents/g of the defatted meal followed by foxtail, little, pearl, and proso millets. Furthermore, finger millet is the richest in condensed tannins (Dykes and Rooney, 2006). A recent report showed that finger millet varieties with darker color from Northern Malawi had higher polyphenols and antioxidant properties in comparison with the lighter ones. It means that the dark-colored finger millet varieties have greater potential for use as functional food ingredients as they possess higher amounts of natural antioxidants than light-colored varieties (Xiang et al., 2019).

Micronutrients

As the name suggests, micronutrients are required in very few quantities for human health. However, micronutrient deficiency and their imbalance cause various age-related diseases in humans like obesity, diabetes, osteoporosis, etc. The minerals and vitamins are called micronutrients. Worldwide, more than 2 billion people are suffering from one or other age-related chronic micronutrient deficiencies (MNDs) whereas more than 800 million people are undernourished (Kumssa et al., 2015; Beal et al., 2017). In millets, the mineral content range is from 1.7 to 4.3 g/100 g which is a comparatively higher side than that of main cereals such as wheat (1.5%) and rice (0.6%). Minerals play a chief role in structure and rigidity, muscular contractions, formation of strong bones and teeth, oocyte activation, blood clotting, oxygen transport, regulation of heartbeat, maintenance of fluid balance, nerve signal transmission, etc. (Kulkarni et al., 2018; Kumar et al., 2018). Calcium and iron deficiencies are mainly observed in developing countries. A large number of people are suffering from osteoporosis in India. The calcium

TABLE 2 | Active ingredients of finger millet and their mechanism of action for enhancing the lifespan.

Active ingredients/bioactive peptides	Experimental model	Efficacy	Mechanism	References
Dietary fiber, phytate, and total phenolics of seed coat matter of finger millet	Streptozotocin-induced diabetes model rat	Hypoglycaemic, hypocholesterolaemic, nephoprotective, and delayed cataractogenesis	Reduced urinary glucose excretion, regulation of postprandial glycemia, reduced the lipid levels and atherogenic index, reduce levels of blood and urinary creatinine and attenuated nephromegaly, anticataractogenic property, lower the levels of AGE, HbA1c, AR activity, and lesser lenticular opacity	Shobana et al., 2010
Diadzene, gallic, coumaric, synringic, and vanillic acids of finger millet	–	Antioxidant activity	Inhibition of oxidation of linoleic acid and reduction of ferrous to ferric iron	Viswanath et al. (2009)
Seed coat matter of black finger millet	Streptozotocin-induced diabetes model rat	Antioxidative and antidiabetic activity	Reduced the serum levels of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) and elevation in catalase (CAT) and superoxide dismutase (SOD) and lowered the thiobarbituric acid reactive substances (TBARS)	Okoyomoh et al., 2013
Formulated finger millet	Alloxan-induced diabetes mellitus model male Wistar albino rats	Hypoglycaemic and hypolipidemic properties	Lower the glucose level, cholesterol, triglycerides, and low-density lipoproteins (LDL)	Nadro and Elkanah, 2017
Ferulic and cinnamic acids of kodo millet	–	Antioxidant activity	Free radical scavenging activity	Sharma S. et al. (2017)
Whole grain and bran of finger millet	High-fat-diet-fed LACA model mice	Antioxidant and anti-inflammatory properties	Prevention of obesity by regulation of obesity-related genes, improved lipid profile and anti-inflammatory status, suppresses oxidative stress	Murtaza et al., 2014
Polyphenol of finger millet	–	Antioxidant activity	Inhibits superoxide, hydroxyl, and nitric oxide radicals	Bindu and Malleshi, 2003
Ethanolic extract from finger Italian millet	–	Antidiabetic and antioxidant activity	Inhibits glycation	Ofosu et al., 2020
Carotenoids and vitamin E of finger millet	–	Antioxidant activity	Free radical scavenging activity	Chethan et al., 2008
Methanolic extract of finger millet	–	Protective role against diabetes mellitus	Inhibits glycosylation of collagen	Hegde et al., 2002
Whole grain extracts of finger millet (Oshadha and Rawana), proso millet, white finger millet, kodo millet, foxtail millet	–	Antidiabetic, antialpha amylase and antiglucosidase activity	Inhibition of early glycation, middle glycation and reversal of antiglycated products	Senevirathne et al., 2021
Free and bound phenolics from finger millet varieties	–	Antioxidant activity	Free radical scavenging activity	Xiang et al., 2019
Proanthocyanidins of finger millet	–	Antioxidant activity	Free radical scavenging activity	Dykes and Rooney, 2006
Benzoic acids				
Protocatechuic acid (PCA) of finger millet seed coat	–	Antibacterial, antiviral, neurological effect, antiatherosclerotic, antifibrotic, antiaging, antiulcer, anticancer	Antiaging ability is induced as it increases the activity of glutathione peroxidase and catalase and decreases the malondialdehyde level.	Zhang et al., 2011; Kakkar and Bais, 2014; Khan et al., 2015
Gallic acid of finger millet seed coat	–	Antioxidant, anticarcinogenic, antimicrobial, anti-inflammatory, antimelanogenic, neuroprotective, hepatoprotective	Induces antiaging effect due to its ROS quenching and antiglycation activity	Panich et al., 2012; Badhani et al., 2015

(Continued)

TABLE 2 | Continued

Active ingredients/bioactive peptides	Experimental model	Efficacy	Mechanism	References
Syringic acid of finger millet seed coat	–	Antimitogenic, antidiabetic	Eradicates free radicals due to their antioxidant potential	Wei et al., 2012; Muthukumaran et al., 2013
Vanillic acid and its derivatives of finger millet seed coat	–	Antimicrobial, antiaging, hepatoprotective, antivenom	Inhibits collagenase and elastase activity	Dhananjaya et al., 2009; Itoh et al., 2009; Widowati et al., 2016
Cinnamic acid derivatives				
p-Coumaric acid of finger millet seed coat	–	Antioxidant,anti-inflammatory, antimutagenic, antiulcer, antiplatelet, and anticancer	Due to its potent antioxidant potential	Pragasam et al., 2013; Pei et al., 2016
Ferulic acid of finger millet seed coat	–	Antioxidant, antimicrobial, anti-inflammatory, antithrombosis, and anticancer activities	Inhibits the expression of some cytotoxic enzymes, such as nitric oxide synthase, caspase, and cyclooxygenase-2	Rao and Muralikrishna, 2002; Ou and Kwok, 2004; Pei et al., 2015
Caffeic acid of finger millet seed coat	–	Antioxidant, anti-ischemia reperfusion, antithrombosis, antihypertension, antifibrosis, antivirus, and antitumor	Potent antioxidant potential	Jiang et al., 2005
Sinapic acid of finger millet seed coat	–	Antioxidant, antimicrobial, anti-inflammatory, anticancer and antianxiety activities	Elastase, tyrosinase and collagenase inhibitory activities	Chen, 2016; Taofiq et al., 2017
Flavonoids				
Quercetin of finger millet seed coat	–	Antioxidant, antiaging, antiobesity, anticarcinogenic, antiviral, antibacterial, and anti-inflammatory effects	Proteasome activator with antioxidant properties that consequently influence cellular lifespan	Chethan et al., 2008; Chondrogianni et al., 2010; Wang et al., 2016
Catechin and its derivatives of finger millet seed coat (gallocatechin, epicatechin, and epigallocatechin)	–	Antioxidant, anti-inflammatory, antimutagenic effects, antiaging, as well as cardiovascular disease-preventive properties.	Eradicates free radicals, stimulates the activity of the regulatory protein SIRT1	Sripriya et al., 1996; Unno et al., 2011; Hong et al., 2013

content of finger millet (ca. 340 mg/100 g) is almost eight times higher than major cereals wheat and rice. Therefore, finger millet is the richest source of calcium to overcome age-related diseases like osteoporosis (Puranik et al., 2017; Sharma D. et al., 2017). The consumption of pearl millet and barnyard millet help in protection from anemia because they have been found as the richest source of iron. The highest content of zinc (4.1 mg/100 g) in foxtail millet as compared with other millets and stable cereals make it special to combat zinc-associated diseases. Foxtail millet is also a good source of iron (2.7 mg/100 g). The immune system is boosted by the intake of zinc and iron (Kulkarni et al., 2018; Kumar et al., 2018). Millets are also the ultimate source of β-carotene and B vitamins like riboflavin, niacin, and folic acid as compared with wheat and rice. The foxtail millet has the richest source of thiamine (0.60 mg/100 g) whereas the barnyard millet has the highest content of riboflavin (4.20 mg/100 g) (**Table 1**). The regular consumption of millets in our diets aids to curb nutritional deficiencies and age-related diseases (Chandel et al., 2017). The presence of antinutrients in millets like phytates, polyphenols, and tannins is responsible for the reduction in mineral bioavailability by chelating multivalent cations such as Ca, Zn, Fe, Mg, and K. Besides this, the rich content of protease and amylase inhibitors in millets also causes reduced digestibility of millet grains. Therefore, food processing techniques are used to enhance nutritional content, increase digestibility, and bioavailability of nutrients with a reduction in antinutrients and increase the accumulation of nutrients in milled grains (Sharma D. et al., 2017; Vinoth and Ravindhran, 2017). The applications of genetic engineering and genome-editing tools are also useful in facilitating nutrient

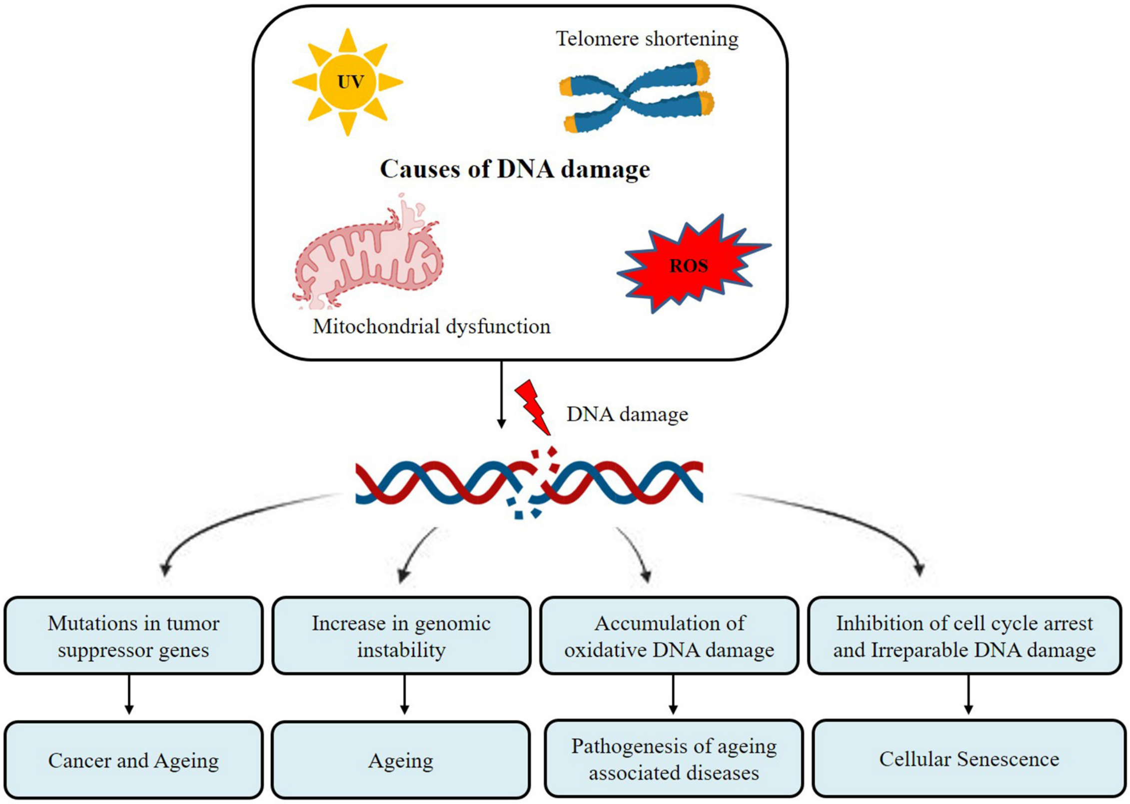

FIGURE 2 | Different types of DNA damage result in aging and associated diseases.

accumulation in grains and the prevention of the synthesis of antinutrients.

HEALTH BENEFITS OF MILLET CONSUMPTION

Millets are better alternatives to common cereal grains due to their nutraceutical properties and immense health-promoting attributes. Millets contain ample amounts of fiber and lesser amounts of simple sugars responsible for their relatively low glycemic index. Millets have been shown to aid low blood sugar levels in comparison with wheat or rice (Kumari and Sumathi, 2002) and thus possess antidiabetic properties. Millets similar to whole grains are abundant in magnesium, which acts as a cofactor for enzymes especially involved in the glucose uptake and secretion of insulin. Magnesium has been reported to reduce the effects of migraines and heart attacks. Niacin in millets is hypocholesteremic in nature. Consumption of millets also reduces the C-reactive protein and unhealthy lipids and thus may prevent cardiovascular diseases. Millets provide plenty of phosphorus which plays an important role in cell structure determination. Aside from constituting the bone mineral matrix, phosphorus is an essential component of adenosine triphosphate (ATP), the energy currency of the cell. Apart from the abovementioned health benefits, the compounds of millets are also known to possess antioxidant and antiaging properties. Previous reports have well-established the antioxidant potential of many millet varieties (Chandrasekara and Shahidi, 2010). Regulation of oxidative stress, through antioxidant administrations, is also capable of controlling different health-related issues such as diabetes, cancer, neurodegenerative disorders, cardiovascular problems, and age-associated diseases. Thus, in the benefit of the current review, the different properties of millets are explained in relation to aging.

BIOACTIVE COMPOUNDS: ANTIAGING AND ANTIOXIDANT PROPERTIES

Antioxidants derived from plants, such as phenolics and flavonoids have numerous biological effects. Antioxidants play a major role in the reduction of lipid peroxidation, a process that plays a key role in cancer and aging (Namikii, 1990). Antioxidants provide stable radical intermediates which prevent the oxidation-induced damage of fatty acids and oils (Lobo et al., 2010). Polyphenols like phenolic acids, flavonoids, and tannins found in the seed coat of millet seeds act as reducing agents such as free radical quenchers, metal chelators, and singlet oxygen quenchers (Banerjee et al., 2012). Potent antioxidant attributes of polyphenols arise as a result of their ability to act as hydrogen

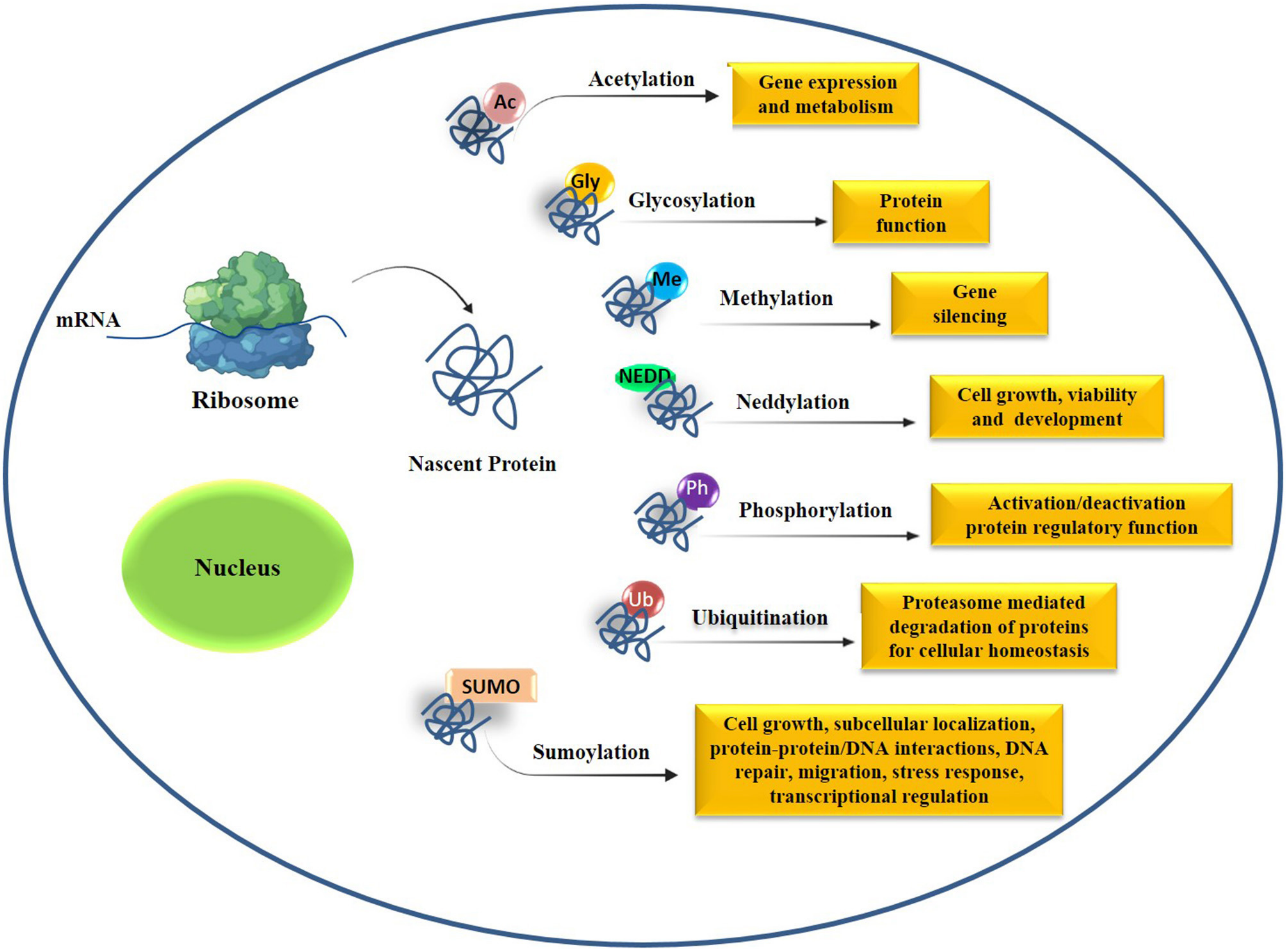

FIGURE 3 | Posttranslational modifications (PTMs) in cells and alteration in PTMs cause premature aging and aging-associated diseases. Abbreviations used in this figure: Ac, acetylation; Gly, glycosylation; Met, methylation; NEDD, neddylation; Ph, phosphorylation; SUMO, sumoylation, Ub, ubiquitination.

atom donors *via* hydroxyl groups on phenol rings to electron-deficient free radicals.

Polyphenols overcome oxidative stress by the maintenance of balance between oxidants and antioxidants. Being antioxidant, these compounds may show different health benefits and primarily may act as antiaging compounds as well. Polyphenols presently have gained the status of "life span essentials" as they play a crucial role in maintaining health (Chandrasekara and Shahidi, 2010). Millet grain is entirely edible, and conventional food recipes are prepared from wholemeals throughout Asian and African countries. This indicates that the phytochemicals of millets such as polyphenols are consumable without any adverse effects on human health. The content and composition of these polyphenols vary depending on the type of millet grain and have received considerable interest because of their antioxidant, antiaging, and other nutraceutical properties (Chandrasekara and Shahidi, 2011). Additionally, some of the other health benefits such as hypoglycemic (Kumari and Sumathi, 2002), hypocholesterolemic (Hegde et al., 2002) and antiulcerative (Tovey, 1994) properties can further be contributed to a large extent to their polyphenol content.

Different types of millets are known to be rich in different polyphenols which may act as potent antioxidants which are critical for exhibiting antiaging properties. For example; different types of millet grains contain dimers and trimers of ferulates which possess comparatively higher antioxidant activity (Chandrasekara and Shahidi, 2011). Very recent findings on phenolics of millet grain varieties that are cultivated in South Korea like millet (M), Italian millet (IM), barnyard millet (BM), and finger Italian millet (FIM) have shown their potent antioxidant potential and antiglycation properties effective against diabetes mellitus which is a main age-related disorder. It has also been reported that the finger Italian millet has the highest total phenolics and total flavonoid content as compared with barnyard millet and Italian millet. Therefore, finger Italian millet is a crucial nutraceutical as it possesses high antioxidant and antidiabetic activity (Ofosu et al., 2020). Colored (brown, reddish, or red) finger millet varieties from the Northern Malawi region exhibit more significant antioxidant activity than

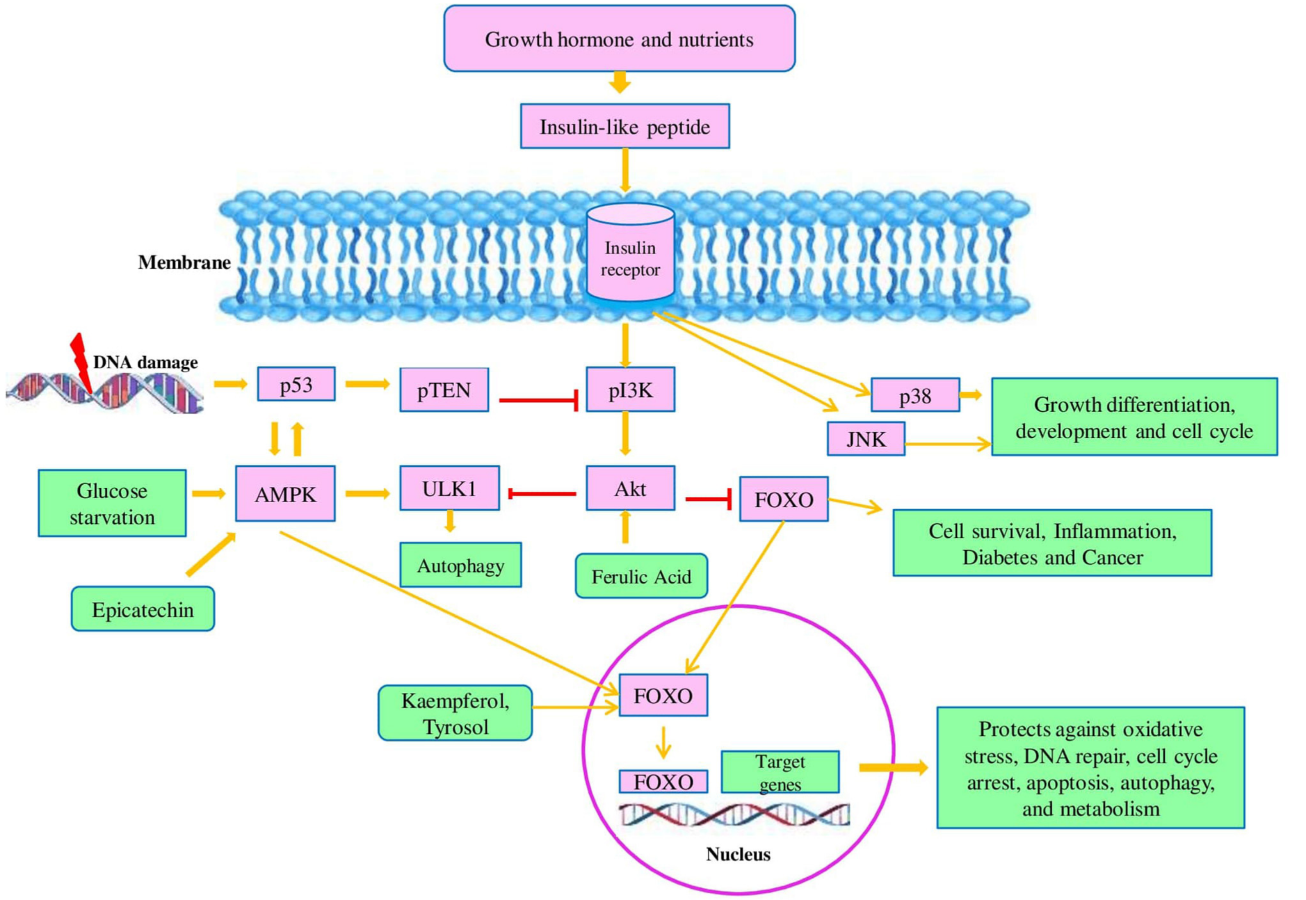

FIGURE 4 | Model of the insulin/insulin-like growth factor signaling (IIS) pathway: in response to growth hormones and nutrients, insulin as a ligand binds to membrane-bound glycoprotein receptors which results in a conformational change in membrane-embedded glycoprotein. This insulin ligand-receptor complex mediates the activation of tyrosine kinases by their phosphorylation. Tyrosine kinase (TK) is responsible for the phosphorylation of IRS family proteins and opens the binding sites for various signaling pathways. TK mediates activation of phosphatidylinositol kinase (PI3K). Moreover, PI3K activated the protein kinase B (Akt) which modulates cellular processes like cell survival and inflammation by phosphorylation and suppression of FOXOs. In response to DNA, damage p53 is activated and accelerates the activity of pTEN which in turn inhibited the PI3K and prevents the activation of Akt- mediated repression of FOXO. The insulin ligand-receptor complex also activates the MAPK family members like p38 and c-Jun-terminal kinase (JNK) which are the chief players of cell growth, development, apoptosis, and inflammation. Under glucose starvation conditions, adenosine $5'$-monophosphate-activated protein kinase (AMPK) is activated and induces autophagy by the acceleration of the ULK1 kinase whereas Akt diminished the activity of ULK1 kinase. AMPK also activates the FOXO and promoted the FOXO transcriptional activity. Additionally, p53 and AMPK activate each other.

the white variety due to the presence of high amounts of phenolic compounds, tannins, and flavonoids in them (Xiang et al., 2019). Amadou et al. (2011) have studied the antioxidant efficiency of defatted foxtail millet (DFMB) and observed that it can scavenge free radicals and superoxide anions.

Watanabe (1999) isolated two flavones viz., luteolin and tricin, with antioxidant activity from Japanese barnyard millet. Luteolin along with its glycosides possesses health-beneficial properties such as antioxidant, anti-inflammatory, cancer preventive, and antiarrhythmic activities (Lin et al., 2008). Tricin is also known for its antitumor and antimetastatic properties (Yue et al., 2020). Finger millet showed the highest total flavonoid content in defatted meals followed by kodo and foxtail millets. Studies were carried out on the natural antioxidants in edible flours of small millets. Ferulic acid exhibits very strong antioxidant, free radical scavenging, and anti-inflammatory activity (Shahidi et al., 1992; Castelluccio et al., 1995).

Free radical production in excess and lipid peroxidation are the major reasons for chronic diseases such as diabetes, cardiovascular disease, cancer, cataract, and aging. Non-enzymatic glycosylation whereby chemical reaction occurs in between the aldehyde group of reducing sugar and the amino group of proteins plays a major role in the complications of diabetes and aging (Rowan et al., 2018). Non-enzymatic glycosylation and cross-linking of collagen are induced by free radicals, whereas free radical scavengers such as polyphenols inhibit these reactions (Fu et al., 1992). Polyphenols act as antioxidant compounds that suppress excessive oxidation responsible for cancer and aging (Namikii, 1990). Thus, any compound which can neutralize these free radicals can in

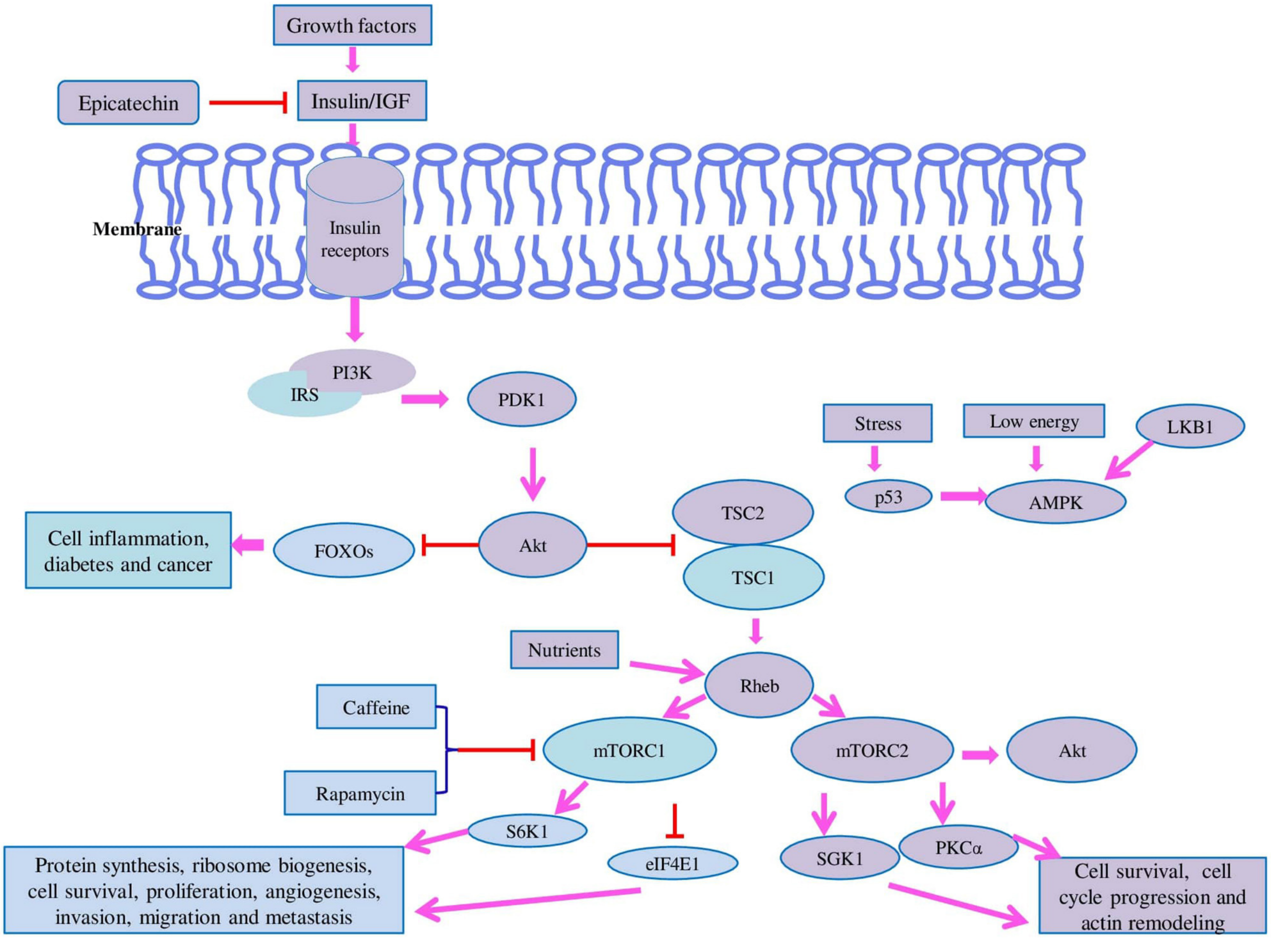

FIGURE 5 | Model of the mammalian target of rapamycin (mTOR) pathway: the mTOR pathway has two major components called TOR complex 1 (TORC1) and TOR complex 2 (mTORC2) which regulate different cellular functions. mTORC1 is sensitive to rapamycin and play role in protein synthesis, ribosome biogenesis, cell survival, proliferation, angiogenesis, invasion, migration, and metastasis whereas mTORC2 is insensitive to rapamycin and regulates cell survival, cell cycle, and actin remodeling. In response to growth nutrients, the mTOR pathway is regulated by the PI3K cascade. mTOR is negatively regulated by tuberous sclerosis proteins called TSC1 and TSC2 which exist as heterodimers. In response to insulin signaling, Akt phosphorylates TSC2 which makes it functionally inactive. Shortage of nutrients mainly amino acid starvation leads to dephosphorylation of well-known substrates of mTORC1 such as ribosomal S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein (4E-BP) whereas in the supply of nutrients, restoration of amino acids enables phosphorylation of S6K and 4E-BP which accelerates protein synthesis. Amino acids activate mTORC1 in two ways either *via* inhibition of TSC1-TSC2 or by stimulation of Rheb. AMPK is activated under low energy (high AMP/ATP ratio) and promotes fatty acid oxidation and inhibits protein synthesis. The tumor suppressor LKB1 is responsible for the activation of AMPK which leads to inhibition of mTORC1-mediated phosphorylation of S6K1 and 4E-BP1. AMPK also directly activates TSC2 *via* its phosphorylation and increases its activity which ultimately leads to repression of mTORC1 signaling. High-energy situation mediates upregulation of mTORC1 activity. Upon stress conditions, p53 is activated and inhibits the mTOR activity by the AMPK-TSC2 mechanism. In contrast to mTORC1, mTORC2 is insensitive to nutrients but responds to growth factors such as insulin which requires PI3K. For the activation of mTORC2, ribosomes are very crucial. mTORC2 binds to ribosomes in an aPI3K-dependent manner and regulates protein kinase B (Akt), glucocorticoid-induced protein kinase 1 (SGK1), and protein kinase C-α (PKC-α). SGK-1 is also activated by mTORC2 and controls the growth and ion transport. mTORC2 regulates the actin remodeling by the activation of PKC-α. mTORC1 regulates protein synthesis, ribosome biogenesis, cell survival, proliferation, angiogenesis, invasion, migration, and metastasis by regulation of S6K1 and 4E-BP1.

turn exhibit antiaging effect. Senevirathne et al. (2021) have very recently observed antidiabetic properties from different varieties of millets such as finger millet, proso millet, white finger millet, kodo millet, and foxtail millet cultivated in Sri Lanka. The inhibition of early glycation, middle glycation, and reversal of antiglycated products by these millets in diabetes make them crucial functional food for delaying the aging process. These millet varieties also showed anti-α-amylase activity, and suppression of this enzyme is required for better carbohydrate digestion and maintains the blood glucose levels for diabetic patients (**Table 2**).

Hegde et al. (2005) studied the effects of the antioxidant properties of millets on oxidative stress and glycemic status in alloxan-induced rats. The alloxan-induced rats when fed with a diet enriched with finger millet showed a significant decrease in blood glucose and cholesterol level. Tail tendon collagen glycation was reduced in the finger millet-fed rats. Diabetic groups fed on finger millet diet showed alleviated

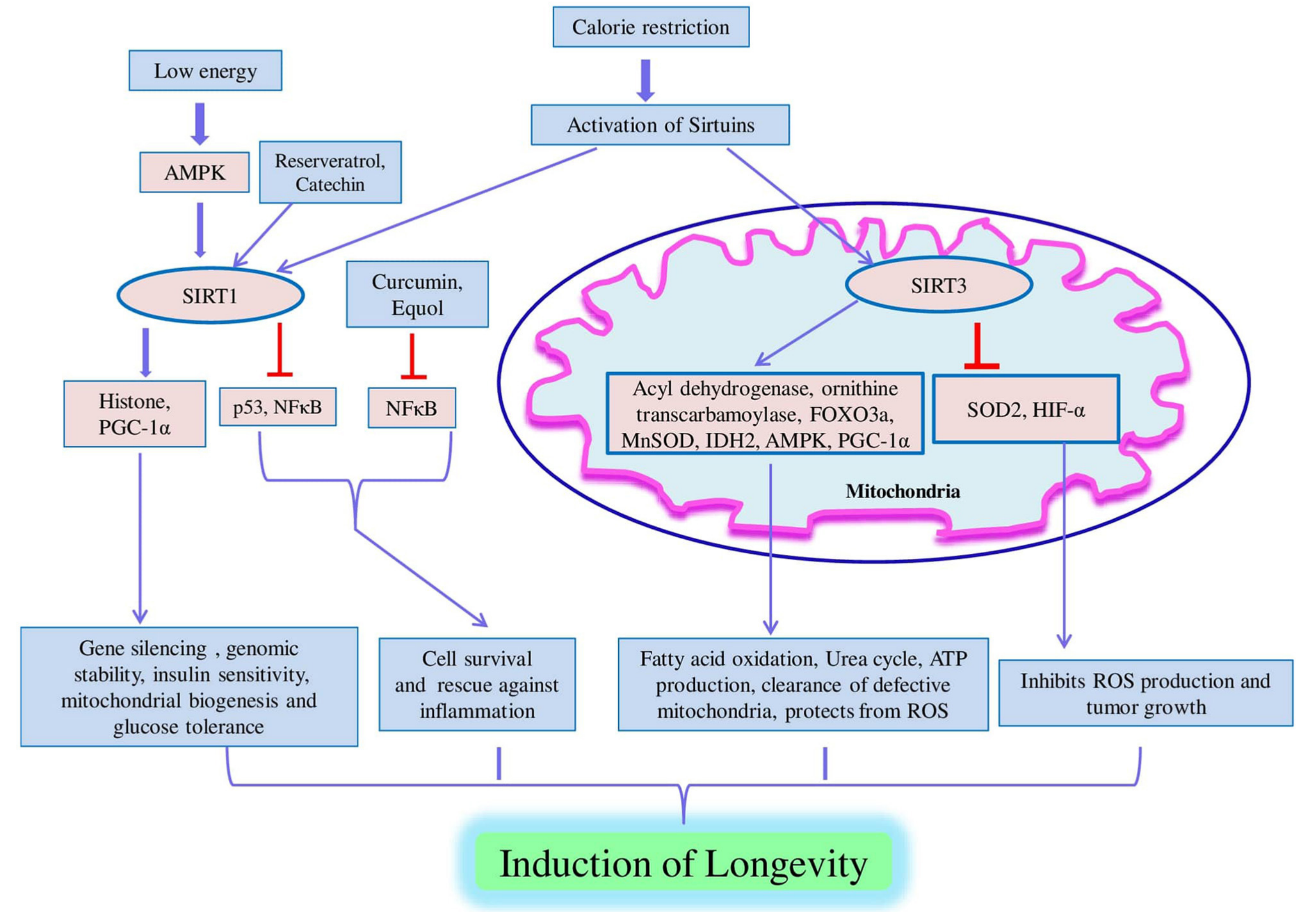

FIGURE 6 | Model of sirtuin pathway. Sirtuin pathway is activated. In response to low energy upon calorie restriction in cells, AMPK is activated which promotes SIRT1-mediated *trans*-activities. SIRT1 regulates key biological processes such as gene silencing, genomic stability, insulin sensitivity, mitochondrial biogenesis, glucose tolerance, cell survival, and protection against inflammation and mediates longevity *via* deacetylation of various substrates like histone, PGC1-α, p53, and NFκB. In response to prolonged calorie restriction, upregulation of SIRT3 occurs in liver and brown adipose tissue which results in the activation of long-chain acyl dehydrogenase and ornithine transcarbamoylase that is associated with β-oxidation of fatty acids and the urea cycle. SIRT3 positively modulates the FOXO3a, MnSOD, isocitrate dehydrogenase 2 (IDH2), AMPK, and PGC-1α; promotes ATP production and clearance of defective mitochondria; and protects against ROS. SIRT3 inhibits ROS production and tumor growth *via* negative regulation of SOD2 and hypoxia-inducing factor-alpha (HIF-α).

levels of enzymatic and non-enzymatic antioxidants along with lipid peroxides. This was associated with the abundant amounts of phenolics, tannins, and phytates present in finger millets. Furthermore, the effects of methanolic extracts of finger millet and kodo millet on glycation and cross-linking of collagen were studied (Hegde et al., 2002). It is thus proposed that finger millet can have a potential therapeutic role as dietary supplements for the prevention of glycation-induced complications, as in diabetes or aging.

Free radicals are regularly generated in our body as a byproduct of normal aerobic respiration and substrate oxidation. However, the excessive accumulation of these radicals causes damage to vital biomolecules such as carbohydrates, lipids, proteins, and DNA, consequently leading to the manifestation of various age-related diseases. Polyphenols are suggested to exhibit their beneficial antioxidant effects either by scavenging free radicals, chelating metal ions, quenching singlet oxygen, or by inhibiting the reactive oxygen species (ROS) producing enzymes such as myeloperoxidase, lipoxygenase, cyclooxygenase, and xanthine oxidase. Chandrasekara and Shahidi (2011) evaluated the antioxidant properties of phenolic extracts from seven millet varieties [kodo, finger (Ravi), finger (local), proso, foxtail, little, and pearl] on HT-29 cells. All varieties used in this study showed a significant reduction in lipid peroxidation in liposomes, singlet oxygen quenching, and DNA scission inhibition to varying degrees. Furthermore, the cytoprotective effects of various types of millet phenolics (catechin, ferulic acid, vanillic acid, and resveratrol) were investigated, and it was observed that they exhibit preventive effects on protein and human erythrocyte peroxidation (Palaniswamy and Govindaswamy, 2017).

Different studies have also given exclusive attention to finger millet for its pharmacological significance. For instance, the study

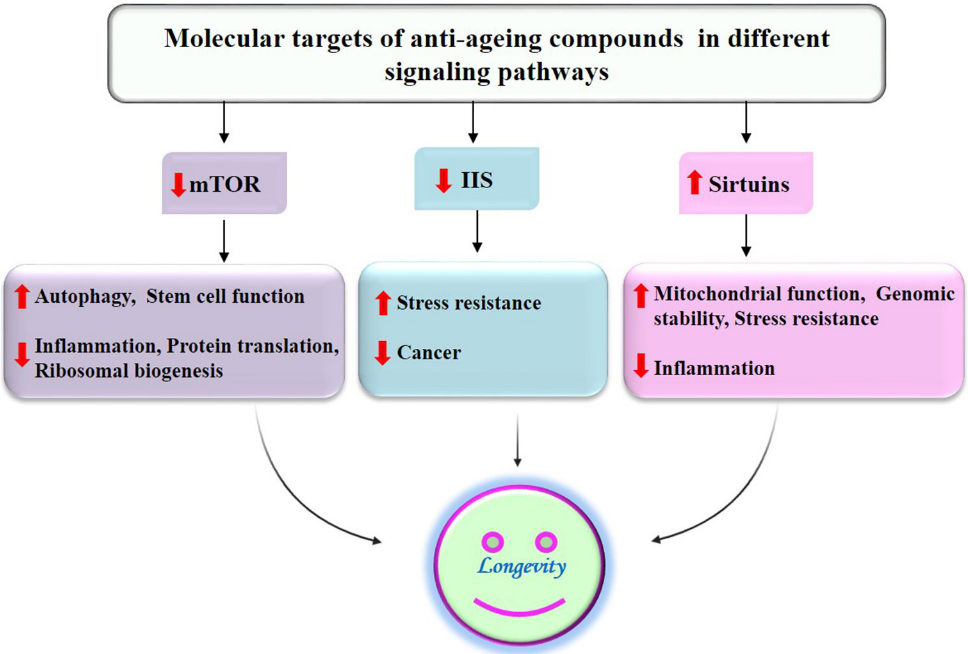

FIGURE 7 | Suppression of aging and age-associated diseases by antiaging compounds of millets through modulation of different signaling pathways by acting on their molecular targets.

conducted on early diabetic rats indicated that the administration of finger millet exhibited its beneficial effects on antioxidant status of the skin, production of nerve growth factor, and wound-healing parameters (Rajasekaran et al., 2004). Ajiboye (2017) also evaluated the antioxidant potential and free radical scavenging ability of whole-grain finger millet. The study revealed that ethanolic extract of whole finger millet effectively scavenged H_2O_2, NO, and DPPH.

Oxidative stress, caused by ROS, is responsible for modulating several pathological conditions during aging. Thus, aging can also be defined as low energy and high oxidative stress state. As studies indicate that the administration of millet may greatly affect the level of reactive oxygen species and hence may also combat age-associated health issues. Though there is a lack of evidence showing the molecular mechanism for antiaging effect of millets, the possible molecular mechanism for antiaging may be explained using different aging theories and preliminary shreds of evidence supporting antiaging effect of millets.

ANTIAGING PROPERTIES AND MOLECULAR INSIGHTS

Natural compounds offer a repertoire of highly diverse structural scaffolds that can offer hopeful candidate chemical entities with antiaging potential. As per a large number of published pieces of literature, it is highly emphasized that consumption of millet may help in reducing aging and age-related complications. Though, its exact mechanism of action is not known. However, based upon these studies, it can be suggested that phytochemicals obtained from millets can play a significant role in the suppression of aging processes and aging-related diseases by influencing genetic repair, protein glycation, and stress-responsive pathways. The effect of millets on all these pathways can be attributed to their antioxidant property and antiaging potential, as explained below by various aging theories.

Free Radical Theory

The free radical theory of aging and the antiaging property stated that progressive accumulation of endogenous oxygen radicals causes damage to macromolecular components of the cell-rendering cells and organs to stop functioning, ultimately leading to death by senescence (Finkel and Holbrook, 2000). With the discovery of the mitochondrial genome, the theory was modified to the mitochondrial theory of aging in the year 1972. This theory stated that reactive oxygen species (ROS) generated during mitochondrial respiration damages macromolecules including mitochondrial DNA (mtDNA) (Muller et al., 2007). Such mutations in mtDNA which accumulate over time result in increased ROS generation and oxidative damage as suggested by studies conducted in a variety of experimental animal models and tissues (Muller et al., 2007). The grains of millet possess natural antioxidants which help to combat damage induced by free radicals and prolong the lifespan of organisms. This theory has been proven by different experiments as listed in **Table 2**.

Telomere Shortening Theory

Excessive oxidative stress leads to the accumulation of oxidative DNA damage in telomeres and is strongly related to premature aging (Lu and Liu, 2010). As per telomere shortening theory, aging occurs due to the loss of telomeres. Cellular senescence is initiated by erosion or improper functioning of telomeres resulting in cell-cycle exit after several cell cycles (Holliday, 2014). In each subsequent cellular division, telomere length gets shorter which obstructs the chromosomal ends from being identified as double-strand breaks. DNA damage response gets triggered due to telomere shortening (Karlseder et al., 1999). Apart from telomere shortening, damage in DNA in regions other than telomere is also responsible for aging. Similarly, DNA damage theory also proposes that loss of its functionality during aging process may stem from the accumulation of unrepaired naturally occurring DNA damages like DNA breaks, cross-linking, and base modifications (Hayflick, 2007). With the accumulation of such mutations over time in aged organisms, there is an increased risk of tumor formation and other age-related disorders (Iyama and Wilson, 2013). As per literature, there are several factors inducing DNA damage which in turn are responsible for aging and associated diseases (**Figure 2**). Naturally occurring compounds in millets act as potent antioxidants which may reduce the level of oxidative stress and hence minimize the rate of telomere shortening.

Protein Translational Modifications

During aging, the functioning of the proteins can be disturbed due to disorganized protein synthesis and aberrant types of posttranslational modifications (PTMs) such as acetylation, phosphorylation, glycosylation, ubiquitination, sumoylation, and neddylation ultimately leading to the accretion of inactive, denatured, misfolded, and mutated proteins in cells of aged organisms (Santos and Lindner, 2017) (**Figure 3**). The posttranslational modifications of proteins are chiefly responsible for the activity, stability, as well as protein-protein interactions, influencing the cellular function and metabolism (Santos and Lindner, 2017). It has been observed that different compounds influence the PTMs of different proteins and help in maintaining cellular function during the aging process (Lan et al., 2016; Peleg et al., 2016). In the recent past, a good number of drugs and natural compounds including millets and millet-based products have been studied for their antiaging potential. It was reported that millet extracts inhibited the glycosylation of collagen protein and thus protects its function (Hegde et al., 2002).

NEW PARADIGM FOR DESIGNING ANTIAGING DRUGS DERIVING INFORMATION FROM MILLETS AND OTHER COMPOUNDS OF PLANT ORIGIN

Various natural products from grains, fruits, and vegetables are studied for their antiaging properties. These natural products or compounds are suggested to delay the aging process by regulating genes involved in different pathways and involved in the aging process. Though the detailed mechanism of action of bioactive compounds of millets has not been explored, preliminary investigations highlight the effect of millet extract on longevity as well as aging-associated diseases. Hence, it is imperative to have detailed investigations of bioactive compounds of millets for their mode of action as antiaging compounds. As per various studies, the premature aging and aging-associated diseases are found to be associated with the fault in the key cellular pathways such as the IIS (**Figure 4**), mTOR (**Figure 5**), and sirtuin (**Figure 6**) molecular networks. Hence, all these crucial pathways involved in the aging process need to be studied focusing on the effect of bioactive compounds of millet extracts on the key molecules of all these pathways in suitable *in vitro* model systems (**Figure 7**). Once the molecular mechanism of action of bioactive compounds obtained from millets is revealed, formulations based on these compounds can be processed and targeted to the specific targets in a nanoencapsulated form.

Millets are a rich natural source of antioxidants and thus hold potential as a nutraceutical and functional food ingredient promoting health and reducing the risk of disease. However, more validation in animal models and human subjects needs to be performed to verify their activity and health benefits. The effect of different cellular processes such as methylation, glycosylation, acetylation, phosphorylation, ubiquitination, etc. on aging has been studied by many scientists to find the mechanism by which these processes affect the extent of aging and associated diseases. Several efforts have been made to design some potential drugs that can bind with the target enzyme of cellular processes associated with aging. Some therapeutics such as nutulin-3, HDAC, and MLN4924 have shown their effectiveness in tackling the aging and associated diseases, but no success story of any drug is available in the market that can be recommended for use to prevent aging and its symptoms. The role of some pathways such as the IGF-1 signaling pathway, mTOR signaling pathway, and sirtuin-related pathways is also associated with the aging process. Modulation of these pathways by the use of some potential therapeutics can also be one of the possible approaches to curb the aging process. A comprehensive network of different processes and pathways associated with aging can be designed to find some potential targets for the treatment of aging and associated diseases. Furthermore, validation studies related to these targets can be carried out by *in vitro* and *in vivo* studies, if not validated earlier. Structure-based drug designing approach can be applied to design and screen some potential candidate drugs that can modulate the aging-associated pathways by specific binding with the drug target. The druggability of these candidate drugs can be predicted using absorption, distribution, metabolism, and toxicity (ADMT) prediction tools to avoid the chance of drug failure in clinical trials.

Many synthetic and natural compounds such as indolepropionaamide (IPAM), acetyl L-carnitine (ALC), spermidine, metformin, lithocholic acid, caffeine, reserveratrol, rapamycin, epicatechin, and quercetin have shown their potential role in the suppression of the aging process by modulating different drug targets. Mechanisms of action for these compounds are also different for different targets by which they generate the antiaging response. It is important to mention that most of such studies related to aging have been performed in *Caenorhabditis elegans*, *Drosophila* *melanogaster*, mice, rat, and worms. The effect of these antiaging compounds needs to be studied in humans to explore the mechanism of action and interaction of compounds with the drug target. Natural ingredients in millets have also shown their antiaging response, but their target and mechanism of action are not explored well scientifically. More studies on the antiaging response of millets are required to validate the target and mechanism of action. Compounds with known antiaging effects can be used as a lead molecule to design more specific and potential candidate drugs for a given drug target. Optimization of lead compounds is very necessary to achieve better bioavailability, specificity, and affinity for the drug target. Computational tools can be more helpful in deciphering the chemical modification required to optimize the activity of a lead molecule. Potential candidate drugs resulted from lead optimization can be recommended for *in vitro* or clinical testing.

CONCLUSION

A balanced diet with definite nutritional supplements plays a key role in promoting the lifespan and health of human beings. Various natural compounds from different plants have been found to curb aging and associated diseases. Millets are a chief source of proteins, carbohydrates, and minerals and are low in fat content which validates their utilization in many healthy diets. Furthermore, millets contain high content of total phenolics, flavonoids, and other phytochemicals that have been reported to possess antiaging potential. Henceforth, millets might be utilized in the formulation of nutraceutical and functional foods that can be consumed daily due to the presence of many bioactive compounds that prevent aging and associated diseases. The prospects include the identification, isolation, and characterization of bioactive molecules present in millets that can slow down the aging process. Once characterized, further validation of their mechanism of action at the molecular level can be explored by the wet lab and *in silico* analysis. The effectiveness of these bioactive compounds requires evaluation in a cell or tissue-specific manner followed by the animal model system for safety concerns and to determine their therapeutic potency.

AUTHOR CONTRIBUTIONS

AK gave the manuscript concept. MR and PS wrote the manuscript. SM, DS, HK, and RKV contributed critically in revising the draft and updating the manuscript for publication. All authors contributed to the article and approved the submitted version.

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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.2021.684318/full#supplementary-material

Supplementary Figure S1 | Structure of major phenolic compounds present in millets.

Supplementary Figure S2 | Basic structure of flavonoids reported in millets.

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Bradyrhizobium Inoculation of Field-Grown Kersting's Groundnut [*Macrotyloma geocarpum* (Harms) Marechal & Baudet] Increased Grain Yield and N_2 Fixation, Measured Using the Ureide, and ^{15}N Natural Abundance Techniques

Mustapha Mohammed[1,2], Glory C. Mbah[1], Elias N. K. Sowley[2] and Felix D. Dakora[3]*

[1] Department of Crop Sciences, Tshwane University of Technology, Pretoria, South Africa, [2] Department of Crop Science, University for Development Studies, Tamale, Ghana, [3] Chemistry Department, Tshwane University of Technology, Pretoria, South Africa

Edited by:
E. O. Deedi Sogbohossou,
Wageningen University and
Research, Netherlands

Reviewed by:
Mark Paul Running,
University of Louisville, United States
Kedir Woliy,
Hawassa University, Ethiopia
Zaiya Arlette,
Institute for Agricultural Research for
Development (IRAD), Cameroon

***Correspondence:**
Felix D. Dakora
DakoraFD@tut.ac.za

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Here, we report on the symbiotic N_2 fixation and water use efficiency ($\delta^{13}C$) of Kersting's groundnut, an important but underutilized grain legume in Africa, in response to inoculation with *Bradyrhizobium* strains 3267 and CB756. The ^{15}N natural abundance and xylem ureide techniques were used to quantify N_2 fixation. The landraces in this study derived variable levels of their N requirements from symbiosis, which translated into marked differences in the amounts of N-fixed by the landrace–inoculant combinations across the test locations. In most instances, the landrace-strain combinations that elicited higher shoot biomass also recorded greater N-fixed and/or ureide-N in xylem sap as well as grain yield. Although some landraces coupled increased grain yield with higher water use efficiency (shoot $\delta^{13}C$), a trait that could be tapped for crop improvement, others recorded lower yields despite eliciting relatively high shoot $\delta^{13}C$ values, indicating genotypic variations in adaptation to the different environments. Grain yield of the test landraces showed marked variation and ranged from 131 to 1349.8 kg ha^{-1} depending on the landrace–strain combination used and the planting location. The high symbiotic dependence by landraces in this study could explain why Kersting's groundnut thrives in the low nutrient soils that are prevalent in its cultivation areas. These results provide more insights into the literature regarding the Kersting's groundnut–rhizobia symbiosis as well as the crop's water use efficiency.

Keywords: legume-rhizobium symbiosis, N-fixed, grain yield, $\delta^{13}C$, water use efficiency, Kersting's groundnut

INTRODUCTION

The N_2-fixing trait of legumes offers them advantage over other species in N-limiting soils as they can reduce atmospheric N_2 into useable forms for plants, through their symbiotic association with rhizobia (Menge and Chazdon, 2016). The symbiotic process can supply all or part of the legume's N needs, and even contribute to the soil N reserves for use by subsequent non-legume crops (Preissel et al., 2015). The Kersting's groundnut is an underutilized African legume that thrives in arid environments of Africa (Mergeai, 1993) and capable of N_2 fixation when in symbiosis with soil rhizobia (Dakora, 1998). As with most tropical and sub-tropical legumes, the products of N_2-fixation in the root nodules of Kersting's groundnut are exported as ureides (allantoin and allantoic acid) to the shoots *via* the xylem stream (Dakora et al., 1992; Dakora, 1998). Thus, the concentration of ureides relative to nitrate and amino-N in readily sampled xylem sap of Kersting's groundnut could be used to assess the crop's dependence on N_2 fixation for its N nutrition (Herridge and Peoples, 1990; Unkovich et al., 2008). Like most neglected grain legumes, the crop can produce substantial grain yield without fertilization even in nutrient limiting environments (Bayorbor et al., 2010; Mohammed et al., 2018). The seeds of Kersting's groundnut contain a high proportion of protein (21.3%), carbohydrates (up to 73.3%), and essential amino acids (Ayenan and Ezin, 2016). The symbiotic, nutritional, and medicinal values of Kersting's groundnut make it an important food security crop (Amuti, 1980; Assogba et al., 2016; Akohoué et al., 2018). Since an earlier report that Kersting's groundnut can nodulate with *Bradyrhizobium* sp. strain CB756 and exhibit nitrate tolerant symbiosis in sand culture (Dakora, 1998), little has been done on the N_2-fixing potential of Kersting's groundnut under field conditions (Mohammed et al., 2018). This is despite the current efforts at harnessing the benefits of symbiotic N_2 fixation for agriculture as well as the renewed interest in rhizobial inoculant production and usage (Stephens and Rask, 2000; Catroux et al., 2001; Hungria et al., 2006; Rebah et al., 2007).

Field inoculation of legumes with rhizobia often aims at increasing plant growth and grain yield at a sustainable cost when compared to the use of fertilizers. A known example of inoculation success is the story of soybean in Brazil, where the government is reported to have saved about US$ 10 billion on fertilizers in one cropping season (Dwivedi et al., 2015). However, there has also been inoculation failures in the field due to the presence of native soil rhizobia with higher competitiveness for nodule occupancy (Ulzen et al., 2016; Mohammed et al., 2018). Legume inoculation in the field often does not yield the desired response in soils where the resident rhizobia are very competitive and not limited by numbers (Catroux et al., 2001). It is therefore necessary to test the response of legumes to inoculation with commercially available rhizobial inoculants in the presence of native soil rhizobia to check the quality of introduced strains in different agro-environments. Kersting's groundnut is an important food security crop commonly grown in rural Africa. There is therefore the need to assess its symbiotic performance in the field in response to inoculation with different rhizobial inoculants.

Although Kersting's groundnut is reported to be drought-tolerant (Mergeai, 1993; Ayenan and Ezin, 2016), little information exists on its water use efficiency measured using reliable techniques. During C3 plant photosynthesis, carbon isotope discrimination against $^{13}CO_2$ in favor of $^{12}CO_2$ (expressed as $\delta^{13}C$) is directly related to water use efficiency (Farquhar et al., 1989). Under water-limiting conditions when there is soil moisture deficit, plants with high water use efficiency exhibit reduced discrimination against $^{13}CO_2$, resulting in higher $\delta^{13}C$ values. Conversely, plants accessing adequate soil moisture tend to display low water use efficiency through increased ^{13}C discrimination, which results in reduced $\delta^{13}C$ values (Farquhar et al., 1989; Wang et al., 2016). Thus, carbon isotope discrimination has become a useful tool for assessing plant water use efficiency in nodulated legumes (Mohale et al., 2014; Mapope and Dakora, 2016), as well as in ecological settings (Wang et al., 2016). In this study, we measured symbiotic N_2 fixation and water use efficiency in field-grown Kersting's groundnut in response to inoculation with *Bradyrhizobium* sp. strains CB756 and 3267. The ureide and ^{15}N natural abundance techniques were used to assess N_2 fixation in the Kersting's groundnut landraces, while the $^{13}C/^{12}C$ isotopic analysis was used to measure plant water use efficiency.

MATERIALS AND METHODS

Experimental Site Description

The study was carried out at three locations (namely, Nyankpala, Savelugu, and Gbalahi) in the Northern Region of Ghana. Nyankpala is located on latitude 9.404 and longitude −0.982, while Gbalahi is located on latitude 9.437 and longitude −0.734. Savelugu, on the other hand, is located on latitude 9.569 and longitude −0.830. The Nyankpala site had been fallowed for over 5 years, while the Savelugu site was cultivated to okra in the previous year. The field at Gbalahi was planted to maize in the year before this experiment. Prior to planting, soil samples were cored at several points across plots, bulked, air-dried, and sieved (2 mm). Soil sub-samples were taken for analysis of pH (H_2O), %C (Walkley and Black, 1934), P (Bray and Kurtz, 1945), B, Ca, Cu, Fe, K, Mg, Mn, Na, and S (ammonium acetate method) and total N using Kjeldahl digestion.

The Northern Region has a unimodal rainfall of 800–1200 mm distributed between April and October each year (Owusu and Waylen, 2013). The total annual rainfall during the study period in 2013 was 1081.5 mm at Nyankpala and 1009.7 mm at Savelugu. Due to broken equipment, rainfall data at Gbalahi (857.3 mm) were collected from January to October 2013. However, the total rainfall from planting till sampling (early podding stage of Kersting's groundnut) was 481 mm at Nyankpala, 471 mm at Savelugu, and 305 mm at Gbalahi. The mean weekly rainfall distribution at the three locations during the study period in 2013 is shown **Figure 1**.

Source of Seeds, Experimental Design, and Planting

The eight Kersting's groundnut landraces [Puffeun, Boli, Dowie, Funsi, Heng Milk Mottled (Heng MM), Heng Red Mottled (Heng RM), Nakori, and Sigiri] used in this study were sourced from the University for Development Studies, Tamale, Ghana. The seeds were originally collected from different villages within the Upper West Region of Northern Ghana (Bayorbor et al., 2010). The landraces together with two *Bradyrhizobium* inoculants (*Bradyrhizobium* sp. strains 3267 and CB756) and an uninoculated control, which gave a total of 24 treatment combinations, were laid in a randomized complete block design at each location with four replicate plots per treatment. Planting

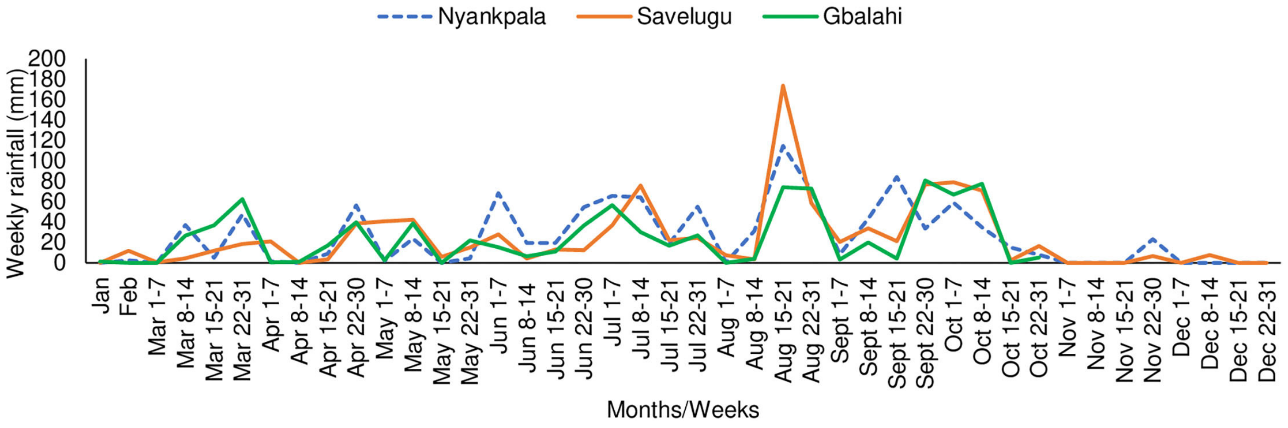

FIGURE 1 | Weekly rainfall distribution at Nyankpala, Savelugu, and Tamale (Gbalahi) during the study in 2013.

was done on July 2, 2013 at Nyankpala, July 10, 2013, at Savelugu, and July 16, 2013 at Gbalahi. Seeds were sown in plots measuring 2.4 m × 2 m at a spacing of 60 cm between rows and 20 cm between plants, with a 1-m path between plots and 1.5 m between blocks. Weeding was done when necessary, using a hand hoe.

Seed Inoculation With *Bradyrhizobium* Strains

Seed inoculation was done in the shade using 10 g inoculant per kg seed, which yielded 10^8 rhizobial cells g^{-1} of inoculant. To avoid cross contamination, uninoculated/control plots were planted first, followed by inoculated treatments with the individual *Bradyrhizobium* strains. *Bradyrhizobium* sp. strain CB756 is a commercial cowpea inoculant strain originally isolated from the root nodules of *Macrotyloma africanum* in Zimbabwe (Blumenthal and Staples, 1993) and was obtained from Stimuplant, South Africa. The *Bradyrhizobium* sp. strain BR 3267 is a commercial inoculant from Brazil, which was obtained from the CSIR-Savanna Agricultural Research Institute, Ghana.

Plant Sampling and Processing

Plants were sampled at early podding at 74 days after planting (DAP) at Nyankpala and Savelugu, and 75 DAP at Gbalahi. Five plants were randomly dug up from each plot at the experimental sites, and separated into shoots, roots, and nodules. The shoots were oven-dried at 65°C for 48 h and weighed to determine shoot biomass. The shoots were finely ground (0.50 mm sieve) for ^{15}N and ^{13}C isotopic analysis. The nodules were counted, similarly oven-dried, and weighed to determine nodule dry matter. Shoots of non-legume reference plants were concurrently sampled from each location, and similarly processed for ^{15}N analysis (**Supplementary Table 1**).

$^{15}N/^{14}N$ Isotopic Analysis of Plant Shoots

Ground shoot samples of both legume and reference plants were weighed (2.0–2.5 mg) and each sample was fed into a Carlo Erba NA1500 elemental analyzer (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finnigan MAT252 mass spectrometer (Fisons Instrument SpA, Strada, Rivoltana, Italy) *via* conflo II open-split device to measure $^{15}N/^{14}N$ isotopic composition, as described by Mapope and Dakora (2016). The $\delta^{15}N$ of shoot samples was calculated as Unkovich et al. (2008):

$$\delta^{15}N(‰) = \frac{[^{15}N/^{14}N]_{\text{sample}} - [^{15}N/^{14}N]_{\text{atm}}}{[^{15}N/^{14}N]_{\text{atm}}} \times 1000$$

where the $^{15}N/^{14}N_{\text{sample}}$ is the abundance ratio of ^{15}N and ^{14}N in the sample, and $^{15}N/^{14}N_{\text{atm}}$, the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

Shoot N

The %N of shoot samples was obtained directly from the mass spectrometer, and shoot N content estimated as the product of shoot dry matter and %N (Pausch et al., 1996).

Percent N Derived From Fixation and N-Fixed

Shoot percent N derived from N_2 fixation (%Ndfa) was calculated as Shearer and Kohl (1986):

$$\%\text{Ndfa} = \frac{\delta^{15}N_{\text{ref}} - \delta^{15}N_{\text{leg}}}{\delta^{15}N_{\text{ref}} - B} \times 100$$

where $\delta^{15}N_{\text{ref}}$ is the ^{15}N natural abundance of reference plant, $\delta^{15}N_{\text{leg}}$ is the ^{15}N natural abundance of legume, and the B value is the ^{15}N natural abundance of the shoot of Kersting's groundnut plants completely dependent on N_2 fixation for their N nutrition. The B value (−3.84‰) used in this study was earlier determined by inoculating the Puffeun and Funsi landraces with *Bradyrhizobium* sp. strain CB756 in the glasshouse (Mohammed et al., 2018). The mean $\delta^{15}N$ of all reference plants collected from each location (**Supplementary Table 1**) was used to calculate the %Ndfa of landraces per study site.

The N-fixed by Kersting's groundnut was calculated as Maskey et al. (2001):

$$N - \text{fixed} = \%\text{Ndfa} \times \text{N content of Kersting's groundnut shoots}$$

The soil N uptake by legume plants was determined as the difference between shoot N content and the amount of N-fixed.

Shoot $^{13}C/^{12}C$ Isotopic Analysis

The ground shoot samples of Kersting's groundnut were similarly subjected to $^{13}C/^{12}C$ isotopic analysis, as described for $^{15}N/^{14}N$. The ^{13}C natural abundance ($\delta^{13}C$) was calculated as Farquhar et al. (1989):

$$\delta^{13}C = \left[\frac{\left({}^{13}C/{}_{12}C \right)_{sample}}{\left({}^{13}C/{}_{12}C \right)_{s\tan dard}} - 1 \right] \times 1000$$

where $^{13}C/^{12}C_{sample}$ is the isotopic ratio of the plant sample and $^{13}C/^{12}C_{standard}$ is the isotopic ratio of PDB, a universally accepted standard from Belemnite Pee Dee limestone formation (Craig, 1957).

Collection of Xylem Sap

Xylem sap was collected from the same plants that were sampled and processed for ^{15}N and ^{13}C isotopic analysis. After decapitating five plants at crown level for shoot samples from each plot, the root-bleeding xylem sap was collected into 2 ml Eppendorf tubes using clean glass Pasteur pipettes. The xylem sap samples were stored at −20°C until analysis for N solutes (ureide-N, nitrate-N, and amino-N).

Measurement of Xylem N Solutes

Ureide Concentration in Xylem Sap

The concentration of ureides in xylem sap was colorimetrically determined, as described by Young and Conway (1942) or Unkovich et al. (2008). Varying concentrations of allantoin (0.1, 0.02, 0.04, 0.1, and 0.15 mM) were prepared as standards for estimating ureides in xylem sap, after colorimetric assay (Young and Conway, 1942).

Nitrate Level in Xylem Sap

Nitrate-N in xylem sap was determined using the salicylic acid method (Cataldo et al., 1975), as described by Unkovich et al. (2008). Again, varying concentrations of KNO_3 (0, 1.25, 2.5, 5, 10, and 15 mM) were used as standards to construct curves for extrapolating the concentrations of nitrate from xylem sap.

Total Amino Acid Determination

Amino acids in xylem sap was measured using the ninhydrin method (Yemm and Cocking, 1955; Unkovich et al., 2008). As with ureide and nitrate determinations, a standard curve was prepared from assaying different known concentrations of the amino acids glutamine and asparagine (0, 0.1, 0.2, 0.4, and 1 mM).

Relative Ureide Nitrogen

The relative ureide-N in xylem sap (or relative ureide abundance) was calculated as Herridge et al. (1990):

$$\text{Relative ureide} - \text{N} = \left(\frac{4a}{4a + b + c} \right) \times 100$$

where *a, b*, and *c* are the molar concentrations of ureides (ureides contain four nitrogen atoms per molecule), nitrate-N, and amino-N, respectively (Unkovich et al., 2008).

Determination of Grain Yield

At maturity, which coincided with 125, 122, and 120 DAP at Nyankpala, Savelugu, and Gbalahi, respectively, 20 plants were harvested from the inner rows of each plot for determination of grain yield. The pods were detached from plants, air-dried to 15% moisture content, and threshed to obtain seeds. The seeds were weighed, and grain yield expressed per hectare.

RESULTS

Chemical Properties of Soils

The bulk soils collected from Nyankpala, Savelugu, and Gbalahi before planting, respectively, had pH (H_2O) 5.93, 6.08, and 6.3, and in that order contained 0.04, 0.01, and 0.08 mg kg^{-1} B, 0.40, 0.029, and 0.41% C, 250, 272, and 460 mg kg^{-1} Ca, 0.30, 0.20, and 0.78 mg kg^{-1} Cu, 42.0, 23.96, and 46.69 mg kg^{-1} Fe, 48, 29, and 58 mg kg^{-1} K, 55.2, 51.6, and 130.8 mg kg^{-1} Mg, 131.4, 66.46, and 65.51 mg kg^{-1} Mn, 7, 4, and 17 mg kg^{-1} Na, 7, 5, and 7 mg kg^{-1} P, 2.7, 2.1, and 2.2 mg kg^{-1} S, as well as 0.023, 0.016, and 0.031% N.

Shoot $\delta^{15}N$ of Non-legume Reference Plants

The $\delta^{15}N$ of reference plants used in estimating %Ndfa by Kersting's groundnut landraces is shown in **Supplementary Table 1**. The $\delta^{15}N$ of reference plants ranged from +1.83‰ to +4.57‰ at Nyankpala (mean = +3.01‰), +2.52‰ to +3.51‰ at Savelugu (mean = +2.95‰), and +2.15‰ to 5.01‰ at Gbalahi (mean = +3.26‰). The mean $\delta^{15}N$ of reference plants from each study site was used to calculate the %Ndfa of Kersting's groundnut plants from that location (**Supplementary Table 1**).

N_2 Fixation and Water Use Efficiency of Kersting's Groundnut at Nyankpala

Main Effect of Landrace on Plant Growth, Symbiotic Parameters, Grain Yield and Shoot $\delta^{13}C$

A two-way ANOVA of data from Nyankpala showed that the main effect of landrace was significant ($p \leq 0.05$) for nodule dry matter, shoot biomass, and N content at the site (**Table 1**). Landraces Heng RM, Boli, Dowie, and Heng MM recorded significantly higher nodule mass compared to the other landraces at Nyankpala (**Table 1**). The landraces Heng MM and Heng RM (with high nodule mass) together with Nakori and Sigiri (with lower nodule mass) recorded greater shoot biomass relative to the other landraces at that site (**Table 1**). The %N and C/N ratio of shoots were however similar for all the Kersting's groundnut landraces, with values ranging from 2.4 to 2.8% for N and 14.7 to 17.1 g g^{-1} for C/N ratio (**Table 1**). Shoot N accumulation (N content) was greater in landraces Heng RM and Nakori, followed by Heng MM, Dowie, and Sigiri (**Table 1**).

From the ^{15}N analysis, the landraces Heng MM and Funsi had the least shoot $\delta^{15}N$ values at Nyankpala, while Boli, Dowie,

TABLE 1 | Plant growth, N_2-fixing parameters, grain yield, and $\delta^{13}C$ of eight Kersting's groundnut landraces planted at Nyankpala in the northern Region of Ghana in 2013.

Treatments	Nodule DM mg plant^{-1}	Shoot DM g plant^{-1}	N conc'n %	C/N ratio g g^{-1}	N content mg plant^{-1}	$\delta^{15}N$ ‰	%Ndfa %	N fixed kg ha^{-1}	Soil N uptake kg ha^{-1}	Grain yield kg ha^{-1}	$\delta^{13}C$ ‰
Landraces											
Boli	8.6 ± 0.91ab	16 ± 1.18bc	2.5 ± 0.08a	16.4 ± 0.45a	389 ± 27.35cd	−1.08 ± 0.16a	60 ± 2.29e	46 ± 2.21c	32 ± 3.77bc	783 ± 55.17c	−29.72 ± 0.08abc
Dowie	8.8 ± 1.22ab	17 ± 0.82bc	2.8 ± 0.13a	14.7 ± 0.84a	486 ± 36.01abc	−1.14 ± 0.16ab	61 ± 2.31de	59 ± 5.38ab	38 ± 3.42ab	627 ± 53.18d	−29.67 ± 0.11ab
Funsi	5.5 ± 0.58d	18 ± 1.33bc	2.4 ± 0.06a	17.1 ± 0.35a	426 ± 35.9bcd	−1.55 ± 0.15de	67 ± 2.13ab	57 ± 5.60abc	28 ± 2.39c	685 ± 39.67d	−29.91 ± 0.09bcd
Heng MM	8.9 ± 0.91ab	21 ± 1.40a	2.5 ± 0.05a	16.6 ± 0.31a	508 ± 38.28ab	−1.66 ± 0.05e	68 ± 0.74a	69 ± 5.12a	32 ± 2.69bc	959 ± 69.72a	−29.48 ± 0.06a
Heng RM	9.4 ± 1.22a	21 ± 0.92a	2.5 ± 0.07a	16.0 ± 0.52a	545 ± 30.94a	−1.29 ± 0.13abc	63 ± 1.92cde	68 ± 3.49a	41 ± 3.82a	878 ± 104.00b	−29.60 ± 0.06a
Nakori	7.8 ± 0.69bc	21 ± 1.51a	2.5 ± 0.07a	16.1 ± 0.49a	529 ± 40.64a	−1.26 ± 0.08abc	62 ± 1.18cde	66 ± 5.91a	39 ± 2.58ab	850 ± 24.82b	−29.71 ± 0.07abc
Puffeun	7.1 ± 1.13c	16 ± 1.11c	2.5 ± 0.07a	15.7 ± 0.72a	389 ± 32.10d	−1.43 ± 0.10cde	65 ± 1.50abc	51 ± 4.75bc	27 ± 2.05c	786 ± 32.56c	−29.93 ± 0.08cd
Sigiri	5.6 ± 0.61d	19 ± 1.15ab	2.5 ± 0.12a	16.4 ± 0.50a	481 ± 47.99abc	−1.39 ± 0.11bcd	64 ± 1.67bcd	61 ± 6.01ab	35 ± 4.07abc	445 ± 10.78e	−29.97 ± 0.09d
Inoculation											
Bradyrhizobium sp. 3267	8.8 ± 0.65a	17 ± 0.49a	2.4 ± 0.04a	16.4 ± 0.34a	418 ± 15.81b	−1.32 ± 0.06a	63 ± 0.94a	53 ± 2.22b	31 ± 1.38b	663 ± 32.27b	−29.72 ± 0.05a
Bradyrhizobium sp. CB756	6.4 ± 0.53c	19 ± 0.74a	2.5 ± 0.06a	16.2 ± 0.31a	476 ± 23.27a	−1.29 ± 0.10a	63 ± 1.42a	59 ± 2.89b	36 ± 2.50a	808 ± 50.01a	−29.80 ± 0.06a
Un-inoculated	7.9 ± 0.58b	20 ± 1.03a	2.6 ± 0.06a	15.8 ± 0.38a	513 ± 28.34a	−1.44 ± 0.07a	65 ± 1.06a	67 ± 3.97a	36 ± 2.06a	784 ± 40.27a	−29.73 ± 0.05a
F statistics											
Landrace (L)	8.98***	4.93***	1.64 ns	1.67 ns	3.46**	5.50***	5.50***	3.77**	3.69**	52.45***	4***
Inoculation (I)	15.47***	4.73 ns	2.10 ns	0.78 ns	5.73**	2.61 ns	2.61 ns	6.93**	3.433*	31.94***	1 ns
L × I	14.95***	2.94***	0.68 ns	0.73 ns	1.95*	7.93***	7.93***	2.20*	3.22***	29.14***	1 ns

*Values (means ± standard errors) with dissimilar letters in a column are significantly different at ***$p < 0.001$, **$p < 0.01$ and *$p \leq 0.05$. Plants were sampled at early podding stage of all landraces. ns, not significant.*

Heng RM and Nakori recorded higher $\delta^{15}N$ values (**Table 1**). As a result, the landraces Heng MM and Funsi with lower shoot $\delta^{15}N$ recorded relatively higher %Ndfa values (68 and 67%, respectively), followed by Puffeun (65%) and then Sigiri (64%) (**Table 1**). However, landraces Heng RM, Heng MM, and Nakori showed much higher amounts of N-fixed due to greater shoot biomass and N content at Nyankpala. Dowie, Sigiri, and then Funsi were next with high N-fixed values (**Table 1**), with landrace Boli showing the lowest N-fixed from its very high shoot $\delta^{15}N$ and low %Ndfa at the Nyankpala site (**Table 1**).

Soil N uptake by landraces at Nyankpala also varied, with higher values found in Heng RM followed by Dowie, Nakori, and Sigiri (**Table 1**). Landraces Puffeun and Funsi recorded the least soil N uptake at Nyankpala. Here, grain yield was highest in landraces Heng MM (959 kg ha^{-1}) followed by Heng RM (878 kg ha^{-1}) and Nakori (850 kg ha^{-1}), and least in Sigiri (445 kg ha^{-1}) (**Table 1**).

The landraces Heng MM and Heng RM, which produced high grain yield, were also found to record greater shoot $\delta^{13}C$ values (−29.48‰ and −29.60‰, respectively) at Nyankpala. Other landraces with high $\delta^{13}C$ values at Nyankpala include Dowie (−29.67‰), Boli (−29.72‰) and Nakori (−29.71‰), while Sigiri had the least $\delta^{13}C$ value (−29.97‰) at that site (**Table 1**).

Main Effect of Inoculation on Plant Growth, Symbiotic Parameters, Grain Yield and Shoot $\delta^{13}C$

The main effect of inoculation was also significant for nodule dry matter, N content, N-fixed, soil N uptake, and grain yield, while the overall shoot dry matter, %N, C/N ratio, and $\delta^{13}C$ were unaltered by inoculation at Nyankpala (**Table 1**). Here, the highest nodule mass was obtained by bacterial inoculation of the landraces with strain 3267 followed by non-inoculation. The least nodule mass was recorded in plots inoculated with the *Bradyrhizobium* sp. strain CB756 (**Table 1**). Shoot N content was however greater in uninoculated plots as well as those inoculated with *Bradyrhizobium* sp. strain CB756 and lowest in plots inoculated with strain 3267. Overall, non-inoculation resulted in greater N-fixed compared to inoculation of the test landraces with either *Bradyrhizobium* strains at Nyankpala (**Table 1**). As observed with shoot N content, the average soil N uptake and grain yield of the plants at Nyankpala were higher in uninoculated plots or plots inoculated with strain CB756 relative to inoculation with strain 3267 at the site (**Table 1**). However, there was no overall effect of inoculation on shoot $\delta^{13}C$ at Nyankpala.

Main Effect of Landrace on the Concentrations of Xylem N Solutes

There was a significant effect of landrace on the concentrations of N solutes in the xylem sap of the test Kersting's groundnuts grown at Nyankpala (**Table 2**). The landraces Heng MM and Nakori (with high N-fixed from ^{15}N natural abundance) as well as Boli (with low N-fixed), all recorded higher concentrations of ureide and amino acids, but lower nitrate-N in xylem sap relative to the other landraces at the site (**Table 2**). As a result,

TABLE 2 | Ureide, nitrate, α-amino-N concentrations and relative ureide-N (RU-N) in xylem sap of eight Kersting's groundnut landraces planted at Nyankpala, Savelugu, and Gbalahi in 2013.

Treatment	**Ureide-N**	**Nitrate-N**	**α-amino-N**	**RU-N**	**Ureide-N**	**Nitrate-N**	**α-amino-N**	**RU-N**	**Ureide-N**	**Nitrate-N**	**α-amino-N**	**RU-N**
	μg ml⁻¹			**%**	**μg ml⁻¹**			**%**	**μg ml⁻¹**			**%**
Landrace	**Nyankpala**				**Savelugu**				**Gbalahi**			
Boli	5.2 ± 0.86a	0.55 ± 0.07d	108 ± 17.48a	9.3 ± 2.05b	84 ± 9.22d	4.9 ± 0.68abc	30 ± 12.24e	83 ± 5.16a	107 ± 6.99a	16.1 ± 1.85a	121 ± 8.60a	58 ± 1.97d
Dowie	2.0 ± 0.31b	1.39 ± 0.25b	58 ± 6.22cd	6.1 ± 1.20cd	102 ± 10.99cd	5.6 ± 0.69ab	74 ± 7.57b	70 ± 2.62de	86 ± 4.04bcd	5.2 ± 0.67d	106 ± 8.32b	60 ± 1.84cd
Funsi	1.8 ± 0.09bc	0.90 ± 0.22c	41 ± 5.15f	8.1 ± 1.47bc	120 ± 13.88bc	6.0 ± 0.45a	74 ± 6.60b	72 ± 1.75cde	91 ± 5.62bcd	5.6 ± 0.44d	86 ± 7.06c	65 ± 1.94ab
Heng MM	5.1 ± 0.97a	0.41 ± 0.01d	63 ± 4.58c	12.2 ± 1.79a	73 ± 10.38d	4.3 ± 0.89bcd	65 ± 10.26bc	68 ± 3.73e	79 ± 4.59d	9.0 ± 1.32bc	131 ± 19.7a7	53 ± 3.87e
Heng RM	2.0 ± 0.49b	0.50 ± 0.05d	45 ± 2.46ef	6.1 ± 1.66cd	116 ± 11.42bc	3.3 ± 0.45d	46 ± 4.08d	80 ± 2.41ab	98 ± 9.90ab	7.6 ± 0.68cd	102 ± 14.58b	62 ± 5.02bcd
Nakori	5.0 ± 1.21a	0.86 ± 0.20c	73 ± 11.20b	12.0 ± 2.66a	102 ± 16.08cd	4.7 ± 0.93bc	86 ± 11.17a	77 ± 4.82bc	85 ± 4.05bcd	7.4 ± 0.75cd	70 ± 6.84de	67 ± 2.29a
Puffeun	1.1 ± 0.19c	0.87 ± 0.20c	48 ± 6.64def	4.1 ± 1.11de	134 ± 16.57b	3.9 ± 0.57cd	71 ± 11.58bc	75 ± 4.03bcd	80 ± 3.49cd	13.9 ± 2.27a	63 ± 8.91e	64 ± 1.54abc
Sigiri	1.3 ± 0.12bc	1.63 ± 0.13a	54 ± 2.94cde	3.4 ± 0.47e	189 ± 19.14a	4.2 ± 0.82cd	60 ± 3.89c	84 ± 1.28a	93 ± 7.28bc	10.1 ± 1.03b	78 ± 3.42cd	65 ± 2.28ab
Inoculation												
Bradyrhizobium sp. 3267	3.7 ± 0.62a	1.13 ± 0.14a	67 ± 8.83a	8.2 ± 1.32b	111 ± 12.28a	3.7 ± 0.35b	67 ± 6.57a	78 ± 2.65a	88 ± 4.05b	9.3 ± 0.86ab	107 ± 8.85a	59 ± 2.02b
Bradyrhizobium sp. CB756	1.9 ± 0.20b	0.77 ± 0.09b	66 ± 4.44a	5.0 ± 0.73c	109 ± 8.63a	4.8 ± 0.43a	72 ± 5.87a	71 ± 2.30b	96 ± 4.49a	10.7 ± 1.33a	77 ± 6.60b	66 ± 1.68a
Uninoculated	3.3 ± 0.52a	0.77 ± 0.12b	52 ± 3.69b	9.7 ± 1.19a	124 ± 9.06a	5.3 ± 0.49a	51 ± 5.26b	80 ± 1.48a	86 ± 3.02b	8.1 ± 0.63b	100 ± 6.06a	60 ± 1.50b
F statistics												
Landrace (L)	51.38***	53.43***	45.91***	20.90***	9.78***	3.94**	21.42***	9.04***	4.95***	22.05***	32.94***	8.42***
Inoculation (I)	36.25***	34.18***	18.84***	29.07***	1.32 ns	9.01***	22.06***	14.87***	3.80*	6.57**	37.02***	17.89*
L × I	32.63***	39.04***	36.93***	23.62***	3.99***	9.88***	25.46***	12.52***	6.83***	8.88***	30.00**	14.81***

*Values (means ± standard errors) with dissimilar letters in a column are significantly different at ***$p < 0.001$, **$p < 0.01$ and *$p \leq 0.05$. Plants were sampled at early podding stage of all landraces. ns, not significant.*

those landraces exhibited higher RU-N in xylem sap at Nyankpala when compared to the other landraces at that site (**Table 2**).

Main Effect of Inoculation on the Concentrations of Xylem N Solutes

There was a significant effect of inoculation on the concentrations of ureide, nitrate, and amino acids in the xylem sap of the test Kersting's groundnuts grown at Nyankpala (**Table 2**). Here, non-inoculation as well as bradyrhizobial inoculation with strain 3267 increased the concentration of ureide in xylem sap relative to inoculation with strain CB756 (**Table 2**). The average concentration of nitrate in the xylem sap of the Kersting's groundnuts was higher with *Bradyrhizobium* strain 3267 inoculation relative to the control or inoculation with strain CB756. Bacterial inoculation with either strains significantly increased the overall concentration of amino acids in xylem sap relative to non-inoculation at Nyankpala (**Table 2**). The uninoculated control showed greater overall RU-N at Nyankpala, followed by plots inoculated with strain 3267 (**Table 2**).

Effects of Landrace × Inoculation Interactions at Nyankpala

The landrace × inoculation interaction effect was significant for all measured parameters at Nyankpala, except for %N, C/N ratio, and shoot $\delta^{13}C$ values (**Tables 1**, **2**). For brevity, the interaction effects are described for shoot biomass, N-fixed, and grain yield for landraces that showed consistent trends for those parameters. As shown in **Figures 2D–F**, non-inoculation led to greater shoot biomass in landraces Dowie, Heng MM, Nakori, and Puffeun relative to inoculation of those landraces with either *Bradyrhizobium* strains (**Figure 2D**). As a result, N-fixed was greater in uninoculated plants of those landraces (although not significant for Nakori) and resulted in higher grain yield relative to bacterial inoculation of those landraces with either strains (**Figures 2E,F**).

N_2 Fixation and Water Use Efficiency at Savelugu

Main Effect of Landrace on Plant Growth, Symbiotic Parameters, Grain Yield, and $\delta^{13}C$

At Savelugu, a two-way ANOVA revealed significant effect of landrace on nodule dry matter, shoot dry matter, N content, $\delta^{15}N$, %Ndfa, N-fixed, soil N uptake, grain yield, and shoot $\delta^{13}C$ of Kersting's groundnut (**Table 3**). The landrace Sigiri recorded the highest dry nodule mass followed by Boli, Heng RM, and Puffeun, which recorded relatively lower but similar nodule mass. The landrace Dowie recorded the least nodule dry matter at the Savelugu site (**Table 3**).

The landrace Heng MM had the highest shoot biomass at Savelugu followed by Dowie, Heng RM, Nakori, and Puffeun, which recorded relatively lower but similar shoot biomass at that site. The least shoot biomass was recorded in Boli and Sigiri despite their relatively high nodule mass at the Savelugu site (**Table 3**).

The shoot N concentration (%N) and C/N ratio were each similar between and among the landraces grown at Savelugu, with values of %N ranging between 2.4 and 2.6%, while C/N ratio

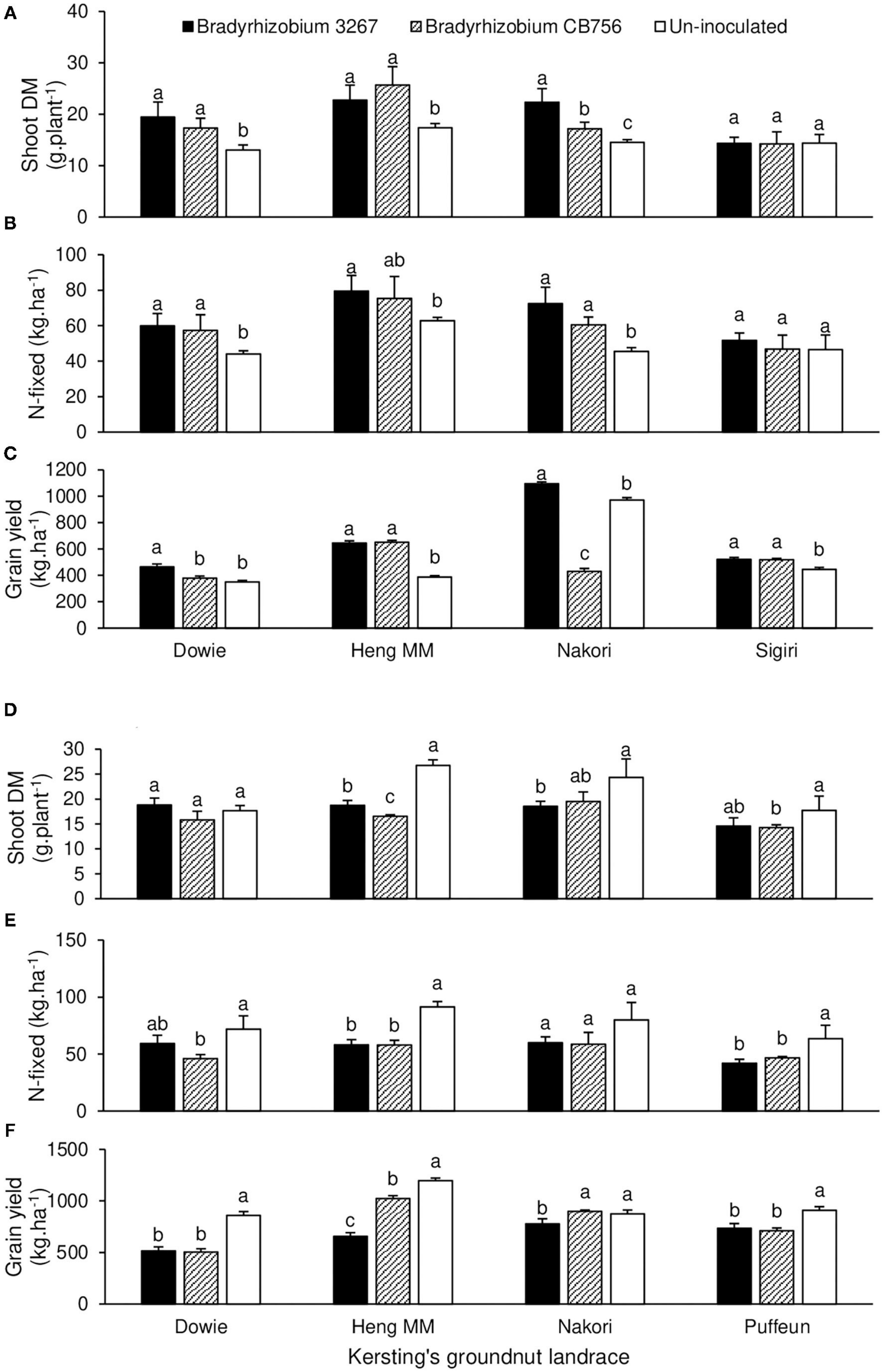

FIGURE 2 | The interactive effect of landrace × inoculation on **(A)** shoot dry matter at Savelugu, **(B)** N-fixed as Savelugu, **(C)** grain yield at Savelugu as well as the interactive effect of landrace × inoculation on **(D)** shoot dry matter at Nyankpala, **(E)** N-fixed at Nyankpala, and **(F)** grain yield at Nyankpala in 2013. For each landrace, bars with dissimilar letters are significantly different ($p \leq 0.05$).

TABLE 3 | Plant growth, N_2-fixing parameters, grain yield, and $\delta^{13}C$ of eight Kersting's groundnut landraces planted at Savelugu in the northern Region of Ghana in 2013.

Treatments	Nodule DM mg plant^{-1}	Shoot DM g plant^{-1}	N conc'n %	C/N ratio g g^{-1}	N content mg plant^{-1}	$\delta^{15}N$ ‰	%Ndfa %	N fixed kg ha^{-1}	Soil N uptake kg ha^{-1}	Grain yield kg ha^{-1}	$\delta^{13}C$ ‰
Landraces											
Boli	9.2 ± 1.33b	15 ± 1.18d	2.6 ± 0.10a	15.2 ± 0.59a	378 ± 29.94cd	−1.59 ± 0.09bc	67 ± 1.35ab	51 ± 4.58bc	25 ± 1.68c	565 ± 17.57d	−29.36 ± 0.16ab
Dowie	6.3 ± 0.66d	17 ± 1.36bcd	2.6 ± 0.05a	15.4 ± 0.29a	425 ± 34.03bcd	−1.38 ± 0.09bc	64 ± 1.37ab	54 ± 4.01bc	31 ± 3.19abc	398 ± 16.43g	−29.92 ± 0.12c
Funsi	7.2 ± 0.41cd	16 ± 0.69cd	2.6 ± 0.07a	15.3 ± 0.39a	400 ± 17.80bcd	−1.62 ± 0.07c	67 ± 1.03a	54 ± 2.43bc	26 ± 1.48bc	522 ± 24.32e	−29.61 ± 0.12abc
Heng MM	7.4 ± 0.77cd	22 ± 1.75a	2.6 ± 0.12a	15.9 ± 0.80a	553 ± 40.42a	−1.53 ± 0.10bc	66 ± 1.53ab	73 ± 5.11a	38 ± 3.54a	561 ± 37.85d	−29.31 ± 0.18a
Heng RM	8.3 ± 0.85bc	19 ± 0.85b	2.6 ± 0.08a	15.5 ± 0.49a	482 ± 27.63ab	−1.34 ± 0.17b	63 ± 2.51b	61 ± 4.82b	35 ± 2.81a	620 ± 28.64c	−29.51 ± 0.06ab
Nakori	7.0 ± 0.59cd	18 ± 1.33bc	2.6 ± 0.07a	15.2 ± 0.33a	463 ± 32.40bc	−1.39 ± 0.05bc	64 ± 0.67ab	59 ± 4.57bc	33 ± 2.01ab	832 ± 88.0a	−29.49 ± 0.08ab
Puffeun	8.2 ± 1.12bc	18 ± 2.09bc	2.4 ± 0.08a	16.2 ± 0.52a	436 ± 51.89bcd	−1.01 ± 0.12a	58 ± 1.79c	49 ± 4.72c	38 ± 5.90a	657 ± 75.39b	−29.67 ± 0.18abc
Sigiri	10.5 ± 1.44a	14 ± 0.35d	2.6 ± 0.09a	16.0 ± 0.58a	369 ± 13.69d	−1.49 ± 0.13bc	65 ± 1.92ab	48 ± 2.42c	26 ± 1.66c	495 ± 12.54f	−29.72 ± 0.07bc
Inoculation											
Bradyrhizobium sp. 3267	9.6 ± 0.81a	17 ± 0.97a	2.7 ± 0.04a	15.0 ± 0.27b	444 ± 24.64a	−1.40 ± 0.05b	64 ± 0.80b	57 ± 3.16a	32 ± 2.00a	615 ± 34.52a	−29.71 ± 0.08a
Bradyrhizobium sp. CB756	6.6 ± 0.43c	18 ± 0.86a	2.5 ± 0.06b	16.1 ± 0.40a	448 ± 21.25a	−1.54 ± 0.06a	66 ± 0.89a	59 ± 3.07a	30 ± 1.48a	564 ± 22.06b	−29.50 ± 0.09a
Un-inoculated	7.9 ± 0.40b	17 ± 0.82a	2.5 ± 0.05b	15.6 ± 0.23ab	423 ± 19.95a	−1.32 ± 0.09b	63 ± 1.35b	52 ± 2.01a	32 ± 2.56a	564 ± 36.30b	−29.51 ± 0.07a
F statistics											
Landrace (L)	9.01***	6.24***	0.41 ns	0.71 ns	4.49***	5.50***	5.50***	4.60***	5.24***	193.8***	2.6*
Inoculation (I)	29.88***	1.24 ns	4.46*	3.77*	0.55 ns	5.14**	5.14***	2.38 ns	0.66 ns	27.09***	2.5 ns
L × I	20.57***	5.49***	1.81 ns	2.35**	3.16***	5.33***	5.32***	2.42**	5.46***	148.08***	1.1 ns

*Values (means ± standard errors) with dissimilar letters in a column are significantly different at ***$p < 0.001$, **$p < 0.01$, and *$p \leq 0.05$. Plants were sampled at early podding stage of all landraces. ns, not significant.*

ranged between 15.2 and 16.2 g g^{-1} at that site. Shoot N content closely mirrored patterns of shoot biomass, with landraces Heng MM and Heng RM recording higher N content at the site and Sigiri the least. Other landraces with lower N content at Savelugu were Boli, Dowie, Funsi, and Puffeun (**Table 3**). Landrace Funsi recorded the least $\delta^{15}N$ value, and hence greater %Ndfa (67%) at Savelugu while Puffeun recorded the highest $\delta^{15}N$ and the least %Ndfa (58%) at that site (**Table 3**). N-fixed at Savelugu was highest in Heng MM due to its greater shoot biomass and high %Ndfa, followed by the landrace Heng RM. The landraces Sigiri and Puffeun recorded the least N-fixed at the site (**Table 3**). Together with Puffeun and Nakori, landraces Heng MM and Heng RM, which recorded higher N-fixed, also showed greater soil N uptake at Savelugu. Here, the lowest values of soil N uptake were recorded in landraces Boli and Sigiri (**Table 3**).

The highest grain yield was recorded in landrace Nakori (832 kg ha^{-1}), followed by Puffeun (657 kg ha^{-1}), Heng RM (620 kg ha^{-1}), Boli (565 kg ha^{-1}), Heng MM (561 kg ha^{-1}), and then Funsi (522 kg ha^{-1}) in that order (**Table 3**). The least values for grain yield at Savelugu were recorded in landraces Sigiri (494.6 kg ha^{-1}) and then Dowie (398 kg ha^{-1}). Here, shoot $\delta^{13}C$ was highest in Heng MM followed by landraces Boli, Heng RM, and Nakori with similar values, and least in Dowie (**Table 3**).

Main Effect of Inoculation on Plant Growth, Symbiotic Parameters, Grain Yield, and $\delta^{13}C$

The main effect of inoculation at Savelugu was also significant for nodule dry matter, %N, C/N ratio, $\delta^{15}N$, %Ndfa, and grain yield but not $\delta^{13}C$ (**Table 3**). Here, plants inoculated with *Bradyrhizobium* sp. strain 3267 had the highest nodule mass followed by the control plants. Seed inoculation with *Bradyrhizobium* sp. strain 3267 increased overall %N in shoots of Kersting's groundnut and decreased C/N ratio relative to non-inoculation or inoculation with strain CB756 (**Table 3**). Non-inoculation or bacterial inoculation with strain 3267 increased shoot $\delta^{15}N$ values and decreased overall %Ndfa of Kersting's groundnut at Savelugu relative to bacterial inoculation with strain CB756. Overall, N-fixed and soil N uptake were unaltered by inoculation relative to the control (**Table 3**). Grain yield was increased by inoculation with *Bradyrhizobium* sp. strain 3267 when compared to non-inoculation or inoculation with *Bradyrhizobium* sp. strain CB756 (**Table 3**).

Main Effect of Landrace on the Concentration of Xylem N Solutes

There were significant differences in the concentrations of N solutes in the xylem sap of the landraces grown at Savelugu (**Table 2**). The landraces Puffeun and Sigiri recorded higher concentrations of ureide-N in xylem sap and relatively lower nitrate-N at the site (**Table 2**). The landrace Nakori exhibited the highest concentration of amino-N in xylem sap at Savelugu despite its relatively lower ureide-N at the site. However, the landraces Sigiri, Boli, and Heng RM recorded greater RU-N at Savelugu relative to the other landraces (**Table 2**).

Main Effect of Inoculation on the Concentration of Xylem N Solutes

There was a significant effect of inoculation on the xylem ureide concentration at Savelugu (**Table 2**). However, together with non-inoculation, bacterial inoculation with *Bradyrhizobium* sp. strain CB756 increased xylem nitrates when compared to inoculation of the landraces with strain BR 3267. On the other hand, bradyrhizobial inoculation with either *Bradyrhizobium* strains resulted in a greater concentration of amino acids in the xylem sap of the Kersting's groundnuts compared to the uninoculated control (**Table 2**). Overall, RU-N was markedly greater in plants sampled from uninoculated plots and those from plots inoculated with *Bradyrhizobium* sp. strain 3267 (**Table 2**).

Effect of Landrace × Inoculation Interactions at Savelugu

At Savelugu, the landrace × inoculation interaction was significant for shoot biomass, N-fixed, grain yield, and all other measured parameters, except for shoot %N and $\delta^{13}C$ values (**Table 3**). Here, bacterial inoculation with either *Bradyrhizobium* sp. strain BR 3267 or *Bradyrhizobium* sp. CB756 increased shoot biomass in landraces Dowie, Heng MM, and Nakori relative to non-inoculation (control), although the parameter was unaltered in landrace Sigiri (**Figure 2A**). As a result, N-fixed was markedly increased by inoculation of those landraces with either *Bradyrhizobium* strains at Savelugu relative to the control (**Figure 2B**). Despite the increased shoot biomass and N-fixed in plants of Dowie inoculated with either bacterial strains, grain yield was only increased in plants inoculated with *Bradyrhizobium* sp. strain BR 3267 (**Figures 2A–C**). However, inoculation with either bacterial strains increased grain yield in Heng MM when compared to non-inoculation. On the other hand, non-inoculation or bacterial inoculation with *Bradyrhizobium* sp. strain BR 3267 increased grain yield in Nakori when compared to inoculation with *Bradyrhizobium* sp. strain CB756 (**Figure 2C**). Although bradyrhizobial inoculation did not markedly alter shoot biomass and N-fixed in Sigiri, it resulted in higher grain yields than the control (**Figures 2A–C**).

N_2 Fixation and Water Use Efficiency at Gbalahi

Main Effect of Landrace on Plant Growth, Symbiotic Parameters, Grain Yield, and $\delta^{13}C$

A two-way ANOVA of the data collected from Gbalahi revealed significant effect of landrace on nodule dry matter, shoot dry matter, N content, $\delta^{15}N$, %Ndfa, N-fixed, soil N uptake, grain yield, and shoot $\delta^{13}C$ (**Table 4**). Here, landrace Sigiri recorded the highest nodule mass followed by Boli, while Funsi and Nakori recorded the least values of the parameter (**Table 4**).

Shoot biomass was highest in the Nakori landrace followed by Dowie, while the remaining landraces had lower but similar shoot biomass at the Savelugu site. There were however no significant differences in shoot N and C/N ratio of Kersting's groundnut landraces at Gbalahi. The %N values ranged between 1.8 and 2.1% while C/N ratio ranged between 17.7 and 20.2 g g^{-1} (**Table 4**). Shoot N content was highest in the landrace Nakori

TABLE 4 | Plant growth, N_2-fixing parameters, grain yield, and $\delta^{13}C$ of eight Kersting's groundnut landraces planted at Gbalahi in the northern Region of Ghana in 2013.

Treatments	Nodule DM mg plant^{-1}	Shoot DM g plant^{-1}	N conc'n %	C/N ratio g g^{-1}	N content mg plant^{-1}	$\delta^{15}N$ ‰	%Ndfa %	N fixed kg ha^{-1}	Soil N uptake kg ha^{-1}	Grain yield kg ha^{-1}	$\delta^{13}C$ ‰
Landraces											
Boli	6.1 ± 0.73b	20 ± 1.45b	1.9 ± 0.15a	18.8 ± 0.83a	370 ± 51.60bc	−1.07 ± 0.09a	61 ± 1.23b	46 ± 6.70bc	28 ± 3.80bc	316 ± 7.59b	−29.51 ± 0.09a
Dowie	4.6 ± 0.67bc	23 ± 1.81ab	2.1 ± 0.16a	17.7 ± 0.90a	480 ± 60.97ab	−1.22 ± 0.18ab	63 ± 2.47ab	58 ± 5.88ab	38 ± 6.55ab	164 ± 9.74e	−29.98 ± 0.07b
Funsi	3.0 ± 0.55c	21 ± 0.74b	2.1 ± 0.10a	17.9 ± 0.72a	436 ± 37.89abc	−1.47 ± 0.08b	67 ± 1.12a	58 ± 5.08ab	29 ± 2.71abc	365 ± 15.51a	−30.04 ± 0.04b
Heng MM	4.3 ± 0.45bc	19 ± 2.39b	1.8 ± 0.18a	20.2 ± 0.81a	359 ± 72.77bc	−1.48 ± 0.09b	67 ± 1.21a	47 ± 8.65bc	25 ± 6.04c	262 ± 28.35c	−29.89 ± 0.09b
Heng RM	4.8 ± 1.23bc	18 ± 1.56b	2.0 ± 0.11a	19.4 ± 0.82a	372 ± 53.57bc	−1.24 ± 0.16ab	63 ± 2.20ab	46 ± 5.53bc	29 ± 5.45bc	248 ± 25.38c	−29.93 ± 0.08b
Nakori	3.9 ± 0.41c	26 ± 3.18a	2.1 ± 0.12a	18.1 ± 0.97a	554 ± 79.89a	−1.31 ± 0.08ab	64 ± 1.07ab	71 ± 9.71a	40 ± 6.40a	319 ± 44.60b	−29.95 ± 0.05b
Puffeun	4.9 ± 0.42bc	21 ± 2.00b	1.9 ± 0.13a	18.0 ± 0.58a	400 ± 57.74bc	−1.22 ± 0.11ab	63 ± 1.59ab	51 ± 7.52bc	29 ± 4.27bc	315 ± 17.35b	−29.97 ± 0.10b
Sigiri	14.0 ± 2.19a	18 ± 1.95b	1.9 ± 0.11a	18.4 ± 1.20a	336 ± 52.81c	−1.10 ± 0.12a	61 ± 1.68b	41 ± 6.55c	26 ± 4.19c	224 ± 16.03d	−29.91 ± 0.06b
Inoculation											
Bradyrhizobium sp. 3267	6.9 ± 1.09a	22 ± 1.39a	2.1 ± 0.08a	17.9 ± 0.49a	474 ± 37.47a	−1.39 ± 0.05b	65 ± 0.70a	61 ± 4.59a	34 ± 3.01a	285 ± 19.27a	−29.94 ± 0.05a
Bradyrhizobium sp. CB756	5.7 ± 0.77b	22 ± 1.21a	2.0 ± 0.09a	18.4 ± 0.53a	437 ± 39.00a	−1.22 ± 0.09ab	63 ± 1.21ab	54 ± 4.59a	33 ± 3.43a	278 ± 9.01b	−29.89 ± 0.06a
Un-inoculated	4.6 ± 0.48b	18 ± 1.01b	1.9 ± 0.08a	19.4 ± 0.56a	330 ± 31.02b	−1.18 ± 0.08a	63 ± 1.09b	41 ± 3.53b	25 ± 2.84b	267 ± 21.86b	−29.87 ± 0.05a
F statistics											
Landrace (L)	26.83***	3.10**	1.07 ns	1.18 ns	3.10**	2.50*	2.50*	3.62**	2.44*	165.68***	5***
Inoculation (I)	8.11***	7.72***	3.03 ns	2.85 ns	8.54***	3.37*	3.37*	11.44***	4.38*	8.05***	1 ns
L × I	7.94***	4.75***	3.70***	2.66**	6.61***	3.50***	0.50***	5.87***	6.98***	131.35***	1 ns

*Values (means ± standard errors) with dissimilar letters in a column are significantly different at ***$p < 0.001$, **$p < 0.01$ and *$p \leq 0.05$. Plants were sampled at early podding stage of all landraces. ns, not significant.*

followed by Dowie while the least N content was recorded in landrace Sigiri (**Table 4**).

The least $\delta^{15}N$ values were recorded in the Funsi and Heng MM landraces, while Boli and Sigiri recorded the highest (**Table 4**). The remaining landraces recorded slightly higher but similar $\delta^{15}N$ to Boli and Sigiri (**Table 4**). As a result, %Ndfa was greater in the Funsi and Heng MM landraces at Gbalahi, and lower in Boli and Sigiri at the site. The remaining landraces recorded relatively lower but similar %Ndfa to those of Funsi and Heng MM (**Table 4**). N-fixed was highest in Nakori followed by the landraces Dowie and Funsi. The least N-fixed at Gbalahi was recorded in landrace Sigiri despite its high nodule mass (**Table 4**).

The landrace Nakori recorded the highest soil N uptake followed by Dowie and Funsi, while Heng MM and Sigiri recorded the least (**Table 4**). Grain yield at Gbalahi was highest in Funsi (365 kg ha^{-1}), followed by Boli (316 kg ha^{-1}), Nakori (319 kg ha^{-1}), and Puffeun (315 kg ha^{-1}) with relatively lower grain yield. The least grain yield at Gbalahi was recorded in the landraces Dowie (164 kg ha^{-1}) and Sigiri (224 kg ha^{-1}). Other landraces with lower grain yield at Gbalahi were Heng MM and Heng RM (**Table 4**). Here, shoot $\delta^{13}C$ was highest in the landrace Boli (−29.51‰) and least but similar in the remaining landraces (−29.89‰ to −30.04‰) (**Table 4**).

Main Effect of Inoculation on Plant Growth, Symbiotic Parameters, Grain Yield, and $\delta^{13}C$

As in the other locations, there was a significant effect of inoculation on nodule dry matter, shoot dry matter, N content, $\delta^{15}N$, %Ndfa, N-fixed, soil N uptake, and grain yield but not $\delta^{13}C$ at Gbalahi (**Table 4**). Whereas inoculation with *Bradyrhizobium* sp. strain BR 3267 increased nodule mass over non-inoculation or inoculation with *Bradyrhizobium* sp. strain CB756, bacterial inoculation with either strains increased shoot biomass and N content over the control (**Table 4**). The overall $\delta^{15}N$ value was however decreased by inoculation with *Bradyrhizobium* sp. strain BR 3267 relative to non-inoculation. As a result, bacterial inoculation with *Bradyrhizobium* sp. strain BR 3267 increased the overall %Ndfa compared to non-inoculation at Gbalahi (**Table 4**). Bacterial inoculation with either strains also increased N-fixed and soil N uptake compared to non-inoculation. There was no marked effect of inoculation on overall shoot $\delta^{13}C$ at Gbalahi (**Table 4**). However, overall grain yield was only increased by bacterial inoculation with strain 3267 compared to non-inoculation or inoculation with *Bradyrhizobium* sp. strain CB756 (**Table 4**).

Main Effect of Genotype on the Concentrations of Xylem N Solutes

There was a marked effect of landrace on the concentrations of xylem N solutes (**Table 2**). The landrace Boli (with relatively lower N-fixed from ^{15}N natural abundance) together with Heng RM recorded the highest concentration of ureide in xylem sap. However, the landrace Boli together with Puffeun recorded greater concentration of nitrate in xylem sap at the site relative to the other landraces (**Table 2**). However, the concentration of amino acids was greater in the xylem sap samples of the landraces Boli and Heng MM, followed by Dowie and Heng RM (**Table 2**). With high N-fixed values from ^{15}N natural abundance, the landrace Nakori also recorded the highest RU-N, followed by landraces Funsi, Sigiri, and Puffeun (**Table 2**).

Main Effect of Inoculation on the Concentrations of Xylem N Solutes

Bradyrhizobial inoculation with strain CB756 increased the concentration of ureides and nitrate in xylem sap samples relative to non-inoculation (control) or inoculation with strain BR 3267 (**Table 2**). However, non-inoculation or bacterial inoculation with *Bradyrhizobium* sp. strain BR 3267 each increased the concentration of amino acids in xylem sap when compared to inoculation with *Bradyrhizobium* sp. strain CB756. Consequently, bacterial inoculation with strain CB756 resulted in greater RU-N due to increased concentration of ureides and reduced concentration of amino acids (**Table 2**).

Effect of Landrace × Inoculation Interactions at Gbalahi

At Gbalahi, there was a significant effect of landrace × inoculation interaction on shoot biomass, ureide concentration in xylem sap, relative ureide-N (RU-N), N-fixed, grain yield, and all other parameters, except for shoot $\delta^{13}C$ (**Tables 2**, **4**). Here, bradyrhizobial inoculation with strain BR 3267 or CB756 each increased shoot biomass relative to the control in landraces Heng MM and Sigiri (**Figure 3A**). However, bacterial inoculation with *Bradyrhizobium* sp. strain BR 3267 increased shoot biomass over inoculation with *Bradyrhizobium* sp. strain CB756 in Funsi, and over non-inoculation or inoculation with strain CB756 in landrace Puffeun (**Figure 3A**). Non-inoculation (control) or bacterial inoculation of Heng RM with *Bradyrhizobium* sp. strain CB756 each resulted in higher shoot biomass relative to inoculation of the same landrace with strain BR 3267 (**Figure 3A**). The landrace/inoculant strain combinations that elicited higher shoot biomass mostly recorded higher ureide concentration and RU-N in xylem sap, albeit a few exceptions (**Figures 3A–C**). For example, despite the lower shoot biomass of control plants of Puffeun and those inoculated with *Bradyrhizobium* sp. strain CB756, ureide-N and RU-N were unaltered by inoculation of the landrace (**Figures 3A–C**). Generally, N-fixed and grain yield by the landraces closely mirrored patterns of shoot biomass. Thus, landrace/inoculant strain combinations that elicited higher shoot biomass were found to record higher N-fixed and grain yield (**Figures 3A–E**).

Three-Way ANOVA Comparison of Symbiotic Parameters, Grain Yield, and $\delta^{13}C$ Between Locations

Except for %Ndfa, the main effect of location was significant for all growth and symbiotic parameters, grain yield, and shoot $\delta^{13}C$ values (**Supplementary Table 2**). Although the overall nodule mass of plants was greater at Nyankpala and Savelugu, shoot biomass was markedly higher in the plants grown at Gbalahi followed by those grown at Nyankpala (**Supplementary Table 2**). The overall %N was higher in the plants grown at Nyankpala and Savelugu, resulting in much lower C/N ratio in those plants when compared to their counterparts grown at Gbalahi. The high shoot

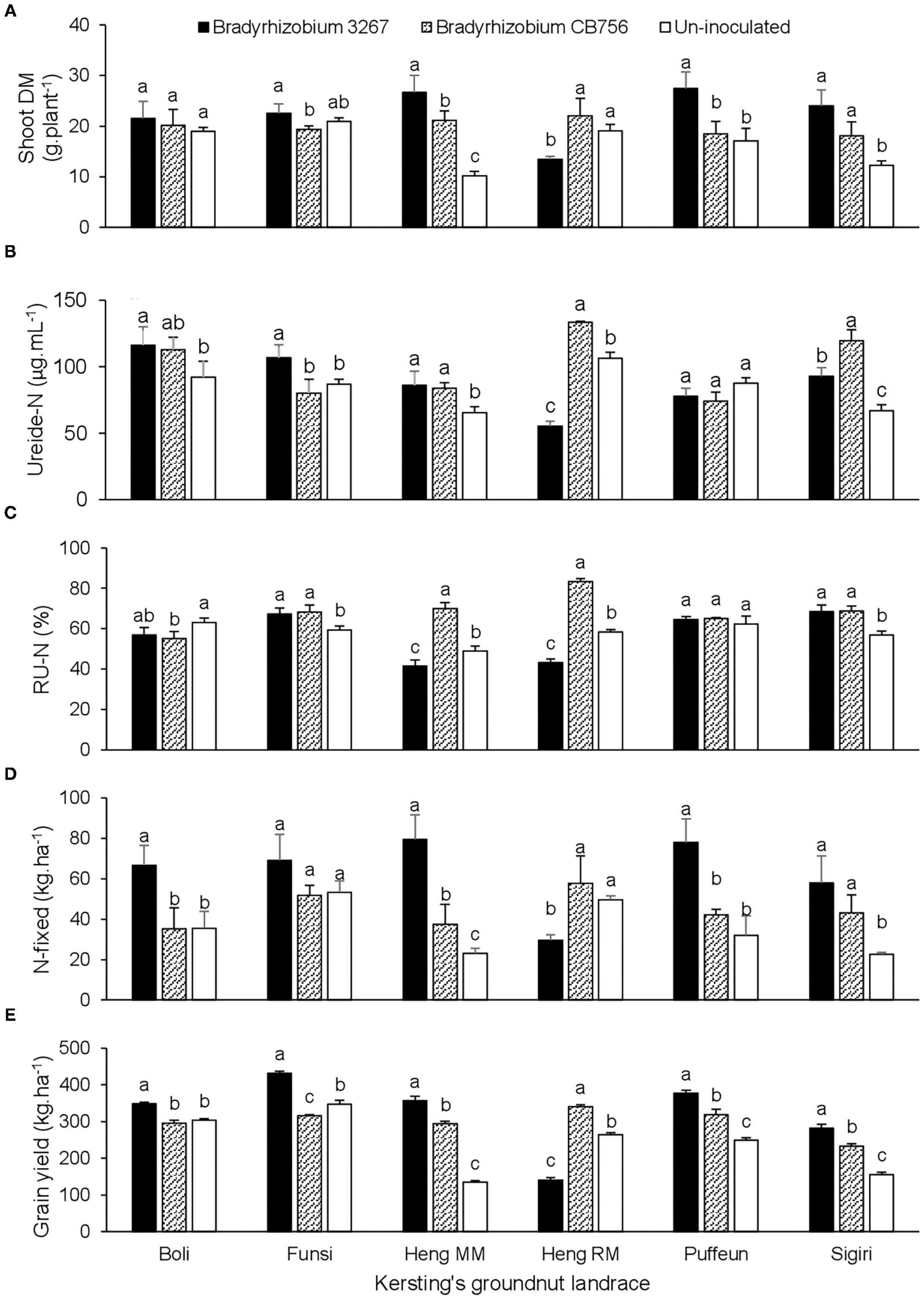

FIGURE 3 | The interactive effect of landrace × inoculation on **(A)** shoot dry matter, **(B)** ureide-N in xylem sap, **(C)** relative ureide-N (RU-N), **(D)** N-fixed, and **(E)** grain yield of six Kersting's groundnut landraces planted at Gbalahi in 2013. For each landrace, bars with dissimilar letters are significantly different ($p \leq 0.05$).

biomass and %N of plants at Nyankpala and Savelugu resulted in greater N content in those plants (**Supplementary Table 2**). Although average shoot δ^{15}N value of plants was markedly lower at Savelugu, %Ndfa was similar between locations due to differences in the δ^{15}N values of reference plants between those locations (**Supplementary Table 1**). However, N-fixed, soil N uptake as well as grain yield closely mirrored patterns in shoot biomass and N content between locations (**Supplementary Table 2**). Overall, shoot δ^{13}C was greater in the plants grown at Savelugu, followed by those grown at Nyankpala and least in the plants at Gbalahi (**Supplementary Table 2**).

There was a marked effect of location on xylem N solutes of Kersting's groundnut in this study (**Supplementary Table 3**). The concentrations of ureides and nitrate were both higher in the xylem sap of the plants grown at Savelugu and Gbalahi, and unexpectedly least in the plants grown at Nyankpala despite their greater shoot biomass and N-fixed at the site. The concentration of amino acids in xylem sap was higher at Gbalahi, and lower but similar between Nyankpala and Savelugu (**Supplementary Table 3**). However, RU-N was higher in the plants at Savelugu followed by those at Gbalahi due to greater overall ureides in the plants grown in those locations (**Supplementary Table 3**).

Correlation Analysis

Correlation analysis revealed that the concentration of nitrate in xylem sap and %Ndfa measured from ^{15}N natural abundance were negatively correlated in landraces Boli, Dowie, and Funsi at Nyankpala (**Figure 4**). The RU-N in xylem sap was also positively correlated with grain yield in all the test locations (**Figure 4**). Moreover, positive correlations were obtained when shoot biomass was plotted against the amount of N-fixed, soil N uptake, and shoot N content in this study (**Figure 5**). Furthermore, there was a positive correlation between %N and %C in shoots of the test landraces (**Figure 6**). On the other hand, shoot δ^{13}C and grain yield were positively correlated at Nyankpala, although not statistically significant at Savelugu (**Figure 6**). The combined data from all the test locations also revealed a direct correlation between δ^{13}C and grain yield (**Figure 6**).

DISCUSSION

Nodulation, Plant Growth, and Symbiotic N_2 Fixation in Field-Grown Kersting's Groundnut Landraces

The ability of Kersting's groundnut to enter symbiosis with *Bradyrhizobium* sp. strain CB756 leading to N_2 fixation was earlier demonstrated (Dakora, 1998). Since then, there has been little report of N_2 fixation by this orphan grain legume (Mohammed et al., 2018), although such studies can provide information much needed for the crop's conservation and improvement. This study assessed symbiotic N_2 fixation and water use efficiency (δ^{13}C) of Kersting's groundnut landraces in response to inoculation with two *Bradyrhizobium* strains (*Bradyrhizobium* sp. BR 3267 and *Bradyrhizobium* sp. CB756) under field conditions in Ghana.

The findings revealed that increased nodulation in some Kersting's groundnut landraces resulted in greater plant growth, whereas the opposite was observed in some instances. For example, increased nodule mass in landraces Heng MM and Heng RM resulted in greater shoot biomass and N accumulation at Nyankpala as opposed to an observed lower shoot biomass in landraces Boli and Sigiri at Savelugu despite their higher nodule mass at the site. Moreover, the fact that the observed increases in plant growth associated with high nodulation at Nyankpala only decreased shoot δ^{15}N and increased %Ndfa in Heng MM (**Table 1**) clearly suggests that the extent of nodulation alone does not always reflect symbiotic performance. However, whether differences in nodule size and/or nodule position on the root might have contributed to the observed trends in symbiotic performance of the test landraces was not assessed in this study. These results agree with earlier reports that enhanced symbiosis is linked to the N_2-fixing efficiency of the nodule symbionts rather than increased nodulation only (Westhoek et al., 2017; Mohammed et al., 2019). The Kersting's groundnut landraces in this study exhibited marked variations in N_2 fixation as evidenced by the differences in their shoot δ^{15}N and %Ndfa values. As expected, lower δ^{15}N by landraces were accompanied by higher %Ndfa in all the study locations, a finding consistent with previous reports that, lower δ^{15}N values in N_2-fixing legumes is due to their greater reliance on atmospheric N_2 fixation (Unkovich et al., 2008; Szpak, 2014). With relatively lower δ^{15}N values, therefore, symbiotic dependence (%Ndfa) was much higher in landraces Heng MM (68%) and Funsi (67%) at Nyankpala, Funsi (67%) at Savelugu, and in Heng MM (67%) and Funsi (67%) at Gbalahi. The high N_2-fixing trait of these landraces is a desirable trait that can confer adaptation to low N environments such as those in this study. Owing to the observed differences in the percent N derived from symbiosis by the test landraces, the amounts of N-fixed also varied markedly, and closely mirrored the patterns of their shoot biomass and N accumulation. Moreover, symbiotic N and endogenous soil N both contributed to enhanced plant growth, a finding further supported by the positive correlations observed when shoot biomass was plotted against N-fixed, soil N uptake, and shoot N content (**Figure 5**). Due to the high symbiotic dependence by Kersting's groundnuts in this study, the amounts of N-fixed by landraces were generally greater than their soil N uptake values, with the amounts of N-fixed ranging from 46 to 69 kg ha^{-1} at Nyankpala, 48 to 73 kg ha^{-1} at Savelugu, and from 41 to 71 kg ha^{-1} at Gbalahi. Legumes exhibiting greater N_2 fixation often show less dependence on soil N since the efficiency of the legume–rhizobia symbiosis diminishes with higher endogenous soil N concentrations (Zahran, 1999; Mbah and Dakora, 2017).

Across the board, the relatively lower overall δ^{15}N values in the plants grown at Nyankpala and Savelugu resulted in higher %N and reduced C/N ratio in those plants probably due to enhanced symbiosis (**Supplementary Table 2**). Although C/N ratio did not markedly vary among the Kersting's groundnut landraces, the values were generally low and ranged between 14.4 and 20.2 g g^{-1} across the three sites, a finding that could be

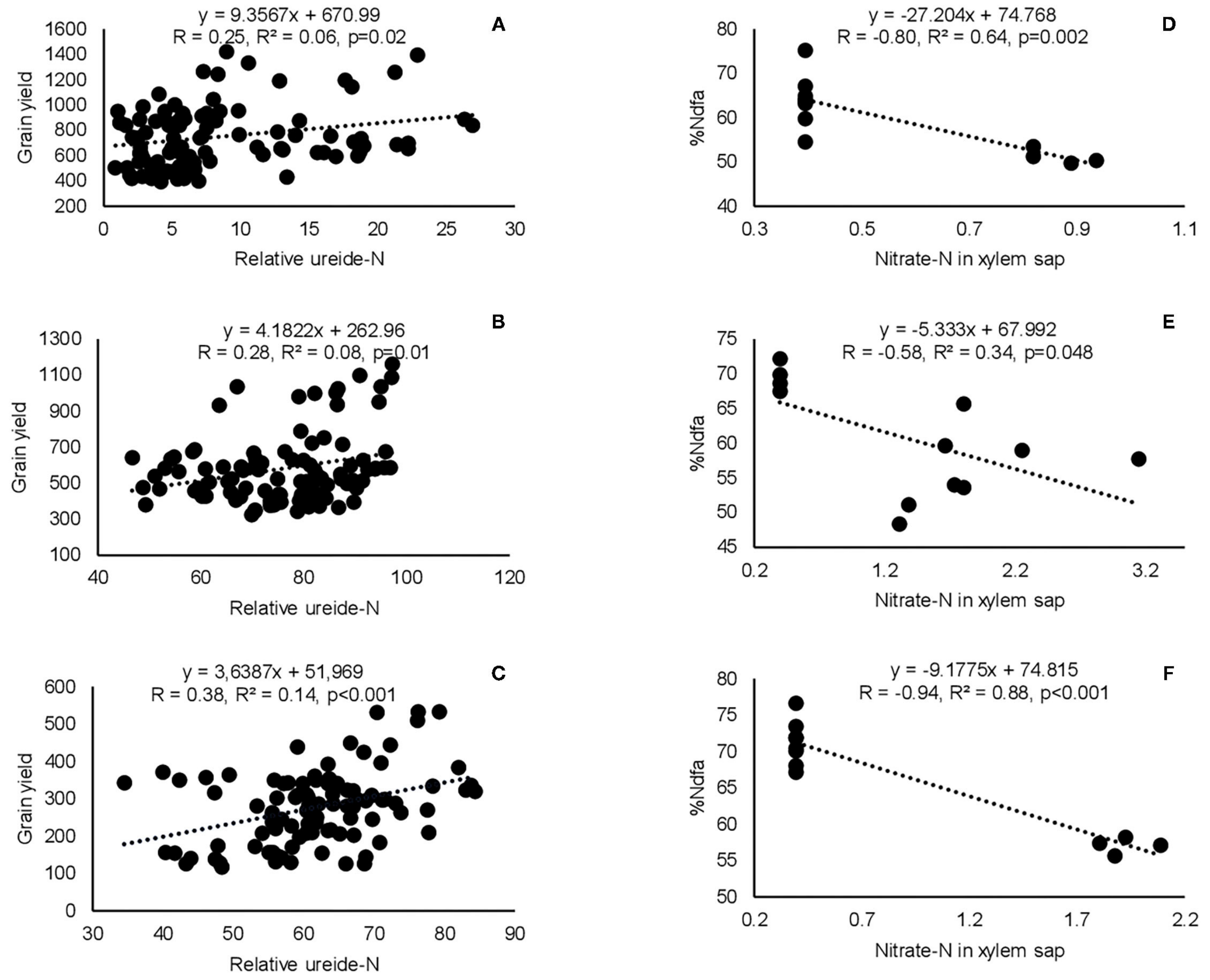

FIGURE 4 | Correlation and regression analysis between xylem nitrate-N and relative ureide-N (RU-N) and grain yield of eight Kersting's groundnut landraces grown at **(A)** Nyankpala, **(B)** Savelugu, and **(C)** Gbalahi, and between xylem nitrate-N and %Ndfa from ^{15}N natural abundance in landraces **(D)** Boli, **(E)** Dowie, and **(F)** Funsi planted at Nyankpala in 2013.

attributed to the reduction of atmospheric N_2 to NH_3 in their root nodules (Hobbie et al., 1998; Wolf et al., 2017). These results agree with earlier reports that legumes exhibit a characteristic low C/N ratio ($<24\,g\ g^{-1}$) due to their N_2-fixing activities as opposed to higher values ($>24\,g\ g^{-1}$) often recorded in non-legumes (Hobbie et al., 1998). The legume–rhizobia symbiosis is such that, rhizobia in root nodules fix atmospheric N_2 in exchange for plant photosynthates (Oldroyd et al., 2011), a functional link that was further illustrated by positive correlations between %N and %C in shoots of Kersting's groundnuts in this study. Thus, the increased supply of carbon compounds from photosynthesis stimulated greater N_2 fixation in root nodules of the test landraces, such that higher shoot N concentration was accompanied by higher C concentration (Mohammed et al., 2018). Nevertheless, although the overall percent N derived from symbiosis was unaltered between locations, the shoot $\delta^{15}N$ values of landraces showed marked variations, and closely mirrored the patterns in the endogenous soil N concentrations between the three sites (**Supplementary Table 2**). Thus, due to the relatively lower N content of soils at Savelugu (0.016%), the plants at the site recorded much lower overall $\delta^{15}N$ value; conversely, the relatively higher endogenous soil N concentrations at Nyankpala (0.023%) and Gbalahi (0.031%) led to higher $\delta^{15}N$ values in the plants at those sites due to a suppression of the symbiosis (Mbah and Dakora, 2017). However, although the observed lower $\delta^{15}N$ in plants at Savelugu were accompanied by greater ureides biosynthesis and hence higher relative ureide abundance in the xylem sap, the RU-N in plants at Nyankpala were unexpectedly very low due to their reduced ureides concentration (**Supplementary Table 3**). Whether the dramatic reduction in the concentration of ureides in xylem sap at Nyankpala was influenced by the dynamics in soil moisture levels at the time

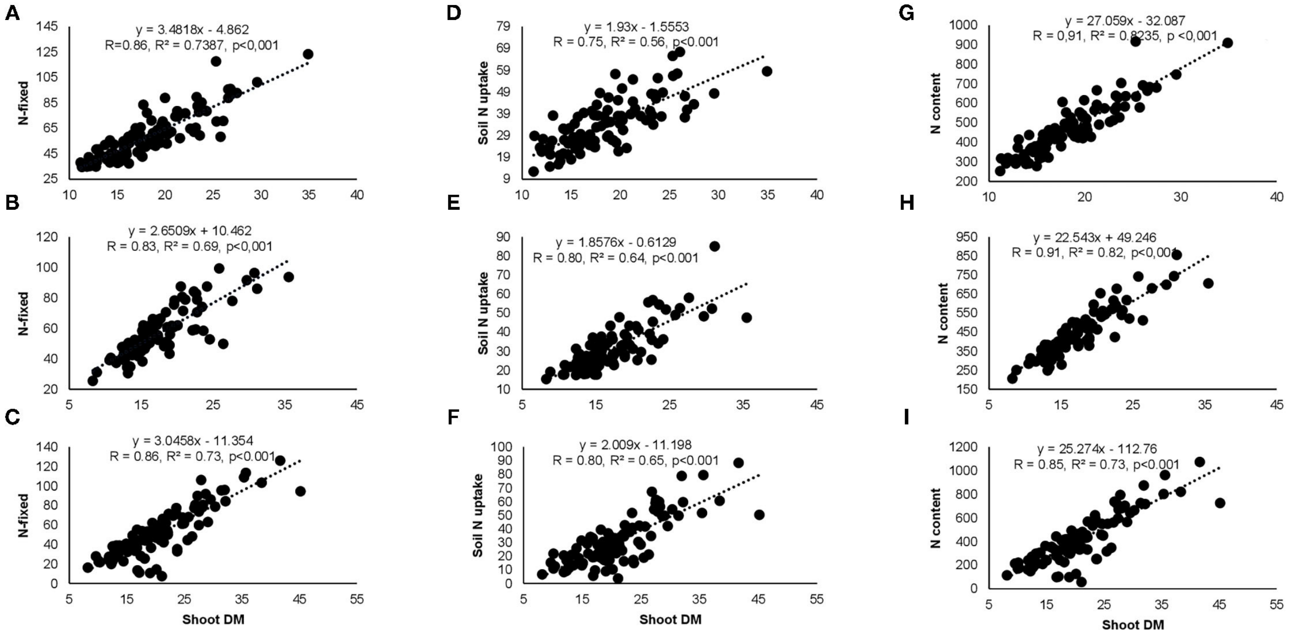

FIGURE 5 | Correlation and regression analysis between shoot DM and N-fixed at **(A)** Nyankpala, **(B)** Savelugu, **(C)** Gbalahi, and between shoot DM and soil N uptake at **(D)** Nyankpala, **(E)** Savelugu, **(F)** Gbalahi as well as between shoot DM and N content at **(G)** Nyankpala, **(H)** Savelugu, and **(I)** Gbalahi.

of sampling was not assessed. Whatever the case, however, the ureide measure of symbiosis is instantaneous and can therefore be influenced by soil/environmental factors prevailing at the time of sampling as opposed to the more integrative ^{15}N natural abundance technique (Unkovich et al., 2008).

Low grain yield of Kersting's groundnut on farmers' fields partly accounts for the decline in its cultivation (Ayenan and Ezin, 2016). In this study, average grain yield ranged between 445 and 959 kg ha^{-1} at Nyankpala, between 398 and 832 kg ha^{-1} at Savelugu, and between 164 and 365 kg ha^{-1} at Gbalahi. The relatively higher grain yield in the plants at Nyankpala and Savelugu could be attributed to adequate soil moisture during plant establishment and pod filling due to timely planting at the site relative to the cropping season (**Figure 1**). The fact that most landraces with greater shoot biomass coupled that with increased N accumulation and grain yield in some instances suggests complementary roles of symbiosis and soil N uptake in promoting the growth and yield of Kersting's groundnut in this study. The grain yield recorded by landraces in this study are generally lower than values (up to 1,876 kg ha^{-1}) reported in earlier studies (Bayorbor et al., 2010; Mohammed et al., 2018). Although planting date was not reported in their study at Nyankpala (Bayorbor et al., 2010), earlier planting could have contributed to the higher yields obtained for the same landraces in that location. However, grain yield was reduced (192–688 kg ha^{-1}) when planting was carried out in July in another experiment (Adu-gyamfi et al., 2012) possibly due to reduced soil moisture during crop development and/or pod filling. Variations in soil moisture resulting from differences in planting dates and/or rainfall distribution could possibly explain the patterns of grain yield across the three locations in this study (**Figure 1**).

Variations in Water Use Efficiency (δ^{13}C) in Field-Grown Kersting's Groundnut Landraces

Even though Kersting's groundnut is reported to thrive well under drought conditions (Mergeai, 1993), there is no available report on the crop's water use efficiency. In this study, shoot δ^{13}C was used as a measure of water use efficiency. The landraces Heng MM and Heng RM, both with high grain yield at Nyankpala also exhibited greater water use efficiency at that site as evidenced by their higher shoot δ^{13}C (−29.48‰ and −29.60‰, respectively), when compared to other landraces. On the other hand, the landrace Sigiri coupled the least grain yield with the lowest water use efficiency at Nyankpala, recording a δ^{13}C value of −29.97‰. These findings clearly indicate variations in water use efficiency among the tested Kersting's groundnut landraces in this study. Nevertheless, the fact that shoot δ^{13}C values of the test landraces were much lower than values reported for some Bambara groundnut (−26.2‰) and soybeans (−25.1‰) elsewhere (Mohale et al., 2014; Mapope and Dakora, 2016) suggests that the plants in this study might have received adequate rainfall during the growth period, leading to an increase in C isotope discrimination and hence lower δ^{13}C values. Among the landraces, Boli exhibited consistently high water use efficiency across the three locations followed by Heng MM and Heng RM, which also exhibited greater water use efficiency at Nyankpala and Savelugu. Interestingly,

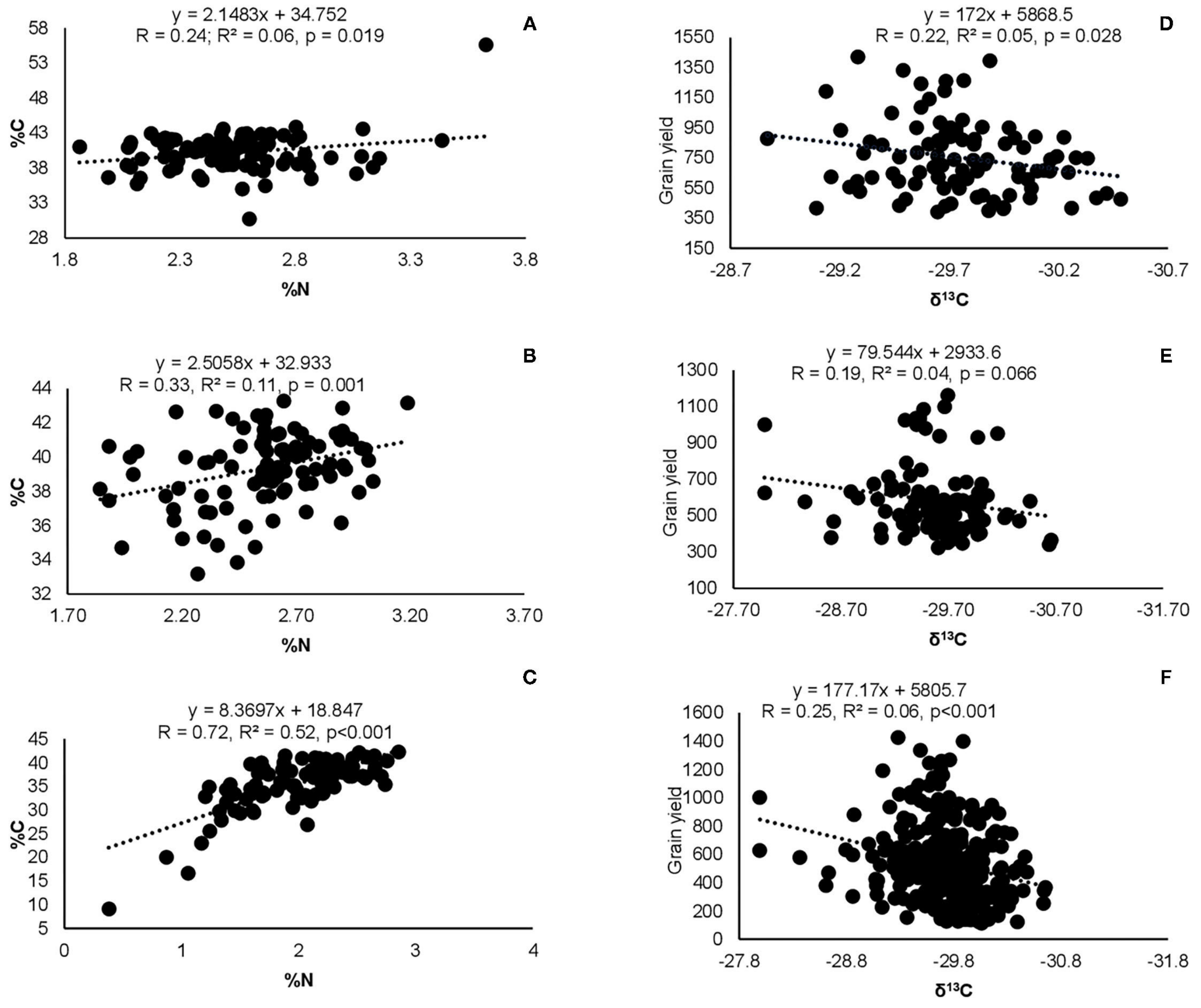

FIGURE 6 | Correlation and regression analysis between shoot %N and %C at **(A)** Nyankpala, **(B)** Savelugu, **(C)** Gbalahi, and between δ^{13}C and grain yield at **(D)** Nyankpala, **(E)** Savelugu, as well as **(F)** between δ^{13}C and grain yield of the combined data from the three locations.

grain yield correlated positively with shoot δ^{13}C at Nyankpala ($r = 0.22$, $r^2 = 0.09$, $p = 0.004$), just as the combined data from all the test locations also revealed a positive correlation between the two parameters ($r = 0.25$, $r^2 = 0.06$, $p < 0.001$), although these relationships were weak. These findings could explain why Kersting's groundnut is able to thrive and produce yield under water-limiting conditions in the African savanna (Mergeai, 1993; Mohammed et al., 2018). The fact that most landraces with higher grain yield in this study coupled that with relatively greater water use efficiency is a trait that could be exploited for increased production of Kersting's groundnut in water-limiting environments.

Aside from the observed genotypic differences, there was also a marked effect of planting location on δ^{13}C of Kersting's groundnut in this study, with plants at Savelugu eliciting the highest overall water use efficiency (shoot δ^{13}C) followed by those planted at Nyankpala and Gbalahi. The observed variations in shoot δ^{13}C values of plants between locations could be attributed to the variations in rainfall distribution and soil moisture during plant growth. Although grain yield at Gbalahi was reduced by decreased soil moisture due to late planting at the site relative to the rains (**Figure 1**), the stress happened at the onset of podding. As a result, the expected greater δ^{13}C (water use efficiency) from water stress (Wang et al., 2016) was not noticed in plants at the site since sampling was done around the same time (early podding) that the water stress started. Nevertheless, the total rainfall from sowing till plant sampling was 481 mm at Nyankpala, 471 mm at Savelugu, and 305 mm at Gbalahi. The lower δ^{13}C in plants at Gbalahi relative to those in the other locations was therefore unexpected since plants exposed to reduced soil moisture are known to exhibit greater water use efficiency

($\delta^{13}C$) (Farquhar et al., 1989; Wang et al., 2016). However, the field at Nyankpala was previously fallowed while the soils at Savelugu were shallow despite being under cultivation. The surprisingly lower $\delta^{13}C$ in plants at Gbalahi might have been due to alterations in soil structure resulting from cultivation practices at the site that might have favored improved water retention capacity. The fact that $\delta^{13}C$ was highest at Savelugu could however be attributed to both reduced rains at the site relative to Nyankpala coupled with lower water retention capacity due to an observed shallow nature of the soils at the site. An earlier study with soybeans also observed reduced $\delta^{13}C$ in some areas with lower rainfall compared to those with higher rains due to differences in tillage systems (Mapope and Dakora, 2016).

The Response of Kersting's Groundnut Landraces to *Bradyrhizobium* Inoculation

Previous studies on legume inoculation in the field have reported variable response due to environmental differences, crop species, and/or genotypes as well as rhizobial strain differences in N_2-fixing efficiency (Ulzen et al., 2016; Kyei-Boahen et al., 2017; Mohammed et al., 2018). In this study, plant growth, symbiotic performance, and grain yield of the test landraces showed variable response to inoculation with the two *Bradyrhizobium* strains. Inoculation response was markedly influenced by the crop landrace, location, and the strain of *Bradyrhizobium* used, indicating differences in host–soil microbiome compatibility. Although interaction effects within study sites were significant for several parameters (**Tables 1–4**), for brevity, the results are discussed for shoot biomass, N-fixed, and grain yield for landraces that showed consistent patterns of interactions for those parameters. The test landraces showed contrasting response to inoculation between the study locations, with bacterial inoculation of landraces Dowie, Heng MM, and Nakori with either *Bradyrhizobium* strains at Savelugu resulting in enhanced plant growth and N-fixed, leading to significant grain yield increases over non-inoculation in the case of Heng MM (**Figures 2A–C**). Despite the reduced shoot growth and N-fixed in uninoculated Nakori plants at Savelugu, however, grain yield was markedly increased when compared to inoculation of the landraces with *Bradyrhizobium* sp. strain CB756 (**Figures 2A–C**). In contrast to the plants at Savelugu that showed positive inoculation response, non-inoculation of Dowie, Heng MM, Nakori, and Puffeun at Nyankpala markedly enhanced plant growth and the amounts of N-fixed, resulting in significant grain yield increases when compared to *Bradyrhizobium* inoculation of those landraces with either inoculant strains at the site (**Figures 2D–F**). The observed contrasting response to inoculation by Kersting's groundnut landraces between the two locations could be attributed to variations in the N_2-fixing efficacy of the native rhizobial populations between the sites. Whereas, the inoculated plants at Savelugu showed better symbiotic performance than their uninoculated counterparts at the site, the uninoculated plants at Nyankpala showed greater symbiotic performance than their inoculated counterparts. Similarly, the contrasting response to bacterial inoculation elicited by the plants at Gbalahi clearly highlights the complex effects of the crop landrace, inoculant strain, and location-specific factors on the Kersting's groundnut–rhizobia symbiosis under field conditions. Previous studies have highlighted the complex nature of legume inoculation response under field settings, a process governed by the N_2-fixing efficiency of the inoculant strains as well as their competitive ability for nodule occupancy relative to the resident/indigenous strains (Catroux et al., 2001; Mohammed et al., 2018). Because the products of N_2 fixation in root nodules of Kersting's groundnut are exported to the aerial portions as ureides *via* the xylem, the landrace/inoculant strain combinations that promoted higher shoot biomass a Gbalahi also recorded greater xylem ureides and amounts of N-fixed (**Figure 3**). Probably due to the instantaneous nature of the ureide measure of N_2 fixation, however (Unkovich et al., 2008), some deviations were observed where higher xylem ureide levels did not translate into greater shoot biomass or N-fixed. Nevertheless, improved plant growth and symbiotic performance resulting from inoculation or non-inoculation at Gbalahi promoted significant grain yield increases, a finding that clearly demonstrates the importance of legume N_2 fixation to plant growth and consequently grain yield production.

The trends in plant growth closely mirrored the patterns of N-fixed and was further illustrated by positive correlations between the two parameters (**Figures 5A–C**). The observed direct link between plant growth and N nutrition as observed with Kersting's groundnut in this study was earlier reported in field-grown cowpea and Kersting's groundnut (Belane and Dakora, 2010; Mohammed et al., 2018). The amounts of N-fixed by Kersting's groundnut in this study also varied interactively, with values ranging from 42 to 91 kg ha^{-1} at Nyankpala, from 44 to 80 kg ha^{-1} at Savelugu, and from 23 to 80 kg ha^{-1} at Gbalahi depending on the landrace/inoculant combinations used in those locations. These N-fixed values clearly indicate the potential role of Kersting's groundnut in sustainable agriculture, where their N contribution to soils from shoot residues after harvest could be substantial. The contribution of legume N_2 fixation to plant growth and grain yield was further evidenced by positive correlations between RU-N in xylem sap and grain yield (**Figure 4**). It is this N_2-fixing trait of nodulated legumes such as Kersting's groundnut that gives them advantage over non-legumes in low-N environments (Menge and Chazdon, 2016). The fact that shoot biomass correlated positively with N-fixed, soil N-uptake, and shoot N content shows that both symbiosis and soil N uptake complemented each other in enhancing the growth of Kersting's groundnuts in this study. Nevertheless, lower soil N uptake values were largely associated with greater dependence on symbiotic N, thus agreeing with known reports that low endogenous soil N stimulates greater symbiotic N_2 fixation and *vice versa* (Zahran, 1999; Mbah and Dakora, 2017; Mohammed et al., 2018).

Depending on the landrace/inoculant strain combinations used, grain yield of the test Kersting's groundnuts varied between 515 and 1,198 kg ha^{-1} at Nyankpala, between 350 and 1,095 kg ha^{-1} at Savelugu, and between 135 and 432 kg

ha^{-1} at Gbalahi. In most instances, the landrace/inoculant combinations that elicited greater plant growth and N-fixed also had high grain yield. Thus, whereas bacterial inoculation with either *Bradyrhizobium* strains generally improved symbiotic performance and grain yield of Kersting's groundnuts at Savelugu and Gbalahi, non-inoculation markedly enhanced N-fixed and grain yield over bacterial inoculation of the plants at Nyankpala, a clear indication of variations in the plant growth-promoting effects of the soil microbiomes between the test sites (**Figures 2**, **3**).

CONCLUSION

This study has explored the N_2-fixing capacity and water use efficiency ($\delta^{13}C$) of the orphan Kersting's groundnut in response to inoculation with two bradyrhizobial strains. The findings revealed high symbiotic dependence by Kersting's groundnut, which was influenced by the landrace/inoculant strain combination and planting location, indicating differences in the N_2-fixing efficacy of native rhizobia between locations. Moreover, the differential performance of the inoculant strains between and among locations indicate possible variations in their competitiveness for nodule occupancy and their ability to persist and fix N_2 in symbiosis with their host in the studied environments. Whereas, symbiosis accounted for a greater part of the N demand by Kersting's groundnut in this study, soil N uptake complemented the symbiotic process in enhancing plant growth, shoot N accumulation, and, in some instances, grain yield. Given that the overall symbiotic dependence by landraces in this study ranged between 58 and 68%, there is huge potential to increase the efficiency of the Kersting's groundnut–rhizobia symbiosis through manipulation of the host legume and its nodule symbionts. This effort could commence through exploring indigenous rhizobia within and without the crop's cultivation areas that are capable of higher N_2 fixation in diverse environments.

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

MM conducted field experiments, analyzed and interpreted data, and prepared the manuscript. GM took part in xylem N solute analyses. ES helped in field experiments. FD funded and coordinated the research and helped in manuscript preparation. All authors contributed to the article and approved the submitted version.

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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.2021.672247/full#supplementary-material

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Morphological Description and Ethnobotanical Review of the Orphan Crop Myin-Hkwa (*Centella asiatica* L.) From Myanmar

Musavvara Khaitovna Shukurova [1†], *Daisy Myint* [2†], *San San Yi* [3], *Ohm Mar Saw* [4] *and Kazuo N. Watanabe* [5*]

[1] *Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan,* [2] *Department of Agriculture, Land Use Division, Ministry of Agriculture, Livestock and Irrigation, Nay Pyi Taw, Myanmar,* [3] *Department of Agriculture, Policy and Planning Division, Ministry of Agriculture, Livestock and Irrigation, Nay Pyi Taw, Myanmar,* [4] *Department of Agricultural Research, Seed Bank, Ministry of Agriculture, Livestock and Irrigation, Nay Pyi Taw, Myanmar,* [5] *Tsukuba-Plant Innovation Research Center, University of Tsukuba, Tsukuba, Japan*

Edited by:
Katherine Steele,
Bangor University, United Kingdom

Reviewed by:
Ayodeji B. Oyenihi,
Cape Peninsula University of Technology, South Africa
Ryoko Machida-Hirano,
National Agriculture and Food Research Organization (NARO), Japan

***Correspondence:**
Kazuo N. Watanabe
watanabe.kazuo.fa@u.tsukuba.ac.jp

[†] *These authors have contributed equally to this work*

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Centella asiatica is a medicinal herb commonly known as Asiatic pennywort or gotu kola. The species is valued for its medicinal and nutritional properties. It is a perennial plant with leaves and stems that can be consumed as a green leafy vegetable. It is used as a folk remedy to cure various mild and chronic diseases due to its anti-rheumatic, antipyretic, antibacterial, antiviral, and anti-inflammatory effects, and as a mental rejuvenator. Although the species is widely distributed throughout the tropics and subtropics, its recognition was limited. The morphological description of *C. asiatica* is not well-documented in Myanmar, in Burmese or other local languages. Plant assessment *via* morphological markers is one of the ultimate methods for the primary description and characterization of their phenotypic traits. The objectives of this study were focused on the description of Myanmarese *C. asiatica* grown in Japan through morphological markers and a brief overview of its ethnobotanical use in Asia. Morphological characterization revealed the quantitative and qualitative differences regarding several traits among assessed *C. asiatica* samples. Furthermore, the study can provide information on the primary *C. asiatica* cultivation system in Japan, as it can be a potentially new and economically important crop for the Japanese market.

Keywords: *Centella asiatica*, myin-hkwa, Asiatic pennywort, gotu kola, underutilized orphan crop, medicinal species, ethnobotanical use, morphological characterization

INTRODUCTION

Consumers are paying more attention to proper nutrition and the health effect of food. The green leafy vegetables (GLVs) make up a great portion of this diet and play a vital role to ensure food security and fulfill daily needs. The growing interest in GLVs is mainly due to health benefits associated with bioactive nutrients such as minerals, vitamins, especially antioxidant vitamins (vitamins A, C and E), dietary fibers, and non-nutritive phytochemicals (Septembre-Malaterre et al., 2018).

Increased migration and ethnic diversity in many regions and countries around the world stimulates the introduction of new plant species for daily consumption. *Centella asiatica* is one of the prominent medicinal herbs that can be introduced for Japanese consumers as a GLV for its nutritional and medicinal values. It is one of the most consumed GLV in Myanmar, Thailand, Northern Laos, Indonesia, Malaysia, Bangladesh, and Sri Lanka (Peiris and Kays, 1996; Hashim, 2011; DeFilipps and Krupnick, 2018; Parker, 2020), prized for its slightly pungent flavor and aroma. Although the species is widespread throughout the tropics and subtropics (Brinkhaus et al., 2000; Parker, 2020), it can be considered orphaned. In Japan, the local people are little aware about this species for daily consumption.

C. asiatica (L.) Urb. (syn. *Hydrocotyle asiatica* L.) is a stoloniferous plant belonging to the genus *Centella*, family Apiaceae (Umbelliferae). It is known as "Asiatic or Indian pennywort," "gotu kola," "penny weed" in English, but it also has over 60 common indigenous names (Engels and Brinckmann, 2011). In Burmese, the species is better known as "myin-hkwa," "myin-khwar pin," "ranjneh-hnah" (Chin State), and "hlahnip-chai" (Mon State), (DeFilipps and Krupnick, 2018). It has found wide application in the orient as a potential remedy to cure-all and the list of its therapeutic properties are exhaustive (Gohil et al., 2010). *C. asiatica* is used in traditional medicine for treating gastrointestinal diseases, skin disorders (burn wounds, rashes, and itchy), sleep disorders, tuberculosis, and diabetes; as an anti-rheumatic, antipyretic, diuretic, anti-bacterial, antiviral, and anti-inflammatory agent; as a memory enhancer; and as a mental rejuvenator (Brinkhaus et al., 2000; Kimura et al., 2008; Gohil et al., 2010; Bylka et al., 2013; Prasad et al., 2014; DeFilipps and Krupnick, 2018).

C. asiatica prefers relatively shady and damp habitats such as wetlands, riversides, ponds, wet meadows, and forests from a 300-to 1,800-m altitude (Gohil et al., 2010; Lansdown, 2019; Parker, 2020). The species is characterized as a perennial, flowering, faintly aromatic, low-growing, creeping herbaceous plant, with prostrate or semi-erect stems and rooting at the nodes, with a height varying between 10.0 and 45.0 cm (Engels and Brinckmann, 2011; Udumalagala et al., 2015; Parker, 2020). The leaves are reniform, shovel-shaped, emerging alternately in rosettes at the nodes (Uddin et al., 2017; Ravi et al., 2019). It is an entomophilous plant and can be reproduced through seeds or vegetative reproduction by far-reaching runners. It has been reported that plants derived from cuttings are more competitive than those developing from seeds (Peiris and Kays, 1996). In Myanmar, it is widely distributed to the cooler regions and found all year near the water's edge (DeFilipps and Krupnick, 2018).

Recently, increasing attention is being given to the nutritional value, medicinal function, and biological activities of *C. asiatica* (Brinkhaus et al., 2000; Matsuda et al., 2001; Siddiqui et al., 2007; Zainol et al., 2008; Chong and Aziz, 2011; Hashim et al., 2011; Bylka et al., 2013; Paudel et al., 2017; Sardrood et al., 2019). Fresh leaves consist of 87.7% moisture, 0.49% soluble dietary fiber, and 5.4% insoluble dietary fiber (Peiris and Kays, 1996; Udumalagala et al., 2015). The plant is also low in the content of fats (0.2%), proteins (2.4%), and carbohydrates (6.7%), but rich in the content of vitamins: vitamin C (7 mg/100 g), vitamin A (738 IU), vitamin B1 (0.09 mg/100 g), and minerals such as Ca (171 mg/100 g), P (32 mg/100 g), K (468.59 mg/100 g) and Fe (5.6 mg/100 g). Numerous conducted studies have pointed out that major bioactive constituents attributing its medicinal value are triterpenes (asiaticoside, asiatic acid, madecassic acid, madecassoside), (Engels and Brinckmann, 2011; Hashim et al., 2011; Belwal et al., 2019), which have also been regarded as its biomarker components (Zheng and Qin, 2007).

The plant genotype integration with different environments (genotype × environment interactions, GEI) can produce a wide range of phenotypes, which leads to its phenotypic plasticity (Baye et al., 2011; Osei et al., 2018). Habitat-related populations of *C. asiatica* show marked variation in morphological traits, such as leaf area, stolon production, petiole length, leaf color, and other traits (Peiris and Kays, 1996). About 20 morphotypes of *C. asiatica* are reported depending on habitat (elevation, terrain), (Gohil et al., 2010). The geographical location, climatic condition, cultural diversity of different tribes, and agricultural approaches led to plant diversification in Myanmar (Jatoi et al., 2008). *C. asiatica* originally from Myanmar is not documented well in Burmese and other local languages. This study is a step toward future studies and promotion of *C. asiatica* originating from Myanmar.

The objectives of this study were focused on the morphological description of the obtained Myanmar *C. asiatica* plant material growing in Japan and a brief overview of its ethnobotanical use. The study will provide information on the cultivation system of *C. asiatica* in Japan, as well as its further promotion and commercialization. It can be a potentially new GLV for Japanese daily consumption, especially during winter when demand for GLVs is particularly high.

MATERIALS AND METHODS

Plant Material and Experimental Design

The original *C. asiatica* plant material was provided by the Vegetables and Fruit Research and Development Center (VFRDC) of Myanmar. The obtained plant material was transferred to Japan *via* the Standard Material Transfer Agreement (SMTA) in 2009 with Myanmar Agriculture Service. The plant material was introduced to the collection of the Gene Research Center of the University of Tsukuba (Japan), (GRC UT). The plant material was cultivated in the pots and maintained as a living collection in the greenhouse of the GRC UT to preserve germplasm.

To conduct this study, *C. asiatica* plant samples were derived from the vegetative multiplication of the mother plant by stem

TABLE 1 | Means, standard deviations, ranges and *p*-values of four quantitative characters assessed on *C. asiatica*.

Characters	Mean ± SD	Range	*p*-value[a]
Leaf length (cm)	6.13 ± 0.79	5.00–7.70	0.01
Leaf width (cm)	7.05 ± 1.00	5.30–9.00	0.18
Leaf area (cm^2)	51.23 ± 13.20	27.69–79.22	0.09
Petiole length (cm)	24.51 ± 3.65	19.50–37.10	0.64

[a] *p-value is derived from ANOVA calculation that was used to determine if there are any significant differences between replications. Significant at* $p \leq 0.05$.

cutting and cultivated in 10.5 × 9.0 cm pots in the greenhouse of GRC UT. The rooted cuttings were then transplanted to open ground in a vinyl house (36°7′28.40″ north latitude and 140°5′44.28″ east longitude) of a local farmer (Tsukuba, Japan). To evaluate the morphological characteristics, a common field test was carried out according to a randomized complete block design with three replications. The observation, visualization (photographing), scoring, and measurement of morphological characters were carried out from August 10 to 15, 2020, when the plant was in its full vegetative and flowering stages.

Morphological Evaluation

C. asiatica originating from Myanmar was characterized using the developed list of descriptors after cultivation at the homogeneous condition in Japan. The qualitative morphological data of this study were compared with herbarium specimen records for *C. asiatica* available on the Flora of Myanmar Database (Flora of Myanmar Database, 2020).

Morphological evaluation was recorded on 10 randomly selected rosettes from each plot, for a total of 30 plant samples. Fifteen qualitative and quantitative markers were chosen for morphological evaluation, as shown in **Supplementary Table 1**. Four quantitative characters, i.e., leaf length, leaf width, leaf area (per one leaf), and petiole length, were measured using a ruler and a graph paper. Eleven qualitative characters were identified visually and recorded directly from measurement using a 1–4 scale. The color of the leaf, petiole, stolon, flowers, and the petiole color at the base was identified according to the RHS color chart and recorded using a 1–4 scale.

Statistical Analysis

The mean values for each quantitative morphological trait were calculated and used for statistical data analysis. The mean value, range value, and standard deviation (SD) were measured for each quantitative character. Single-factor analysis of variance (ANOVA) was calculated using SPSS software package version 24.0 (IBM Corp., Armonk, NY, USA) to determine the significance of variation for all quantitative morphological characters. The quantitative data (**Table 1**) are expressed as a mean ± SD value, range value, and with probability level of significance of 5% ($P \leq 0.05$).

RESULTS

Quantitative Morphological Traits

The developed quantitative morphological markers included four traits as follows: leaf length (cm), leaf width (cm), leaf area (cm^2), and petiole length (cm). Mean, standard deviation, range, and ANOVA were calculated to determine the significance of the variation accordingly (**Table 1**). The quantitative data were then categorized based on the descriptor list and combined with the qualitative data (**Supplementary Table 1**). Leaf size varied from 5.00 to 7.70 cm in length and from 5.30 up to 9.00 cm in width. Leaf area ranged between 27.69 and 79.22 cm^2. Petioles were long and their length ranged between 19.50 and 37.10 cm. The leaf length showed significant variation compared to four other measured quantitative traits. The variation in leaf width, leaf area and petiole length were not significant, but the range of minimum and maximum values varied considerably for the leaf area and petiole length (**Table 1**).

Qualitative Morphological Traits

In this study, *C. asiatica* exhibited a semi-erect and prostrate growth habit (**Figure 1A**). The plant stem is weak and glabrous, and consists of long creeping stolons rooting at the nodes (**Figure 1B**). The thin and fragile stem grew along the surface. The stolons are long internodes of mixed colors: from milky-white in young developing stolons, to light-green, green-pinkish, or dark-pink (purplish) in developed ones (**Figures 1B,D**, **2A**). The roots are adventitious and hairy, growing vertically down and white-creamish in color. Leaves are simple, emerging alternately on slender petioles and arranged solitary or in a cluster of two to three at each node (**Figures 1D**, **2A**). The leaf lamina is wide, peltate, with orbicular shape (**Figure 1C**), and cordate at base. Lamina's margin is slightly crenate with shallow lateral sinuses, glabrous abaxially and adaxially. The leaf margin is brought together and united at the lamina base, although there are sometimes leaves with the margin cleft at the base of the leaf blade. The flowers are actinomorphic, inconspicuous, <3.0 mm in size, white color, and arranged in small rounded whorls (umbels) that are born on slender pedicels with different lengths (19.50–37.10 cm), (**Figures 3A,B**). Flowers contain five corolla lobes, with five stamens per flower, which are attached to the glandular disc and bent inside the flower when young (**Figure 3C**). The gynoecium is syncarpous and consists of two carpels; the lower two-celled ovary at the apex passes into the sub-column (or stylopodia), often called the glandular or nectar disc. The calyx teeth are invisible and underdeveloped. Seeds have not been observed.

DISCUSSION

Taxonomic Identification

This study was conducted on morphological characterization of *C. asiatica* plant originating from Myanmar to overview plant adaptation and growth habits in Japanese environmental condition.

C. asiatica (L.) Urb. is reported as a very variable species and having over 40 synonyms but only *Hydrocotyle asiatica* L. is still

FIGURE 1 | (A) General view (habit) of *C. asiatica*. **(B)** Stem of *C. asiatica* plant with the creeping stolons. **(C)** Leaf lamina. **(D)** Part of stem with young leaf and stolon of white color.

occasionally used (Parker, 2020). The leaf size is a quite unstable character and considerably varies in width and length (Alfarra and Omar, 2013). In Myanmar, *C. asiatica* is widely distributed to the cooler regions and found all year near the water's edge, paddy fields (DeFilipps and Krupnick, 2018). It grows wild but it is also widely cultivated as it is much used. It is characterized as a slender herbaceous plant with long creeping stems coming off from the leaf axils (Medicinal Plants of Myanmar, 2007).

C. asiatica is poorly recorded in Myanmar. We used the records of herbarium specimens on *C. asiatica* available at the Flora of Myanmar Database (2020) for taxonomic identification and comparative morphological assessment. In total, Flora of Myanmar Database (2020) contains five records on specimens for the *Centella* genus collected from Myanmar and deposited in herbaria of the National Museum of Nature and Science, Tokyo (TNS) and the University of Tokyo (TI) for the period 2000–2017.

On closer inspection of the images available for these specimens, the phenotypic differences were detected regarding leaf shape compared with plant samples used in this study. Four herbarium specimens (TI00072253–TI00072256) possess reniform or orbicular-reniform leaves and are determined as *C. asiatica*. One specimen (TNS01300836) displays peltate leaves and was determined as *Centella* species (specific epithet absent). The leaves clustered simultaneously at the nodes and visually different in size. The lamina margins are crenate. Two specimens—TI00072253 (Shan State) and TI00072255 (Chin State)—visually possess long internodes, whereas specimens TI00072254 and TI00072256 (Mandalay Division) are characterized with short internodes and dense leaf arrangement.

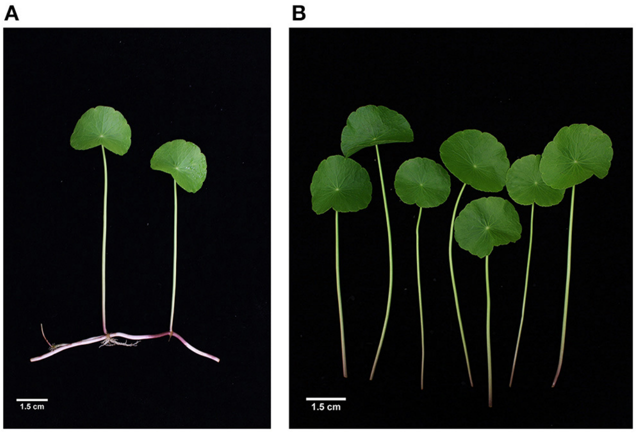

FIGURE 2 | (A) Part of the stolon with developed single leaves. **(B)** Leaves with petioles.

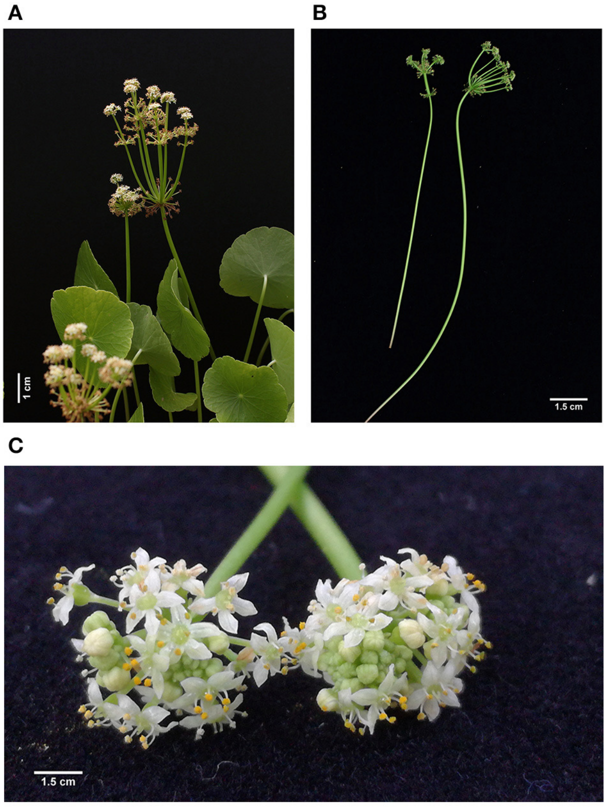

FIGURE 3 | (A) General view of *C. asiatica* inflorescence. **(B)** Inflorescence with pedicel. **(C)** Close-up view of flowers.

Morphological Characterization

Among four quantitative morphological traits, the significant variation was observed only in leaf length ($P < 0.05$). The other three morphological traits, i.e., leaf width, leaf area, and petiole length, did not display any significant differences.

Upon close observation and characterization, we found phenotypic differences and similarities in qualitative traits such as plant's growth habit, leaf shape, leaf margin, and leaf color from those that have been previously reported (Peiris and Kays, 1996; Prasad et al., 2014; Alqahtani et al., 2017; Ravi et al., 2019; Flora of Myanmar Database, 2020). In the current study, plants displayed a semi-erect growth habit with thin and fragile horizontally grown stem along the soil surface (**Figure 1**), peltate shaped leaves, cordate at the base (**Figures 1**, **2**), glabrous and glossy, corresponding with those that have been reported from India by Ravi et al. (2019) and from Australia by Alqahtani et al. (2017). The erect growing plants are ideal for maximum and uniform exposure to sunlight resulting in increased dry matter production and, subsequently, yield, compared with semi-erect and prostate types (Uddin et al., 2017). According to the leaf shape characteristic, and petiole and pedicel length, only one *C. asiatica* herbarium specimen (TNS01300836, Shan State) from Myanmar (Flora of Myanmar Database, 2020) matched our result.

Leaf color was another phenotypic trait that displayed notable variation ranging from light green to dark green. Plants growing under full sun, in dry non-enriched soil with low moisture, had thin, mat light-green colored leaves (data has not shown), whereas the plants that were growing under the shade, in a sufficiently moist and enriched soil, had fleshy, glossy, and dark green colored leaves (**Figure 1**). According to Peiris and Kays (1996), plants growing under the shade develop a profuse canopy of leaves with long petioles, while high light intensity produces more leaves, clonal offspring, a greater leaf area, and dry matter content. The leaf size itself directly determines the photosynthetic efficiency of the plant, influencing its growth, yield, synthesis, and accumulation of secondary metabolites. At the same time, the nutritional composition in *C. asiatica* can be varied somewhat with growth location and environmental conditions (Peiris and Kays, 1996).

Since the phenotypic traits are genetically attributed, a set of environmental conditions (soil nutrition, moisture supply, and light), cultivation method, cultivation duration (mutation collection caused by the vegetative reproduction), and plant interaction with the environment may influence the phenotypic expression of plants during growth and development. There are different morphotypes of *C. asiatica* reported from Sri Lanka (Udumalagala et al., 2015; Dissanayake et al., 2016), Madagascar (Rahajanirina et al., 2012), India (Prasad et al., 2014; Ravi et al., 2019), and Australia (Alqahtani et al., 2017) based on leaf morphology and growth pattern. In this regard, we assume that observed phenotypic expression in leaf color, termed phenotypic plasticity, was determined by the environmental conditions, i.e., soil nutrition, moisture, and light intensity. Although *C. asiatica* grows easily under full sun, it is beneficial to provide plants with adequate soil nutrients and moisture for their initial establishment and subsequent growth (Peiris and Kays, 1996).

Ethnobotanical and Culinary Use

The practical uses of plants in particular regions and indigenous cultures found their reflection in different ethnobotanical studies and are well-described in ancient oriental medicinal tracts such as Indian Ayurveda, Chinese herbology medicine, and Japanese Kampo medicine. Nearly 1,500 plant species are incorporated into official Indian ayurvedic pharmacopeia for medicinal purposes (Kumar et al., 2017). About 11,000 plant species are listed in Chinese pharmacopeia, medicinal botany textbooks, and ancient Chinese medical texts (Zhang and Yang, 2012). A total of 148 different herbal formulations are approved to be used as official Kampo remedies, and 294 formulations are approved for non-prescription Kampo products in Japan (Arai and Kawahara, 2019; Watanabe et al., 2019). Plant-based medications and additives are synchronized with the food and extensively used in both developing and developed countries for daily healthcare. It is evidenced by numerous accumulated studies highlighting the enormous potential of herbs utilized for medicinal and nutritional purposes (Gohil et al., 2010; Engels and Brinckmann, 2011).

C. asiatica has a long history of use as a GLV and medicinal herb in oriental medicine in Asian countries (Stafford et al., 2008; Zainol et al., 2008; Gohil et al., 2010; Hashim, 2011) including Myanmar (DeFilipps and Krupnick, 2018). It is cultivated all over the world for consumption as a fresh vegetable (Kosaka et al., 2013). All parts of the plant are used for medicinal purposes in ethnomedicine in Asian countries (Gohil et al., 2010). The leaves are slightly bitter and eaten raw or cooked as a vegetable and/or as a soft drink. It is also available as a dried herb or extract, in teas, tablets, capsules, ointments, tinctures, and cosmetic preparations (Engels and Brinckmann, 2011). Most of all, *C. asiatica* is valuable due to its healing effects to cure various mild and chronic disorders such as hepatitis, anemia, skin diseases (Kimura et al., 2008; Gohil et al., 2010), diarrhea, ulcers, fever, and amenorrhea (Brinkhaus et al., 2000; Gohil et al., 2010; Engels and Brinckmann, 2011), and as an antioxidant, antibacterial, antiviral, and anti-cancer agent (Bylka et al., 2013; Belwal et al., 2019). In Western medicine, it is popular as a "brain tonic" agent to revitalize the brain and nervous system, increase attention span and concentration, and combat aging, as well as for its neuroprotective activities (Matsuda et al., 2001; Gohil et al., 2010). The list of therapeutic properties of *C. asiatica* is huge and it has been used in ancient cultures and tribal groups with diverse prescriptions (Alfarra and Omar, 2013).

Known as a "*myin-hkwa,*" in Myanmar (DeFilipps and Krupnick, 2018), *C. asiatica* is utilized both as a leafy vegetable and a medicinal herb. Information on the ethnobotanical use of *C. asiatica* in Myanmar was provided in personal communication with Daisy Myint and according to DeFilipps and Krupnick (2018). *Myin-hkwa* young leaves are more valuable as a leaf vegetable and consumed raw in salads, deep-fried ("tenpura"), as a separate soup, and in combination with Roselle (*Hibiscus sabdariffa*) leaves fried or in a soup. The plant is used to treat diabetes, skin diseases (eczema, leprosy, itching, rashes, and sores), and nervous system and blood problems; to control phlegm; and to treat dysentery and urine retention, painful urination, blood in the urine, and syphilitic affections; it is also used as a poison neutralizer. The leaf extracts, together with sugar and honey, are consumed daily as a restorative product to treat colds and fever. The dried leaves are used as a tea to relieve hypertension and to treat severe sore eyes and hypersensitivity to strong light. The fresh green leaves, crushed and wrapped in a thin cloth, are used as an eye mask. The dried powdered leaves mixed with an equal amount of honey are used against insomnia, and mixed with water used to treat coughs and tuberculosis in children or applied to the chest as a warm compress.

C. asiatica is known as "snow plant" (Engels and Brinckmann, 2011) for its cooling properties. Historically, it has been called one of the "miracle elixirs of life" and for the first time was mentioned in the Chinese Shennong Herbal (1st—2nd century of CE) because of a Chinese herbalist named Li Ching-Yun, who some believe lived to the age of 197 and used gotu kola regularly (Engels and Brinckmann, 2011). In traditional Chinese medicine, dried gotu kola, known as *ji xue cao* (at a dosage of 15–30 g), or fresh plant (at a dosage of 30–60 g) is prescribed to use for treatment various disorders (Emboden, 1985). The plant possesses diuretic properties and is used for treating looseness of the bowels, heaving, jaundice and scabies, Hansen's ailment (disease), urinary troubles, nosebleeds, breaks, tonsillitis, measles, and tuberculosis (Menglan and Watson, 2005).

C. asiatica was incorporated into the Indian Pharmacopeia in the 19th century (Engels and Brinckmann, 2011). In Ayurveda, *C. asiatica* is known as a mental rejuvenator, or *medhya rasayana*, a tonic used to reduce mental fatigue and improve mental clarity (Premila, 2006). In Ayurveda and Unani ethnomedicine systems, it has been used to cure different ailments like ulcers and body aches, stomach disorders, asthma, leprosy, leukorrhea and urethritis, loose bowels, and dysentery, and for enhancing memory power, in maternal healthcare (Sidhu et al., 2006; Das et al., 2009; Uddin et al., 2017). *C. asiatica* is an important part of Ayurvedic formulation known as *Brahma Rasayana*, a complex mixture of herbs and fruits in a paste form, taken with warm milk as a cerebral tonic for mental exhaustion, nervous weakness, insomnia, and memory loss (Engels and Brinckmann, 2011).

In Nepal, all parts of *C. asiatica* are used in the form of teas for diarrhea and hypertension, as a detoxicant and diuretic, and for urinary tract infections and poor memory (Ahmad and Ismail, 2003). Smashed leaf and root concentrate are used to eliminate germs from wounds. Around four teaspoons of leaf juice obtained from 50 leaves by squeezing is taken orally in the morning during 2–3 weeks to cool the body and stomach (Mahato and Chaudhary, 2005). Crushed leaf and root extract are applied to kill germs from wounds (Uddin et al., 2017).

Kavirajes, traditional medicinal practitioners from the Chalna area of Bangladesh, use the whole plant of *thankhai* (local name of *C. asiatica*) to treat multiple ailments like dog bite, asthma, carminative, itching, leucorrhea, malaria, tumors, and wounds (Rahmatullah et al., 2010).

C. asiatica is commonly known as *pegaga* in Malaysia and *pegagan* or *kaki kuda* in Indonesia (Alfarra and Omar, 2013). In Malaysia and Indonesia, the whole plant is eaten fresh as a vegetable in a salad, soup, or appetizer (Hashim et al., 2011). It is always cooked with the addition of coconut milk or shredded coconut to soften the plant's mild bitterness. Kadazandusun communities (Malaysia) use the plant as a tea for treating hypertension, diarrhea, and urinary tract infections; as a detoxicant and diuretic; and to lower blood pressure and decrease heart rate (Uddin et al., 2017).

In Thailand, the fresh leaves are eaten with northern food such as sour chopped meat salad or fried noodles (JIRCAS, 2020). The fresh prepared green juice is rich in vitamin A and is commonly taken for thirst quenching purposes or as a cooling drink to reduce "inner heat" (Hashim, 2011). For medicinal purposes, the fresh plants are used to soothe headaches or heat-burns, and as a diuretic, anti-syphilitic, astringent, and expectorant.

Although the therapeutic potential of *C. asiatica* is obvious, some precautions are needed to avoid side effects occurring with high doses of the herb. Reported side effects may include skin allergy, burning sensations (with external use), headache, stomach upset, nausea, dizziness, and extreme drowsiness (Orhan, 2012). No toxicity was reported within the recommended doses as well as negative drug–herb interactions between *C. asiatica* with other medications. The reported daily dose is about 600 mg of dried herb in the form of an infusion, single-dose capsules (300–680 mg, thrice daily), or a 10-mg concentrated extract, for a period of time up to 6 weeks (Gohil et al., 2010).

The ethnic communities are custodians of the traditional knowledge of plant consumption, plant's healing properties, and formulations accumulated empirically and passed down from generation to generation. Their knowledge can be implemented in modern science and serve as a base for the investigation of the new biologically active compounds from important orphan crop species that can cure a variety of diseases.

Future for *C. asiatica* in Japan

As part of our future research promotion, we have conducted a preliminary evaluation of one of the important phytonutrients—carotenoids in *C. asiatica*. The result revealed the high intensity of the lutein and beta-carotene constituents (data unpublished). Lutein and beta-carotene are carotenes found in many fruits and vegetables, most notably in GLVs like kale and spinach (Ranard et al., 2017). Beta-carotene is the most common carotene found in plants and acts as a precursor for vitamin A in humans and animals (Grune et al., 2010). Lutein and its isomer zeaxanthin are the only carotenoids that accumulate in the fovea of the human retina that play a role in the visual system (Ranard et al., 2017; Alvarado-Ramos et al., 2018).

There are no data on the market-oriented production of *C. asiatica* in Japan. The plant possesses unique nutritional and health attributes and can be a potentially new and economically important plant for the Japanese market, especially during the winter season when there is a huge demand for leafy vegetables. Generally, *C. asiatica* is easy to cultivate. With the correct provision of the necessary conditions for growth, the first harvest can be possible in about 90 days from planting with subsequent 60-day intervals of harvesting over 2–3 years (Peiris and Kays, 1996). As a first step, a consumer acceptance test in Japan may be worth considering to help to define subsequent steps for future market production. Combined with molecular and tissue culture techniques, the nutritional and medicinal values of the plant can be increased as well as new varieties promoted and conserved in the future.

CONCLUSION AND FUTURE PROMOTION

This study is the first to report on the morphological description of *C. asiatica* originating from Myanmar, which is not well-documented in Burmese or other local languages. The evaluation revealed that *C. asiatica* originating from Myanmar is different considering its morphological characteristics. The morphological diversity of the foliar traits and growth pattern can serve as a taxonomic marker for primary *C. asiatica* differentiation/identification in Myanmar. It is assumed that different morphotypes of *C. asiatica* may exist in Myanmar. Moreover, different morphotypes can considerably differ by both morphological characteristics and biochemical composition. Future studies are needed to elucidate this assumption.

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

The experimental idea was based on KW with a future contribution on guidance, project administration, funding acquisition, review, and editing of the original draft. MS prepared the original draft. KW, MS, and DM contributed to conceptualization and including the design of the experiment. MS and DM equally contributed to methodology and data curation, including formal and statistical analysis, investigation, and visualization. Plant material resources and validation of the obtained data were carried out by KW, SY, and OS. All authors have read and agreed to the publication of this version of the manuscript.

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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.2021.680862/full#supplementary-material

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Farmer and Field Survey in Cassava-Growing Districts of Rwanda Reveals Key Factors Associated With Cassava Brown Streak Disease Incidence and Cassava Productivity

Chantal Nyirakanani [1,2*†], ***Jean Pierre Bizimana*** [1,3*†], ***Yves Kwibuka*** [4,5], ***Athanase Nduwumuremyi*** [3], ***Vincent de Paul Bigirimana*** [2], ***Charles Bucagu*** [3], ***Ludivine Lassois*** [1], ***Eléonore Malice*** [6], ***Nicolas Gengler*** [7], ***Sébastien Massart*** [4], ***Claude Bragard*** [8], ***Michael Habtu*** [9], ***Yves Brostaux*** [10], ***Cécile Thonar*** [1,11] ***and Hervé Vanderschuren*** [1,12*]

[1] Plant Genetics and Rhizosphere Processes Laboratory, TERRA Teaching and Research Center, University of Liège, Gembloux Agro-Bio Tech, Gembloux, Belgium, [2] Department of Crop Science, School of Agriculture, University of Rwanda, Musanze, Rwanda, [3] Department of Research, Rwanda Agriculture and Animal Resources Development Board, Huye, Rwanda, [4] Integrated and Urban Plant Pathology Laboratory, TERRA Teaching and Research Center, University of Liège, Gembloux Agro-Bio Tech, Gembloux, Belgium, [5] Departement des Sciences Agronomiques, Université Catholique de Bukavu, Bukavu, République Démocratique du Congo, [6] Unité Santé des Plantes et Forêts, Centre wallon de Recherches agronomiques, Gembloux, Belgium, [7] Numerical Genetics, Genomics and Modeling Laboratory, TERRA Teaching and Research Center, University of Liège, Gembloux Agro-Bio Tech, Gembloux, Belgium, [8] Phytopathology Laboratory, University of Louvain, Louvain-la-Neuve, Belgium, [9] Department of Public Health, School of Health Sciences, Mount Kenya University, Kigali, Rwanda, [10] Modelisation and Development, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium, [11] Agroecology Lab, Université Libre de Bruxelles (ULB), Brussels, Belgium, [12] Tropical Crop Improvement Laboratory, Department of Biosystems, Katholieke Universiteit Leuven, Heverlee, Belgium

Cassava (*Manihot esculenta* Crantz) is a vital crop in Rwanda where it ranks as the third most consumed staple. However, cassava productivity remains below its yield potential due to several constraints, including important viral diseases, such as cassava brown streak disease (CBSD). Because various factors can be addressed to mitigate the impact of viral diseases, it is essential to identify routes of virus contamination in the cassava agrosystems from the seed system to farmer's practices and knowledge. The present study aimed at (1) assessing the current cassava seed system and farmers' practices and their knowledge of the biotic constraints to cassava production, (2) determining the status of CBSD as well as critical factors associated with its spread through the seed system channels, and (3) determining factors that influence cassava productivity in Rwanda. A cross-sectional study was carried out from May to September 2019 in 13 districts of Rwanda. A total of 130 farmers and cassava fields were visited, and the incidence and severity of CBSD were evaluated. CBSD was detected in all cassava-producing districts. The highest field incidence of CBSD was recorded in the Nyanza district (62%; 95% CI = 56–67%) followed by the Bugesera district (60%; 95% CI = 54–65%), which recorded the highest severity score of 3.0 ± 0.6. RT-PCR revealed the presence of CBSD at the rate of 35.3%. Ugandan cassava brown streak virus was predominant (21.5%) although

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Katherine Steele,
Bangor University, United Kingdom

Reviewed by:
Makeshkumar Thangaraj,
Central Tuber Crops Research Institute (ICAR), India
Hale Tufan,
Cornell University, United States

***Correspondence:**
Chantal Nyirakanani
Chantal.Nyirakanani@uliege.be
Jean Pierre Bizimana
Jean.Bizimana@uliege.be
Hervé Vanderschuren
herve.vanderschuren@kuleuven.be;
herve.vanderschuren@uliege.be

† These authors have contributed equally to this work

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cassava brown streak virus was 4% and mixed infection was 10%. An informal cassava seed system was dominant among individual farmers, whereas most cooperatives used quality seeds. Cassava production was found to be significantly influenced by the use of fertilizer, size of the land, farming system, cassava viral disease, and type of cassava varieties grown ($p < 0.001$). Disease management measures were practiced by a half of participants only. Factors found to be significantly associated with CBSD infection ($p < 0.05$) were the source of cuttings, proximity to borders, age of cassava, and knowledge of CBSD transmission and management.

Keywords: cassava, seed system, CBSD, field survey, cassava production, Rwanda

INTRODUCTION

Cassava (*Manihot esculenta* Cranzt) ranks as the sixth most important food crop worldwide and the fourth after rice, maize, and wheat among developing and emerging countries (Otekunrin and Sawicka, 2019; Saranraj et al., 2019). In Rwanda, cassava is the third most important crop after banana and sweet potato (Night et al., 2011). Because of its importance in several tropical regions and its relatively good performance on marginal lands under suboptimal climatic conditions (Burns et al., 2010), cassava is recognized as a subsistence crop to overcome food insecurity for the fast-growing population in areas prone to important climatic changes (El-Sharkawy, 2004; Chavez et al., 2005; Lobell et al., 2008; Burns et al., 2010). Although cassava plays an important role as a food security crop in sub-Saharan Africa, it is also used as a cash crop in various cassava-growing regions (Spencer and Ezedinma, 2017; Munganyinka et al., 2018).

The yield potential of cassava under optimum conditions is about 90 tons of fresh roots per hectare, which is equivalent to 30 tons of cassava dry matter per hectare (El-Sharkawy, 2004). More than half (61%) of cassava production is taking place in sub-Saharan Africa; however, cassava yield in tropical countries is still far below its production potential. Indeed, in 2017, the world cassava yield was about 11.08 tons of fresh roots per hectare, and the top cassava producer (Nigeria) had an average yield of 8.75 tons per hectare, followed by the Democratic Republic of Congo with 8.14 tons per hectare (FAO, 2019; Otekunrin and Sawicka, 2019). Cassava production in Rwanda varied between 3,000 and 3,701 Mt of fresh roots per year from 2015 to 2018 with a reported average yield of about 14.5 tons per hectare (FAO, 2018; Rwanda Agricultural Board[1]). Despite its resilience under adverse environmental conditions, the production of cassava remains constrained by several abiotic and biotic factors. The former includes postharvest deterioration, infertile soils, planting unimproved traditional varieties, and inadequate farming practices, whereas the latter includes green mites, mealy bug, cassava bacterial blight, and viral diseases (Bull et al., 2011; Kombate et al., 2017).

As a consequence of viral diseases and the lack of resistant varieties, cassava yields have drastically decreased in many countries. Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the most economically important cassava diseases, causing yield losses of more than US$1 billion yearly globally (Legg and Thresh, 2003; Legg et al., 2006; Patil et al., 2015; Rey and Vanderschuren, 2017).

CBSD has so far only been reported in sub-Saharan Africa. CBSD is particularly devastating because it negatively impacts cassava tuberous roots both quantitatively and qualitatively, causing important economic losses to African farmers (Mohammed et al., 2012). For decades, CMD has been managed through dissemination of resistant varieties, but unfortunately, the distributed CMD-resistant varieties were found to be sensitive to CBSD in Rwanda and in many other African countries (Legg et al., 2001; Thresh and Cooter, 2005; Bua, 2017; Nyirahorana et al., 2017). CBSD is caused by two species of single-stranded RNA viruses of the family *Potyviridae,* Genus *Ipomovirus*; Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) (Mbanzibwa et al., 2009). In Rwanda, CBSD was first reported in 2009 in the Southern province (Muhanga district). CBSD has since spread to reach most cassava-producing regions in the south (Kamonyi, Ruhango, Nyanza, Gisagara and Muhanga Districts) and east of the country (Bugesera, Nyagatare, Gatsibo, Kirehe, Kayonza and Ngoma Districts) (Nyirahorana et al., 2017). A study conducted in Rwanda in 2014 reported a distribution of CBSD incidence as follows: 74.2% UCBSV, 15.3% CBSV, and 10.5% mixed infection (Munganyinka et al., 2018).

The CBSVs are transmitted by either the whitefly *Bemisia tabaci* and/or exchange of infected planting materials between farmers. Plant pathologists and extension services recognize the importance of establishing a disease-free seed system to mitigate the spread of CBSD (Mbanzibwa et al., 2009; Patil et al., 2015; Maruthi et al., 2017). In Rwanda, formal distribution of clean planting seeds usually involves the whole production chain from the Rwanda Agricultural and Animal Resources Development Board (RAB) where researchers produce basic clean seeds. Basics seeds are then distributed to seed multipliers across different regions for further multiplication, and before their dissemination to farmers, a quality seed certification agency is involved to ensure the quality of the planting materials (Broek and van den Byakweli, 2014; Andrade-Piedra et al., 2016). Quality seed refers to the seed preferred by farmers and consumers with good health (virus free), genetic purity, appropriate physiological age, and physical quality (Andrade-Piedra et al., 2016).

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[1] Available online at: https://www.newtimes.co.rw/business/what-holding-back-cassava-yield.

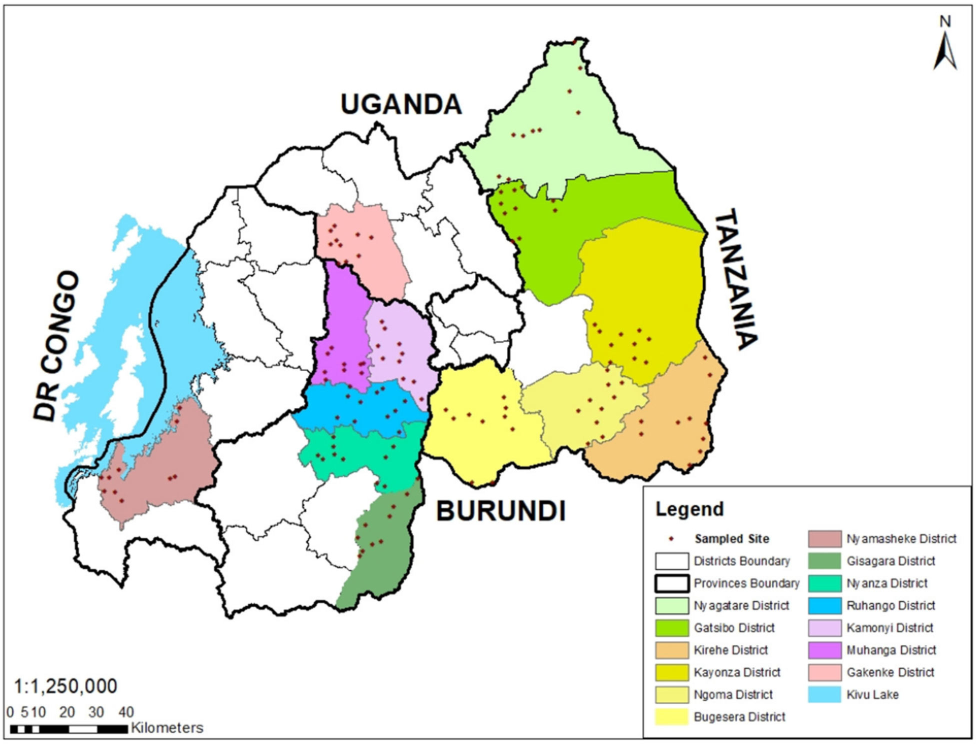

FIGURE 1 | Map of Rwanda showing surveyed districts. The red dots represent the location of the cassava fields assessed in the study.

The concomitant existence of an informal seed system involves production and dissemination of seeds by farmers usually without quality seed certification, which tends to favor disease dissemination and maintenance in cassava agrosystems (Muthoni and Nyamongo, 2008). Because of its prominence in most cassava agrosystems worldwide, the informal seed system plays a major role in the rapid spread of diseases (Andrade-Piedra et al., 2016; Maruthi et al., 2017).

Various management measures are applied to reduce CBSD impact, including investigation and breeding of CBSD-tolerant varieties (Masumba et al., 2017; Anjanappa et al., 2018; Sheat et al., 2019; Shirima et al., 2020) as well as the dissemination of disease-free planting material to farmers (Alicai et al., 2016). Conversely, farmers who reuse cuttings from their own fields will not escape the disease as this tends to maintain 30–50% of infection, especially in CBSD hot spots (Rwegasira and Rey, 2011; Patil et al., 2015). Unfortunately, the seed system sustainability remains fragile and needs to be consolidated to provide appropriate healthy planting cassava material to farmers. Despite the emergence of CBSD in cassava fields in Rwanda, there has been limited information about the CBSD distribution and factors associated with its transmission as well as factors that affect cassava productivity in the country.

The present study aimed at assessing the impact of farmers' practices and their knowledge of the biotic constraints on cassava production as well as determining the status of CBSD and the critical factors associated with its spread through the seed system channels.

MATERIALS AND METHODS

Study Area

The study was conducted in 13 cassava-growing districts of Rwanda during 2019. Districts in the southern and eastern provinces are considered to be major cassava-producing areas. In the south, five districts were surveyed, namely, Gisagara, Nyanza, Ruhango, Muhanga, and Kamonyi, whereas in the east, six districts were surveyed, namely, Bugesera, Nyagatare, Kayonza, Gatsibo, Kirehe, and Ngoma. In addition, two districts from the western and Northern provinces, Nyamasheke and Gakenke, respectively, were included in the study (**Figure 1**).

Farmer and Field Selection

A multistage sampling method was applied to select cassava farmers and fields. In the first stage, 13 districts, representing both major and minor cassava-growing areas, were selected. In the second stage, according to the information provided by district agronomists and RAB, five main cassava-growing sectors were purposely selected within each district. Sectors in which cassava is marginally cropped were not kept in the selection because they may not be representative of current limitations encountered by cassava growers. In the third stage, as cassava farmers are classified into two categories (either individual farmers or belonging to cooperatives) (Miklyaev et al., 2021), two farmers per sector, one individual farmer and one farmer belonging to a cooperative, were selected from a sampling frame provided by sector agronomists using a simple random-sampling approach, making a total of 10 farmers per district and 130 interviewed farmers for the 13 districts surveyed. Selected participants were always heads of household, either a man or woman, depending on their availability. In case both were available, simple random sampling was applied to select one of them.

Furthermore, for each participant, a field with cassava plants older than 6 months was also visited for disease evaluation. The distance between two cassava fields visited was around 10 km. Within the selected fields, 30 plants were selected for leaf and stem CBSD symptom examination including five plants of the two diagonals and five of the four sides (Rwegasira et al., 2011). The field incidence per district was recorded as the percentage of symptomatic plants out of the total examined. The 10 plants examined at the two diagonals were further pooled and used for CBSVs indexing by RT-PCR.

Farmer Interviews

Primary data used in the study were collected using a structured questionnaire (**Supplementary Datasheet 1**), semistructured interviews and observations on five key subject areas (such as demography, social economics, agronomy, seed accessibility, and availability factors and disease aspect) relevant to cassava production. For farmers, quality seed was defined as seed certified by a seed-quality inspection agency (Rwanda Agriculture Board) that may be obtained from a professional seed multiplier. District and sector agriculture extension officers liaising with local community leaders were involved in the mobilization of farmers. Permission to conduct research in the area was sought from the administration of the study area (district and sector agronomists) through official communication by RAB authorities. Participants were told the purpose of the research and that participation was voluntary. Oral consent was given before starting the interview and field visit. All records were identified by study identification number to keep participant privacy and confidentiality.

Cassava productivity among participants was considered as cassava yield (kg fresh matter per hectare) in the present study, and income generated from cassava was estimated without considering price variation between districts. The price of cassava was estimated as the average of the cassava root prices recorded during the survey, which was 90 Rwandan francs (Rwf) per kilogram (Max = 95 Rwf, Min = 85 Rwf, std = 4.3 Rwf). Therefore, the income was estimated as the yield times the estimated price of cassava. To learn the factors influencing cassava productivity, the benefit generated from cassava per hectare was calculated as the total income minus the cost of production. Variables collected during the survey are listed in **Supplementary Table 1**. Biotic variables observed and measured in the visited fields are listed in **Supplementary Table 2**.

Disease Severity Assessment

A 1–5 CBSD symptom scale (Gondwe et al., 2003) was used to measure the degree of severity of CBSD aerial symptoms in the fields. The scale used was 1 = no apparent symptoms; 2 = slight leaf feathery chlorosis with no stem lesions; 3 = pronounced leaf feathery chlorosis, mild stem lesions, and no dieback; 4 = severe leaf feathery chlorosis, severe stem lesions, and no dieback; and 5 = defoliation, severe stem lesions, and dieback (Gondwe et al., 2003). The average degree of severity was calculated omitting the score of 1, which represent asymptomatic plants to provide a true picture of the severity in the fields assessed (Sseruwagi et al., 2004). An average of disease severity per district was calculated based on the observation of $30 \times 10 = 300$ plants.

Sampling Test Materials for RT-PCR

Samples were collected from May to September 2019. In each cassava field assessed, 10 cuttings from 10 plants examined along the two diagonals were collected per field and established in the screenhouse. In total 1,300 plants were grown in the screenhouse from 130 fields. Five-month-old plants from collected cuttings were used for molecular analysis. In the laboratory, 20 leaf samples resulting from growth of cuttings collected on the same field were pooled (lower and middle leaves were used per plant); thus, a total of 130 samples were tested for CBSVs using RT-PCR.

Molecular Analysis

RNA Extraction

Total RNA was extracted from ∼0.2 g cassava leaf using the cetyltrimethylammonium bromide protocol previously described (Abarshi et al., 2010).

cDNA Synthesis and RT-PCR

Synthesis of cDNA was done using a ProtoScript II Reverse Transcriptase kit (BioLabs, UK) following the manufacturer's instructions. Briefly, a Master Mix containing 1 µl $d(T)_{23}$ (50 mM), 2 µl of buffer, 1 µl of 0.1 mM DTT, 0.5 µl Protoscript II RT, 0.5 dNTP Mix, and 3 µl of nuclease-free water was prepared. Then, 2 µl of RNA template was subsequently added, making 10 µl per reaction. The reaction mixture was incubated in a PCR thermocycler at 42°C for 1 h for primer annealing and cDNA synthesis, followed by 20 min at 65°C for inactivation of the ProtoScript II Reverse Transcriptase. The resulting cDNA samples were stored at −20°C.

The synthesized cDNA was subjected to polymerase chain reaction using a Taq G2 Hot Start Master Mix from Promega. The primer pair F: 5′-CCTCCATCWCATGCTATAGACA-3′ and R: 5′-GGATATGGAGAAAGRKCTCC-3′ that amplifies ∼703 bp of CBSV and ∼800 bp of UCBSV isolates was used (Elegba, 2018). The 10 µl PCR reaction contained 5 µl G2 Mix, 0.4 µl each primer (0.4 µM final concentration), 1 µl cDNA and the volume was brought to 10 µl with nuclease-free water. PCR conditions were as follows: predenaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30′′ and elongation at 72°C for 50′′ and final elongation at 72°C for 5 min.

An internal control gene from cassava called *Manihot esculenta* protein phosphatase 2 A (*MePP2A*) was detected in parallel using a pair of primers F: 5′-TGCAAGGCTCACACT TTCATC-3′ and R: 5′-CTGAGCGTAAAGCAGGGAAG-3′ that amplifies 150 bp of *Me*PP2A to ensure the accuracy of the PCR results by ruling out any false negative results (Moreno et al., 2011). PCR amplification was checked by loading 10 µl of PCR products in 1% (w/v) agarose gel stained with Gel red in 1X Tris-acetate-EDTA (TAE) buffer for 1 h at 200 V to allow the separation of amplicons from the two isolates. The PCR products were visualized on a UV gel documentation system and photographed.

Data Analysis

Raw data were transferred into Statistical Package for Social Sciences (SPSS) version 22 for analysis. Frequency and proportions for categorical variables were computed to describe the basic attributes of the respondents (farmers) as well as the occurrence of cassava infection (defined as the presence of CBSV or UCBSV after PCR) in sampled fields. A bivariate analysis with chi square tests was used to determine factors associated with cassava virus infections (categorical variable). Then, multivariate logistic regression analyses were performed by considering together all significant factors during bivariate analysis. The goodness of fit was assessed using Hosmer–Lemeshow test. In all statistical tests, differences were considered statistically significant at $p < 0.05$.

Similarly, descriptive statistics using mean and standard deviation for continuous variables were used. After testing for normality, cassava benefit was not normally distributed, and Sqrt transformation was used to ensure the normality of the cassava benefit distribution. Then, an ANOVA test was used to compare means to estimate the effect of sociodemographic, economic, and agronomic variables on cassava productivity. Multiple linear regression analyses were performed to assess the independent factors affecting cassava productivity. Ten factors were considered together, and upon fitting all against cassava productivity using multiple linear regression and specifying a *"stepwise"* method, five factors remained independently affecting cassava productivity. To validate the regression analysis, the histogram and P-P plot presented in **Supplementary Figure 3** were used to show the reasonable normality of data. Furthermore, multi-collinearity problems were assessed using tolerance and variance inflation factors (VIFs) as well as Durbin Watson was used to check for autocorrelation that the residuals from linear regressions are independent.

RESULTS

Sociodemographic Characteristics of Participants, Source of Cassava Cuttings, Trust, and Disease Management

Among the 130 farmers interviewed, a majority of them were individuals (58.5%), men (64.6%), and married people (66.9%). The percentages of men and women in the participants also reflects the gender distribution for cash-crop growers in Rwanda (Gender Monitoring Office, 2017; Munganyinka et al., 2018). Most of the participants (72.3%) had attended primary school. The respondents' age was in three categories with 45 years and above being slightly higher (36.2%) than the other two categories (**Supplementary Table 3**). The majority of participants (62.1%) reported that they obtain planting material from their own fields and use the same materials over many seasons. A minority of interviewed farmers (25.1%) acquired planting materials from seed multipliers every season. Among those, a large proportion (86%) were cooperative members (**Figures 2A,B**).

Although most farmers use seeds from their fields, about 45% questioned their quality and feared that their cassava fields may succumb to diseases. A gap in cassava viral disease management was noted through the study as 50.3% of interviewed farmers took no action to control viral disease and only 19.5% declared roguing out infected plants from their field (**Figures 2C,D**).

CBSD Incidence and Severity Score of Observed Symptoms

Field incidence of CBSD and severity of aerial symptoms were evaluated in the 13 districts. The highest incidence (62%; 95% CI = 56–67%) was recorded in the Nyanza district, and the lowest (12%; 95% CI = 8–16%) was observed in Gakenke district. Disease mean severity scores varied from district to district, ranging from 3.0 ± 0.6 recorded in Bugesera to 2 ± 0.2 recorded in Nyamasheke. **Table 1** shows field incidence and mean severity score of observed CBSD symptoms in the examined fields.

Molecular diagnostics was performed on a total of 130 samples collected from 13 districts. Samples were analyzed by RT-PCR for detection of CBSV and UCBSV. The overall incidence of CBSVs was 35.3%. Among the positive samples, 61% (28/46) were UCBSV whereas 11% (5/46) were CBSV, and 28% (13/46) had mixed infection of both CBSV and UCBSV (**Figure 3**), **Supplementary Figure 1** shows RT-PCR detection of CBSVs in field samples.

All 13 districts surveyed were found to be affected by CBSVs based on RT-PCR results, and the highest incidence (60%) was recorded in the south, in Nyanza district followed by Gisagara and Bugesera districts, both displaying an incidence of 50%. A single infection of UCBSV was found in all districts except Muhanga, whereas a single infection of CBSV occurred in Muhanga, Bugesera, Gatsibo, and Gakenke. Mixed infections were recorded in most districts except in Ngoma, Gatsibo, Gakenke, and Nyamasheke (**Figure 3**).

Bivariate Analysis of Factors Associated With CBSD Incidence

Fields From Farmers Working in Cooperatives Display Lower CBSD Infection Rate

Using data collected during the survey, we tested whether sociodemographic characteristics influence the level of cassava infection. Bivariate analysis (using Chi square test) of sociodemographic factors of 130 farmers (for whom cassava fields were visited) stratified by CBSV infection revealed that there is a significant association between category of respondents and cassava infection, and individual farmers had more infected fields than farmers in cooperatives ($p = 0.023$) (**Supplementary Table 3**). Farmer's age also significantly influenced the level of cassava infection of their field ($p = 0.043$). All the other sociodemographic factors had no influence on levels of cassava infection.

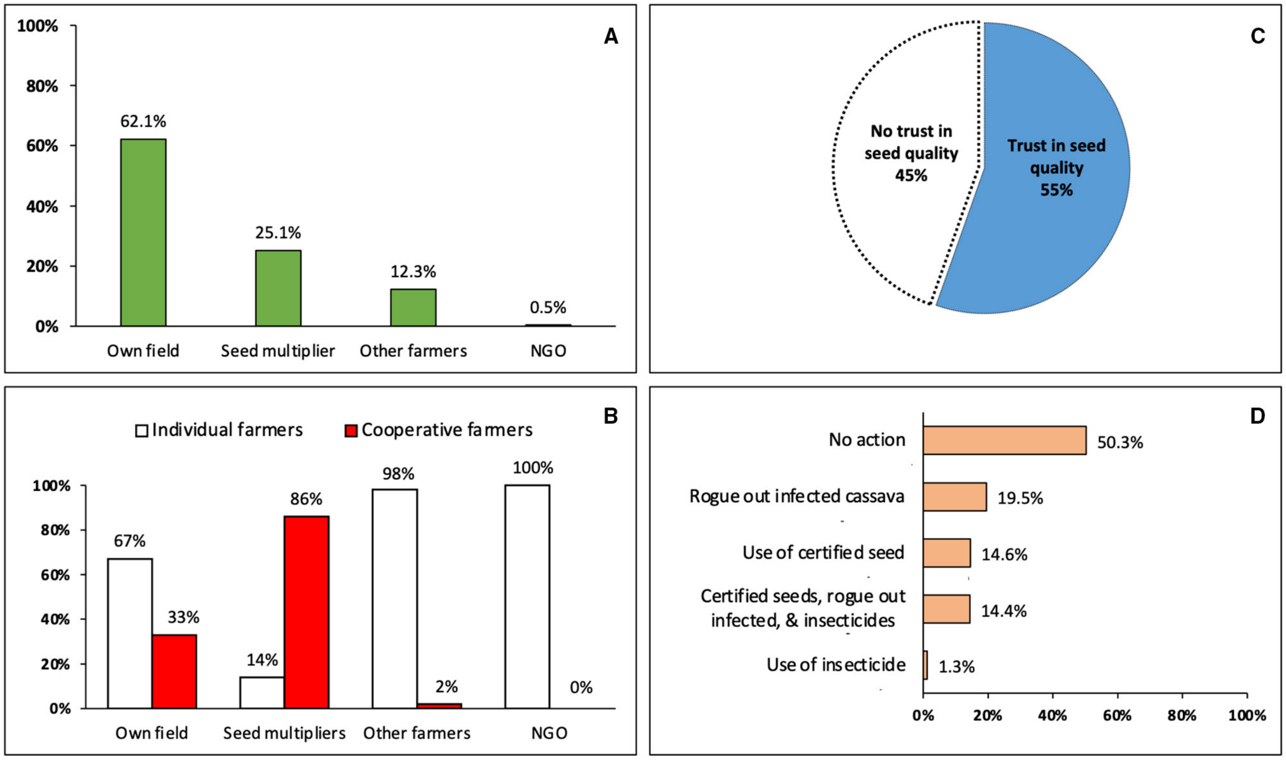

FIGURE 2 | Sources of cassava planting materials, trust, and disease management among farmers in Rwanda during 2019. **(A)** Sources of planting materials, **(B)** categories of farmers vs. sources of seeds, **(C)** level of trust in seed quality among participants, **(D)** disease management methods applied by participants (NGO, non-government organization).

TABLE 1 | Field incidence, severity, and frequency of cassava plants showing aerial CBSD symptoms in Rwanda, 2019.

Districts	Field incidence % (95% CI)	Mean severity score	Frequency of severity score (%)				
			1	2	3	4	5
South							
Gisagara	58.3 (52–63)	2.7 ± 0.8	42	29	19	8	2
Nyanza	62 (56–67)	2.9 ± 0.8	38	18	33	7	4
Ruhango	35.6 (30–41)	2.1 ± 0.3	61	35	4	0	0
Muhanga	30 (24–35)	2.2 ± 0.4	70	24	6	0	0
Kamonyi	25.3 (20–30)	2.2 ± 0.6	72	23	2	3	0
East							
Bugesera	60 (54–65)	3 ± 0.6	37	11	44	5	3
Kayonza	32.3 (27–37)	2.1 ± 0.3	68	28	4	0	0
Gatsibo	18 (13–22)	2.4 ± 0.5	82	10	8	0	0
Nyagatare	30 (24–35)	2.5 ± 0.6	70	16	12	2	0
Ngoma	20.6 (16–25)	2.2 ± 0.5	80	16	3	1	0
Kirehe	50 (44–55)	2.6 ± 0.5	50	22	26	2	0
North							
Gakenke	12 (8–16)	2.1 ± 0.3	87	11	2	0	0
West							
Nyamasheke	14.3 (10–18)	2.0 ± 0.2	86	13	1	0	0

Three hundred plants per district were examined. CI, confidence interval.

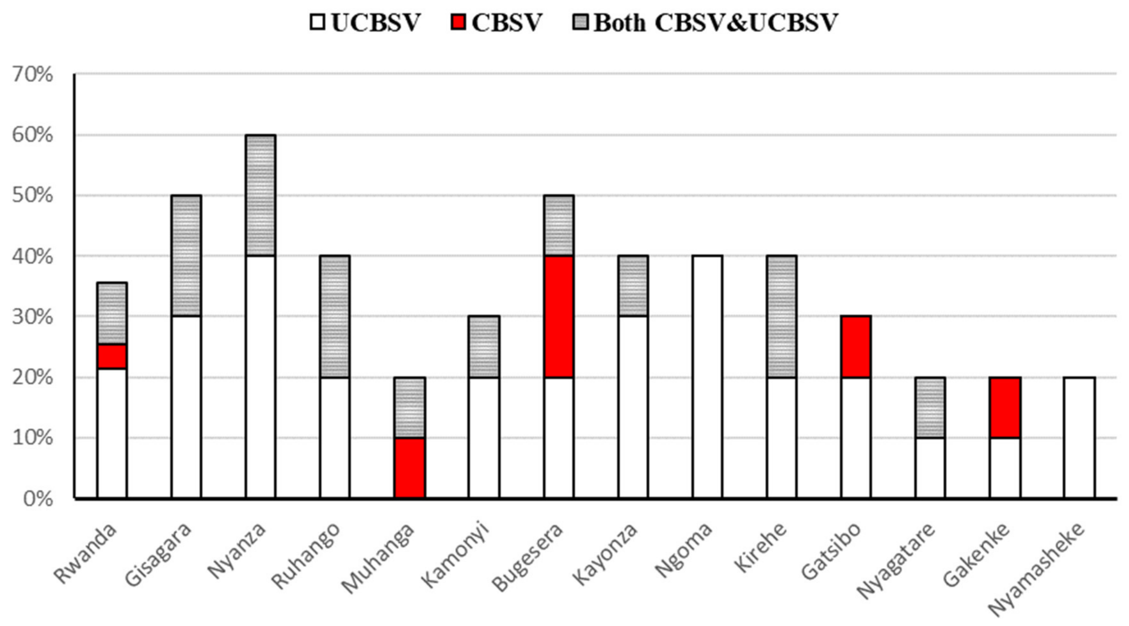

FIGURE 3 | Incidence of CBSD based on RT-PCR in different districts of Rwanda, 2019. The highest incidence was recorded from Nyanza, followed by Gisagara and Bugesera districts. UCBSV, Ugandan cassava brown streak virus; CBSV, Cassava brown streak virus.

TABLE 2 | Impact of accessibility of planting materials on cassava infection.

Variables	Total, *n* (%)	CBSVs Positive, *n* (%)	Chi square value	df	*p**
Source of cassava cuttings					
Seed multiplier	30 (23.0)	5 (10.9)	14.733	2	**0.001**
Own field	79 (60.7)	28 (60.9)			
Other farmers	21 (16.2)	13 (28.2)			
Distance to the source (Km)					
<1	70 (53.8)	26 (56.5)	4.032	3	0.258
1–4	28 (21.5)	7 (15.2)			
4–8	24 (18.5)	8 (17.4)			
>8	8 (6.2)	5 (10.9)			
Proximity to the tarmac road (Km)					
<1	14 (10.8)	4 (8.7)	1.732	3	0.630
1–4	24 (18.5)	11 (23.9)			
4–8	27 (20.8)	10 (21.7)			
>8	65 (50.0)	21 (45.7)			
Proximity to RAB (Km)					
<10	42 (32.3)	23 (50.0)	13.445	3	0.070
10–20	21 (16.2)	3 (6.5)			
20–30	21 (16.2)	4 (8.7)			
>30	46 (35.4)	16 (34.8)			
Proximity to the border (Km)					
<10	53 (40.8)	25 (54.3)	9.7	3	**0.021**
10–20	23 (17.7)	10 (21.7)			
21–50	10 (7.7)	3 (6.5)			
51 and above	45 (34.6)	8 (17.4)			

**Significant at $p < 0.05$ bolded; df, degree of freedom.*

TABLE 3 | Impact of agronomic variables on cassava infection.

Variables	Total, *n* (%)	CBSVs positive, *n* (%)	Chi square value	df	*p**
Age of plants in months					
<8	57 (43.8)	9 (19.5)	19.65	2	**<0.001**
8–10	56 (43.1)	24 (52.2)			
>10	17 (13.1)	13 (28.2)			
Type of cassava varieties grown					
Improved	85 (65.4)	29 (63.0)	7.2	2	0.067
Local	9 (6.9)	0 (0.0)			
Both improved and local	36 (27.7)	17 (37.0)			
Farming system used					
Monoculture	60 (46.2)	16 (34.8)	3.7	1	0.054
Polyculture	70 (53.8)	30 (65.2)			
Using fertilizers to grow cassava					
Yes	75 (57.7)	18 (39.0)	10.05	1	**0.002**
No	55 (42.3)	28 (61.0)			
Access to extension services					
Yes	108 (83.1)	37 (80.4)	0.35	1	0.552
No	22 (16.9)	9 (19.6)			
Extension services benefited by farmers					
Visit of cassava field	42(32.3)	17(37.0)	2.06	4	0.725
Advice on diseases management	7(5.4)	3(6.5)			
Advice on farming practices	31 (23.5)	9 (19.5)			
None	22 (16.9)	9 (19.5)			
Field visit and advice on GAP	28 (21.5)	8 (17.4)			

**Significant at $p < 0.05$ bolded; df, degree of freedom.*

Fields Established With Planting Material From Seed Multipliers Have a Lower Probability to Be CBSD Infected

We further investigated the link between cassava seed accessibility and CBSV infection. Our analysis showed that the source of cassava cuttings and proximity to the border had a significant impact on cassava infection with *p* values of 0.001 and 0.021, respectively (**Table 2**). Farmers who used seeds from their own field were more likely to have infected fields (60.9%) than those who got seeds from seed multipliers (10.9%). It was also noted that farmers near the country's border had more infected fields (54.3%) (**Table 2**).

A majority of cassava fields surveyed were more than 8 months old (56.2%). Most participants grew improved varieties (65.4%) and had access to extension services (83.1%). In fact, the analysis revealed that there was a significant association between age of the plant and cassava infection ($p < 0.001$) where the plants aged <8 months (57.1%) were significantly more likely to be healthy than to be infected (19.5%). Furthermore, a significant association was noted between use of fertilizers and cassava infection ($p = 0.002$) as the farmers using fertilizers (68%) were significantly more likely to have healthy than infected fields (39%) (**Table 3**).

Farmers' Awareness of Cassava Viral Diseases Is Associated With Lower CBSD Incidence

Although all farmers were aware of at least one cassava viral disease's existence, 34.6% of them did not know the symptoms of cassava viral diseases. Symptoms of CMD were easily recognized by 31.5% followed by 20.8% who recognized both CMD and CBSD (**Supplementary Figure 2** shows symptoms of CMD and CBSD). Farmers who were not aware that the viruses can be transmitted had more infected fields (65.2%) ($p < 0.001$), and likewise, those who did not know disease management had more infections in their fields (67.4%) compared with those who knew the management techniques ($p < 0.001$) (**Supplementary Table 4**).

Multivariate Analysis of Risk Factors Associated With CBSD

Eight factors that showed significant association ($p < 0.05$) during bivariate analysis (including source of cassava cuttings, proximity to the border, age of the plants, use of fertilizers, category of respondents, age of farmers, knowledge of cassava viral disease transmission, and knowledge of cassava disease management) were considered together in a multivariable analysis to identify the variables associated with cassava infections. Upon fitting the factors using multiple logistic regression and specifying the *"backward conditional"* method with removal at $p < 0.05$, five factors remained in the final analysis as shown in **Table 4**. After testing the goodness of fit using the Hosmer–Lemeshow test, the chi-square value was 4.80 with degree of freedom 6, and *p* value was 0.570, which indicates that the fitted model was adequate.

TABLE 4 | Factors associated with CBSD.

Variables	AOR	95% CI		p*
		Lower	Upper	
Category of respondents				
Individual farmers	0.43	0.12	1.52	0.191
Cooperative	Ref			
Farmers' age in years				
25–35	0.74	0.22	2.43	0.617
35–45	0.26	0.08	1.22	0.080
45 and above	Ref			
Source of cassava cuttings				
Seed multiplier	Ref			
Own field	7.31	1.52	35.06	**0.013**
Other farmers	10.1	1.73	58.81	**0.010**
Proximity to the border (Km)				
<10	4	1.33	12.05	**0.014**
10–20	4.24	1	17.97	**0.050**
21–50	1.7	0.27	10.65	0.571
51 and above	Ref			
Age of plants in months				
<8	Ref			
8–10	4.76	1.69	13.39	**0.003**
>10	18.47	3.93	86.78	**<0.001**
Using fertilizers to grow cassava				
Yes	Ref			
No	2.44	0.93	10.25	0.127
Knowledge of cassava viral diseases transmission				
Yes	Ref			
No	3.97	1.46	10.83	**0.007**
Knowledge of cassava viral diseases management				
Yes	Ref			
No	2.94	1.08	7.96	**0.034**

*Ref, reference, *Significant at $p < 0.05$ bolded; AOR, adjusted odds ratio; 95% CI, confidence interval.*

Farmers who use cuttings from their own fields or from other farmers' fields had a more than seven-fold higher risk than those who used cuttings from a seed multiplier ($p < 0.05$). Respondents located near the border had a four times higher risk of having CBSVs than those located far away ($p < 0.05$). Cassava plants <8 months old had fewer risks of infection compared with the older ones ($p < 0.05$). Similarly, those who were not aware of the disease transmission and management had nearly a three times higher risk of having the infected plants ($p < 0.05$) (**Table 4**).

Effect of Sociodemographic Variables on Cassava Productivity

Although diseases are among the main constraints to cassava yield and production (FAO, 2015; Rey and Vanderschuren, 2017), socioeconomic factors might also be associated with suboptimal cassava yields. Taking advantage of the farmer survey, we performed an ANOVA to assess the effect of different sociodemographic variables on cassava productivity. The demographic variables that significantly affected cassava productivity were district, level of education and farmer category. The district of Ruhango had a significantly higher average of cassava benefit per hectare than other districts ($p < 0.001$). The mean of cassava benefit was significantly higher among respondents with a secondary level of education compared with illiterate farmers ($p < 0.001$). Similarly, cooperative farmers had significantly more average cassava benefit than individual farmers ($p < 0.001$) (**Supplementary Table 5**).

Effect of Economic and Agronomic Variables on Cassava Productivity

During bivariate analysis using ANOVA, all agronomic and economic factors tested showed that they significantly influence cassava productivity ($p < 0.001$). The mean cassava benefit was significantly higher among farmers with fields displaying less viral disease (after PCR), larger size of land, growing improved varieties, practicing monoculture, applying fertilizers, and using seeds from seed multipliers (**Table 5**).

Multiple Linear Regression for Cassava Productivity

A multiple linear method was used to determine which variables had the most significant impact on cassava productivity. The results show that 5 out of 10 factors predict cassava productivity. The 10 variables considered in the model were all those variables significant at the bivariate analysis using the ANOVA test (see **Table 5**; **Supplementary Table 5**). Upon fitting these 10 factors against the dependent variables using multiple linear regression and specifying the *stepwise* method, five variables (*using fertilizer*, *size of the land*, *farming system*, *cassava viral disease*, and *type of cassava varieties grown*) remained affecting the productivity of cassava (**Table 6**). There were no collinearity issues found in this study between the different outcome and independent variables as tolerance was above 0.1 and VIFs were below 10. Durbin Watson was also used to check for autocorrelation that the residuals from linear regressions are independent. Durbin Watson with zero indicated positive autocorrelation, and four indicated negative autocorrelation while around two indicated that the residuals are uncorrelated.

Using fertilizer was the main positive effect in the first place and explained 57% ($R^2 = 0.57$) of the changes in cassava benefit. In the second model, size of the land is added, which led to 67% ($R^2 = 0.67$) of the changes in the cassava benefit. In the third model, farming system is added, which led to 69% ($R^2 = 0.69$). In the fourth model, viral disease led to 71% ($R^2 = 0.71$), and type of cassava varieties grown in the fifth model led to 72% ($R^2 = 0.72$) variation in cassava benefit (**Table 6**).

DISCUSSION

The present study used a comprehensive cross-country survey to assess the current cassava seed system, farmers' practices, and their knowledge of the biotic constraints, the status of CBSD, and critical factors associated with its spread throughout the cassava

TABLE 5 | Effects of economic and agronomic variables on cassava productivity.

Variables	*N*	Mean (benefit/Ha)	Std. deviation	Std. error	95% CI for mean		F	ANOVA
					Lower	Upper		*p*
Cassava infection								
Positive	46	482.58	125.01	18.43	445.46	519.71	10.56	**0.001**
Negative	84	648.27	332.70	36.30	576.07	720.47		
Size of land used to grow cassava (Ha)								
<1	45	425.34	126.12	18.80	387.45	463.23	14.90	**<0.001**
1–5	31	556.81	96.95	17.41	521.25	592.37		
6–10	19	836.95	521.52	119.64	585.59	1,088.32		
>10	35	695.73	244.03	41.25	611.90	779.55		
Type of cassava varieties grown								
Improved	85	665.45	315.45	34.22	597.41	733.49	9.65	**<0.001**
Local	9	435.47	165.57	55.19	308.20	562.74		
Both improved and local	36	449.20	143.01	23.84	400.81	497.59		
Farming system used								
Monoculture	78	694.76	321.21	36.37	622.34	767.18	32.25	**<0.001**
Polyculture	52	431.98	109.14	15.14	401.59	462.36		
Using fertilizers to grow cassava								
Yes	75	710.33	317.06	36.61	637.38	783.27	40.68	**<0.001**
No	55	425.08	112.70	15.20	394.62	455.55		
Access to extension services								
Yes	108	620.19	300.65	28.93	562.84	677.54	7.54	**0.007**
No	22	439.71	144.64	30.84	375.58	503.84		
Source of cassava cuttings								
Seed multiplier	79	552.51	211.12	23.75	505.22	599.80	9.14	**<0.001**
Own field	30	768.35	433.75	79.19	606.39	930.32		
Other farmers	21	474.04	145.93	31.84	407.62	540.47		

**Significant at $p < 0.05$ bolded; Std, Standard; 95 CI, confidence interval; F, F test for continuous outcome.*

seed system as well as factors that influence cassava productivity in Rwanda.

The current findings confirmed the occurrence of CBSD (both CBSV and UCBSV) in Rwanda. The disease was found in all 13 districts surveyed, indicating that it has spread out in all major cassava-growing regions, including Kirehe and Nyagatare, where CBSVs were not detected in previous studies (Munganyinka et al., 2018).

In our survey, we found that districts located near the border displayed a higher rate of CBSD incidence. The highest field incidences and severities were recorded in the three districts, namely, Nyanza, Bugesera, and Gisagara, bordering Burundi. In an earlier study carried out by Munganyinka et al. (2014), Nyanza and Gisagara districts also displayed the highest CBSD incidence, which confirms them as hot spots for CBSD (Munganyinka et al., 2018). This observation might correspond to the informal movement of cassava cuttings across countries that leads to the importation of infected cuttings or use of genetic material that is more susceptible to CBSVs. Furthermore, the high CBSD incidence in those districts could be because since its first report in 2009, the virus could have flourished in those areas season after season due to the relatively warm environments that favor proliferation of whitefly vectors (Campo et al., 2011).

A CBSD survey performed in Burundi previously reported an average incidence and severity of 15.3 and 2.3%, respectively (Bigirimana et al., 2011). A decade ago, UCBSV was the only viral species that was associated with the disease in Burundi although it was already present in Tanzania (Rwegasira et al., 2011).

Based on RT-PCR diagnostics, the overall CBSV incidence was found to be less than CBSV incidences reported elsewhere in East-Central Africa (Kenya, Tanzania, Malawi, Zambia) (Rwegasira et al., 2011; Mbewe et al., 2014; Koima et al., 2018; Mulenga et al., 2018). This difference might be due to a later introduction of CBSVs in Rwanda. UCBSV was prevalent across the country, indicating that it is the commonest cause of CBSD in the country. Similar findings were reported in the survey conducted in 2014 among major cassava-growing regions (Munganyinka et al., 2018). It should be noted that our study revealed an increased rate of mixed infections from 10% in 2014 to 28% in 2019, highlighting the dynamism of disease spread over time either through vectors or exchange of unhealthy cuttings among farmers.

Despite farmers being aware of the importance of quality seed to optimize output, it was observed that most farmers have difficulty identifying quality seed as was reported earlier (Minot et al., 2007).

TABLE 6 | Multiple linear regression for cassava productivity.

Model	Independent variable	Coefficients						Model summary and ANOVA test					Durbin Watson test
		Unstandardized Coefficients	Standardized Coefficients	*t*	Sig.	Collinearity Statistics							
		B	Beta			Tolerance	VIF	*R*	R^2	Adj. R^2	F	Sig.	
1	(Constant)	212.77		7.84	<0.001								
	Using fertilizers to grow cassava	212.31	0.75	12.92	<0.001	1.00	1.00	0.75	0.57	0.56	166.91	<0.001	1.347
2	(Constant)	198.98		8.29	<0.001								
	Using fertilizers to grow cassava	157.15	0.56	9.26	<0.001	0.73	1.38	0.82	0.67	0.66	127.04	<0.001	
	Size of land	43.09	0.37	6.20	<0.001	0.73	1.38						
3	(Constant)	156.56		5.95	<0.001								
	Using fertilizers to grow cassava	136.10	0.48	7.78	<0.001	0.63	1.58	0.83	0.69	0.69	95.25	<0.001	
	Size of land	30.32	0.26	3.94	<0.001	0.5	1.83						
	Farming system used	65.92	0.23	3.35	0.001	0.5	1.97						
4	(Constant)	227.38		5.86	<0.001								
	Using fertilizers to grow cassava	134.86	0.48	7.85	<0.001	0.63	1.585	0.84	0.71	0.70	75.75	<0.001	
	Size of land	31.22	0.27	4.13	<0.001	0.54	1.83						
	Farming system used	52.71	0.19	2.63	0.01	0.47	2.12						
	Cassava infection	−36.80	−0.13	−2.45	0.016	0.87	1.14						
5	(Constant)	177.62		4.07	<0.001								
	Using fertilizers to grow cassava	129.18	0.46	7.58	<0.001	0.61	1.61	0.85	0.72	0.71	63.90	<0.001	
	Size of land	26.06	0.23	3.37	0.001	0.50	1.99						
	Farming system used	49.75	0.18	2.52	0.013	0.46	2.13						
	Cassava infection	−36.82	−0.13	−2.49	0.014	0.87	1.14						
	Type of cassava varieties grown	29.23	0.13	2.35	0.02	0.74	1.34						

B, beta coefficient; t, student t test for continuous variables; Sig., significance; VIF, variance inflation factor; R, correlation coefficient; Adj. R^2, adjusted correlation coefficient squared; F, F test for continuous outcome.

Our present study indicates that a majority of farmers (76.9%) use informal ways to get cuttings for free from either their own fields or from neighbors. Because the supplied planting material often suffers from a lack of quality control, farmers are more likely to plant virus-infected cuttings, which can lead to low yield and reduced benefits. The informal seed system might be further maintained by the lack of knowledge about cassava viral diseases as identified in the survey, highlighting the need to increase farmers' awareness of using quality seed and also to mobilize the private sector to invest in the commercial cassava seed business. Previous studies have already highlighted the need to promote farmers' awareness (Chipeta et al., 2016; Nduwumuremyi et al., 2016; Bentley et al., 2017) as farmers using cassava planting materials from appropriate sources (research institutions, NGOs, etc.) appear to have fields with reduced CBSD infection (Gondwe et al., 2003).

Despite differences in CBSV incidence between districts, farmers who used quality seeds kept having lower CBSV infection compared with those reusing seeds from their own field or from other farmers. This observation also highlights the importance of the human factor (transport and exchange of unhealthy cuttings), contributing to the propagation and dissemination of CBSD (Patil et al., 2015; Maruthi et al., 2017).

The ultimate goal of cassava farming is to optimize yields for food security and income. Taking into account that cassava is a climate-resilient crop essential in fighting food insecurity for the fast-growing population (El-Sharkawy, 2004; Chavez et al., 2005; Burns et al., 2010), crop-intensification programs have been launched in Rwanda and other African countries to improve agricultural production (FAO, 2016). Despite that cassava production is a profitable investment in Rwanda (Gasangwa, 2013), its production still remains constrained by several factors.

The present study indicates that high yield and income generation from cassava logically increase with the use of fertilizers, the size of the land, monoculture, and improved cassava varieties, whereas incidence of viral diseases is associated with a decrease in the income. Although the use of fertilizers is known to increase yields of cassava storage roots (Munyahali et al., 2017), cassava continues to be seen as a resilient crop with limited or no requirement for fertilization. Therefore, promoting adequate use of fertilizers in cassava should remain a priority in future crop-intensification programs.

The survey also indicates that cooperatives nearly double their benefits as compared with individual farmers. This observation could be explained by the fact that most cooperatives exploit bigger land and better comply with good agriculture practices (including the use of fertilizer, improved quality seeds, and practicing monoculture) than individuals. A recent assessment of the benefits associated with cultivating improved high-yielding varieties shows that yields can be increased more than four-fold as compared with the cultivation of local varieties in Africa (Khonje et al., 2015). Thus, farmers should be encouraged to group into cooperatives and to practice good agriculture practices (GAP) to meet the high yield and food security objectives of crop intensification programs.

Following the emergence of CBSD in 2009, efforts made by governmental entities to combat cassava viral diseases, have helped reduce their impact on cassava production. However, CBSD remains a major challenge to cassava production despite past and ongoing efforts to breed for virus-resistant varieties, to distribute clean planting material, and to promote GAP (Catholic Relief Services, 2012). Although time and resource constraints prevented us from performing a more extensive survey, the random approach operated in the cassava-growing sectors makes our survey representative of the cassava-growing areas.

Our study reveals that viral diseases remain a constraint to cassava productivity with a disease prevalence that has increased to 35.3% in Rwanda. Therefore, there is a need to continue efforts to introgress virus-resistance traits into farmer-preferred varieties and to establish a cassava seed system enabling sustainable and affordable supply of clean planting material to farmers. The strengthening of the cassava seed system also requires the development of important capacities for virus diagnostics (Mukasa, 2015; Wossen et al., 2020). To sustain the implementation of GAP, there is also a need to increase farmers' awareness of cassava diseases and the immediate benefit of using quality seeds.

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.

ETHICS STATEMENT

Ethical review and approval was not required for this study with human participants, in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

HV, CBr, CBu, NG, CN, and JB contributed to the conception and design of the study. CN, JB, AN, VB, SM, CT, and HV contributed to the methodology design. HV, SM, CBr, CT validated the study. MH, YB, CT, CN, and JB performed the statistical analysis. CN and JB wrote the first original draft. YK, YB, CT, LL, SM, EM, AN, and HV revised and edited the manuscript. HV provided resources and supervision. All authors contributed to the article and approved the submitted version.

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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.2021.699655/full#supplementary-material

Supplementary Figure 1 | Detection of CBSV and UCBSV by reverse transcription-PCR in samples collected from **(A)** Nyanza and **(B)** Bugesera districts. Expected PCR product sizes were CBSV (703 bp) and UCBSV (800 bp). From left to right, 100 bp DNA ladder; C+ correspond to positive control for both CBSV and UCBSV, whereas C– corresponds to negative control; lanes 1–10 represent pool of samples from fields 1–10; faster DNA ladder. **(C)** Detection of the internal control *Me*PP2A for samples in **(A)**; **(D)** detection of the internal control *Me*PP2A for samples in **(B)**; expected PCR product sizes was 150 bp. From left to right, 100 bp DNA ladder; Lanes 1–10: samples; internal control was detected in all tested samples.

Supplementary Figure 2 | (A) CBSD symptoms on cassava leaves; **(B)** CBSD symptoms on cassava stem; **(C)** CBSD symptoms on cassava roots; **(D)** CMD symptoms on cassava leaves.

Supplementary Figure 3 | Histogram and P-P plot normality tests for cassava productivity linear regression analysis.

Supplementary Table 1 | List of collected variables during the survey.

Supplementary Table 2 | List of biotic variables recorded or measured during the survey of the 130 visited fields.

Supplementary Table 3 | Socio-demographic factors stratified by cassava infection.

Supplementary Table 4 | Disease awareness variables stratified by cassava infection.

Supplementary Table 5 | Effects of demographic variables on cassava productivity.

Supplementary Datasheet 1 | Questionnaire.

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REVIEW
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A 20-Year Journey Through an Orphan African Baobab (*Adansonia digitata* L.) Towards Improved Food and Nutrition Security in Africa

Achille E. Assogbadjo [1]****, Flora Josiane Chadare*** [2]***, Leonard Manda*** [1,3] ***and Brice Sinsin*** [1]

[1] Laboratoire d'Écologie Appliquée, Universit'e d'Abomey-Calavi, Cotonou, Benin, [2] Laboratoire de Sciences et Technologie des Aliments et Bioressources et de Nutrition Humaine, Ecole des Sciences et Techniques de Conservation et de Transformation des Produits Agricoles, Centre Universitaire de Sakété, Université Nationale d'Agriculture, Cotonou, Benin, [3] Biological Sciences Department, Mzuzu University, Mzuzu, Malawi

The African baobab (*Adansonia digitata* L.) is a multipurpose orphan tree species of the semi-arid and sub-humid Sub-Saharan Africa where it plays an important role in rural livelihoods. Its wide distribution and dense nutrition properties make it an important species for food and nutrition security in Africa. However, despite the increasing interest in the species over the past two decades, the full potential of baobab remains underexploited. This review highlights strides made over the past 20 years (2001–2020) towards harnessing and unlocking the potential values of baobab in Benin, West Africa, to contribute to food and nutrition security. Challenges and threats are identified, and next steps suggested to guide research and development initiatives for orphan tree fruit species like baobab to address hunger and malnutrition in Africa.

Keywords: African baobab, *Adansonia digitata*, orphan species, food security, nutrition security, West Africa

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****Correspondence:***
Achille E. Assogbadjo
assogbadjo@yahoo.fr

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INTRODUCTION

Despite their status, orphan species, which are also popularly known as neglected and underutilised species (NUS) (Jamnadass et al., 2020), are treasure troves for livelihood improvement in the developing countries (Jamnadass et al., 2011; Tadele, 2019; Ulian et al., 2020). Indeed, owing to the strong cultural links between plants and nature (Díaz et al., 2018), orphan species like baobab have a special niche in the dietary habits of rural communities in Africa (Buchmann et al., 2010), where they offer a large untapped resource to support food security and agriculture (Ulian et al., 2020). As such, they could be important for food and nutrition security in these societies, in addition to representing the regional identity of human communities (Bedigian, 2018). The continued neglect of these species, which include 477 fruit tree species (Awodoyin et al., 2015), could be one of the reasons Africa is still characterised by poverty, hunger and malnutrition (Jamnadass et al., 2020; Ulian et al., 2020). Recently, the Lancet Commissions (Willett et al., 2019) reported that over 820 million people worldwide were food insecure and many more were malnourished, the majority of which were in Africa, thus calling for enhancing agro-biodiversity to improve food and agriculture.

The growing recognition in the use of orphan species to address local challenges (Borelli et al., 2020), coupled with the increasing urgent need for transformation towards healthy diets and sustainable agro-ecosystems (Willett et al., 2019; Vermeulen et al., 2020), points to increasing attention and utilisation of orphan species. This is could lead to the improvement of both the ecological and human well-being in Africa, where a substantial diversity of orphan species is found. Owing to their dense nutritional properties and low input requirements for their production (Tadele, 2019), broad gene pool and adaptability to niche marginal areas (Mabhaudhi et al., 2019), orphan species have a great stake in the strategies for transformational diets and agro-ecosystems in Africa through crop improvement, expansion into new agro-ecological zones and building resilience of marginal cropping systems (Mabhaudhi et al., 2019; Jamnadass et al., 2020; Ulian et al., 2020).

The African baobab (*Adansonia digitata* L.) (**Figure 1**) is a multipurpose orphan tree species, native to the dry savanna of the sub-Saharan Africa (SSA), where it plays an important role in the food security and livelihoods of rural communities (Kamatou et al., 2011), and is inextricably linked with cultural identity and local beliefs (Buchmann et al., 2010). Evidence points to West Africa as the origin of the African baobab, from which it became widely distributed to the rest of the savannas of Africa and Madagascar (Pock Tsy et al., 2009). All baobab parts (bark, flowers, fruits, leaves, roots, seeds) are useful and edible (Kaboré et al., 2011), even if the fruit pulp, leaves and seeds are the parts mostly used for food and nutrition security (Assogbadjo et al., 2008; Rashford, 2018). Over 300 baobab uses have been reported in West Africa alone spanning across 11 ethnic tribes (Buchmann et al., 2010). This demonstrates the diversity and cultural importance of baobab in the sustenance of livelihoods in Africa. Baobab is also valued at international markets such as Europe, where an estimated 300 baobab-derived products have been recorded (Gebauer et al., 2016). The breakthrough of baobab onto the international market follows the acceptance of baobab products as "novel products" at the European and American markets (Buchmann et al., 2010). This signifies the potential role baobab could play in addressing regional and global challenges such as food insecurity and malnutrition. Additionally, and as is common with a number of orphan plant species, baobab is well-adapted to local environmental conditions, and thrives in marginal areas and soils (Sidibe and Williams, 2002). These environmental characteristics make baobab an important species for agro-ecosystems in the SSA, where over 80% of the arable land has low soil fertility (Hengl et al., 2015).

FIGURE 1 | Part of a baobab trunk showing leaves and drooping immature fruits.

Recognition of baobab as an important resource dates back to the 1800s with reports of baobab leaves used as a vegetable (Wickens, 2007). More interest rose in the 1980s and 1990s with notable work on ecology, pollination and floral biology (Baum, 1995, 1996). Later, more comprehensive work on baobab was done by Sidibe and Williams (2002), who described a wide range of areas including biogeography, taxonomy, reproduction and domestication of baobab. Since then, several laudable efforts have been made on baobab addressing various aspects such as conservation genetics, ethnobotany, pharmacology, domestication, and baobab biology. However, these are littered in various publications. This makes it difficult for fledgling researchers, development practitioners, and policy makers to utilise the information generated to harness and unlock the potential benefits of baobab in addressing poverty, food and nutrition insecurity in Africa (Gebauer et al., 2016). According to Schumann et al. (2012), packaging of fundamental information at a small-scale level (national or regional), is critical for the development of utilisation and management strategies for orphan species like baobab.

Accordingly, this study is a review of the work carried out on baobab in the last 20 years (2001–2020) in Benin, West Africa. It aims to guide research and development agenda in harnessing and unlocking the potential benefits of baobab to address food and nutrition insecurity, ultimately contributing to the attainment of the 2030 Sustainable Development Goals (Goal 2) and Agenda 2063 aspirations in Africa (Aspiration 1). First, the study outlines the research strides made on baobab in Benin from 2001 to 2020 towards sustainable food and nutrition security. Second, it identifies challenges and constraints. Third, it proposes and discusses the next steps, by drawing lessons from the region and elsewhere. Finally, the study draws the conclusions. In all these steps, the study attempted to consider the food system dimensions (Timmer, 2014), namely, access, availability, utilisation, and sustainability of baobab resources.

METHODOLOGY

Benin is located between 6°25′ N to 12°30′N and 0°45′E to 4°00′E and has a total surface area of about 115,762 km^2. It has three contrasting climatic zones: (i) the Guinean in the south—humid zone with bimodal precipitation averaging 1,200 mm per year and an annual temperature range of 25–29°C; (ii) Sudano-Guinean in the centre—sub-humid zone with a tendency to unimodal precipitation averaging 900–1,110 mm per year and an

TABLE 1 | Summary of achievements on baobab based on research findings and their relationship with food security dimensions.

Achievements	References	Relation to food and nutrition security
Baobab is distributed throughout Benin	Codjia et al. (2003), Assogbadjo et al. (2005a), Chadare et al. (2008), Djossa et al. (2015), and Dossa et al. (2015)	Accessibility
Baobab food resources are available almost all the year round in Benin	Assogbadjo et al. (2005a), Chadare et al. (2010), and De Caluwe and Van Damme (2011)	Availability
Elite baobab trees with locally preferred traits have been identified in Benin	Assogbadjo et al. (2008, 2009)	Selection for domestication, thus ensuring accessibility, availability and sustainability
Wide genetic and morphometric variations are found within baobab populations across climatic zones of Benin	Assogbadjo et al. (2005b, 2006)	Selection for domestication, thus ensuring accessibility, availability and sustainability
Baobabs in Benin have wide and dense food properties in pulp, leaves and seeds	Chadare et al. (2008, 2014) and Assogbadjo et al. (2012)	• Utilisation • Catalyst for industrial growth of baobab food products, thus improving access and availability
Baobab is revered by local communities and is a biocultural keystone species in the country	De Caluwé et al. (2009), Chadare et al. (2008, 2010)	• Utilisation • Catalyst for industrial growth of baobab food products, thus improving access and availability • Motivating factor for domestication, thus ensuring access, availability, and sustainability
Background information on the baobab value chain is available for Benin	De Caluwe and Van Damme (2011)	• Accessibility • Availability
Baobab is threatened by climate change but is amenable to domestication	Assogbadjo et al. (2011) and Sanchez et al. (2011)	Selection for domestication, thus ensuring accessibility, availability and sustainability
Baobab productivity is improved by fruit bats	Djossa et al. (2015)	Planning for its conservation in the landscape, thus ensuring accessibility, availability, and sustainability

annual temperature range of 25–29°C; and (iii) the Sudanian in the north—semi-arid zone with strictly unimodal precipitation averaging <1,000 mm per year and an annual temperature range of 24–34°C.

This study is not a fully-fledged systematic review but rather a traditional review that used some elements of the systematic approach (Haddaway et al., 2015). It used 16 peer reviewed articles on baobab published between 2001 and 2020 whose work has a footprint in Benin. Forty nine additional papers on baobab from elsewhere other than Benin and 37 articles on agroforestry/indigenous fruit trees/orphan species were used to augment the discussions and draw lessons. Three online search engines were used to select papers on baobab: Google scholar, Science Direct and African Journals Online (AJOL). A combination of terms "African baobab," "baobab," and "*Adansonia digitata*" with "food security," "nutrition security," and "Benin" or "Africa" were used. An advanced search option was used for the Google scholar. Records totalling 6,297 on baobab (Google scholar [5,824], Science Direct [353], AJOL [120]) were collected in the first week of January 2021. After scanning through the topics and removing duplicates, 315 publications were retained. Reading through the abstracts and keywords of these publications yielded a total of 65 articles on baobab (16-Benin; 49-elsewhere) which were retained for this review. These were publications that focused on food and nutrition, indigenous knowledge, value chain, domestication and conservation (genetics, population dynamics, and climate change effects on baobab). General articles on agroforestry, indigenous fruit trees and/or orphan species were randomly selected from the literature, whilst a couple others were suggested by anonymous reviewers of the draft manuscript.

STRIDES MADE TO UNLOCK AND HARNESS THE POTENTIAL VALUE OF BAOBAB FOR IMPROVED FOOD AND NUTRITION SECURITY

Table 1 summarises the strides that have been made in the last 20 years towards unlocking and harnessing the potential value of baobab to contribute to improved food and nutrition security in Benin in West Africa.

Baobab Is Distributed Throughout Benin

Several studies addressing various aspects of baobab and using different methods have demonstrated that baobab is widely distributed in the country, spanning all the three climatic zones. These include: ethnobotanical studies e.g., local edible vegetables (Codjia et al., 2003); indigenous knowledge and baobab food products (Chadare et al., 2008); ecological studies e.g., ecological diversity and productivity of baobab (Assogbadjo et al., 2005a); characterisation of natural populations of baobab (Dossa et al., 2015); and reproductive ecology (Djossa et al., 2015). For example, Assogbadjo et al. (2005a) showed that Benin has a baobab density of 1–5 trees per km^2, with the Guinean zone having a relatively higher density than the other two zones. Further, the authors observed that baobab density tends to decrease with increasing distance from farms and/or villages i.e., more baobabs are on farms or closer to the villages. The results may mean that, for millennia, baobab saplings close to the villages might have been protected and tendered by humans as opposed to baobab populations away from the villages that may have been subjected to damage by several factors including wild animals. Further, the wide distribution and closeness to villages imply baobab is available and can be easily accessed by the local communities in Benin for their utilisation.

The spatial dependence of baobabs on human settlements, albeit worrying recruitment rates, has also been reported in many parts of Africa including Mali (Duvall, 2007), Namibia (Lisao et al., 2018) and South Africa (Venter and Witkowski, 2010, 2011), buttressing the evidence of the long historical association between baobab and human cultures in Africa, considering that baobab is a very long-lived species (>1,000 years) (Patrut et al., 2015).

Baobab Food Resources Are Available Almost All the Year Round in Benin

Flowering, fruiting and leaf shedding are seasonal phenomena in baobab. In Benin, according to Assogbadjo et al. (2005a), flowering in baobab coincides with the peak of the rainy season (July–August) in the Sudanian Zone, and the peak of the two rainy seasons in the Guinean Zone. The peak of fruiting, however, corresponds with the onset of the dry season (October) with subsequent fruit maturity extending from December to March (dry season). On the other hand, leaves are generally retained throughout much of the year, except during episodes of leaf shedding at the peak of the dry season between October and April. During this time, variations are observed with a mixed of early, medium and late leaf shedding trees giving way to new tender shoots (Assogbadjo et al., 2005a). Generally, fruit availability in baobab is reported to be between April and October across Africa (Omotayo and Aremu, 2020).

Given that maturation of fruits and availability of leaves also coincide with the peak of the dry season, baobab thus becomes an important food species especially when field crops are in off-season. The overlapping of baobab resources with time of food shortage has also been reported in many countries (Sidibe and Williams, 2002; Jäckering et al., 2019). It would seem that subsequent storage of fruits and leaves (often harvested in bulk during the peak period), beefed up by the storage and distribution of a wide range of processed baobab products (Chadare et al., 2008, 2010; De Caluwe and Van Damme, 2011), make baobab food resources become available and accessible almost all the year round in Benin. The seasonal variations among climatic zones of Benin also work to the advantage for constant supply and distribution of baobab from one area to another virtually throughout the year, albeit low availability. This trend in the availability of baobab resources and the practise by communities to store baobab products, so as to spread over periods of food shortage is a common practice in Africa (Wanjeri et al., 2020).

Elite Baobab Trees With Locally Preferred Traits Have Been Identified in Benin

Local communities in Benin have demonstrated a great depth of knowledge that enables them identify baobab morphotypes with preferable traits. According to Assogbadjo et al. (2008), local communities use a matrix of 21 criteria to differentiate baobab morphotypes in Benin. The matrix criteria relate to leaf, fruit, bark and tree traits. The most distinguishing characteristics by farmers are: leaf taste, pulp taste, capsule size and shape, ease of bark harvest, and fertility of trees, even if leaf taste was found the dominant characteristic (Assogbadjo et al., 2008). According to Assogbadjo et al. (2008), farmers use this combination of traits as a guide when collecting baobab products. For instance, farmers could tell the taste of the pulp and leaves from the ease of bark harvest (ease of bark harvest = tasty pulp and leaves). Likewise, the taste of the pulp could be guaged from its texture (slimy pulp = tasty pulp), and from the orientation and arrangement of the fruit capsule (closely longitudinally marked fruit capsules = tasty pulp). Following this folk classification, eight morphotypes were identified in Benin, although a follow-up genetic fingerprinting analysis did not correlate with the traditional morphological identification (Assogbadjo et al., 2009). It is suggested that the traits used in folk classification could be influenced by environmental factors, hence the differences in the findings between the two studies (Assogbadjo et al., 2009).

Interestingly, somewhat similar desirable traits and selection criteria have been reported among the farmers of the Blue Nile State and North Kordofan State in Sudan (Gurashi et al., 2017), probably further indicating long historical use and sharing of indigenous knowledge associated with the widely distributed and important species among rural communities. The fact that there are morphotypes with desirable traits to local communities, presents a springboard for participatory selection and domestication of baobab, so as to increase access to, and availability of, baobab resources.

Moreover, it is well-documented that the likelihood of success for conservation activities is high if they: (1) build on the local knowledge and cultural context (Waylen et al., 2010); (2) foster the daily livelihoods of local communities (Teuea and Nakamura, 2020); and (3) support for choice of the right species for given environmental conditions and established purposes (Kettle et al., 2020). Indeed, exclusion and non-active participation of local communities in decision-making processes are among the many factors that have contributed to the failure of laudable efforts in agroforestry programmes in many African countries (Leakey et al., 2012; Leakey, 2017, 2019), with most of the efforts failing to go beyond academic work (laboratory or field experiments). To reset the agro-ecosystems in Africa and achieve food and nutrition security, it is believed that “Cinderella” species, selected based on the local community criteria (Leakey, 2019), should be enhanced through participatory approaches (Leakey, 2017) and that science ought to embrace the needs and aspirations of smallholder famers (Nature, 2020).

Wide Genetic and Morphometric Variations Are Found Within Baobab Populations Across Climatic Zones of Benin

Studies have confirmed the presence of high genetic and morphological diversity within baobab populations in Benin. Assogbadjo et al. (2006) showed that baobabs in the country are highly polymorphic (78.34% polymorphism), with 82.37% of the total variation within populations and 17.63% among populations ($P < 0.001$). Genetic diversity is higher within than between populations, with the mean gene diversity within population ($HS = 0.309 \pm 0.000$) and average gene diversity between population ($DST = 0.045 \pm 0.072$). The authors further identified tree height, number of branches and capsule thickness as the three most distinguishing morphological traits. Related to productivity with respect to ecological zones, the Sudano-Guinean Zone is the most productive, producing the highest yield of pulp, seeds and kernels followed by the Sudanian Zone and lastly the Guinean Zone (Assogbadjo et al., 2006; Table 7, p. 827). Similar findings were made in an earlier study (Assogbadjo et al., 2005b; Table 2, p. 53).

Trait variations between baobab provenances have been reported elsewhere in Africa (Munthali et al., 2012, 2013; Wiehle et al., 2014), and these may be due to physical isolation and the resulting genetic structuring (Assogbadjo et al., 2006). Given that morphological diversity and genetic diversity are not completely correlated with each other (Assogbadjo et al., 2008), it was suggested that variations in baobab phenotypic traits are a consequence of environmental factors (Assogbadjo et al., 2011). Provenance variations due to environmental factors such as water stress have also been implicated in other wild fruit species such as *Sclerocarya birrea* (Jama et al., 2008).

The variability of both genetic and morphological parameters, especially capsule production in various climatic zones, imply availability of adequate precursor material for selection of elite trees of baobab to address the needs of the rural populations in Benin and Africa. The findings also provide a strong argument for *in situ* conservation of baobab populations to preserve the genetic integrity of the species for posterity.

Baobabs in Benin Have Wide and Dense Food Properties in Pulp, Leaves and Seeds

That baobab is nutritious is no longer questionable. Several studies have underscored this property in baobab (Sidibe and Williams, 2002; Chadare et al., 2009; Habte and Krawinkel, 2018). Probably, what is contentious is the variability of the nutritive properties (Habte and Krawinkel, 2018), which seems to be influenced by a number of factors including genetic and environmental factors, although more evidence is pointing towards edaphic physicochemical properties (Assogbadjo et al., 2012).

According to Assogbadjo et al. (2012), the dominant soils in Benin are rich in carbon, clay, fine silt and organic matter, and these correlate positively with the concentration of iron, potassium, vitamin C, carbohydrates, zinc, proteins and lipids in baobab. Further, the concentrations of proteins, lipids, carbohydrates and fibres in baobab parts do not vary among the three climatic zones in Benin. Furthermore, baobab seeds contain high levels of proteins and lipids but low levels of carbohydrates and fibres, with seeds containing 11 times and two times more proteins, respectively, than the pulp and leaves. In addition, seeds are ∼three times lower than pulp and leaves in carbohydrate concentration (Assogbadjo et al., 2012; Table 1, p. 96). The authors showed this by sampling baobab parts (pulp, leaves, seeds) from genetically different populations and soils in the three different climatic zones of Benin.

Regarding climatic zones, the authors reported that baobab pulp and leaves from the Guinean zone were relatively rich in iron, potassium, vitamin C, carbohydrates but had a low concentration of magnesium. It should be noted that pulp and leaves were taken from baobab trees that were established on soils rich in carbon, clay, fine silt, organic matter, with a high pH water and C/N ratio, but a low concentration in gross silt (Assogbadjo et al., 2012; Table 4; Figure 1, p. 98). On the other hand, pulp and leaves of the baobabs from the Sudanian and Sudano-Guinean zones were reported relatively rich in calcium and vitamin A, but had a low concentration of zinc, proteins and lipids. Samples for these came from baobab trees growing on soils with high levels of gross silt but poor in carbon, clay, fine silt, and having low pH water and C/N ratio (Assogbadjo et al., 2012; Table 4; Figure 1, p. 98).

In a follow-up study, Chadare et al. (2014) assessed mineral and carotenoids contents of different categories of baobab leaves and their *in vitro* solubility towards cooking. Calcium levels in raw leaves ranged from 1,371 (slimy, young) to 3,310 mg/100 g dm (bitter, old). The iron content of different types of raw young and old sweet baobab leaves was quite similar (9–10 mg/100 g dm). However, raw, bitter leaves contained a higher quantity of iron (16 mg/100 g dm). The zinc contents of different types of baobab leaves were lower than those of iron. Zinc in bitter leaves was lower than in sweet leaves, which contained 3–5 mg/100 g dm (Chadare et al., 2014; Table 2, p. 11). The main carotenoids detected in baobab leaves were lutein, trans-, and cis (1 and 2)—betacarotene (Chadare et al., 2014; Table 3, p. 14). Chadare et al. (2009) suggested that the variations in nutritional value and biochemical composition may be due to a number of factors. These are: the quality of the sample (mixture of samples, or samples obtained from markets or samples from individual trees), sample provenance, sample age, treatment and analytical methods, the storage conditions, the processing method, genetic variation, and the soil structure and its chemical composition.

The biochemical and nutritional trait variations in baobab based on plant tissue and place of origin have been reported from different countries across Africa by a number of authors (Parkouda et al., 2012; Muthai et al., 2017; Braca et al., 2018). For instance, in their pan-African study involving 17 baobab provenances from six countries (Kenya, Malawi, Mali, Tanzania, Zambia and Zimbabwe), Muthai et al. (2017) found marked variations in pulp moisture, protein, fibre, ash and elemental content among the studied provenances, although provenance variations at national level were also found. For example, regarding crude protein, the West African provenances had the lowest contents while the East African provenances had the highest (Muthai et al., 2017). This compelled the authors to suggest the influence of soil other than climate alone, since Mali (West Africa) is characterised by soils that are poor in nitrogen, nitrates, or nitrites which essentially make up proteins in plant tissues (Muthai et al., 2017).

These results confirmed the earlier suggestion by Assogbadjo et al. (2012) that the biochemical composition of baobab tissues in Benin may be a reflection of the microsite soil properties on which the species are found, which, according to Muthai et al. (2017), is dependent on a number of factors including resource mobilisation, translocation and redistribution within the tree species as influenced by local environmental factors. It can also be argued that this is an interactive process between genetics of the species and climatic conditions resulting into phenotypes with varying capabilities of mining available nutrients for their metabolic processes, which may further explain the observed within-population variations in the biochemical compositions. The influence of soil properties on the nutritional traits of plants has also been reported in other indigenous fruit trees such as tamarind (*Tamarindus indica* L.) in Mali where marked genetic variation was also observed (van den Bilcke et al., 2014).

Recognising the influence of environmental factors such as edaphic characteristics on nutritive value can aid in selecting not only super plus trees for breeding (van den Bilcke et al., 2014), but also sites for cultivation and domestication. This is a critical step towards enhanced research and development for the expansion (increasing accessibility and availability) and utilisation of orphan species like baobab. For instance, to increase utilisation of iron and vitamin C in Benin, Assogbadjo et al. (2012) recommended the use of baobab populations from the Guinean zone, whereas those from the Sudanian Zone or Sudano-Guinean Zone should be targeted for calcium and Vitamin A utilisation.

The combined richness of iron and Vitamin C in the morphotypes from the Guinean zone presents an interesting opportunity for the utilisation of baobab, as it provides a double punch to the fight against malnutrition. Consumption of Vitamin C enhances iron bioavailability, and in this regard, baobab Vitamin C could improve non-heme iron absorption in the most vulnerable populations (Evang et al., 2020).

Baobab Is Revered by Local Communities and Is a Biocultural Keystone Species in the Country

A biocultural keystone species is a species which has disproportionally critical roles in local cultures (Shackleton et al., 2018). Culture identifies a group of people, and this is often reflected in the utilisation of food products (Bedigian, 2018; De Caluwe and Van Damme, 2011). Baobab plays a crucial role in the food culture of Benin, as it is used in the daily diets, influenced by age, gender and ethnic tribes (Codjia et al., 2003; De Caluwé et al., 2009).

In Benin, Chadare et al. (2008) identified 35 baobab food products processed from leaves, pulp, seeds and kernels (**Figures 2A–D**). The products have varying cultural importance and they include dough, gruel, drinks, sauces, snacks and flavouring agents. Local communities also ferment some products e.g., *Dikouanyouri* from seeds ($pH = 6.5$), *Tayohounta* from kernels ($pH = 7$), and *Mutchayan* from pulp ($pH = 4.2$). The authors reported that fermentation in *Dikouanyouri* is induced by *Bacillus* spp. (8.5 Log cfu/g), so too is *Tayohounta* (9.5 Log cfu/g), but *Mutchayan* is induced by lactic acid bacteria (8.1 Log cfu/g) and yeasts (7.2 Log cfu/g). While *Dikouanyouri* and *Tayohounta* are used as snacks and flavouring agents in sauces or drinks, *Mutchayan* is used as a beverage and main dish (Chadare et al., 2008, 2010).

FIGURE 2 | (A–D) Traditionally processed baobab products: pulp powder **(A)** leaf powder **(B)** *Tayohounta* from kernels **(C)** and *Mutchayan* from pulp **(D)**.

The wide range of baobab products, as well as the associated knowledge and practises, provide an opportunity for research and development including technologies aimed at increasing access, availability and utilisation of baobab products in Benin and Africa. Further, premised on these findings, enhanced agro-technologies can be used to add value to the local products and minimise post-harvest handling losses, thus increasing access and availability of baobab food products. Besides, it is a considered view that the cultural context and existing local values of a particular species are effective springboards for collaboration between local communities and scientists (Waylen et al., 2010; Kettle et al., 2020). This is particularly important for the success and sustainability of research and development projects targeting the species upon which the communities rely for their livelihoods (Waylen et al., 2010; Allendorf et al., 2018).

Background Information on the Baobab Value Chain Is Available for Benin

Relating to non-timber forest products, Jensen (2009) considers a value chain analysis as a process of tracing the information flow from harvesting to consumers, aiding in clarifying the dynamics in the product value chain especially in light of commercialisation. The author further states that the principal aim of a value chain analysis in this regard is to elicit patterns of value addition and identify actors and domains of value-appropriation while showing responses to increased harvesting and scarcity of the resource. Indeed, value chain analyses help identify opportunities and constraints for the industry upon which recommendations could be drawn for various actors including decision makers to optimise the market and its enabling environment (Jäckering et al., 2019).

Accordingly, De Caluwe and Van Damme (2011) investigated the value chain of baobab by conducting interviews with stakeholders along the value chain in Benin and Mali. As is common with other countries such as Kenya (Jäckering et al., 2019), baobab products in Benin are harvested by smallholder farmers, mostly women, using traditional harvesting techniques which require low-cost tools and manual labour. Further, baobab products are made from low-cost and simple technologies, and are mostly sold at farm gates by women, contributing to an estimated 11% of the total income of the poorest household (De Caluwe and Van Damme, 2011). This implies that the baobab market is at a smallholder scale level in Benin. Further, it suggests that in addition to household consumption, baobab products help smallholder farmers earn considerable income which can help them diversify food products, thus enhancing access to food. The use of income generated from sales of baobab products to diversify food for households is common in Africa (Venter and Witkowski, 2013b; Wanjeri et al., 2020). In Nigeria alone, Onyekwelu et al. (2015) estimated that households could earn between US$ 300 and US$ 1,300 (representing 20–60% of annual household income varying along the value chain) from the sale of indigenous fruit tree products.

The high participation of women in the baobab value chain has also been recently reported in Kenya (Jäckering et al., 2019), where 72% of the respondents in the value chain analysis were women. The fact that women make the majority of the stakeholders in the baobab value chain and other indeginous fruit trees (Sanou et al., 2019a), offers an opportunity for enhancing women economic emancipation and reducing social inequalities in Benin and Africa, thereby contributing to the UN Sustainable Development Goal 5 (gender inequality) and Goal 10 (reduced inequalities), respectively.

Recent studies from Kenya (Jäckering et al., 2019) and Malawi (Darr et al., 2020) have shown that an improved baobab value chain could raise baobab products from the status of neglected and underutilised to being invaluable and premium–priced products in Africa and beyond. Through value addition, product diversification, good packaging and storage, wastage could substantially be reduced, thereby improving the accessibility, availability, utilisation and sustainability. Likewise, the baobab value chain in Benin also appears to be improving in the recent years, especially in processing and packaging, as evidenced by an influx of value-added products such as juices in the formal and informal markets.

Baobab Is Threatened by Climate Change but Is Amenable to Domestication

The need to domesticate baobab spans several years, and Gebauer et al. (2016) summarised a number of studies that are in agreement with this cause. The need arises from the increasing threats on baobab resources including wild animals and land use change such as forest clearing for agriculture (Wickens, 2007), commercialisation (Buchmann et al., 2010), as well as climate change (Sanchez et al., 2011; Birhane et al., 2020). These are expected to negatively affect accessibility, availability and sustainability of baobab resources.

According to Cuni Sanchez et al. (2011), only 5 to 48% of the current suitable habitat for baobab in West Africa would be suitable under future climatic conditions (c.f. 27 to 69% for East Africa), with Benin showing a marked contraction towards the south (Cuni Sanchez et al., 2011; Figure 2, p. 238). Further, no single current protected area in Benin is anticipated to host baobab in the future distributions (Cuni Sanchez et al., 2011; Table 3, p. 241). The wide range (5–48 %) and the marked variations between West and East African baobab populations may be an indication of how variable baobab populations will respond to climate change in West Africa and across Africa. Recent findings from East Africa of contractions in the baobab populations (41–100%) (Birhane et al., 2020), further support the evidence for baobab vulnerability to climate change. It is thus pertinent that conservation planning and utilisation strategies take these findings into consideration for the sustainability of baobab genetic resources.

Given that local communities in Benin regard baobab in high esteem, such that incipient management seems to be protecting baobab on farms (Assogbadjo et al., 2005a), provides an opportunity for domestication. This requires propagation materials, and different techniques exist (Sidibe and Williams, 2002; Verheij, 2006), which can be verified under local conditions. Accordingly, trials investigating the adaptable response of baobab populations to some of these techniques have been done in Benin. These include seed germination and vegetative propagation (e.g., grafting, stem cuttings, layering) (Assogbadjo et al., 2011).

Baobab seeds could be sown without scarification, as non-scarified seeds had registered the best germination rate (57%) by day 25 from the date of sowing, particularly from the Guinean zone (Assogbadjo et al., 2011). The authors recommended seed germination on sand substrate first before transferring the seedlings to the substrate with organic matter for further growth. The authors further recommended use of autochthonous seeds within specific climatic zones, as one measure towards maintaining genetic diversity.

Trials on vegetative propagation (Anjarwalla et al., 2017; Jenya et al., 2018), a review study on the same (Agbohessou et al., 2020) and use of seeds to produce edible root tubers (Jansen et al., 2020), all provide evidence of baobab amenability to domestication. These findings may be useful in the multiplication and domestication programmes of baobab to increase access to, and availability of baobab resources, thereby promoting sustainability of the baobab wild populations.

Besides, unlike seed propagation, vegetative propagation has advantages of reducing the fruiting delay in baobab from more than 10 years in the natural stands to <five years (Anjarwalla et al., 2017), and maintains valuable traits of the mother tree (Awodoyin et al., 2015). It is believed that improvements in earlier fruiting will create incentives to cultivate indigenous fruit

trees such as baobab (Omotayo and Aremu, 2020), since this will provide early rewards and therefore reduce investment costs on the part of the farmer (Mwase et al., 2015).

Baobab Productivity Is Improved by Fruit Bats

Baobab is a sexually reproducing species. Therefore, the importance of pollination ecology cannot be overemphasised: no pollination, no fruits! Thus, understanding what pollinates baobab is critical for the conservation of the genetic diversity of this species, particularly in view of the impacts of land-use change on the spatial distribution of species (Salako et al., 2019). The question regarding the pollination in baobab has eluded researchers for long, with both cross-pollination (fruit bats, wind, moths and hymenopterous insects) and self-pollination implicated (Sidibe and Williams, 2002; Wickens, 2007).

The contribution of fruit bats to pollination success in baobab was investigated in Benin by comparing pollination success in caged and non-caged flowers using freshly opened flowers (Djossa et al., 2015). Caging was by small nets that excluded bats, and fruit bat flower visitation was monitored for 2–8 weeks. The study was conducted in three sites: Comé-Houéyogbé district (Guinean Zone), Dassa district (Sudano-Guinean Zone) and Matéri district (Sudanian Zone). According to the authors, Dassa had the lowest visitation rate, suggesting low fruit bat abundance in the area, and non-caged flowers had higher pollination success than caged flowers (Djossa et al., 2015; Figure 5, p. 286). Since fruit-set and production were observed in both treatments, the authors did not rule out self-pollination and the involvement of other agents (sphingid moth species). Moreover, caged flowers showed a tendency to higher fruit abortion, compelling the researchers to suggest self-incompatibility owing to limited pollination success as a result of caging (Djossa et al., 2015).

Surprisingly, pollination success rates were the same among the populations, in spite of the observed significant differences in visitation rates (Djossa et al., 2015). This further compelled the authors to suggest probable differences in pollination efficiency by the fruit bats, with those of Dassa (lowest visitation rate) considered the most efficient. The involvement of fruit bats as pollinating agents and high fruit abortion rates in baobab has been reported elsewhere on the continent (Munthali et al., 2012; Taylor et al., 2020).

Although Djossa et al. (2015) did not solve the mystery surrounding pollination in baobab, but their findings suggest that fruit bats ought to be given space in the landscape for the conservation and sustainable utilisation of baobab genetic resources.

CHALLENGES AND CONSTRAINTS

In spite of the registered successes, there are a number of factors that are constraining the exploitation of the full potential of baobab to contribute to improved food and nutrition security in Benin. The challenges and constraints are drawn from Benin and also from lessons in other countries across Africa. It should also be noted that the identified factors tend to overlap and influence each other.

Low Consumption

Despite the generation of information on the nutritive values of baobab products (fruit pulp, leaves, seeds, and processed products), there appears to be low consumption levels of the products among other sectors of the society. Prevalence of low consumption of fruits and vegetables below the recommended daily intakes is a persistent phenomenon in developing countries (Okop et al., 2019).

For baobab, this could be due to many factors including: (i) low quality of baobab products (De Caluwe and Van Damme, 2011), which may put off other consumers and result into reduced market potential, both local and international (as discussed in the subsequent section); (ii) cultural beliefs, which may affect consumer preferences and therefore undermine utilisation and markets, as is the case in Kenya, where baobab food products like candies "mabuyu sweets" are considered the poor man's food (Kiprotich et al., 2019); (iii) lack of familiarity with baobab products and/or limited knowledge of preparation as reported in Nigeria (Omotesho et al., 2013) and Kenya (Kiprotich et al., 2019); (iv) negative attitudes towards wild foods and/or low interest in wild fruits (Darr et al., 2020), which may also result from low or limited consumer awareness and understanding of the various benefits of fruit consumption (Borelli et al., 2020); and (v) affordability, which may be influenced by fluctuating prices for baobab products and limited disposable income of consumers, especially in the urban areas, with larger households routinely consuming less baobab products (Kiprotich et al., 2019).

Indeed, according to Jamnadass et al. (2011), the current marketing systems of indigenous fruits are weak, poorly structured and coordinated, and characterised by high fruit prices for urban consumers, thereby limiting availability and accessibility of baobab products to consumers. Low fruit consumption among limited-resource urban consumers has also been reported by different authors (Bvenura and Sivakumar, 2017; Okop et al., 2019).

While affordability may affect consumption among stakeholders other than producers, it is also possible that producers themselves may consume less of baobab products due to high levels of poverty in the rural areas. Farmers may be forced to sell whatever little they harvest of baobab to earn an income, in view of the fact that trading of indigenous fruits or their derivatives is a lucrative enterprise in Africa (Onyekwelu et al., 2015; Omotayo and Aremu, 2020).

Put together, this shows that low consumption, which may arise from various socio-economic traps including cultural beliefs, limited knowledge on the benefits of fruit consumption and poverty, could partly explain why belts that are rich in baobab and other wild fruits are also synonymous with perpetual food and nutrition insecurity (Maruza et al., 2017; Momanyi et al., 2019). It has been shown in Malawi, though, that consumers' negative perceptions of baobab can be overcome by, among other things, proper design of the enterprise development that meets different segments of the consumers, capitalising on the changing preferences for natural, healthy and authentic food products by the urban middle class (Darr et al., 2020). Further, it has been suggested that awareness raising on the potential health and nutritive benefits of baobab could address many other constraints related to consumption, thereby enhancing the market demand for baobab which seems to be fast growing (Buchmann et al., 2010; Jäckering et al., 2019).

Aspects of limited knowledge on the preparation may lower utilisation of baobab products in several ways such as limiting the available dietary micronutrients and bioactive compounds, as well as wastage through poor preparation methods (Tembo et al., 2017). In this regard, Tembo et al. (2017) tested the effect of thermal treatment and storage of selected quality attributes of baobab juice. The study has shown that vitamin C can be retained by thermal pasteurisation (72ºC, 15 s) with an extended shelf-life under refrigeration (6ºC). If incorporated and up-scaled in baobab product development, these findings would go along away in increasing the consumption by way of increasing accessibility and availability of baobab products, especially to the urban populace and for export markets.

An additional aspect of perception, which is often opaque in the literature, is the perceived health risk associated with the consumption of baobab seed oil caused by cyclopropenoid fatty acids (CPFAs) (Msalilwa et al., 2020). This perception has the potential to lower the market demand, especially for the urban and export market (both regional and international) where such issues may become more glaring. Interestingly, using gas-liquid chromatography (GLC) in Tanzania, Msalilwa et al. (2020) found that the major breakdown of CPFAs occurs at 200°C. The authors have thus recommended the refining of baobab crude oil at higher temperatures (200–250ºC) to reduce CPFAs. These findings too, if well-incorporated into the baobab enterprise development, would improve the market base for baobab, thereby increasing utilisation.

Low Quality of Products

Currently, the baobab industry is still largely rudimentary, characterised by low product quality (De Caluwe and Van Damme, 2011; Jäckering et al., 2019). Low product quality affects consumer demand with consequent poor economic returns to the producers. Considering that consumers have different preferences, improving the product quality can be a catalyst for better market development for orphan species like baobab. For instance, in Kenya, Meinhold and Darr (2020) have shown that establishment of community-based enterprises through multi-stakeholder engagement could promote the production of high-quality baobab products such as baobab powder and oil, thereby contributing to enhanced baobab management, increased acceptability by consumers and subsequent increased consumption and improved economic returns to the farmers.

Likewise, the development of vibrant local market chains of baobab in Benin would improve baobab product quality and subsequently provide a fertile ground for regional and international markets (De Caluwe and Van Damme, 2011). Low product quality, which is associated with low acceptability and therefore poor market, is considered a threat to orphan species and has been implicated in the displacement of orphan species by introduced species (Awodoyin et al., 2015).

Inadequate Investment in Formal Research and Development for Baobab Species Improvement

The existing investment in research and development for improvement of orphan species in general, and baobab species in particular, is generally low and almost nil in Benin. As such, most research is done at the mercy of teams of individuals who have dedicated their time to the plight of orphan species. This could be a consequence of local policy failure, or inadequate provision in strategies for orphan species as important species that could help mitigate the challenges of hunger and malnutrition. Currently, the existing food and nutrition regulatory frameworks in Benin such as the Strategic Plan for Development of Food and Nutrition (PSDAN) and Strategic Plan for Agricultural Sector Development (PSDSA) (2016–2021) do not put emphasis on orphan species in the fight against these challenges. As such, the national direction on research for orphan species is hazy and investment for research and development in such species is almost non-existent.

Consequently, harvesting of baobab is still from the wild, the whole value chain is still rudimentary, and the baobab species improvement remains a long, far-fetched dream. Inadequate investment in formal research and development for the improvement of orphan species including baobab is widely considered a challenge for many developing countries (Awodoyin et al., 2015; Marunda et al., 2019; Omotayo and Aremu, 2020). It is not surprising, therefore, that Africa has not yet started benefiting fully from baobab and other indigenous fruit trees. This is because rigorous research and development is key to addressing the numerous knowledge gaps such as those in production and/or harvesting, processing and marketing that constrain the unlocking of potential benefits from indigenous fruit trees (Awodoyin et al., 2015; Omotayo and Aremu, 2020).

Given that trading in indigenous fruits seems a lucrative adventure (Omotayo and Aremu, 2020), one would expect the private sector to invest in the baobab value chain. However, this is not the case for many countries in Africa (Marunda et al., 2019). This could be explained by the inadequacies in the national regulatory frameworks such as nutritional security, livelihood and poverty alleviation strategies that fail to embrace orphan species like baobab. The private sector is profit-oriented and requires an enabling business environment (e.g., stable markets), which is largely dictated by policy provisions. The impact of disabling policies and regulatory frameworks in failing to encourage production and consumption of orphan species through various aspects including limiting investment in research and development has been reported elsewhere (Borelli et al., 2020).

Limited Propagules for Domesticating Baobab

Insufficient and ineffective seed supply systems are some of the key constraints to smallholder agroforestry programmes based on native species like baobab (Kettle et al., 2020). In Africa, inadequate quality tree seedlings available to smallholder farmers is one of the barriers to adopting fruit cultivation

(Takoutsing et al., 2012; Mwase et al., 2015). This constraint is further compounded by an ineffective seed supply and technical backstopping system (Kettle et al., 2020), thereby limiting production and consumption of indigenous fruit products like baobab.

In Benin, for instance, although trials on seedling establishment and selected vegetative propagation techniques for baobab have been done (Assogbadjo et al., 2011), and preferred morphotypes have been identified in a participatory manner with the farmers (Assogbadjo et al., 2008), baobab is yet to be cultivated. This is due to a number of factors. Chief among them are limited investment into orphan species improvement and inadequate policy provision (as earlier discussed), as well as limited availability of quality planting material. However, in the event that planting materials are made available, the lack of a national research agenda on orphan species or indeed non-inclusion of orphan species in the national food security and nutrition strategies would affect the distribution of planting materials and associated extension messages, since such efforts would require major changes in the existing extension services (Sidibe and Williams, 2002). This suggests the need for concerted effort from all stakeholders and political will to multiply and make available planting materials of baobab for cultivation and/or domestication.

In this regard, the use of Agroforestry Rural Resource Centres (RRCs) (Takoutsing et al., 2012) could become useful for baobab. For instance, through RRCs, farmers in the Western Highlands of Cameroon are trained to multiply tree planting materials and also distribute planting materials (Takoutsing et al., 2012; Essougong et al., 2018). The centres also serve as platforms for knowledge exchange, thereby bridging the extension service gap (Takoutsing et al., 2012). According to Takoutsing et al. (2012), the approach has effectively improved the timely dissemination, accessibility, affordability and availability of quality planting materials. Through the RRCs, farmers also network with various stakeholders in commodity value chains such as financial lending institutions (Wouapi et al., 2019), which is important for farmer capital investment into fruit tree production.

The use of RRCs in Benin for baobab could help strengthen the seed system for baobab, through multiplication and distribution, given the current limited public extension service facilities. However, before rolling out the RRCs, it would be wise to conduct a feasibility study and/or pilot study to determine the practicability and acceptability of such innovations in the context of Benin. Such a study would be important in view of the fact that perceptions, preferences and attitudes, which may be influenced by socio-cultural factors, are among the strong determinants for farmer adoption of agroforestry technologies (Sanou et al., 2019b).

Low Production Levels

Since baobab, as for other indigenous fruit trees, is not domesticated, products are harvested from naturally growing and often low yielding trees. This undermines not only the quantity, but also the quality and stability of baobab products to meet the urban consumer demand and export market. It is recognised, though, that production may not increase if demand is low. However, evidence abound that the demand for baobab products is growing (Buchmann et al., 2010; Jäckering et al., 2019), but what is critical is the diversity and quality of products to meet consumer preferences, and market stability to stimulate more demand.

Moreover, collection from the wild is not sustainable considering that wild baobabs in Africa are characterised by low yields (averaging 80 kg fruits, 14 kg pulp, 23 kg seeds and 130 kg leaves annually per tree) (Habte and Krawinkel, 2018), with some trees consistently being very poor fruit producers (Venter and Witkowski, 2019), thus putting into question the baobab market stability.

Several factors have been suggested for the low productivity in baobabs, these include: old age for most trees (Sanchez, 2011); low pollination rates in baobab (Venter et al., 2017) mainly due to rarity of pollinators (Venter et al., 2017; Taylor et al., 2020), owing to sparse distribution of baobab as a result of land-use changes (Assogbadjo et al., 2005a; Venter and Witkowski, 2010); and high rates of fruit abortion (Djossa et al., 2015) due to maternal regulation (Verheij, 2006). Besides, high rates of predation (>85% loss) of young fruits by wild animals have also been reported (Venter and Witkowski, 2011), thus further compounding the low availability of harvestable fruits. All these put together, support the need for cultivation and domestication of baobab to increase production levels so as to meet the increasing urban and export market demand.

However, as pointed out earlier, baobab domestication is still at infancy in Africa. In Benin, for instance, in a study to investigate the local communities' motivations for plant domestication (Vodouhè and Dansi, 2012), reported that domestication of baobab was at stage two—naturally growing wild species maintained in the fields during land preparation. Therefore, cultivation could improve the production levels and somehow stabilise the market. It may also improve quality standards right from the production phase of the baobab value chain, thereby stimulating demand for baobab products.

To increase production through cultivation would require planting materials, which are currently inadequate (as discussed herein above). Moreover, poor farm management practises is one of the constraints to smallholder farmers' involvement in fruit cultivation in the SSA (Jamnadass et al., 2011). Again, RRCs could help in this case. Through RRCs, baobab farmers could gain technical skills in baobab cultivation, postharvest handling and marketing, so as to improve the availability and stability of baobab products even for export market.

The institutional organisation of farmers could also strengthen the farmers' bargaining power, which is currently weak owing to the weak marketing systems which disproportionate farmers with low farm- gate prices (Jamnadass et al., 2011). Consequently, farmers are unable to invest in fruit production because they cannot afford the high input investment costs which may unlikely be recouped (Jamnadass et al., 2011). However, as suggested herein above, a feasibility study through participatory approaches ought to be done to determine the practicability and acceptability of RRCs in this regard.

NEXT STEPS

Increase Awareness on the Health Benefits and Nutritive Values of Baobab

Attitudes have a long history in the use of wild food, and knowledge can play a crucial role in attitudinal change (Bvenura and Sivakumar, 2017). In this regard, increasing evidence of the nutritive value of orphan species like baobab can eventually lead not only to access (Borelli et al., 2020), but also proper utilisation, thereby helping address food and nutrition insecurity.

Malnutrition is a global public health concern, with global undernutrition as manifested in childhood stunting, wasting and underweight estimated at over one million deaths per year, and Africa having comparably the highest rates (Ssentongo et al., 2021). In Benin, anaemia is estimated at 72 % in children under the age of 6–59 months, and nearly 58% in women of reproduction age; 5% of the children under 5 years suffer from wasting; stunting is at 7.4%, whereas 12.4% of the population is diabetic (Hounkpatin et al., 2020). Therefore, raising the profile of nutritious dense plant species like baobab may enhance utilisation, consequently contributing to the fight against micro-nutrient deficiencies in Benin and Africa. This could be done by way of increasing awareness to all stakeholders in the baobab value chain including consumers and decision makers using evidence-based information. Recently, Kiprotich et al. (2019) found awareness levels had positive influence on the utilisation of baobab in Kenya. The need for increased public awareness to enhance the utilisation of orphan species for improved food and nutrition security in Africa has been echoed by many other scholars (Sogbohossou et al., 2018; Borelli et al., 2020).

Further, appropriate packaging of nutritive benefits from baobab targeting decision-makers may facilitate inclusion of baobab and other orphan species in relevant national policies and/or strategies as species of importance for ameliorating food and nutrition insecurity. Moreover, putting orphan species on the national agenda is a strategy many countries like Brazil, Kenya, Sri Lanka and Turkey are keenly pursing (Hunter et al., 2019) and lessons can be learnt from such countries.

Furthermore, increasing awareness to relevant decision makers including government and private sectors may also foster improved investment by way of financial investment, technological development and market creation. This would subsequently lead to improved research and development in the area of orphan species such as baobab, ultimately leading to increased accessibility, availability, utilisation and sustainability of agrobiodiversity for improved food and nutrition security.

Create Markets for Orphan Species

Deliberate policies ought to be made by the public and private sector to create markets for orphan species, which are already playing critical roles in rural livelihoods and food and nutrition security. Apart from access and availability, markets can help smallholder farmers earn income and motivate them to domesticate orphan species like baobab as is the case with "mainstream" crops (Borelli et al., 2020). One option for policy consideration in the market creation could be to use baobab as a novel species to help reduce iron deficiency in Benin and Africa, a challenge which is currently a public health concern in the view of the alarming prevalence rates (Hounkpatin et al., 2020). Consideration may be given to use some baobab food products in school feeding programmes, and as mandatory nutrient supplements for pregnant and lactating women.

Mandatory use of local agrobiodiversity in national feeding programmes like School feeding Programme has shown promising outcomes in Brazil (Wittman and Blesh, 2017; Resque et al., 2019), where government has decreed the use and public procurement of local agrobiodiversity in the national feeding programmes. Benin can borrow a leaf from these developments. Besides, this approach has triple effects: addressing malnutrition, improving income and livelihoods of smallholder farmers, and conservation of local agro-biodiversity for posterity.

Currently, Benin has the National Integrated School Feeding Programme and the Coordinated Early Childhood Nutrition and Development Project as the main interventions through which the government is striving to address malnutrition (Hounkpatin et al., 2020). According to Hounkpatin et al. (2020), these initiatives are showing positive outcomes this far. Therefore, incorporating baobab products as mandatory supplements in these programmes could go a long way in the fight against malnutrition, as the race against time to achieving Zero Hunger (Goal 2) of the 2030 Sustainable Development Goals is also becoming intense.

However, deciding the appropriate recipes and product formulations for both acceptability and positive outcomes will be some of the key aspects for consideration in such feeding programmes. It is apparent, though, that most African meals are cereal-based, and therefore baobab fruit pulp could enhance provitamin A carotenoid bioavailability in cereal formulations (Debelo et al., 2020).

Enhance Technological Development

Enhanced technologies could help improve the baobab value chain, considering that currently it is mostly rudimentary and at the smallholder level across Africa (De Caluwe and Van Damme, 2011; Jäckering et al., 2019), thus negatively impacting access, availability, utilisation and sustainability. The technologies and innovations may be critical to unlock and harness the potential benefits of baobab as these may be important in reducing most of the constraints currently faced along the value chain from production, harvesting, processing, storage, to research and development.

The technologies and innovations may include: (i) genes and crop development technologies, such as those that can facilitate integration of baobab into the production systems, and reduce farm labour requirements (Dawson et al., 2019); (ii) efficient and effective processing technologies, such as those that can help retain nutrients, enhance nutrient-bioaccessibility, and value addition technologies—those that lead to the development of high-quality food products in line with consumer demand; (iii) storage technologies, such as those that can increase the shelf life; and (iv) organisational and marketing innovations, such as traceability of raw materials along value chains. However, for adoptability, farmer input into the designs of the various technologies and innovations should be sought and gender dimensions ought to be considered (wherever applicable).

Scale-Out Multiplication of Planting Materials for Cultivation Programmes

Several studies have indicated the need to cultivate and domesticate baobab (Gebauer et al., 2016). The ultimate aim is to increase access and availability, as well as to promote sustainability of baobab resources which are currently threatened in the wild. However, the agroforestry adoption rate is currently low across Africa (Mwase et al., 2015; Sanou et al., 2019b). Various factors for this trend have been reported including gender and household farm-size (Mwase et al., 2015; Sanou et al., 2019b), local community needs and preferences influenced by local traditional practises and cultural context (Waylen et al., 2010; Poole et al., 2016), as well as inadequate planting material (Mwase et al., 2015). Therefore, to ensure adoptability and sustainability of baobab, multiplication and domestication programmes should consider these dynamics, and should build on the existing local decision-making tools (Kettle et al., 2020).

Incipient management of baobab is already high among communities in Benin. Similarly, farmers in other countries such as Niger have shown high preference for agroforestry systems based on baobab species (Agúndez et al., 2020), thus providing potential for adoptability of baobab cultivation programmes that would eventually require planting materials. As stated earlier, RRCs as used in Cameroon could become useful platforms for multiplication and vehicles for distribution of baobab planting materials in Benin to scale-out cultivation of the species. They could also be used for awareness raising on various ecosystem services that could be accrued from incorporating baobab as an agroforestry tree, thereby increasing the adoption rate, eventually contributing to increased availability and accessibility of baobab.

Address Research Gaps in the Reproductive Ecology, Sustainable Harvesting, and Effect of Drought Stress on Quality of Baobab

From the foregoing, it is apparent that there are a couple of research gaps in baobab to enhance its sustainable utilisation for improved food and nutrition security. For instance, although fruit bats are coming out strongly as pollinating agents of the baobab (Sidibe and Williams, 2002; Djossa et al., 2015; Taylor et al., 2020), but the role of arthropods such as hawk moths remains a mystery (Sidibe and Williams, 2002; Taylor et al., 2020), and the general knowledge of the baobab reproductive biology including mating systems is evidently lacking (Venter and Witkowski, 2019). A clear understanding of the dynamics of pollination/reproduction ecology in baobab is critical for the design and planning of conservation programmes for the sustainability of baobab resources. Some of the research questions in this regard may include: (1) Do baobabs have mixed mating systems which are site specific? (2) What is the degree of fertility in baobab populations; (3) What percentage of ovules in baobab fruit can be attributed to lack of fertilisation, abortion and pre-dispersal seed predation?

Research on sustainable harvesting (leaves and fruits in this context) is also an important area for the sustainable utilisation of baobab. For instance, leaf harvesting, according to Dhillion and Gustad (2004) causes mutilation in baobab, consequently resulting in fruit reduction; whereas, fruit harvesting impacts dispersal and establishment (Shackleton et al., 2018). Similarly, while baobab is generally considered drought tolerant (Sidibe and Williams, 2002), but there are variations between populations (Cuni Sanchez et al., 2010), and poor seedling establishment due to infrequent rainfall has been reported in South Africa (Venter and Witkowski, 2013a), suggesting baobab vulnerability to the looming reduced precipitation (Dai, 2013). Understanding the impact of drought stress on the quality of pulp and leaves will therefore aid in planning for breeding programmes for enhanced access, availability and sustainability. In this regard, answers are needed to these questions: (1) What are the seasonal variations in the yield of baobab leaves and pulp across ecological climatic zones? (2) What quantities can be considered sustainable harvest, and how could these be achieved? (3) How does drought stress affect the biochemical and nutritional composition of baobab pulp and leaves?

To reduce competition for land in the agro-ecosystems, given the small household land holding size in Benin (<2 ha) (Adjimoti, 2018), investigations on how exactly baobab fits into these agro-ecosystems would be important. For instance, it would be important to know: (1) Which crops compete with baobab, and which ones are either outcompeted or facilitated by its presence? (2) What are the appropriate planting densities that would allow both the establishment of viable baobab populations and ecosystem services enhancement on farm? Further, given that baobab has variant tree shapes (Gebauer et al., 2016) and local communities use a matrix of criteria to identify baobab morphotypes with preferred qualities (Assogbadjo et al., 2008), it would also be worth investigating the correlation between these morphotypes (e.g., tree shape) with the preferred qualities of baobab leaves and fruit (Rashford, 2018).

Baobab Breeding

Cultivation using seedlings (to increase variability) and vegetative or clonal material (to maintain the preferred traits) may serve as a short-and medium-term strategy to enhance access to, and availability of baobab resources. Whereas, breeding for improvement should be considered as a long-term strategy. Since fruits, leaves and seeds are used for food and nutrition, a national research agenda ought to be made: whether the breeding programme should focus on leaves or fruits, or both. In this regard, Ræbild et al. (2011) recommended the testing, selection of super plus material and multiplication of young baobab plants for leaf production breeding programmes, and the testing and evaluation of mature trees for fruit character breeding. Considering that baobab pulp has more export potential and leaves are more important for the daily household needs in West Africa (Jensen et al., 2011), and given the existing variations in the cultural importance of leaves and fruit products in Benin and West Africa (De Caluwé et al., 2009; Buchmann et al., 2010; De Caluwe and Van Damme, 2011), it would be wise if both approaches were pursued to meet the diverse needs and market demands.

In either case, breeding focusing on reducing the maturity period, tree size (dwarfism) and yield increase is recommended. The huge size of baobab is a limiting factor not only for management (Awodoyin et al., 2015), but also access to baobab resources (fruits, seeds and leaves), as harvesting is usually difficult from very tall trees, and often leads to poor quality harvest (Verheij, 2006).

It is evident that in Africa, unlike in other continents, smallholder farmers lack better practises for fruit tree management on farm (Jamnadass et al., 2011) and indigenous fruit tree improvement programmes are either inadequate for some species or non-existent for others. Perhaps this is because for a long time, research efforts on orphan plant species have mostly been related to species ecology and biology, genetics, ethnobotany, and chemical/nutrient composition. It is only until the late 1980s that researchers began to seriously consider elements of domestication and plant breeding (Jamnadass et al., 2011), and more recently commercialisation (i.e., markets, products, value chains) (e.g., De Caluwe and Van Damme, 2011; Jäckering et al., 2019). This might explain why Africa is where it is now with regard to indigenous fruit tree cultivation and/or domestication and enterprise development.

Encouragingly, laudable efforts are being made by the African Orphan Crops Consortium, which is currently empowering African plant breeders in the use of advanced genetic approaches to tailor-breed orphan species including baobab to the needs of the local producers, processors and consumers in Africa and beyond (Jamnadass et al., 2020). These efforts dovetail nicely with the aspirations for baobab breeding in as far as this study is concerned.

CONCLUSIONS

As for many other orphan species, research on baobab has for many years focused on its biology, genetics, ecology, ethnobotany, propagation, and chemical and/or nutrition composition. Commercialization, domestication and plant breeding of the species have, until recently lagged behind, largely due to inadequate capacity in these subject areas among African scientists. This has undermined baobab product development, subsequently limiting accessibility to, and availability, utilisation and sustainability of baobab resources. Encouragingly, capacity has increasingly grown over the past decade, thanks to the support from cooperating partners such as the World Agroforestry Centre (ICRAF) and many others.

To date, substantial information has been generated and practical solutions along these subject areas have been developed that could inform decision making regarding unlocking and harnessing the potential benefits of baobab for improved food and nutrition security in Benin and Africa. The use of evidence-based information to unlock the potential values of orphan species is considered paramount, as this can help change attitudes regarding orphan species across all divides of stakeholders (Borelli et al., 2020).

Various factors constrain the full potential of baobab to contribute to improved food and nutrition security. These include low consumption, low production levels, and inadequate investment in research and development for baobab species domestication and improvement. These are essentially limiting accessibility to, and availability, utilisation and sustainability of baobab resources. There is need, therefore, to increase awareness on the nutritive values of baobab, enhance domestication and technological development considering gender dimensions, define the national research agenda on the role of orphan species in nutrition, and increase investment in research and development. Practical solutions from Cameroon and Brazil that could promote the cultivation and consumption of baobab, are worth emulating going forward.

To enhance and unlock the potential benefits of orphan species like baobab to contribute to improved food and nutrition security requires active participation of all stakeholders along the product value chain including farmers, researchers and policy makers. Indeed, with stronger synergies among interested stakeholders and better connection between science and policy (Hunter et al., 2019), orphan species such as baobab could transform the food systems and livelihoods in Africa.

AUTHOR CONTRIBUTIONS

AA and LM conceived the idea. AA designed the methodology and manuscript outline and gathered the publications. LM drafted the manuscript. FC and BS critically reviewed the manuscript. All authors read and approved the submitted version.

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REVIEW
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Profiling Cultivars Development in Kersting's Groundnut [*Macrotyloma geocarpum* (Harms) Maréchal and Baudet] for Improved Yield, Higher Nutrient Content, and Adaptation to Current and Future Climates

Mariam Coulibaly [1,2], Guillaume Bodjrenou [1], Félicien Akohoue [1], Eric Etchikinto Agoyi [3], Fustelle Michée Merinosy Francisco [1], Chaldia O. A. Agossou [1], Mahamadou Sawadogo [2] and Enoch G. Achigan-Dako [1]*

[1] Laboratory of Genetics, Biotechnology and Seed Science (GBioS), Faculty of Agronomic Sciences, University of Abomey-Calavi, Abomey-Calavi, Benin, [2] Laboratory of Biosciences, Faculty of Earth and Life Science, University of Ouaga I Pr. Joseph Ki-Zerbo, Ouagadougou, Burkina Faso, [3] Non-timber Forest Products and Orphan Crop Species Unit, Laboratory of Applied Ecology, Faculty of Agronomic Sciences, University of Abomey-Calavi, Abomey-Calavi, Benin

Edited by:
Avtar Krishan Handa,
Purdue University, United States

Reviewed by:
Shivali Sharma,
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India
A. Amarender Reddy,
National Institute of Agricultural Extension Management (MANAGE), India
Tendai Polite Chibarabada,
WaterNet Trust, Botswana

***Correspondence:**
Enoch G. Achigan-Dako
e.adako@gmail.com

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Kersting's groundnut [*Macrotyloma geocarpum* (Harms.) Maréchal and Baudet], Fabaceae, is an important source of protein and essential amino acids. As a grain legume species, it also contributes to improving soil fertility through symbiotic nitrogen fixation. However, the crop is characterized by a relatively low yield (≤500 kg/ha), and limited progress has been made so far, toward the development of high-yielding cultivars that can enhance and sustain its productivity. Recently, there was an increased interest in alleviating the burdens related to Kersting's groundnut (KG) cultivation through the development of improved varieties. Preliminary investigations assembled germplasms from various producing countries. In-depth ethnobotanical studies and insightful investigation on the reproductive biology of the species were undertaken alongside morphological, biochemical, and molecular characterizations. Those studies revealed a narrow genetic base for KG. In addition, the self-pollinating nature of its flowers prevents cross-hybridization and represents a major barrier limiting the broadening of the genetic basis. Therefore, the development of a research pipeline to address the bottlenecks specific to KG is a prerequisite for the successful expansion of the crop. In this paper, we offer an overview of the current state of research on KG and pinpoint the knowledge gaps; we defined and discussed the main steps of breeding for KG' cultivars development; this included (i) developing an integrated genebank, inclusive germplasm, and seed system management; (ii) assessing end-users preferences and possibility for industrial exploitation of the crop; (iii) identifying biotic and abiotic stressors and the genetic control of responsive traits to those factors; (iv) overcoming the cross-pollination challenges in KG to propel the development of hybrids; (v) developing new approaches to create variability and setting adequate cultivars and breeding approaches; (vi) karyotyping and draft genome analysis to accelerate cultivars development and increase genetic gains; and (vii) evaluating the adaptability and stability of cultivars across various ecological regions.

Keywords: adaptability, biotic and abiotic stresses, climate change, cultivars development, Fabaceae, genomics, karyotyping, Kersting's groundnut

INTRODUCTION

Agriculture is a major economic, social, and cultural activity that is highly weather and climate-dependent (Yohannes, 2015). Climate change is the worldwide environmental threat that would seriously cause shifts in crop production and affect mankind in several ways, due to more unpredictable and hostile weather patterns (Enete and Amusa, 2010; Cheng et al., 2017) including changes in average temperatures (heat and cold stress), distribution of rainfall (drought and floods) with an important impact on soils erosion and fertility, and increased occurrence of biotic stresses (pests and diseases) (Padi and Ehlers, 2008; Sileshi et al., 2010; Tirado and Cotter, 2010). The shifts in agricultural production also affect the food systems of consumers and the nutritional quality of many crops around the world (Gobu et al., 2020).

To cope with varying weather conditions, farmers adopted new cultivation practices including changes in the cropping calendar, weeding and fertilization regimes, crops varieties, and species (Snapp et al., 2018). To ensure ecosystem resilience, future agriculture systems should necessarily focus on species that can ensure both agriculture sustainability and food' nutritional quality (Mabhaudhi et al., 2019). Orphan species can contribute to developing a more resilient and nutritionally dense future agriculture in arid and semi-arid regions. Resilient alternative crops such as underutilized legumes are examples of such species that have the potential to contribute more to agriculture fitness. Among those orphan legumes, key species produced in sub-Saharian Africa included Bambara groundnut [*Vigna subterranea* L. (Verdc.)], Yam bean [*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms.)], Faba bean (*Vicia faba* L.), and Kersting's groundnut [*Macrotyloma geocarpum* (Harms.) Marechal and Baudet].

Kersting's groundnut (KG) is a diploid with $2x = 2n = 22$ (Miège, 1954) or $2x = 2n = 20$ (Odo and Akaneme, 2021). It is a geocarpic crop like Bambara groundnut, grown by smallholder farmers throughout West Africa. Kersting's groundnut is well-adapted to natural and agricultural conditions, making it a suitable surrogate to the major crops (Achigan-Dako and Vodouhè, 2006). Kersting's groundnut is a high value source of protein for many people in West Africa (Ajayi and Oyetayo, 2009). It is also a rich source of minerals and crude fiber and low crude fat content (Obasi and Agbatse, 2003; Aremu et al., 2011). In Benin, the grains are sold in rural, semi-urban, and urban markets and provide substantial incomes to many smallholder farmers of its cultivated areas, where scarcity periods can cause prices to rise by as much as 10 dollars per kg (Assogba et al., 2015; Akohoué et al., 2018). In addition to being an agronomically and nutritionally beneficial supplement to cereal crops (Tamini, 1995), it fixes atmospheric nitrogen and contributes to soil fertility (Mohammed et al., 2018, 2019). Unlike other legumes like groundnut (*Arachis hypogea* L.), soybean [*Glycine max* (L.) Merr.], cowpea [*Vigna unguiculata* (L.) Walp.] that have received considerable scientific and financial supports, less attention has been devoted to this crop by mainstream research and development institutions, perhaps due to a lack of awareness about its values. It is grown as landraces, which are mixtures of lines that have been naturally selected across agroecologies where they have been grown for perhaps thousands of years and are likely to have low yields and are/become less suitable to climate variations. Furthermore, the crop still lacks adequate germplasm management strategy, suitable seed systems, and optimal agronomic practices. Therefore, the absence of high yielding cultivars with tolerance/resistance to biotic and abiotic stresses is causing a progressive decline in areas where it is cultivated (Akohoué et al., 2018; Coulibaly et al., 2020). Other production challenges include the lack of marketing channels that limit crop promotion. Fortunately, researchers' increasing interest in underutilized crops to ensure food security has breathed new life into this long-forgotten crop.

Because climate change is expected to induce extreme weather conditions, particularly with negative consequences for agriculture in tropical areas (Lane and Jarvis, 2007; Burke et al., 2009; Bellon and Van Etten, 2014), there is a clear need to select crop genotypes that can tolerate severe environmental conditions. For KG, farmers in Benin, Burkina Faso, and Ghana, for instance, modified their cropping calendars to accommodate the weather issues by planting earlier (in June) or later (in August) (Adu-Gyamfi et al., 2011; Assogba et al., 2015; Akohoué et al., 2018; Coulibaly et al., 2020). However, given the rapid deterioration of climate conditions, cultural practices alone will likely not be enough to ensure the endurance of the crop landraces. Hence, high yielding and stress-tolerant cultivars development are required for the promotion and sustainable production of KG (Akohoué et al., 2018; Coulibaly et al., 2020).

Initial researches on KG included ethnobotanical studies, analysis of genetic diversity to assist breeding programs in selecting diverse parental material based on morphological (Bayorbor et al., 2010; Assogba et al., 2015; Akohoue et al., 2019) and biochemical markers (Pasquet et al., 2002). More recently, molecular markers including SSRs and SNPs were used to harness the genetic diversity among and within KG landraces (Mohammed et al., 2018; Kafoutchoni et al., 2021a), and analyze the marker-trait association and genomic prediction accuracy (Akohoue et al., 2020). However, basic and accurate knowledge about physiology, resistance/tolerance to biotic and abiotic stresses, and the genetic basis underpinning traits of interest is still lacking for this species. In addition, the crop lacks an appropriate breeding pathway, genetic and genomic resources [i.e., breeding populations, inbred lines (ILs), and reference genome] that could be exploited to accelerate cultivars development and make its production profitable to smallholder farmers.

This review provides an overview of the background and the recent research progress into the West-African grain legume, KG; it highlights the existing gaps that need to be addressed and gives a pathway for future breeding and promotion. The main objectives of this paper are (i) to provide an overview of the background on the current research on KG; (ii) to pinpoint knowledge gaps hindering the crop improvement; and finally, (iii) to suggest and discuss future research and breeding programs on KG. The following questions are addressed throughout this review: where do we stand in the research on KG? What are the research gaps hindering the production and promotion of KG? What should be the key components of a successful breeding program for KG to overcome the identified limits?

BASIC KNOWLEDGE ABOUT KERSTING'S GROUNDNUT

Origin, Distribution, and Production

Kersting's groundnut is an underutilized legume species that thrives in West Africa, cultivated across the savannah zone from Senegal to Nigeria and Cameroon and probably, in Tanzania and Mauritius. Although its origin is still uncertain, northern Togo and central Benin were suggested as the source of the crop (Achigan-Dako and Vodouhè, 2006). The crop is grown in contrasting environments, ranging from arid and milder environments of Sudanian zones to more humid environments of Guinean agroecological zones (Baudoin and Mergeai, 2001). Recent investigations and wide germplasm collection by Akohoué et al. (2018) and Coulibaly et al. (2020) revealed the Southern Sudanian zones of West Africa as the centre of diversity for the crop. Kersting's groundnut was found by Hepper (1963) in its wild form *M. geocarpum* var. *tisserantii*, in Cameroon and the Republic of Central Africa. However, Pasquet et al. (2002) found a high genetic distance between the var. *geocarpum* and var. *tisserantii* suggesting that they should be assigned to two different species.

Six different landraces were identified in KG based on the seed coat colour are grown across West Africa and include the White/Cream, White mottled with black eye, White mottled with greyed orange eye, Black, Brown, and Red landraces (Adu-Gyamfi et al., 2011; Assogba et al., 2015; Akohoué et al., 2018; Coulibaly et al., 2020).

Kersting's groundnut is grown annually during the cropping season, as sole crop, in rotation, intercropped with cereals, with generally low yields (500 kg.ha^{-1}) (Amujoyegbe et al., 2007; Adu-Gyamfi et al., 2011; Assogba et al., 2015; Akohoué et al., 2018; Coulibaly et al., 2020), which is far less than the yields reported for other legume crops like Bambara groundnut (Nedumaran et al., 2015) and groundnut (Abady et al., 2019; Konate et al., 2020). The low yield levels observed in KG are attributed to various stresses such as biotic (pests such as pulses beetles and rodents, and diseases including fungi and viruses) (Badii et al., 2013; Assogba et al., 2015; Agoyi et al., 2019) and abiotic (drought, high humidity, and low soil quality). Furthermore, farmers are cultivating unimproved varieties, at a small scale (cultivated areas <1 ha), using poor agronomic practices. The crop can grow and fix nitrogen in drought-prone environments where many other crops can hardly survive (Dakora and Keya, 1997). Prolonged water stresses (droughts and excess water) may result in changes in crop growth, development, and grain yield losses (Akohoué et al., 2018; Coulibaly et al., 2020). Improving KG farming systems through integrated approaches could help to enhance crop productivity. However, improving the cropping system alone would not be sufficient to face the evolving climate. Hence, research investigations in KG should focus on both, development of high yielding cultivars, and establishment and promotion of best agronomic practices.

Nutritional, and Market Benefits

Kersting's groundnut is cultivated for its palatable seeds, an important source of nutrients including protein (12.9–21.3%), fiber (2.01–10.9%), minerals (zinc, calcium, and magnesium), and essential amino acids (Ajayi and Oyetayo, 2009; Aremu et al., 2011; Abiola and Oyetayo, 2015). The comparative analysis between KG and some legume crops grown in Sub-Saharan Africa (**Supplementary Table 1**) revealed a higher level of arginine content (4.1–15.9% of crude protein) in KG than in the other legumes. Arginine is the main amino acid recommended for pediatric growth (Ajayi and Oyetayo, 2009) and pregnant women. Kersting's groundnut seeds are low in crude fat content compared to many other legumes such as African yam bean, chickpeas (*Cicer arieticum* L.), and soybean. The seeds of KG can be used as a complementary food to other crops like maize (*Zea mays* L.), rice (*Oryza sativa* L.), and cassava (*Manihot esculenta* Crantz) (Aremu et al., 2011; Awolu et al., 2015, 2020; Awolu and Osigwe, 2019). Kersting's groundnut haulms are also used to feed livestock. Moreover, KG's protein can be used in juice industries to improve juice quality and storage properties (Osungbade et al., 2021). Seeds of KG are reported to exhibit relatively high content in tannins and polyphenols compared to other legumes and have antioxidant properties (Akpavi et al., 2008). However, the anti-nutritional properties of these compounds can bind to nutritional components making them completely or partially unavailable for digestion (Obasi and Agbatse, 2003). Fortunately, treatments such as dehulling, soaking, cooking, and fermentation can potentially reduce antinutritional factors and improve the bioavailability of nutrients (Obasi, 1996; Ijarotimi and Esho, 2009). In addition, KG exhibits several medicinal and therapeutic benefits according to local communities; the decoction of its seeds (mainly the Black and Brown landraces) or leaves is used to treat stomach aches (Tamini, 1995; Amujoyegbe et al., 2007; Akohoué et al., 2018). The economic importance of the crop for local populations was reported in Benin where its price can rise from 2 to USD 7–10 per kg in a scarcity period (Assogba et al., 2015).

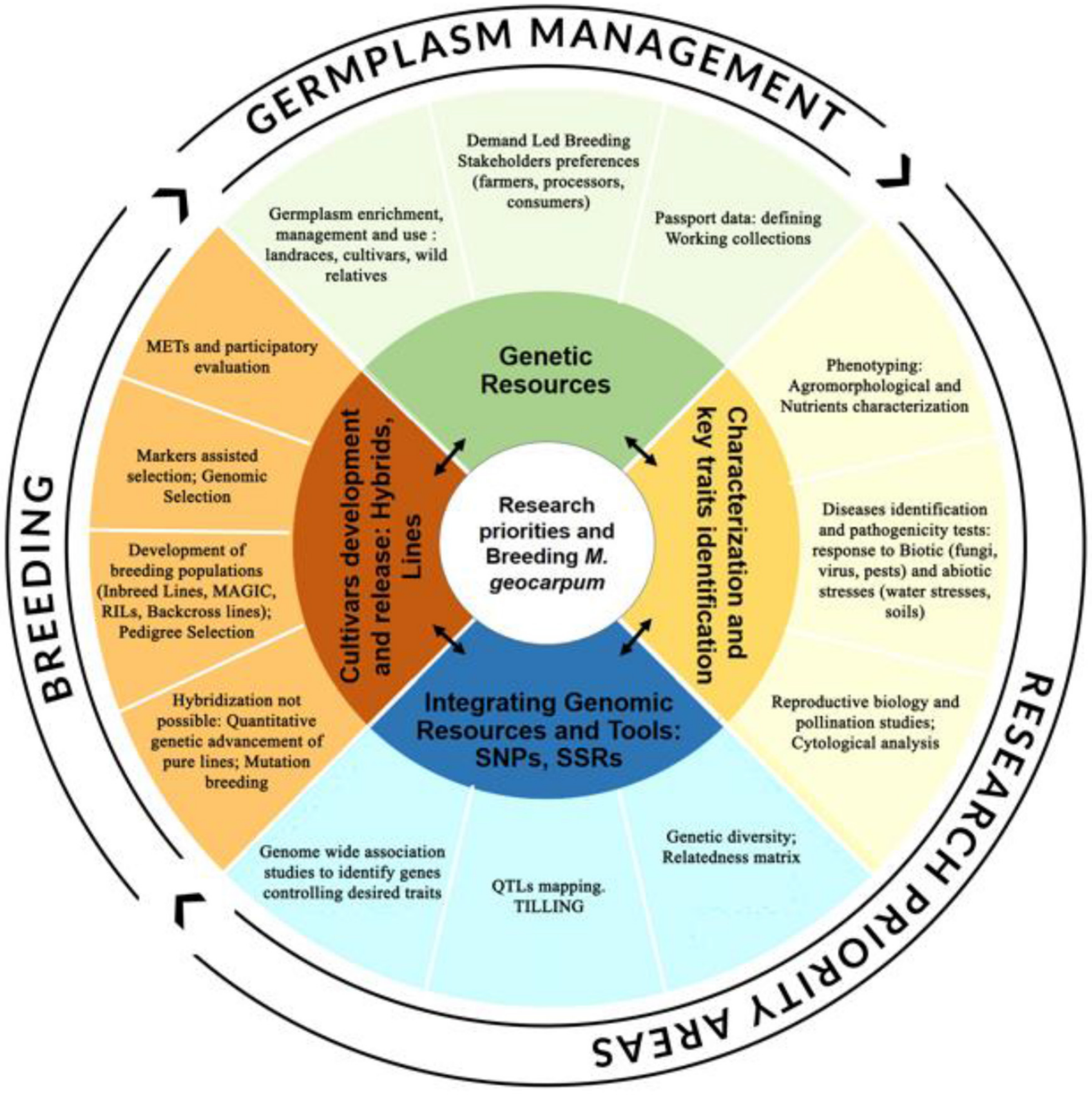

FIGURE 1 | Breeding cycle for Kersting's groundnut.

In other countries such as Burkina Faso, Ghana, and Nigeria, the changes in cropping patterns and the introduction of new cash crops such as groundnut, cowpea, cotton (*Gossypium hirsutum* L.), have negatively impacted the market value of KG's seeds (Tamini, 1995; Amujoyegbe et al., 2007). Therefore, promotion actions would be necessary for those countries to encourage stakeholders and policymakers toward sustainable production of the crop.

DEVELOPING A BREEDING PROGRAM FOR KERSTING'S GROUNDNUT

The development of improved cultivars in KG that meet farmers' and end-users' preferred traits requires the design of a comprehensive approach. Such an approach should integrate (i) germplasm assembly, characterization, evaluation, and definition of breeding objectives, (ii) parental lines and hybrids selection, and (iii) participatory breeding through multi-location evaluation trials (METs) of lines and on-farm participatory evaluations (**Figure 1**). Successful germplasm screening and selection of best KG genotypes requires accurate and high throughput phenotyping across contrasting environments. The target characteristics are measured over multiple growing cycles throughout different environments and stress conditions. Field evaluations of genotypes require the use of appropriate experimental designs, data collection techniques, and interpretation to make accurate decisions. The cultivars development pathway can also integrate tools such as marker-assisted breeding (MAB) and genome-wide selection.

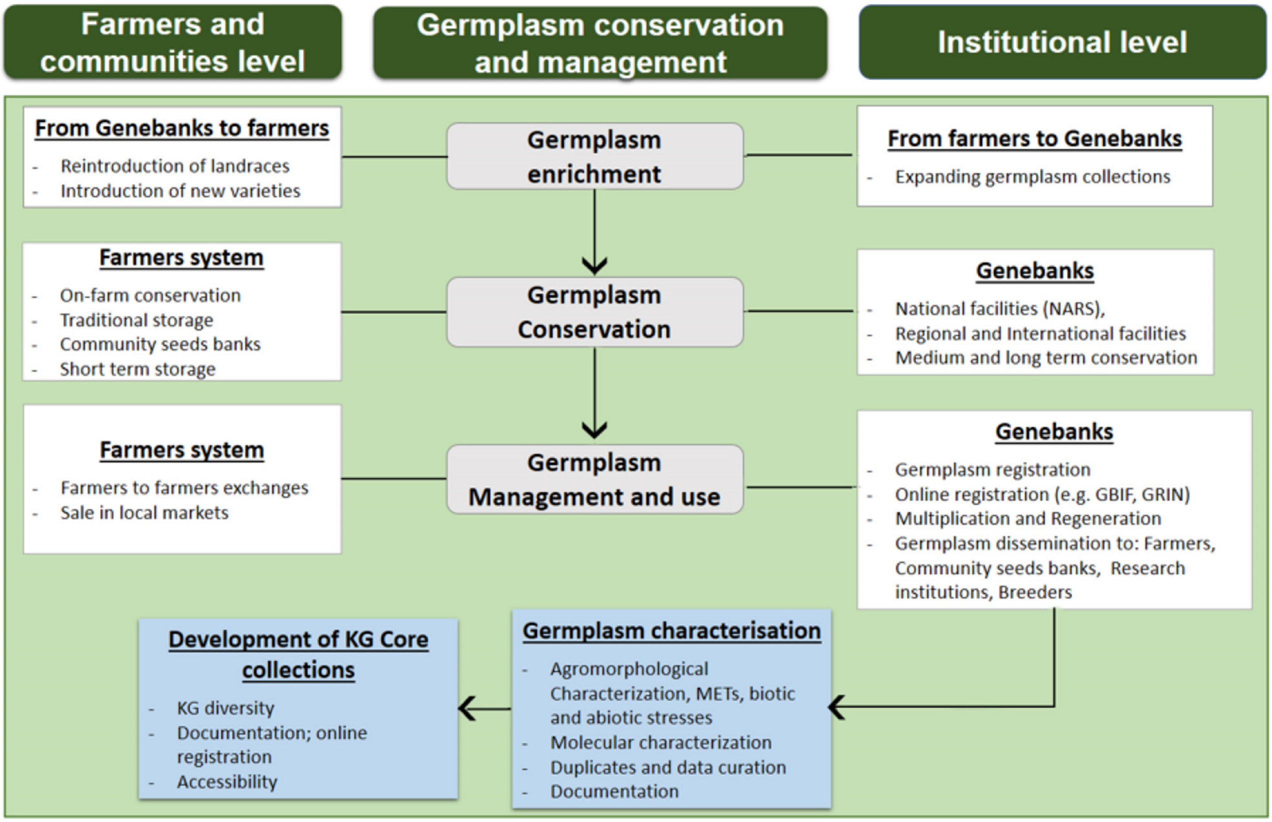

FIGURE 2 | A general scheme of an inclusive germplasm management system for Kersting's groundnut. NARS, national agricultural research systems; GBIF, global biodiversity information facility; GRIN, germplasm resources information network; METs, multienvironment trials; KG, Kersting's groundnut.

Genetic Resources Management for Kersting's Groundnut Breeding and Utilization

Germplasm Conservation and Management

The availability of various genetic resources is the prerequisite for initiating genetic improvement in any crop. The adequate and sustainable conservation and use of KG resources might be categorized into three main steps including the additional collections, conservation, and proper germplasm management and use (**Figure 2**).

In recent decades, the conservation of the genetic resources of orphan crops, including *M. geocarpum*, has attracted more attention because of their role in facing the climate change scenario (Kamenya et al., 2021) and food security. Efforts were made in the collection of KG germplasm and assessment of its on-farm diversity in West Africa (Adu-Gyamfi et al., 2011; Akohoué et al., 2018; Coulibaly et al., 2020). These collections have been further utilized in KG research programs, are sources of useful genes for the development of improved varieties. Overall, about 700 accessions including six landraces based on seed coat color were collected across Benin, Burkina Faso, Ghana, Togo, Nigeria, and Ivory Coast. These accessions are safeguarded and maintained, *ex-situ* at seven national genebanks located in four countries (**Table 1**). In Benin, the University of Abomey-Calavi holds the largest KG germplasm, with more than 600 accessions representing six countries of origin. In Burkina Faso, 32 accessions were collected from two countries and conserved at the INERA. The SARI in Ghana holds 16 accessions, collected in Ghana. However, efforts are still required when compared to the genetic resources accessible *ex-situ* for most of the other legume crops like Bambara groundnut (Massawe et al., 2005, 2007; Aliyu et al., 2016; Mayes et al., 2019), chickpea (Raina et al., 2019; Jha et al., 2020; Kushwah et al., 2020), horse gram (Singh et al., 2014; Chahota et al., 2020), mungbean [*Vigna radiata* (L.) Wilczek] (Ha and Lee, 2019; Mogali and Hegde, 2020; Shanthala et al., 2020), pigeon pea [*Cajanus cajan* (L.) Millsp.] (Foyer et al., 2016; Sharma et al., 2020). Moreover, in Nigeria and Ivory Coast, there is no clear information about the crop genetic resources collection and conservation in genebanks. Although KG production was mentioned in other countries outside West Africa, in Cameroon, Tchad, Mauritius, Tanzania, and Fiji (Achigan-Dako and Vodouhè, 2006), no proper germplasm collection has been undertaken in those countries. Considering the threats to KG, the first and urgent action is to expand germplasm collection mainly across countries and locations not yet explored. This is essential for preserving germplasm and for the setup of a formal seed system for KG. *In-situ* and *ex-situ*

TABLE 1 | Kersting's groundnut germplasm collection available in national genebanks in West Africa.

Country	Institution	Num of accessions	Num of landraces*	References
Benin	Laboratory of Genetics, Biotechnology, and Seed Sciences (GBioS)—UAC	409	6	Akohoué et al., 2018; Coulibaly et al., 2020
Benin	Laboratory of Applied Ecology (LEA)—UAC	217	5	Kafoutchoni et al., 2021a
Benin	Laboratory of Biotechnology, Genetic Resources, and Plant and Animal Breeding (BIORAVE)—UAC	32	3	Assogba et al., 2015
Burkina Faso	Institute of Environment and Agriculture Research-Farako-Ba (INERA-FBa)	32	3	Coulibaly et al., 2020
Ghana	Savanna Agricultural Research Institute (SARI)	16	3	Adu-Gyamfi et al., 2011
Nigeria	University of Agriculture, Makurdi, Nigeria	No data	No data	Obasi and Agbatse, 2003
Total		**706**	**6**	

**: Landraces are based on seed coat color; UAC: University of Abomey-Calavi.*

conservations are the most efficient methods to conserve the genetic diversity of KG resources and minimize losses through time. Although the resources of KG are more abundant in Benin, apparently there is no National plan for *in-situ* conservation of the crop genetic diversity, nor in other countries of production. Unfortunately, many cultivation areas of KG are threatened with the loss of invaluable genetic resources. This is more critical for the wild relatives of the species for which the genetic diversity collection and conservation are not well-documented. To restore lost or reduced crop resources in these areas, the re-introduction or restoration of the species would be essential. This approach can be effective through farmers' fields school, participatory on-field characterization, and evaluation of accessions, and promotion of KG's products with added values.

Farming communities have been preserving or conserving their local crop and varieties in small stores (e.g., clay pots, gourds, underground pits) that represent a "de facto" *ex-situ* conservation system that is likely more dynamic than the conventional one. Moreover, farmers consistently maintain seeds as a security net to provide a backup in case of crop failures to thrive, as well as for sowing the next season. This traditional seed system, mainly based on the farmers' varieties, is an important backup to agricultural crop production in a country (Halewood, 2016). However, both public and private sectors are reluctant to invest in underutilized crops such as KG. In this context, a possibility for an establishment of a community-based seed system, as a complementary measure (Stolton et al., 2006) would be explored toward integrated and inclusive genetic resources management of KG. An example of this system is the community seeds banks (CSB) (Vernooy et al., 2015). Depending on how they are organized, CSB serves functions including conservation and reintroduction of germplasm, access to quality seeds, and enhancing seed and food sovereignty (Vernooy et al., 2015). Moreover, These institutions may facilitate linkages between genebanks and local seed banks considering biodiversity use policies. Genebanks or scientists can also interact, distribute, and organize KG datasets and resources through biodiversity research-data portals, which have proliferated in recent decades. Several specialized portals (PROTA: Plant Resources of Tropical Africa, GBIF: Global Biodiversity Information Facility; GRIN: Germplasm Resources Information Network, POWO: Plants of theWorld Online, etc.) and platform (NARS: National Agricultural Research Systems), collect, disseminate, and promote particular data types for a large number of species, including legumes (Legumes of the World Online, LOWO). However, the use of such platforms in KG germplasm management and use still lacks or is very limited. Indeed, KG remains absent and not documented in the Legumes dataset portals. The recent characterization including agromorphological and molecular information (Assogba et al., 2015; Akohoue et al., 2019, 2020; Kafoutchoni et al., 2021a), can also be incorporated into these online platforms and continuously updated for use by breeders and genetic resources managers. Moving forward, the development of core collections (Paredes et al., 2010; Upahyaya, 2015) using characterization, evaluation, and further collections datasets is the importance of paramount for the effective future management of KG genetic resources, to make germplasm more accessible for interested scientists for breeding purposes or scientific studies. Moreover, these collections could be used for association mapping and genes discovery for targetting material into the original germplasms collected (Gupta et al., 2019). The accessions that have been highly characterized within the collections can be used to inform decisions in breeding programs (Zhang et al., 2019; Abdi et al., 2020).

Assessing Farmers and End-Users' Preferences for New Cultivars Development

Agronomic and processing qualities are important criteria for breeding crops, which must meet specific quality parameters. Kersting's groundnut resources are being managed and maintained by farmers over centuries for their own needs. They have been the growers, germplasm managers, and main users of the species. Thus, to ensure the efficiency of KG new cultivars development, adoption, and sustainable use, access farmers' preferences sought in new varieties is fundamental in defining breeding objectives and priorities, and implementing a successful breeding program. In this context, Coulibaly et al. (2020) investigated farmers' desired traits and found that high yield, resistance/tolerance to pests and diseases, and abiotic stresses were major traits sought in KG new varieties. Among the abiotic stresses, heat, drought, and moisture, are most prevalent

TABLE 2 | Genetic characterization in KG using morphological and molecular markers.

References	Number of accessions	Landraces used	Origin	Markers involved	Genetic variability
Pasquet et al., 2002	20 (2 wild and 18 cultivated)	White (White with black eye), Black, and Gray seeds (White)	Cameroon; Togo, Burkina Faso	19 allozymes encoding 32 putative loci	No variation among domesticated accessions, within and between the two wild accessions. Very high genetic distance between wild and domesticated accessions
Bayorbor et al., 2010	12	Black, White (White with black eye), mottled (Brown)	Ghana	Morphological traits: PHT, CDM, LAI, FSW, DSW, FRW, DRW, NDN, DFF, YLD, HSW	Two clusters
Adu-Gyamfi et al., 2012	16	Black, White (White with black eye), mottled (Brown)	Ghana	Morphological traits: LAI, FSW, DSW, NDN, NPD, YLD, HSW	Variation between landraces and genotypes for LAI, HSW, YLD
Assogba et al., 2015	32	Black, White, Red	Benin	Morphological traits: PHT, DIP, LEL, LEW, PEL, DFF, DTM, NPP, YPP, YLD, HSW, SEL, SEW, PTC, FPC, SCC	Three different clusters for LEW, DFF, YLD
Mohammed et al., 2018	5	Black, White with black eye, mottled (Brown)	Ghana	12 single sequence repeats markers (SSRs) derived from cowpea	Eight monomorphic bands in KG; high genetic variation among landraces
Akohoue et al., 2019	297	White, Black, White with black eye, Red	Benin, Togo	Morphological traits: PHT, DIP, LEL, LEW, PEL, DFF, DTM, NPP, YPP, YLD, HSW, SEL, SEW, PTC, FPC, SCC	Four different clusters for all traits except for SEW
Akohoue et al., 2020	281	White, Black, White with black eye, Red	Benin, Togo	- 493 Single nucleotide polymorphisms (SNPs) - 15 quantitative traits	- Four different clusters defined based on seed coat color and; - Two genetic populations; - 10 significant SNPs related traits, with six SNPs consistent across environments; - Moderate to high prediction accuracies
Kafoutchoni et al., 2021a	227	Cream (White), Cream seed with black eye (White with black eye), Black, Brown mottled, Brown	Benin, Burkina Faso, Ghana, Togo, and Nigeria	886 Single nucleotide polymorphisms (SNPs)	Eight different clusters based on collection sources

PHT, plant height; CDM/DIP, canopy diameter or diameter of plant; LEL, leaflets length; LEW, leaflets width; PEL, petiole length; LAI, leaf area index; FSW, fresh shoot weight; DSW, dry shoot weigh; FRW, fresh root weight; DRW, dry root weight; NDN, nodulation; DFF, number of days to 50% flowering; DTM, days to maturity; NPD, number of pods; NPP, number of seeds per plant; YPP, yield per plant; YLD, grain yield; HSW, hundred seed weight; SEL, seeds length; SEW, seeds width; PTC, petiole color; FPC, fresch pod color, SCC, seed coat color.

in KG production. They found that farmers' preferences varied with social factors such as sociolinguistic membership. This suggests that the genetic improvement of the crop should consider not only agroecologies conditions but also socio-economic factors during the cultivars development process. Moreover, integrating end-users' (processors and customers) preferences is also important to better direct plant breeding objectives (Brouwer et al., 2015; Ragot et al., 2018; Dufour et al., 2021; Tchokponhoue et al., 2021). Hence, further investigations must be carried out across the cultivated areas, involving all stakeholders toward its efficient genetic improvement and end products dissemination. Once desired traits are defined, breeders can then collect genotypes that have the attributes required through characterization and screening of the available germplasm collections.

Research Priority Areas for KG Cultivars Development

Genetic Resources Characterization and Evaluation

Genetic Diversity Analysis and Evaluation

Kersting's groundnut is a small legume species with a maximum spread of 50 cm and a height of up to 40 cm. Unlike other geocarpic legumes such as peanuts, KG branches are coiled and interspersed in a spiral form lying above the ground. On the other hand, the leaves are erected on the branches, giving the plant a bushy growth habit. Kersting's groundnut accessions were characterized and evaluated for various agromorphological traits (Bayorbor et al., 2010; Assogba et al., 2015; AVRDC, 2015; Akohoue et al., 2019) (**Table 2**). Major descriptors included growth habit, flowering and maturity times, plant height, spread diameter, grain yield, and yield components.

All morphological markers were reported to be significantly affected by environmental factors, except growth habit traits (Adu-Gyamfi et al., 2012; Assogba et al., 2015; Akohoue et al., 2019). Adu-Gyamfi et al. (2012) reported significant variation among the White mottled with black eye (White), Black, and Brown (Mottled) landraces of Ghana, based on their agro-morphological performance. Similarly, in Benin, the agromorphological evaluation showed that the White, Black, and Red landraces were significantly different for agronomic performance (Assogba et al., 2015). Still, in Benin, Akohoue et al. (2019) analyzed the diversity in four landraces of KG (White, Black, White mottled with black eye, and Red) using morphological markers and found four clusters based on genotypes performance. The first three clusters were mainly composed of the White landrace while the fourth cluster included the other coloured-coat ones. In terms of performance, individuals in clusters 2 and 4 exhibited higher performance and were intermediate and early maturing genotypes, respectively. Although there is a relatively increasing genetic and phenotypic data on traits, in-depth phenotypic characterization through multi-trait and multi-environmental trials should be conducted using the available germplasm, as a whole, to shed light on the trait variations in the characterized germplasm as well as the performance of landraces grown by farmers. In addition, investigating the response of these landraces under biotic and abiotic stress conditions could be relevant for the improvement of KG. Low yields were obtained in KG by farmers, as well as by researchers, hence, breeding activities must focus on improving yield and tolerance to biotic and abiotic stress factors. However, genotype by environment interactions (GEI) affects yield, making it challenging to select genotypes with wide adaptation, resulting in delayed cultivar release (Abady et al., 2019). Crop breeding strategies for higher yield and disease tolerance can be accelerated through the use of high throughput phenotyping (Shakoor et al., 2017). This technique was successfully used in phenotyping groundnut for the total oil and high oleic acid contents (Sundaram et al., 2010; Awada et al., 2018). Although the high-throughput phenotyping technique is an emerging approach, and its application in crop breeding is still very limited, its utilization in KG breeding could be explored. In 2002, Pasquet used isoenzymes to assess the diversity within and between cultivated KG and its wild form. He found a low variability within each group and high genetic divergence between the cultivated and the wild types. Mohammed et al. (2018) assessed the transferability of cowpea-derived Simple Sequence Repeat markers (SSRs) to KG and revealed genetic variability among the landraces studied. More recently, Akohoue et al. (2020) and Kafoutchoni et al. (2021a) applied SNP markers to assess KG genetic diversity and population structure and found low variation within landraces and relatively high genetic distance between landraces. Furthermore, Akohoue et al. (2020) analyzed marker-trait association and genomic prediction accuracy for main agronomic traits of KG. They found markers related to plant morphological traits, flowering time, maturity, yield, yield components, and seed characteristics. Their results also showed low prediction accuracies for yield and related traits and high prediction accuracies for flowering time, maturity, and 100 seeds weight traits. The findings of these different researches showed the existence of genetic variability in KG and provided the first insight into the relationships of phenotype-to-genotype in KG.

Proximate and Anti-nutrient Contents in Kersting's Groundnut

Quantifying the biochemical properties in KG can serve as a guide to exploit its potential and benefits for human and animal nutrition. Proximate compositions of KG (**Supplementary Table 1**) showed that crude protein content varied from 12.90 to 22.95%, while total fiber ranged between 2.01 and 10.90%, and crude carbohydrate of 57.87–81.00%. Results also indicated a low crude fat in KG with a proportion of 1.00–5.29%. The proteins of KG exhibit interesting essential amino-acid profiles (32.7–44.1%) that make the crop attractive for smallholder farmers. It has a higher arginine proportion compared to many other legumes such as Bambara groundnut (0.064–5.48%, Aremu et al., 2017; Oyeyinka et al., 2017), cowpea (3.5–8.52%, Khattab et al., 2009; Eashwarage et al., 2017), common bean (*Phaseolus vulgaris* L.) (1.17–7.59%, Junkanti et al., 2012; Bouchenak et al., 2013). These findings showed high variability in the levels of nutrients and anti-nutrients in KG, which is potentially due to the biochemical analysis techniques, seed quality, environments, as well as landraces used. Akpavi et al. (2008) compared the proteins and antinutrient contents of two landraces of KG (White and Black) and found a difference between the landraces. Moreover, Badii et al. (2011) suggested that the higher tannins content in the Black and Brown landraces compared to the White ones conferred them more resistance to pulse beetles. Based on these results, we can hypothesize that the varying content of these compounds among landraces is genetically determined. Such genetic variations in KG seed composition offer possibilities for the improvement of related traits through intraspecific crosses. Therefore, accurate information about the proximate and antinutrient compositions of each landrace has become essential for the development of cultivars with high-quality nutrient content.

Analysis of Biotic and Abiotic Stresses in Kersting's Groundnut

Abiotic stresses affecting KG production include drought, high relative humidity, heat, and low soil quality. Although the crop is known to be drought-tolerant (Baudoin and Mergeai, 2001), its yield can be reduced significantly when exposed to long and extreme water stresses. Thus, more resilient varieties and agricultural systems are required for the promotion of KG in the era of global climatic change.

The biotic stresses associated with KG's production include insect pests, rodents, and diseases (Agoyi et al., 2019). In the fields, leaves and pods are destroyed by grasshopper (*Locustra* spp), and Millipede (*Myriapoda* spp). In storage, bruchid weevils (*Callosobruchus maculatus* F.) cause serious damage to the grains. Badii et al. (2011) analyzed the susceptibility of KG seeds to this insect and found the proportion of weight loss in seeds ranged from 8.0 to 14.4% and susceptibility index varying between 4.3 and 12.5. They also found that the effects of these pests on KG seeds differed among landraces; the White landrace showed higher susceptibility to *C. maculatus* than the Black and Brown ones. To minimize grain losses in storage, farmers stored KG as pods or used chemical products or extracts of plants (Assogba et al., 2015; Akohoué et al., 2018; Coulibaly et al., 2020). Badii and Nyarko (2013) and Badii et al. (2013) proposed the extracts from *Hyptis spicigera* (Lam.) and diatomaceous earth for protecting KG against *C. maculatus* infestation. Although the application of biopesticides and insecticides can help farmers in controlling those pests, the development of pest and disease-resistant cultivars remains non-explored and is needed. Drought followed by heavy rain can raise the risk of floods, thereby creating conditions suitable for fungal infestations (Tandzi and Mutengwa, 2020). Agoyi et al. (2019) observed the wilting of the aerial parts, mold, rust, and viruses diseases in KG. A clear knowledge of the pathogenicity of these diseases and the response of KG to the pathogens still lacks and needs to be thoroughly investigated for the development of integrated pests and diseases management, and breeding strategies. In general, legume crops, are attacked in the field by more than one disease and pest at a time (Reddy, 2009), therefore the development of multiple disease resistant varieties is needed and should be explored in KG.

Integrating Molecular Tools and Resources in Breeding Kersting's Groundnut

The availability of genomic information and modern technologies offers a unique opportunity for efficiently improving crops species (Xu and Crouch, 2008; Varshney et al., 2013; Pandey et al., 2016). Many crops' genes and gene activities are constantly being studied and characterized to maximize agricultural production and feed the world's rising population (Ibrahim Bio Yerima and Achigan-Dako, 2021). These studies added value to plant breeding scheme by opening the door to Marker-assisted breeding (MAB). MAB has been successfully used in several legumes' selection programs such as cowpea and soybean; it has accelerated the selection process, and improved genetic gains (Boukar et al., 2016; Omoigui et al., 2017; Ojiewo et al., 2018). Developing and applying genomic tools in legume breeding is therefore of particular relevance to facilitate their promotion and improvement for sustainable production. In KG, Akohoue et al. (2020), Kafoutchoni et al. (2021a) provided the first insights into population structure and the existence of genetic diversity among landraces or morphotypes using SNP markers. In addition, the application of genomic tools such as genome-wide association study (GWAS) and genomic selection (GS) lead to high prediction accuracies (∼0.79) and the identification of 10 significant SNP-traits associations (Akohoue et al., 2020). These studies revealed the high potential and perceived impact of the application of genomics tools for the improvement of KG. Unfortunately, the absence of the reference genome of KG represents a major challenge for proper and precise association and prediction studies, and the application of marker-assisted selection (MAS) within the species (Akohoue et al., 2020). Moreover, low alignment of SNP markers with sister species such as Adzuki bean [*Vigna angularis* (Willd.) Ohwi and H.Ohashi], Bambara groundnut, the common bean was reported in previous studies (Akohoue et al., 2020; Kafoutchoni et al., 2021a). Thankfully, the whole-genome sequence of horsegram (*Macrotyloma uniflorum*), a closely related species, was recently released for the first time by Shirasawa et al. (2021) and offers a unique opportunity for phylogenetic and comparative genomic analyses in KG. Comparative genomic analysis between the two species will enable the identification of genes coding for quantitative and complex traits of interest in KG. Furthermore, transcriptomic analysis can also be performed to identify stress-responsive expressed sequence tags to develop KG cultivars with multiple resistances to abiotic and biotic stresses. Therefore, future efforts for in-depth studies for KG include: (i) the whole genome sequence assembling, (ii) QTLs identification and validation for farmers and end-users preferred traits, (iii) integration of validated QTLs into MAB programs, and (iv) validation of GS and prediction for accelerated cultivar development. Once the KG complete genome is sequenced, backcrossed inbred lines (BIL), recombinant inbred lines (RILs), and multiparent advanced generations inter-cross (MAGIC) breeding populations can be developed to refine the construction of genetic linkage maps (QTLs mapping) and the discovery of genes associated with desired traits (Pandey et al., 2012; Priyadarshan, 2019). Likewise, by developing an F2 population, the bulk segregant analysis (BSA) can be also a useful tool for detecting significant SNPs and identifying candidate genes in plants (Quarrie et al., 1999; Magwene et al., 2011). This is particularly useful for species like KG for which association panels are not readily available. However, the development of such populations in the crop can only be possible if hybridization barriers are unlocked.

Overcoming the Cross-Pollination Challenges in KG

Surveying the Pollination System

The development of breeding populations through intraspecific hybridization is required to efficiently address the absence of improved cultivars in KG (Ayenan and Ezin, 2016; Akohoué et al., 2018; Coulibaly et al., 2020; Kafoutchoni et al., 2021a). For a successful hybridization in the crop, the flower biology and structure, as well as the pollination patterns have to be well-understood. Hence, floral anatomy and physiology, floral and fruiting phenology, and reproductive biology were at the heart of the rising research interest in KG. This is timely, as limited knowledge of reproductive biology is a hindrance to the improvement of most orphan crops (Cullis et al., 2019). Such knowledge has represented breakthroughs in the improvement of many plants, thus contributing to the Green Revolution (Singh et al., 2010; Whitford et al., 2013; Jaiswal et al., 2016). For instance, the proper knowledge of the flowering stage at which emasculation should be performed and the adequate time to pollinate is the basis in achieving a high rate of successful hybridization in peanut (Chu et al., 2016), *Capsicum annuum* L. (Kivadasannavar et al., 2013; García-Tierrablanca et al., 2015).

In the efforts to improve grain yield in KG, plant breeders and geneticists encounter several challenges including the high rate of flower abscission, which represents a major source of continuous failure in attempts to cross KG and low productivity of the crop (Obasi, 1989). As a response to that, three main studies investigated the reproduction systems in KG. Amuti (1980) gave the first description of KG floral biology and deduced from their observations that the crop is a self-pollinated plant with white or purple flowers. Obasi and Ezedinma (1994) focused on floral biology while Kafoutchoni et al. (2021b) went further to study the floral and fruiting phenology, stigma receptivity, pollen viability, and germinability, to devise insights to designing hybridization protocol that guarantees maximum success in the development of breeding lines. Similar research efforts in pigeon pea, which previously had a low success of artificial hybridization (Kalve and Tadege, 2017), has led to the development of hybrid varieties with 25–69% yield superiority over the local cultivars in India (Saxena, 2015).

Allen and Allen (1981) reported that KG has a cleistogamous flower type while Pasquet et al. (2002) classified the crop as chasmogamous. Lord (1981) described the "pre-anthesis cleistogamous" phenomenon as when bud pollination occurs before anthesis, which contributes to increasing the selfing rate. Such a floral structure is known to promote spontaneous selfing (Freitas and Sazima, 2009; Kumari and Sharma, 2017). On the other hand, bud pollination occurs after anthesis in chasmogamous species, which may allow a relatively low rate of allogamy. Based on the changes observed in flower color and size, Kafoutchoni et al. (2021b) described six floral phenological stages followed by six fruiting stages for KG, viz initiated flower (S1), young bud (S2), developed bud (S3), mature bud (S4), opened flower (S5), and wilted flower (S6) for flower development and beginning peg (F1), beginning pod (F2), full pod (F3), beginning seed (F4), full seed (F5), and mature seed (F6) for pod development. Although studies of reproductive biology on KG have provided useful insights into flower and reproductive description and physiology, further investigations are needed to deepen the understanding of cross-pollination processes. Particularly, the information about the species flower category, whether KG is chasmogamous or cleistogamous must be established. A clear knowledge of the pollination of KG would be very useful in determining the handling procedures and the possible strategy for artificial hybridization to increase hybrids' production efficiency.

Reducing Genetic and Environmental Barriers for the Development of a Hybridization Protocol

The small size of flowers, and climate conditions are potential barriers to successful hybridization in KG. These barriers make it necessary to proper timing, skilfully and delicately operating while emasculating and pollinating flowers of KG. Kumar and Singh (2005), reported also the relatively low efficiency of hand emasculation in species with smaller flowers such as sorghum and rice compared to species with larger flowers such as cotton and okra. Moreover, Tamini (1997) showed that variations in weather conditions influence KG flowering cycle, by delaying or accelerating the flowering time. In Bambara groundnut, for instance, temperatures of 33–36°C, adversely affect pollen viability and germination Dhanaraj (2018). The evaluation of thermo-tolerance of pollen is hence recommended before any hybridization activity. However, the physiological effects of environmental parameters on pollen viability and stigma receptivity in KG are not yet known.

In self-pollinating crops, various crossing methodologies such as mechanical emasculation, genetic male sterility, the use of chemical hybridizing agents (CHAs), and genetic transformation have been used for the development of breeding lines and hybrid seeds (Veerappan et al., 2014). However, hand emasculation combined with hand pollination which remains the most widely used technique is tedious, time-consuming, labour-intensive, and costly (Fu, 2014). Moreover, it requires proper skills and delicate operations, especially when flowers are of small size or present several physical and physiological barriers. Therefore, emasculation techniques such as hot-water treatment, anther aspiration (McDonald, 1994), plastic-bag method (Schertz and Clark, 1967), alcohol emasculation, and cold treatment using CHAs, genetic emasculation (Salgare, 2004; Sleper and Poehlman, 2006; Mohammed et al., 2019) need to be potentially explored to cope with pollination issues in KG.

Analyzing the Chromosomes Number in KG

Understanding the appropriate karyotype is important for characterizing genomes of a species and for identifying closely related species (Saensouk and Saensouk, 2018; Senavongse et al., 2018). Kersting's groundnut is a diploid species for which, different numbers of chromosomes were reported for the wild and cultivated types (Pasquet et al., 2002). Miège (1954) evaluated the karyotype of the cultivated KG and found a chromosome number of $2n = 22$. However, According to Hepper, (1963), the chromosome number of KG wild type was $2n = 20$. More recently, Odo and Akaneme (2021) used six accessions and found a chromosome number of $2n = 20$ in the cultivated KG. The clarification in the chromosomal number will certainly be fueling for further investigations particularly relevant to understand the compatibility phenomenon of reproductive organs in intraspecific cross-pollination toward accelerating KG breeding.

Cultivars Development

Defining Breeding Objectives for Kersting's Groundnut

The non-existence of improved varieties for KG despite its economic and nutritional importance is a wake-up call for an improvement program. Identifying plants with desirable traits among existing plant resources, or developing new phenotypes is the initial and most important step in plant breeding. Because the preferences of both growers and consumers are essential in setting up breeding objectives and product targets, they should be investigated at an early stage of the breeding program to guide germplasm collection and characterization strategies. Ethnobotanical studies have been conducted by Tamini (1995), Amujoyegbe et al. (2007), Akohoue et al. (2019), and Coulibaly et al. (2020), and reported on factors constraining the production of KG. In addition, farmers' preferred traits for KG new cultivars were also reported by Coulibaly et al. (2020). Thus, defining and prioritizing breeding objectives for *M. geocarpum* must take into account the identified constraints and stakeholders' desired traits. **Table 3** provides an overview of some of the

TABLE 3 | Breeding objectives for Kersting's groundnut according to farmers, processors, and consumers' preferences.

End-user	Constraints	Traits of interest	Performance desired compared to current variety	Name of landraces to be improved
Farmer	Low yields	Economic yield and related traits	Dry grain yield 1,500–2,000 kg.ha^{-1}	White, White with black eye
	Small seed size	Big seed size	>Current landraces	White, White with black eye
	Biotic stresses	Diseases resistance: fungi (crop wilting) and viruses	<3 (CIAT scale, degree of infestation)	All
	Abiotic stresses	Abiotic stress tolerance: drought, heat, high humidity	Medium tolerance—at reproductive and maturation stages	All
	Post-harvest loss	Tolerance to storage pests: pulses beetles	Medium tolerance—whether stored in pods or shelled in grain	White, White with black eye
	Long crop cycle duration	Early maturation	<90–105 days after planting (dap)	White, White with black eye
Processor/Consumer	Taste: less sweet	Organoleptic properties (Palatability and good taste)	Sweet taste	Black, Brown
	Appearance	Seed coat color	Uniformity of seeds: White and White mottled with black eye	White, White with black eye
	Food preparation	Facility to cook seeds	Short cooking time (90–120 min)	All

breeding objectives; improved cultivars are expected to possess five major traits: (1) early maturing attribute, (2) high yield (>500 kg/ha), (3) resistance to pathogen attacks (viruses, and fungi), (4) resistance to pulse beetles infestations (5) tolerance to abiotic stress especially to drought and high humidity. Yield in KG can be improved indirectly through genetic resistance/ tolerance to biotic and abiotic stressors. Important yield components in KG that could be improved include: number of seeds per plant, days to 50% flowering, and 100 seeds weight (Akohoue et al., 2019). Moreover, according to farmers, water stresses during the reproductive stage in KG are critical as they directly affect pod yield and quality by causing pods rots (Coulibaly et al., 2020). To cope with this situation, farmers modified the planting periods of the crop (Akohoué et al., 2018; Coulibaly et al., 2020). However, changes in rainfall patterns are unpredictable and random. Hence, developing water use efficient cultivars should be an important target trait. In addition, breeding for early maturing varieties for KG, as an escape mechanism to avoid water stress should be explored. Moreover, KG breeding objectives should also include value-adding traits such as higher nutrient-rich seeds, fast cooking time, and market class/seed colour. For the successful breeding purpose, short-term, medium-term, and long-term improvement goals should be set according to the urgency of the impact and traits to be improved. Also important is the participatory definition of breeding objectives with all stakeholders and the establishing the future KG breeding products profile.

Breeding Approaches for Cultivars Development

The objective of KG breeding programs is to develop improved lines with high yield potential, tolerance to abiotic factors, resistance to biotic stresses, adaptation to major agroclimatic conditions, taking into account consumers' and producers' desired traits. The germplasm available in different genebanks must be characterized and screened for the identification of the source of genes for those traits. Improved cultivars can be pure lines, ILs, hybrids, or mutant lines. Several techniques are used to increase or create genetic variability within species (Bhandari et al., 2017) and could be applied to KG. The hybridization technique (e.g., interspecific or intraspecific) is the most and widely used in breeding to increase genetic variability in a plant. Genetic variability is also created through mutation breeding (Reha-Krantz, 2013).

Selection of Pure Lines

Kersting's groundnut is naturally a highly self-pollinated crop (Allen and Allen, 1981) with a narrow genetic basis (Pasquet et al., 2002), suitable for pure-line cultivars development (**Figure 3**). Improved varieties can be developed mainly through mass selection, pedigree breeding, and backcross methods, using the available landraces. Landraces are a valuable source of genetic diversity and possess useful traits for breeding (Lopes et al., 2015). For instance, the average yield in the research stations ranged from 77 to 1,548 kg.ha^{-1} (Assogba et al., 2015) and from 126.89 to 1444.29 kg.ha^{-1} (Akohoue et al., 2019). Hence, there is evidence that crop landraces can potentially produce high yields. Moreover, the genetic differentiation among KG landraces was found to be moderately high, suggesting a possible low rate of outcrossing. In that case, for the development of pure lines, landraces should be purified through subsequent selfings and selections for a minimum of five generations (Acquaah, 2007; Singh et al., 2015) to reduce unwanted alleles (Ahmar et al., 2020). The evaluation of selected genotypes in different environments/agroecologies is required to fix the homogeneity, stability, and adaptability of new lines. However, pure line cultivars may not respond effectively to producers and consumers

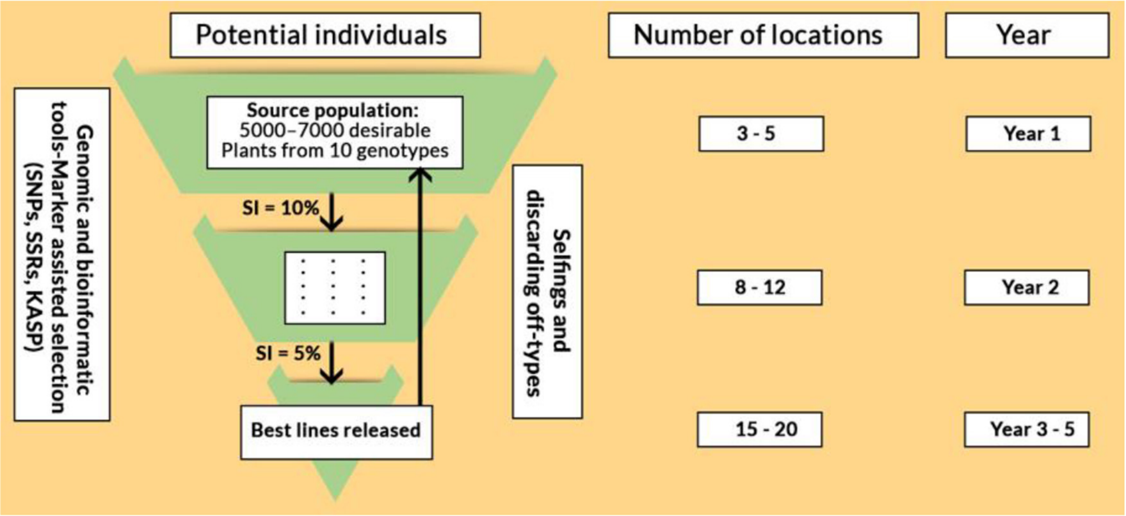

FIGURE 3 | A generalized steps for pure-lines selection in Kersting's groundnut. SI, selection intensity (%).

desires for many reasons: (1) pure lines have very low adaptability due to their narrow genetic base, the selection is powerless to bring changes in hereditary factors i.e., to develop new genotype (Acquaah, 2015); (2) pure lines are poor candidates for multiple trait selection because of the difficulty of finding all the desired traits in a single genotype (Priyadarshan, 2019), (3) Pureline selection requires more time, space, and expensive yield trials (Acquaah, 2015). These lines can often be used as parents in the production of other types of cultivars or breeding populations such as BILs, ILs, RILs, MAGIC, or mutants. Developing such populations in KG would create an avenue to unravel the genetic potential in the crop.

Mutation Breeding

Mutation breeding is an alternate method to conventional plant breeding for increasing genetic variability and conferring specific improvement without influencing the crop phenotype expression (Kulthe and Kothekar, 2011). Whether chemical or physical, the use of mutagenic agents in the creation of genetic variability is becoming increasingly important in plant breeding (Reha-Krantz, 2013). This technique was very successful in the genetic improvement of several leguminous species such as common bean, groundnut, pigeon pea, soybean, pea (*Pisum sativum* L.), cowpea, mungbean, Bambara groundnut, for which the improved traits were different and consist in: disease and pest resistance, earlier or later flowering, higher yield, higher protein content, or less toxic compounds (Adu-Dapaah and Sangwan, 2004). Given the success of this technique in the legumes listed above, the application of mutation breeding may be an alternative to improve the traits of interest in KG. However, mutation induction is a random process and does not always guarantee an ideotype variety ready for commercialization; it just provides a large population of mutants each with specific characteristics (Micke, 1993). It is often difficult and rare to obtain after mutation induction, a mutant possessing all the characteristics of interest. Thankfully, the possibility to combine the chemical mutagenesis with the Targeted Induced Local Lesions in Genomes (TILLING) tools is important progress for accelerating the mutation breeding and enhancing selection accuracy of mutant desired products. In addition, hybridization can be used as a complementary method to mutation breeding insofar as it can support the transfer of the genes in a traditional way between the mutants or lines used as parents (Solanki et al., 2011).

Intraspecific and Interspecific Hybridization

The genetic characterization of KG revealed that there exists a moderate genetic distance between the White landrace and the other landraces which exhibited higher performances. However, landraces with White and White with black eye seed coat color are the most preferred and widely grown by farmers for consumption (Coulibaly et al., 2020). The opportunity for intraspecific hybridization can be exploited to transfer genes between KG landraces, particularly from coloured landraces to the White landrace. Moreover, the interspecific crosses for enhancing genetic variability and introgressing useful genes into KG from closely related species could be explored. Interspecific hybridization involves two different species and is widely used in breeding programs (Da Motta et al., 2020; Pratap et al., 2021). Although this technique is widely used, it remains challenging in the case of KG for several reasons. It seems that the wild form of KG is still unknown and is not found in any genebank (Ayenan and Ezin, 2016). However, Pasquet et al. (2002) had reported using two wild accessions from Cameroon in genetic diversity study. No studies on the agronomic potential and reproductive biology of any wild species have been published to date. Furthermore, possibilities of hybridization of KG with Horsegram can be considered to create early maturing hybrids resistant to drought and pest attacks (Chahota et al., 2013; Amal et al., 2020). In this case, a study of crossability barriers must be carried out to understand the factors of success or failure of a crossing between the two species (Akkerman and Bakker, 2011; Martins et al., 2019; Ferreira et al., 2021).

Testing the Adaptability and Stability in KG Cultivars Across Various Agroclimatic Zones

Stability and adaptability have always been considered as important topics in plant breeding but will be more crucial due to the continuous variations in climatic conditions. Conducting multi-environment trials would help to ensure accurate evaluation of new cultivars' performance to respond to climate change, as well as KG market demand. Thus, multi-location evaluations should be carried out throughout the breeding process by prioritizing end-users preferences for the newly developed varieties. High-yielding, stable, and well-adapted cultivars are much desired in the KG breeding program to ensure sustainable agriculture of the crop. The choice of locations for cultivars' performance evaluation should be based on the range of agroclimatic conditions under which the species is cultivated. Kersting's groundnut is grown during the rainy season (rain-fed cultivation system only), and its production shifts into three main agroclimatic zones of West Africa including Northern and Southern Sudanian zones and the Northern Guinean zone. Through these evaluations, KG cultivars would be successfully and reliably selected to respond to target growth areas. The GEI study is used to optimize the selection of cultivars across testing environments and can be used in breeding KG. Several approaches (METs and statistics) (Resende and Thompson, 2004; Gauch, 2006; Meyer, 2009) and tools (Coe, 2002; Aparicio et al., 2019) were developed in breeding crops, to examine the GEI for specific traits such as yield.

Furthermore, recent, studies showed the importance of the applications of genomics and bioinformatics tools in improving the understanding of GEI and the effects of climate change in species (Heslot et al., 2014; Gotelli and Stanton-Geddes, 2015; Monteverde et al., 2019). These methods allowed the analysis of the phenotypic plasticity of crops by incorporating the environment covariables to understand the relationship between environmental parameters and genes expression, at the specific developmental stage of the plant. Phenotypic plasticity refers to the ability of a single genotype to exhibit different phenotypic expressions across environments and ecological settings (Oostra et al., 2018; Klingenberg, 2019). Virtually, biotic stresses and any variation of environmental factors can induce the plastic response of genotypes, resulting in different phenotypic performances across environments. The extend of phenotypic plasticity in a crop relates to its capacity to active physiological mechanisms to adapt to external biotic and abiotic stimuli. In KG, several abiotic stresses such as drought, high soil moisture, temperature, and biotic stresses including fungal pathogens and insects are major factors contributing to the high yield gap within the crop (Assogba et al., 2015; Akohoué et al., 2018; Coulibaly et al., 2020). Understanding the magnitude of phenotypic plasticity in KG is therefore required to inform breeding options and strategies with regards to disease resistance and abiotic stresses tolerance of genotypes. The use of METs combined with modeling approaches in breeding KG will enable a better analysis of GEI for the selection of potential parental lines and superior cultivars (lines or hybrids) that meet end-users' requirements.

DEVELOPING AN INTEGRATED SEED SYSTEMS MANAGEMENT

Kersting's groundnut is traditionally produced and managed by local communities without an established formal seed system. Farmers grow seeds they purchase from markets, sometimes for high prices but low quality. This is mainly leading to the low yields of currently cultivated landraces, combined with their susceptibility to pulses beetles in storage which decrease the quality and quantity of stored products (Badii et al., 2011; Assogba et al., 2015; Akohoué et al., 2018). Moreover, farmers use their previous harvests as seeds or get from other farmers (Akohoué et al., 2018; Coulibaly et al., 2020). On the other hand, for the sake of increasing the crop yields, farmers increase plant density, raising, therefore, the production costs (Akohoué et al., 2018). Thus, farmers identified the non-availability of seeds as an important constraint limiting KG production. In fact, in most of the growing areas where the crop production was declining or disappeared, farmers lost their seeds and could not renew their seeds stock (Coulibaly et al., 2020). Without a formal seed supply system, management, and distribution, farmers' seeds demand remains unsatisfied. To ensure the availability of genetic resources for farmers and breeding programs, it is critical to creating an integrated seed system for KG resource management and use (**Figure 4**). The development of an organized and formal seed supply system appears as one of the biggest challenges for sustainable and improved KG production (Assogba et al., 2015; Akohoue et al., 2019). Improving KG productivity calls for research and institutional efforts concerning the development and release of new varieties in close partnerships with national and/or private seed companies.

CONCLUSION

The last few years witnessed in West Africa an increasing interest in Kersting's groundnut which represents an excellent source of income for many households in marginal areas. The crop is disappearing in many countries because of the lack of knowledge about best cultivation practices, the lack of improved varieties, climate hazards, and biotic constraints. Despite this increased interest, many bottlenecks still hamper the wider dissemination and exploitation of the crop. Major drawbacks include the lack of improved cultivars that are high-yielding, drought-tolerant, pests and disease-resistant, and compliant with farmers' and consumers' needs. The development of such varieties still requires extensive investigations such as increase and maintenance of genetic resources, evaluation against biotic and abiotic factors, pollination and hybridization studies, development of molecular markers. Furthermore, breeding activities should include conventional and modern approaches.

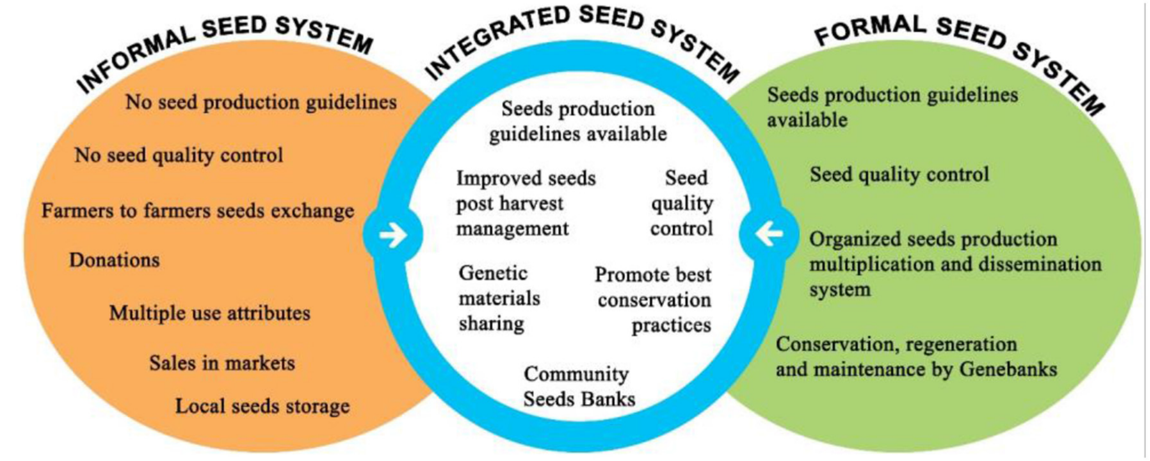

FIGURE 4 | Convergence of informal and formal seed systems into an integrated system in Kersting's groundnut.

A limiting factor is low skill and weak knowledge about the pollination system for successful hybridization that will unlock the opportunities to create genetic variability. Current studies are also focusing on mutagenesis that can allow the creation of more diversity and mutant lines. Meanwhile, breeders can develop open-pollinated varieties but also select high-yielding parents as pure lines. The growth of KG' market in Africa leans on the acceleration of the genetic gains in the crop as well as defining a suitable environment for increased production. Furthermore, access to research funds and technology (genomic and phenomic technologies), will aid in the mapping of KG genetic resources for rapid selection and breeding in West Africa. The development of research collaboration and partnerships with potential institutes working on legumes improvement is needed for successful breeding programs.

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MC and EA-D: conceptualization and writing—review & editing. GB, FA, EA, FM, and CA: writing—review & editing. MS: review & editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REVIEW
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Indigenous African Orphan Legumes: Potential for Food and Nutrition Security in SSA

Michael Abberton[1]*, Rajneesh Paliwal[1], Benjamin Faloye[1], Tchamba Marimagne[1], Azeez Moriam[2] and Olaniyi Oyatomi[1]

[1] Genetic Resources Center, International Institute of Tropical Agriculture, Ibadan, Nigeria, [2] Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria

In Sub-Saharan Africa (SSA), both crop production and the hidden hunger index (HHI, a combination of zinc, iron, and vitamin A deficiency), continue to be worse than the rest of the world. Currently, 31 out of 36 countries of SSA show the highest HHI. At the same time, several studies show climate change as a major constraint to agriculture productivity and a significant threat to SSA food security without significant action regarding adaptation. The food security of SSA is dependent on a few major crops, with many of them providing largely only an energy source in the diet. To address this, crop diversification and climate-resilient crops that have adaptation to climate change can be used and one route toward this is promoting the cultivation of African orphan (neglected or underutilized) crops. These crops, particularly legumes, have the potential to improve food and nutrition security in SSA due to their cultural linkage with the regional food habits of the communities, nutritionally rich food, untapped genetic diversity, and adaptation to harsh climate conditions and poor marginal soils. Despite the wide distribution of orphan legumes across the landscape of SSA, these important crop species are characterized by low yield and decreasing utilization due in part to a lack of improved varieties and a lack of adequate research attention. Genomic-assisted breeding (GAB) can contribute to developing improved varieties that yield more, have improved resilience, and high nutritional value. The availability of large and diverse collections of germplasm is an essential resource for crop improvement. In the Genetic Resources Center of the International Institute of Tropical Agriculture, the collections of orphan legumes, particularly the Bambara groundnut, African yambean, and Kersting's groundnut, have been characterized and evaluated for their key traits, and new collections are being undertaken to fill gaps and to widen the genetic diversity available to underpin breeding that can be further utilized with GAB tools to develop faster and cost-effective climate-resilient cultivars with a high nutrition value for SSA farmers. However, a greater investment of resources is required for applying modern breeding to orphan legume crops if their full potential is to be realized.

Keywords: orphan crops, climate change, genetic resources, genomic-assisted breeding, Sub-Saharan Africa, Bambara groundnut, African yambean, Kersting's groundnut

Edited by:
E. O. Deedi Sogbohossou,
Wageningen University and Research, Netherlands

Reviewed by:
Deepranjan Sarkar,
Banaras Hindu University, India
Dinesh Joshi,
Indian Council of Agricultural Research (ICAR), India

***Correspondence:**
Michael Abberton
m.abberton@cgiar.org

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INTRODUCTION

At present, Sub-Saharan Africa (SSA) is facing multiple challenges including hunger due to low agriculture productivity, a malnourished population, high population growth rate, and climate change (Eriksson et al., 2016; Rippke et al., 2016; IFPRI, 2017; Hendre et al., 2019; Giller, 2020). The food security of Africa is largely dependent on ten staple crops: cereals (maize, pearl millet, rice, and sorghum), tubers (cassava, yam, banana/plantain, and sweet potato), and legumes (cowpea and beans) (Eriksson et al., 2018). However, their average yield is low compared to the average world production and far lower than North America and Europe (Eriksson et al., 2018). There are several factors, such as low soil fertility, rainfed agriculture, insect and pest attacks, and biotic and abiotic stresses, that can significantly reduce the crop yield. In SSA, the commonly used dose of nitrogen, phosphorous, and potassium (NPK) fertilizers by farmers is an average of 8 kg/ha compared to the recommended 30–120 kg/ha. In the past decades, the increased agricultural production of SSA was largely achieved by the cultivation of a larger area rather than an increase in productivity (Sanchez, 2002). However, a remarkable increase in the agriculture productivity of Ethiopia was noticed in statistical data due to the utilization of high-yielding improved cultivars and efficient implementation of the Agriculture Growth Plan of the Ethiopian government (Abate et al., 2015; van Dijk et al., 2020). By 2050, the population of SSA is predicted to increase 2.5-fold and achieving a zero-hunger target under SDG2 (Sustainable Development Goal 2) in SSA is a most essential and difficult challenge. The objective of SDG2 in SSA is reducing hunger, improving nutrition, and ensuring food security through promoting sustainable agriculture. The occurrence of undernourishment in Africa has increased from 17.6 to 19.1% of the population from the year 2014 to 2019, which is more than twice the world average and the highest globally (FAO, 2020). At the same time, numerous climate studies and simulation reports have confirmed that climate change is a major constraint, significantly negatively impacting on agriculture productivity livelihoods and food security (IPCC, 2012; Ramirez-Villegas et al., 2017; Girvetz et al., 2019). The CIMP5 project with the GCM model predicted a rise in the environmental temperature of Africa of 1.7°C, 2.7°C, and 4.5°C by the years 2030, 2050, and 2080, respectively (Girvetz et al., 2019). A meta-database of 16 studies was used for a study on future crop yield prediction, in which the results indicate large changes in yield, ranging from −50 to +90%, with a median yield loss near −11% for the thirteen major staple crops of African countries including West Africa (Roudier et al., 2011). The crop production of SSA is predicted to be among the most significantly reduced, with average production losses between 20 and 40% by 2050 (Ramirez-Villegas and Thornton, 2015). At the same time, biodiversity is declining (Ringler et al., 2010). There is a need to improve nutrition in many rural and urban communities and to develop food systems that are more resilient to climate change. Underutilized or orphan crops have an important role in this (Chivenge et al., 2015; Tadele, 2018). Orphan legume crops have typically been cultivated in a particular region and gone through natural and farmer selection over many years (Dawson et al., 2007). They serve as a means of income generation for the local subsistence farmers in the rural areas (Dawson et al., 2007). Orphan legumes are often rich in micronutrients that are capable of ameliorating nutrient deficiencies.

Modern breeding methods including genomics-assisted breeding (GAB) are playing an increasing role in improving the yield and nutrition content in staple crops that cannot be easily and rapidly achieved by conventional breeding (Cerrudo et al., 2018; Mbanjo et al., 2021; Prasanna et al., 2021). Genomic approaches have been utilized in crops for exploring the diversity of collections (Hiremath et al., 2012; Tamiru et al., 2020); precise parental crossing; dense and comprehensive linkage genetic maps (Hiremath et al., 2012; Paliwal et al., 2012; Saxena et al., 2012); quantitative trait loci (QTL) discovery for biotic, abiotic, and nutritional traits (Varshney et al., 2009; Sarvamangala et al., 2011; Paliwal et al., 2012; Jaganathan et al., 2015; Tamiru et al., 2020); and development of improved lines through the introgression of QTL into elite breeding lines (Saghai-Maroof et al., 2008; Varshney et al., 2015). In maize, the genetic gain for heat and drought increased from 4.4 to 19.4% using GAB tools (Cerrudo et al., 2018). The genetic gain of 19.4% under drought is very significant and indicates that GAB tools could play an important role in developing climate-resilient improved maize lines. The marker-assisted selection (MAS) for root yields, dry matter content and pro-vitamin A, cassava green mite, and cassava mosaic virus disease (CMD) resistance have been applied in the cassava breeding program at the IITA to develop improved varieties using GAB tools (do Carmo et al., 2015; Mbanjo et al., 2021). GRC conserves 6,747 accessions of orphan legumes, including African yambean (AYB), Kersting's groundnut (KG), and Bambara groundnut (BG), which are important indigenous African crops. This diverse collection of orphan legumes has a significant potential to provide genetic resources to initiate crop breeding for essential agronomical traits, including climate-adaptive traits and nutritional traits. However, despite their impressive climate resilience and nutritional profiles, the utilization of conserved collections in crop improvement has been limited due to a lack of knowledge on diversity information, both phenotypic and molecular. Presently, several genomic research activities, including genetic diversity, linkage mapping, and marker–trait association, are in progress using genotyping by sequencing at the GRC in BG, AYB, and KG. This review summarizes the economic potential, utilization, and constraints to yield production in AYB, BG, and KG crops. We also focus on the available germplasm collection and its gap analysis and genomics progress toward the improvement of these African orphan legumes.

AFRICAN YAMBEAN

AYB [*Sphenostylis stenocarpa* (Hochst ex. A. Rich.) Harms] (**Figure 1a**) is an African underutilized legume with a significant potential to enhance food security. It produces edible seeds that are rich in protein and underground swollen roots (NRC., 2007).

FIGURE 1 | Field images of African orphan legumes **(a)** African yambean, **(b)** Kersting's groundnut, **(c)** Bambara groundnut.

Legumes are the major sources of protein in many developing countries of the world (Allen and Allen, 1981). The genus *Sphenostylis* is very small and has species with a growth habit, which can be prostrate, climbing, or erect (Potter, 1992). The AYB is the most cultivated and economically important species out of the seven in the genus *Sphenostylis* (Potter, 1992; Potter and Doyle, 1992). It is one of the most important tuberous legumes among indigenous African food cultures.

Origin and Domestication

AYB is mostly cultivated in West and Central Africa (Bhat and Karim, 2009; Toyosi et al., 2020) especially in Nigeria, Ghana, Cote d'Ivoire, Cameroon, and Togo. Its domestication cannot be traced to a single locality as the occurrence of the wild relatives has been reported in different parts of Africa (Idowu 2014; Potter and Doyle, 1992). The different possibilities of the origin have been proposed (Dalziel, 1937; Harlan, 1971; Kay, 1973; Okigbo, 1973; Potter and Doyle, 1992). One of the hypotheses proposed a single domestication event in Central Africa (Potter, 1992), West Africa (Murdock, 1959; Purseglove, 1976), or Ethiopia (Dalziel, 1937), after which the domesticated races were dispersed by humans till date. Okigbo (1973) proposed two events of domestication and suggested that the domestication of the cultivated seed races might have occurred in West Africa, while the domestication of tuber-cultivated races may have occurred in Central Africa. Based on the chloroplast DNA, isozyme, and morphological variation, Potter and Doyle (1992) suggested two hypotheses: (a) it was domesticated independently in West and Central Africa, but its selection involved a single restricted gene pool; (b) a single domestication event happened in one of the two regions, and later, human dispersal occurred to the second regions prior to the dispersal within either region. Similar hypotheses were reported by Harlan (1971), which suggested that the AYB is not assignable to a single center of origin, and its center of origin should be classified based on its ecological zones in which it was domesticated (West and Central Africa).

Germplasm Collections

The majority of the holders of the AYB germplasm are aged female subsistence farmers. Therefore, preventing the genetic erosion of AYB requires a concerted effort on the exploration, collection, and conservation of the germplasm; the GRC, in collaboration with national gene banks and other national research institutes, has consistently been carrying out a germplasm exploration of AYB. The center currently conserves a diverse collection of over 450 accessions of the AYB landraces collected from Ghana, Nigeria, and the Democratic Republic of the Congo and over 50% of the accessions collected from Nigeria. The phenotypic characterization of this diverse collection is in progress according to the AYB crop descriptor at GRC, IITA (**Figure 2**).

Economic Potential and Utilization

AYB is an efficient nitrogen-fixing legume (Assefa and Kleiner, 1997; Oganale, 2009). It is a dual food crop that produces seed and underground tubers that are like potatoes (Adewale and Dumet, 2010). The nutrient density of AYB gives it potential as a crop to mitigate the challenge of malnutrition faced in SSA. It has a crude protein content of 21–29% in its seed (Edem et al., 1990; Eromosele et al., 2008; Abioye et al., 2015; Duodu and Apea-Bah, 2017; Anya and Ozung, 2019; Adegboyega et al., 2020). The protein content of AYB compares favorably with other legume crops including common bean, chickpea, BG, and pigeon pea (Toyosi et al., 2020). Ajibola et al. (2016) found that albumin and globulin are the most common proteins that are present in AYB seeds. Its seeds are also rich in carbohydrates (Oshodi et al., 1997; Klu et al., 2000; Ndidi et al., 2014; Ajibola and Olapade, 2016), dietary fiber (Ndidi et al., 2014; Baiyeri et al., 2018; Anya and Ozung, 2019), and high levels of essential amino acid present in the protein, especially lysine and methionine levels (Okigbo, 1973; NRC., 2007; Ade-Omowaye et al., 2015; Chinonyerem et al., 2017). The AYB is also a source of essential minerals, namely, calcium, iron, magnesium, and zinc, in which the content is either higher or comparable to soybean and common bean (Adamu et al., 2015). Adeyeye and Agesin (2007) reported that protein, carbohydrate, and mineral concentrations were found in the hull of AYB samples, suggesting higher concentrations of minerals, protein, and carbohydrates in AYB seeds with its hull than in the dehulled AYB seeds.

Aside from its nutritional richness, AYB is also a source of bioactive and phytochemical compounds (Ade-Omowaye et al., 2015; Uchegbu, 2015; Soetan et al., 2018), which are secondary metabolites that may provide health benefits by decreasing the risk for several lifestyle diseases including degenerative disease linked with an antioxidant imbalance in the human body and cardiovascular disease (Oboh, 2006; Oboh et al., 2009; Ade-Omowaye et al., 2015). Phenolic acids and flavonoids are the two most essential bioactive compounds that were reported in the AYB (Oboh, 2006; Oboh et al., 2009; Ade-Omowaye et al., 2015; Soetan et al., 2018).

The crop also has possible medicinal importance (Potter, 1992), as the paste prepared from its seeds is used as a cure for stomachaches and traditionally for the treatment of acute drunkenness (Asuzu, 1986). High levels of antinutritional factors (ANFs) and long cooking time (Fasoyiro et al., 2006) as well as low seed yield (Saka et al., 2004), agronomic demand for stakes, long maturation period, and photoperiodic sensitivity (Okpara and Omaliko, 1995) have greatly limited the utilization of the AYB.

Constraints to Yield Production

AYB has a lot of potential in terms of its nutritional contents and resilience capabilities, but the AYB production has been limited by many constraints, ranging from the lack of improved varieties due to little or no research attention on the crop to high ANFs, long cooking time (Fasoyiro et al., 2006), low seed yield (Saka et al., 2004), laborious staking requirement, photoperiodic sensitivity, non-synchronized pod maturity, and long maturation period (Okpara and Omaliko, 1995). These factors have been the major setbacks for the commercialization of the AYB, particularly in the SSA where the climatic condition for AYB production is mostly favorable. Adequate research attention is therefore necessary to overcome these challenges.

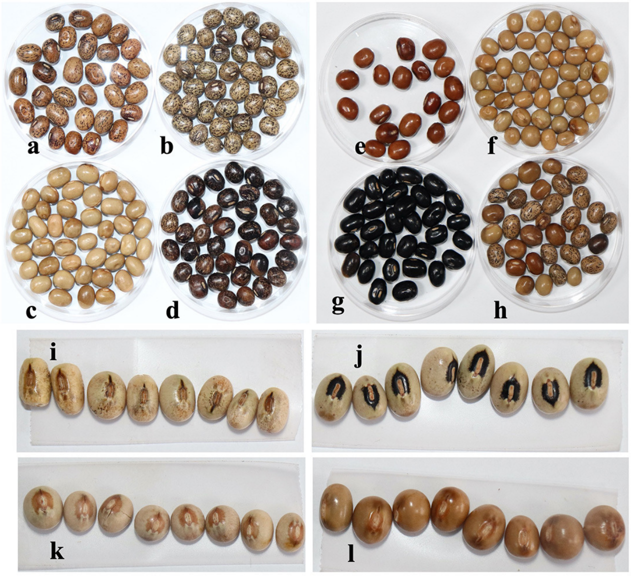

FIGURE 2 | Genetic varaition of seed triats in African yambean landraces for seed basel color **(a)** TSs-24 brown basal color, **(b)** TSs-27 Cream basal, **(c)** TSs-30 not variegated, **(d)** TSs-67 black basal; type of seed shape **(e)** TSs-39 Oval, **(f)** TSs-1 Rhomboid, **(g)** TSs-66 Oblong, **(h)** TSs-62 Round/globular; and seed eye color **(i)** TSs-81A clean eye color of white seed, **(j)** TSs-47 black eye color of white seed, **(k)** TSs-11 brown eye color of white seed, **(l)** TSs-1 non-white seed.

KERSTING'S GROUNDNUT

KG [*Macrotyloma geocarpum* (Harms) Maréchal & Baudet] is also known as the geocarpa groundnut or ground bean (Dako and Vodouhè, 2006; **Figures 1b**, **3**). KG [*M. geocarpum* (Harms) Maréchal and Baudet] is a leguminous crop cultivated on a small scale in the western part of Africa (Pasquet and Vigna, 2001). It is an annual herbaceous legume that is grown in arid and semi-arid regions. It is the third subterranean legume after the groundnut and BG (Adu-Gyamfi et al., 2011). It is an indigenous, high-protein, and herbaceous legume that is grown in arid and semi-arid areas of West Africa (Aremu et al., 2006). Studies have shown that it is mainly grown for its seeds (Buah et al., 2006).

Origin and Domestication

The center of origin of KG is not precisely known, but it is believed to be in northern Togo or central Benin (Dako and Vodouhè, 2006). KG is cultivated in Mali, Nigeria, Burkina Faso, Ghana, Togo, and Benin with low morphological diversity, manifested only in the seed color (Pasquet and Vigna, 2001). Outside West Africa, it is cultivated in Tanzania, Mauritius, and Fiji (Aremu et al., 2006; Dako and Vodouhè, 2006). Harms Kersting, a German colonial civil servant, first named it *Kerstingiella geocarpa* Harms from the domesticated material collected in Togo during the years 1905 and 1907 (Harms, 1908). Two years later, Chevalier described it as Voandzeia poiaaoni A. Chev. from the materials collected in Benin, but, a few months later, Chevalier recognized that his plant was identical to the one described by Kersting. KG was found to have cogeneric characters with Macrotyloma and, therefore, given a new name, *M. geocarpum* by Marechal and Baudet (Amujoyegbe et al., 2007). The studies carried out by Baudoin and Mergeai (2001) showed that KG is a typical West African plant whose wild ancestry was discovered in Cameroon and Central African Republic on the banks of the Ubangi river.

Germplasm Collection

Many African indigenous legumes are almost forgotten and no longer widely cultivated; hence, there is an urgent need to capture the diversity to avoid genetic erosion. KG is one of the important but neglected indigenous legumes that are almost going into extinction. GRC conserves only 28 accessions of KG from Nigeria and Ghana. Out of 28 accessions, 22 accessions were recently collected from farmers in the 3 western states of Nigeria where the crop is being majorly grown; these limited number of accessions call for the urgent need for exploration of its germplasm to pave way for the genetic improvement of the crop. In January 2019, a GRC team in collaboration with the National Center for Genetic Resources and Biotechnology embarked on a mini exploration of KG (*Macrotyloma geocarpum)* in the areas historically known for the cultivation of the crop in the Ondo and Benue state of Nigeria (**Figure 4A**). Two local government areas (LGAs) of the Ondo and Benue states were selected for germplasm exploration based on the predominance of KG

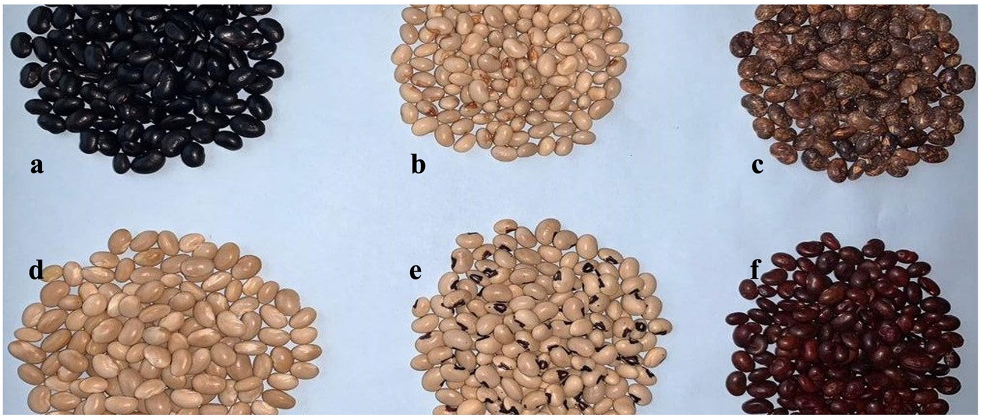

FIGURE 3 | Kersting's groundnut seed coat colors **(a)** Black, **(b)** White mottled with grayed orange eye, **(c)** Brown mottled with grayed orange eye, **(d)** White, **(e)** White mottled with black eye, **(f)** Brown. Source: Coulibaly et al. (2020).

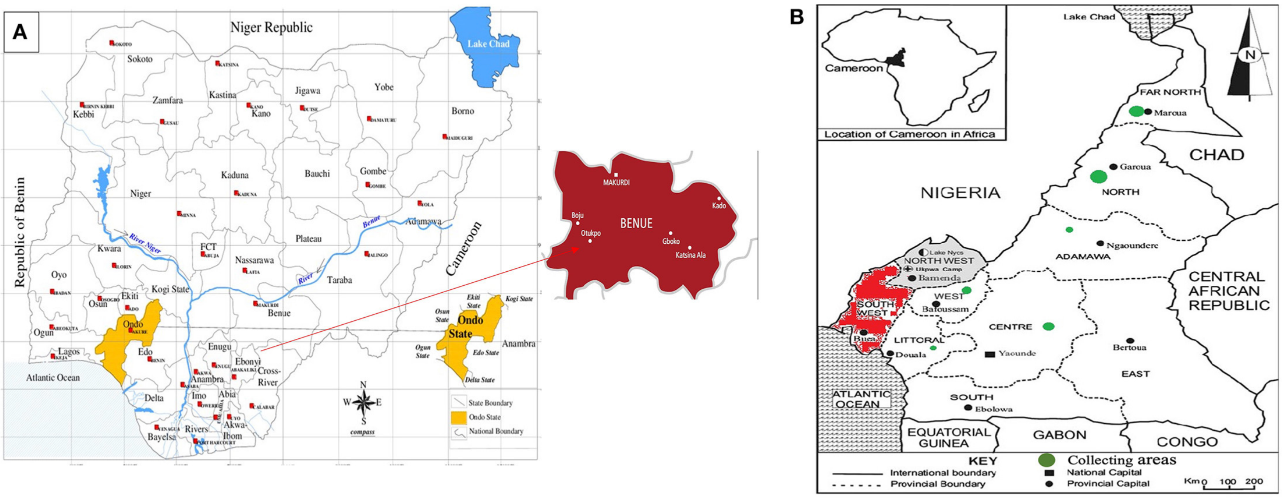

FIGURE 4 | Germplasm collection collected in year 2019 for African orphan legumes; **(A)** Map of geographic locations in Nigeria showing Ondo and Benue state where Kersting's groundnut germplasm were collected, **(B)** map of geographic locations in Cameroon where Bambara groundnut germplasm collected (The map is adapted from Henry and Roger, 2012). The collecting areas of Bambara groundnut are shown in green circles in the map.

production in those areas. The KG collection was carried out in eight LGAs of the state (Akoko Northwest, Akoko Northeast, Akoko Southwest, Akoko Southeast, Owo, Ose, Akure South, and Akure North) which covers about 2,465.6 km^2 and is situated at an altitude between 270 and 2,750 m above sea level. The Benue state lies within the Lower River Benue Trough in the middle belt region of Nigeria. Five villages from each local government were selected based on their involvement in KG farming. A total of twenty-two accessions of KG consisting of the different cultivars that are predominately being grown in these areas were collected directly from farmers for morphological and molecular characterization.

Economic Potential and Utilization

KG is well-adapted to drought regions and seeds with high nutritional values. It is used as a multipurpose plant in which its seeds are utilized for food, animal feed, and traditional medicine, while its leaves and vines are also used for animal feed (Agoyi et al., 2019). KG is currently being cultivated on a relatively small scale by local farmers in the West African savanna zones of Cameroon and Chad, The global production statistics for KG are not available but there have been reports of its production in Fiji, Mauritius, and Tanzania (Amuti, 1988). Its seeds are orthodox, which can be stored in a dry condition (15–20% relative humidity) as well as in −20°C (Ayenan and Ezin, 2016). The seeds are highly nutritious and contain protein (21.3%), crude fiber (6.2%), many essential amino acids, particularly arginine, histidine, methionine, and lysine, vitamins, ash (3.2%), and carbohydrates (61.53–73.3%) (Oyetayo and Ajayi, 2005; Ajayi and Oyetayo, 2009; Aremu et al., 2011). Arginine is the most common amino acid in its seeds and well-studied for arginine's role in the growth of children

(Pittari et al., 1992; Ajayi and Oyetayo, 2009; Van-Vught et al., 2013). Its seeds also provide many important minerals, namely, Ca, Fe, Na, P, Mg, and K (Aremu et al., 2006). They have low fat content, which offers an optional food for those who wish to lose weight (Ajayi and Oyetayo, 2009). Its fresh green leaves are used either in vegetables or added in soup (Mergeai, 1993). Many people boiled its seeds with water to drink as a remedy of diarrhea and also mixed its dry seed powder with hot water to use as an emetic in case of poisoning (Amuti, 1988; Ayenan and Ezin, 2016). As an orphan crop, KG is known as hardy and climate resilient, which adapts in drought regions where it can be sustained in low rainfall (500–600 mm) (Mergeai, 1993; Agoyi et al., 2019) and poor sandy and acidic soils (Agoyi et al., 2019). It can be a potential crop option in a vulnerable climate scenario, particularly for intra-season drought in West African regions (Masih et al., 2014; Ologou, 2015).

Constraints to Yield Production

Several reasons for the decrease of KG were reported by the farmers interviewed during the survey conducted by the GRC in 2019, among which are high costs of inputs (93.6% of responses), susceptibility to high soil moisture (68.1%), and high labor requirement (85.1%) (**Table 1**). It is usually planted between June and August, and most farmers do not intercrop it. This makes the cost of production very high, which is one of the major reasons why many farmers tend to abandon the cultivation of the crop.

Farmers use various traditional conservation tools (buckets, sacks, jar, calabash, metal drums, etc.) to preserve the seeds. KG seeds are very susceptible to insect infestation (Eric et al., 2019), particularly weevils; therefore, different preventative methods are employed by farmers to minimize insect infestation, among which are using cotton insecticide (27.6% respondents), wood ash (14.9% respondents), and the plant extract from the neem tree (*Azadirachta indica*) (6.4% respondents). The storage period ranges from 5 to 12 months, depending on the type of preservation method used. The survey revealed that most of the farmers that are still cultivating KG in Nigeria are women above 50 years old; this corroborated the reports of Amujoyegbe et al. (2010) and Bampuori (2007). Most of the farmers cultivate the crop mainly for subsistence purposes due to the high cost of production and post-harvest handling (Assogba et al., 2016). There is an urgent need to capture the available genetic resources, particularly in West Africa, where there are few KG farmers to prevent genetic erosion and the possible extinction of this important legume.

TABLE 1 | Reasons for decrease in Kersting's groundnut production based on IITA genebank survey in year 2019 in Ondo and Benue States of Nigeria.

Categories	Reasons	Percentage of responses (%)
Economic	High cost of production	93.6
	High labor requirement	85.1
	Lack of good market for seed supply	93.6
Agronomic	Susceptibility to high soil moisture	68.1
	Inadaptability to all types of soil	63.8
	The yield is lower than other leguminous crops	61.7
	Difficult postharvest storage	70.2
Technological	Difficulty of harvest	72.3
	Difficulty of seed hulling	61.7

BAMBARA GROUNDNUT

BG [*Vigna subterranea.* (L.) Verdc.] belongs to the Leguminosae family, it is a popular crop in many African countries (Ahmad et al., 2013; **Figure 1c**). It is similar to the groundnut (*Arachis hypogea*) in terms of the nutritional value and vegetal and culinary attributes (Boateng et al., 2013). It is known to be capable of tolerating adverse weather conditions as it can tolerate a range of environmental conditions and durations of stress (Feldman et al., 2019). BG has a chromosome number $2n = 2x = 22$ (Heller et al., 1997). Two botanical varieties of BG exist, namely, *V. subterranea* var. *spontanea*, which includes the wild types found in Cameroon, Sudan, and some parts of Nigeria and *V. subterranea* var. *subterranea*, which is widely cultivated in SSA (Basu et al., 2007).

Origin and Domestication

BG [*V. subterranea* (L.) Verdc] is believed to have originated from Central Africa prior to the introduction of groundnut (*Arachis hypogea*) from South America (Goli, 1997). Hillocks et al. (2012) reported Nigeria to be the center of origin of BG. BG has two botanical varieties: *V. subterranea* var. *spontanea*, which is comprised of the wild types in Sudan, Cameroon, and parts of Nigeria, and *V. subterranea* var. *subterranean*. BG is one of the underutilized legumes with a lot of potential for resilience and possible climate-smart crop for the future. BG is mainly cultivated for its edible seeds (Olukolu et al., 2012).

Germplasm Collection

GRC conserves over 1,700 accessions of BG from 28 African countries, which makes it the most diverse collection of BG crops globally. Most of the BG collection (68%) belonged to Nigeria (310 accessions), followed by Zambia (278 accessions), Zimbabwe (235 accessions), Cameroon (205 accessions), and Togo (134 accessions), respectively. Although IITA has the largest accessions in conservation as reported by Olukolu et al. (2012), a gap analysis based on passport information has been carried out. This analysis identified Cameroon as the target gaps and hotspot areas of high diversity for BG. In Cameroon, the hotspot regions of diversity were mapped out and then a collection of sixty-one BG samples was made in the year 2019. All these samples were collected from the West, Littoral, Central, Adamawa, North, and the Far-North regions of Cameroon (**Table 2**, **Figure 4B**). This collection will help to enhance the genetic diversity of the existing BG collection and provide an additional genetic diversity from Cameroon for future breeding research. The field characterization of vast BG collection is in progress for different phenotypic traits according to the BG crop descriptor at the GRC, IITA (**Figure 5**).

Economic Potential and Utilization

BG is an indigenous orphan crop that is the most widely cultivated as a legume food source after the cowpea in many countries of the SSA because it provides balanced food with

TABLE 2 | Bambara groundnut samples collected in four different regions of Cameroon in the year 2019.

Regions	Samples
Central	7
North	42
West	8
South	4
Total	61

FIGURE 5 | Genetic varaition of seed triats in Bambara groundnut landraces for seed color **(a)** TVSu-1749 others type, **(b)** TVSu-1918 black, **(c)** TVSu-1574 tan brown, **(d)** TVSu-1981 cream, **(e)** TVSu-1399 light brownish red, **(f)** TVSu-2032 dark red and seed shape type **(g)** TVSu-1412 round type, **(h)** TVSu-2014 other type, **(i)** TVSu-1397 oval type.

high nutritional composition and has a climate-resilient nature (Mayes et al., 2019; Paliwal et al., 2020, 2021). It is well-known as hardy and climate-resilient crop that can survive in poor soils and drought regions, particularly in the semi-arid zones with low rainfall (<800 mm) of the SSA where other crops fail to survive (Gunjal et al., 2009; Mubaiwa et al., 2018; Feldman et al., 2019; Mayes et al., 2019). Its major climate adaptive trait is drought resistance which make it more popular in local populations of SSA (Adzawla et al., 2016a,b; Olayide et al., 2018). Nigeria is the highest producer of BG (100,000 MT), followed by Burkina Faso (44,000 MT), Niger (30,000 MT), Mali (25,000 MT), and Cameroon (24,000 MT) (Hillocks et al., 2012; Aviara et al., 2013). In Africa, the average BG yield was reported as 0.85 t ha^1, while its yield production ranged between 0.5 and 3.0 t ha^1 (Azam-Ali et al., 2001; Mayes et al., 2019). Under drought, BG can produce a reasonable seed yield from 1.3 to 2.1 t ha^1 (Mwale et al., 2007); that yield is higher than chickpea and drought-tolerant groundnut varieties (Leport et al., 1999; Collino et al., 2000). Its biological nitrogen fixation (BNF) activity helps it to survive in poor soils with marginal input (Paliwal et al., 2020). BG is rich in protein, carbohydrates, dietary fiber, and minerals, which make it a cheap complete balanced food (Mubaiwa et al., 2018; Paliwal et al., 2020). Several reports on nutritional composition have shown that its seeds per 100 g of dry weight may provide 15–20% protein, 49–63.5% carbohydrates, 5.2–6.4% dietary fiber, 0.70% ash, and 4.5–7.4% fat (Onimawo et al., 1998; Murevanhema and Jideani, 2013). BG milk is more preferred than other legume's milk because of its color, flavor (Goli, 1997), and especially for its nutritional content with high protein (15–16%) compared to 4% protein of soymilk (Murevanhema and Jideani, 2013). Its seeds are also rich in mineral content, namely, Ca, Fe, Zn, and K (Karikari et al., 1997). Interestingly, Muimba-Kankolongo (2018) indicated that red-seed cultivars have almost double Fe content compared to the cream seed cultivars and these red-seed cultivars could be used for cultivation in the iron-deficiency regions of SSA. The characterization of the phytochemical properties of BG has been initiated, and it is reported that its antioxidant potency is equal to the other legumes such as chickpea, lentils, and common beans (Nyau et al., 2015). Many other secondary metabolites such as flavonoids, tannins, and alkaloids have been identified in BG and a study also shows that the alkaloids possess analgesic properties (Olaleye et al., 2013). The antimicrobial and antioxidant properties of BG could be utilized in the treatment of many ailments (Udeh et al., 2020). There are still more efforts needed toward examining the antimicrobial and nutraceutical values of BG (Udeh et al., 2020) against the pathogens of economic importance.

Constraints to Yield Production

BG has constraints that reduce not only the yield but also the acceptability due to the long cooking time and ANFs. A successful hybridization of the available landraces can make a difference in addressing various bottlenecks in BG, especially the yield and adaptation to diverse environments with respect to climate change. The constraints such as the long cooking time discourage consumers as it takes more fuel to cook BG, especially the dried types. Moreover, the beany taste after cooking, when prepared as a meal, is another constraint surrounding its acceptability; this is due to the ANFs present in BG. It will be quite interesting to assess the ANF level in diverse BG genotypes conserved in the gene bank for its improvement. An assessment of the available germplasm can show variation in BG ANFs and the discovery of genotypes with low ANF and short cooking time, which might improve nutrition and acceptability. A BG meal preparation varies from one country to another. In Nigeria, it is mostly consumed either as a snack by boiling the fresh pods with salt as refreshment or prepared as a meal called okpa. This meal is very common in the middle belt and eastern part of the country. Okpa is prepared by soaking the dried BG seeds in water overnight, dehulling them, and adding some ingredients like cray fish and pepper and grinding the mixture to become paste, red oil, or vegetable oil. Other condiments are added, and the mixture is thoroughly mixed and divided into small containers or wrapped in leaves and steamed by cooking in a pot for about 20–30 min.

Another constraint is uneven germination, which can cause yield reduction. BG is mostly cultivated in a dry environment, and the of soil moisture stress, especially from flower production and at other reproductive stages, is another major production constraint (Smart, 1985; Begemann, 1990; Linnemann and Azam-Ali, 1993; Sesay et al., 1999; Hampson et al., 2000), although the effect varies from landraces (Massawe et al., 2005). There is a need for a concerted research effort to address these constraints using selected parental and promising lines that have adapted different agro-climatic zones. Till date, no improved varieties of BG are available, neither are there any research centers for the improvement of this crop. To improve the yield and to address the bottlenecks in BG, the artificial hybridization of this crop is crucial (Massawe et al., 2005). The outcome of past works using morphological and molecular characterization and high-throughput molecular markers to evaluate BG has brought about the discovery of some BG landraces with useful traits for further breeding work. The University of Nottingham, United Kingdom and Crops For the Future Research Center, Malaysia have characterized and evaluated some landraces using high-throughput molecular markers and a shelter house to study various BG genotypes with diverse origin. The origin of these selected landraces spans through Africa and Southeast Asia (IITA Nigeria, Namibia, Botswana, Ghana, and Indonesia). This work has resulted in the identification of some landraces that can be used as a parent stock to address BG improvement for biotic and abiotic stresses through hybridization (Massawe et al., 2005; Sesay et al., 2010; Mayes et al., 2013; Gao et al., 2020).

INDIGENOUS AFRICAN ORPHAN LEGUME CROP BREEDING

Currently, the farm-level selection of these orphan crops has been carried out where farmers evaluate and multiply the seeds of existing landraces and store them for next-cycle crop production (Massawe et al., 2005). Because of the absence of the targeted breeding of these crops, there are few improved cultivars developed. One of the significant challenges of orphan crop breeding is their low yield production compared with other major crops (Ademola et al., 2020; Sidibe et al., 2020; Temegne et al., 2020). The low crop production may be due to poor seed germination and/or no fertilizer. In BG, the available genetic resources show significant variation for important breeding traits like the flower number, days to maturity, photoperiod sensitivity, leaf number, pod development, pod number, pod weight, seed color, cooking time, and yield (Massawe et al., 2005). These traits could play a critical role in developing farmer-preferred high-yielding improved lines in BG. The days to maturity should be taken into account to develop early- and late-maturity cultivars to avoid overstaying the pod in the soil after the pod maturity. Several reports indicate that BG is less affected by insects and pests than other legumes (Tanimu and Aliyu, 1990; Purseglove, 1992). BG is also infested by several fungal and viral diseases (Brink et al., 2006; Hema et al., 2014; Fourie et al., 2017). Kosini and Nukenine (2017) reported that Bruchids (*Callosobruchus maculatus*) are significant pests attacking the BG seeds during crop storage. In AYB, the flowering time, crop maturity, photoperiod sensitivity, pod shattering, cooking time, antinutritional factors, seed color, pod yield, pod number, and seed yields are observed as important traits during the field characterization at GRC-IITA. These traits could be considered for developing improved high-yielding cultivars under AYB crop breeding programs. In these orphan crops, yield loss from biotic stresses is less than abiotic stresses, particularly heat and drought, which are the major yield reduction factor causing unstable yield. The breeding research on nutritional traits and antinutritional characteristics is limited in these orphan legume crops. Under climate change, the breeding should focus on high-yielding, climate-resilient traits, the cooking time, nutrition, and ANF traits in these orphan African legume crops.

GENOMIC APPROACHES TOWARD GENETIC IMPROVEMENT OF INDIGENOUS AFRICAN ORPHAN LEGUMES

The breeding of these crops has been very limited and based mainly on landrace selection (Zeven, 1998; Mayes et al., 2019). The breeding and genomic research of indigenous African orphan legumes lag far behind other major legume crops, including cowpea, soybean, common bean, pigeon pea, and chickpea. Very limited molecular studies have been carried out. Because of the advances in next-generation sequencing (NGS), the genetic improvement of crops, including major legume crops, has accelerated in recent years through the integrated utilization of conventional and genomic-assisted breeding tools. Recently, high-throughput sequencing genotyping is highly demanded and globally applied on crop molecular breeding for the genotyping of large volumes of DNA samples faster, cost-effectively, and with better genome-wide coverage (Elshire et al., 2011; Poland and Rife, 2012). The application of these cutting-edge genomics tools has been used in crop breeding research for the speedy genetic gain of climate resilience traits and nutritional traits (**Tables 3, 4**).

Advances in NGS technology have enhanced our knowledge of the genomes of over 236 plant species (Chen et al., 2018), including indigenous African orphan legume crops. The African Orphan Crops Consortium (AOCC; http://www.africanorphancrops.org) targeted 101 African orphan crops, to sequence, assemble, and annotate the genomes that contribute to traditional African food supplies (Hendre et al., 2019). Recently, both the nuclear (550 Mbp) and mitochondrial (152,015 bp) genomes of BG have been sequenced using high-density Illumina short-read data (Chang et al., 2018a,b; Liao et al., 2019). The whole-genome sequencing of AYB is in progress using a hybrid sequencing approach (Illumina short-read data and Oxford Nanopore) under the Alliance for Accelerated Crop Improvement in Africa (https://acaciaafrica.org/bioinformatics-community-practice/full-genome-sequencing-and-annotation-of-the-african-yam-bean/) at the Bioscience eastern and central

Africa-International Livestock Research Institute (BecA-ILRI) Hub, Nairobi, Kenya. The complete genome sequencing of KG is also in the list of sequencing crops of 101 species and is yet to be begun by the AOCC. The genome sequencing results will help single-nucleotide polymorphism (SNP) analysis, leading to a better understanding of the genetic diversity, syntenic relationship with other legume crops, development of linkage map, precise QTL/gene discovery, and enhanced molecular breeding.

Exploring the knowledge of genetic diversity enables the selection of parental lines for population development. Different generations of molecular markers [AFLP (amplified fragment length polymorphism), isozyme, RAPD (random amplified polymorphic DNA), ISSR (inter simple sequence repeat), SSR (simple sequence repeat), DArT (diversity arrays technology), and SNP] have been used to explore the genetic diversity and population structure analysis (**Table 3**). Initially, isozyme markers were utilized in BG to explore the genetic diversity between domesticated and wild accessions and reported higher diversity in the wild ($Ht = 0.087$) than cultivated ($Ht = 0.052$) accessions, while the intrapopulation diversity was vice versa (Pasquet et al., 1999). The genetic diversity analysis of 363 accessions using RAPD and ISSR markers showed a close genetic relationship between Cameroon, Nigerian, and other West African accessions (Rungnoi et al., 2012). They reported two population structures where most of the accessions belonged to the first group and the remaining eleven accessions in the second subpopulation structure. Many other genetic diversity studies based on the SSR and SNP markers also reported similar results and indicate two major populations, namely, the West and Central African and South and East African populations (Somta et al., 2011; Molosiwa et al., 2015), while Redjeki et al. (2020) reported three populations in which the third subpopulation was from an Indonesian collection.

In AYB, several types of DNA markers, namely, RAPD (Moyib et al., 2008), AFLP (Ojuederie et al., 2014; Adewale et al., 2015), SSR (Shitta et al., 2016), and ISSR (Nnamani et al., 2019), have been utilized and showed that the wide genetic diversity in the genetic variation of different populations was mostly clustered in 3–4 clusters for genetic diversity (Adewale et al., 2015; Shitta et al., 2016; Nnamani et al., 2019) and population structure analysis (Ojuederie et al., 2014; Nnamani et al., 2019).

In KG, a collection of 281 accessions was grouped into four clusters (neighbor-joining tree method) based on white, red, black, and white with black eye seeded accessions, and it was grouped into two subpopulations with high differentiation across ecological regions that indicate the effect of geographic origins on the genetic diversity (Akohoue et al., 2020). Interestingly, another study with 217 accessions was grouped in eight clusters using the discriminant analysis of principal components and NJ tree method, based on the Edwards distance (Kafoutchoni et al., 2021). Cultivated landraces could be used as founder lines for intercrossing with diverse landraces to build a broader genetic base for future breeding use.

The domesticated/adapted landraces of African orphan legumes are widely cultivated across Africa in the absence of improved varieties. Genomic tools, namely, linkage mapping, QTL discovery using both biparental mapping and a genome-wide association study (GWAS), MAS, and genomic selection (GS), have been utilized in the recent past to speed up major legume crop (soybean, cowpea, chickpea, and common bean) breeding programs to develop high-yielding cultivars. The use of these genomic tools in African orphan legumes has been initiated and is in the early stage of progress. An integrated genetic linkage map has been reported for BG using two mapping populations (IITA686 × Ankpa4; F2 263 lines and Tiga Nicuru × DipC; F3, 71 lines) that generated 11 linkage groups with DArT Silico (presence/absence) and DarTseq SNP makers (Ho et al., 2017). The total distance covered by both linkage maps ranged from 1,376.7 (F3) to 1,395.2 cM (F2), which was higher than the earlier-reported 608.3 cM distance of 21 linkage groups by Ahmad et al. (2016). These mapping populations have been used for QTL discovery and identified several QTLs for morphological, growth-habit, and yield-related traits (**Table 4**; Ahmad et al., 2016; Ho et al., 2017). The SNP position of internode length QTL showed a syntactic relationship with other legume genomes, namely, cowpea, mung bean, and adzuki bean (Ho et al., 2017), a possibility of discovery for potential conserved candidate genes in close-related species genomes for the underlying traits of interest. There is no GWAS study available in BG, but it is in progress for different yield-related traits, drought, and nutritional traits at GRC. Recently, in the AYB, a GWAS study reported on the marker–trait association for protein, starch, and oil traits (**Table 4**; Oluwole et al., 2020). They identified 31 linked QTLs for protein, 11 QTLs for starch, and 8 QTLs for oil traits. So far, there is no linkage and the QTL mapping report is available in the AYB. The development of mapping populations for yield traits and abiotic stresses has been initiated, and its advancement (F2 generation) is in progress at the GRC. Similarly, there is no study reported for linkage and QTL mapping in KG, while recently, a marker–trait association research was reported by Akohoue et al. (2020) for morphological traits (days to 50% flowering and days to maturity) and yield-related traits including 100 seed weight and grain yield. GS in African orphan legumes is only reported for KG where GS revealed a moderate-to-high prediction accuracy using the stratified sampling technique (Akohoue et al., 2020). They found a strong correlation between the observed and predicted breeding value of 100 seed weight (0.62), days to 50% flowering (0.79), and days to maturity (0.72).

Molecular studies (**Tables 3**, **4**) in African orphan legumes are in the very early stage and need more efforts to develop different types of mapping population (F2, recombinant inbred lines, backcross, double haploid mapping population, and near-isogeneic lines) including MAGIC (multi-parent advanced generation inter-cross) population and its QTL mapping, GWAS, and GS, which will provide the opportunity to identify more precise and stable QTL/genes (Huang et al., 2015; Varshney et al., 2018) for yield and climate-adapted traits that could be further used in MAS to speed up the development of improved climate-resilient lines. GWAS and GS approaches could be more helpful for the crop genomics research of BG because

TABLE 3 | Plant genetic resources characterized using DNA markers in indigenous African Orphan legumes.

Crops	Genetic resource	Markers employed	Studies conducted	References
Bambara groundnut	79 domesticated and 21 wild Bambara groundnut accessions	Isozymes markers	Isozyme diversity in Bambara groundnut	Pasquet et al., 1999
	25 African accessions	RAPD markers	Genetic diversity	Amadou et al., 2001
	12 African landraces	RAPD markers	Genetic diversity	Massawe et al., 2003a
	363 accessions	RAPD and ISSR markers	Genetic diversity and structure analysis for five different geographical regions	Rungnoi et al., 2012
	16 cultivated accessions	AFLP markers	Genetic diversity among cultivated landraces	Massawe et al., 2003b
	100 landraces	AFLP markers	Genetic diversity among Tanzanian landraces	Ntundu et al., 2004
	240 landraces collection	SSR	Genetic diversity and structure analysis among Ghanaian landraces	Somta et al., 2011
	80 landraces	SSR	Genetic diversity among Ghanaian landraces	Aliyu and Massawe, 2013
	123 landraces	SSR	Genetic diversity among African and Indonesian landraces	Molosiwa et al., 2015
	105 landraces	SSR	Genetic diversity among Kenyan landraces	Odongo et al., 2015
	78 landraces	SSR	Assessment of genetic diversity and structure in South African collection	Minnaar-Ontong et al., 2021
	40 landraces	DArT marker	Genetic diversity among diverse accessions	Olukolu et al., 2012
	170 landraces	SSR and SNP	Genetic relationship among Indonesian landraces and their origin	Redjeki et al., 2020
African yambean	24 accessions	RAPD	Genetic variation within Nigerian AYB collection	Moyib et al., 2008
	77 accessions	AFLP	Genetic diversity in AYB collection (developed based on agro-morphologically)	Adewale et al., 2015
	40 accessions	AFLP	Assessment of Genetic diversity of AYB accessions	Ojuederie et al., 2014
	67 accessions	SSR derived from cowpea	Analysis of genetic diversity of AYB accessions	Shitta et al., 2016
	17 accessions	ISSR	Genetic diversity study of some AYB accession of Ebonyi state, Nigeria	Nnamani et al., 2019
	40 accessions	Seed protein marker	Evaluation of genetic diversity of Nigerian AYB accessions	Usoroh et al., 2019
	250 accessions	SNP marker	Genetic diversity analysis	Paliwal et al., 2019
Kersting's groundnut	20 domesticated and wild accessions	Isozyme markers	Genetic relationship among Togo and Burkina Faso KG accessions	Pasquet et al., 2002
	5 landraces based on seed color	SSR derived from cowpea	Genetic variation among the Ghana landraces	Mohammed et al., 2018
	281 landraces	Genome-wide GBS-SNP markers	Genetic diversity study of KG accessions collected from a wide range of agro-ecology namely Sudano-Guinean, Guinean, and the Sudanian regions of Togo and Benin	Akohoue et al., 2020
	217 landraces	Genome-wide GBS-SNP markers	Genetic diversity and population structure analysis in regional collection of Benin, Burkina Faso, Ghana, Nigeria, and Togo	Kafoutchoni et al., 2021

developing different types of mapping population is not easy as compared to AYB and KG due to the reproductive biology of the BG crop. Several reports indicate that the attempts made at crossing by hand pollination among parental lines were not successful in a natural field condition (Goli, 1997; Suwanprasert et al., 2006). In BG, QTL mapping could be useful in the mapping populations that are developed under the artificial crossing method, optimized in the United Kingdom (University of Nottingham) and Thailand under the BAMLINK initiative project funded by the European Union for BG research (https://cordis.europa.eu/project/id/15459/reporting). More efforts are required for the whole-genome sequencing and resequencing of many diverse lines to develop the pan-genome reference, which will increase the accuracy of SNP discovery and identify structural variation, comparative genomics with other close-relative legume species, and breeding efforts (Tao et al., 2019; Yang et al., 2019) to increase the commercial value. A recent advanced biotechnological tool known as gene-editing

TABLE 4 | QTLs detected for different traits in indigenous African orphan legume crops.

Crops	Trait associated (No. of QTL loci/MQTL)	Markers	Chromosome/LG	Mapping population	R^2 %**	References
Bambara groundnut	Plant morphological traits: Terminal leaflet length (1 QTL), Terminal leaflet width (2 QTL), Plant Spread (2 QTL), Node no./stem (3 QTL), Internode length (1 QTL), peduncle length (1 QTL)	SSR and DArT markers	LG 1, 3, 4, 5, 8	F2 and F3 (controlled environment) F3-Field (*DiPC* × *Tiga Nicuru*)	11.6–49.9	Ahmad et al., 2016
	Yield traits: Double seeded pods/plant (2 QTL), Pod weight (1 QTL), Pod length (1 QTL), pod width (1 QTL), Pod length of double seeded (1 QTL), Pod width of double seeded (1 QTL), seed weight (1 QTL), 100 seed weight (1 QTL), Biomass dry weight (2 QTL)	SSR and DArT markers	LG 1, 7, 11, 12	F2- and F3 (controlled environment) F3-Field (*DiPC* × *Tiga Nicuru*)	17.3–32.7	Ahmad et al., 2016
	Shelling % (2 QTL)	SSR and DArT markers	LG 7, 12	F2- and F3 (controlled environment (*DiPC* × *Tiga Nicuru*)	19.4–26.3	Ahmad et al., 2016
	Days to emergence	SSR and DArT markers	LG 1, 5, 13, 17	F2 (controlled environment) F3-Field (*DiPC* × *Tiga Nicuru*)	Sig*	Ahmad et al., 2016
	Growth habit	SSR and DArT markers	LG 4, 10, 14, 18	F3 (controlled environment) F3-Field (*DiPC* × *Tiga Nicuru*)	Sig*	Ahmad et al., 2016
	Eye pattern around Hilum	SSR and DArT markers	LG 12, 18	F3 (controlled environment) (*DiPC* × *Tiga Nicuru*)	Sig*	Ahmad et al., 2016
	Internode length	SNP and DArT markers	LG 2	F3 (*DiPC* × *Tiga Nicuru*)	33.4	Ho et al., 2017
African yambean	Protein (31 QTL)	SNP	Non reference SNPs	GWAS population (137 accessions)		Oluwole et al., 2020
	Starch (11 QTL)	SNP	Non reference SNPs	GWAS population (137 accessions)		Oluwole et al., 2020
	Oil (8 QTL)	SNP	Non reference SNPs	GWAS population (137 accessions)		Oluwole et al., 2020
Kersting's groundnut	Morphological Traits: Days to 50% flowering (5 QTL), Days to maturity (4 QTL)	SNP	Non reference SNPs	GWAS population (281 accessions)	10.59–61.41	Akohoue et al., 2020
	Yield traits: Number of pods/plant (1 QTL), Number of seeds/plant (2 QTL), 100 seed weight (2 QTL), Grain yield (1 QTL)	SNP	Non reference SNPs	GWAS population (281 accessions)	4.12–95.80	Akohoue et al., 2020

*Sig *, Kruskal-Wallis analysis; R^2 % **, The percentage of phenotypic variation explained by each QTL.*

technology could play a vital role in indigenous African orphan legume crop breeding. The gene-editing technology has utilized different types of sequence-specific nucleases such as zinc finger nucleases, transcription activation-like effector nucleases, and CRISPER-Cas (clustered regularly interspaced short palindromic repeats) in gene-editing research. The CRISPER-Cas gene-editing tool is widely used in crop research for editing the genes for complex traits like yield, biotic and abiotic stresses, and undesirable traits in different crops (Makhotenko et al., 2019; Wang et al., 2019; Bouzroud et al., 2020; Zeng et al., 2020; Zheng et al., 2020; Li et al., 2021; Liu et al., 2021) including the legumes soybean (Zhang et al., 2020), alfalfa (Meng et al., 2015), and cowpea (Ji et al., 2019). In groundcherry, three ortholog genes (SP, SP5G, and CLV1) that control the plant architecture and flower production and fruit development were introduced from tomato to improve major productive traits in groundcherry (Lemmon et al., 2018). This technology could play a crucial role to edit the gene control flower morphology in BG to make the hand-made hybridization crossing technique easy and successful. Therefore, it could also be utilized to improve yield, climate-resilient traits, and antinutritional traits in the future crop breeding of indigenous African orphan legumes.

BIOLOGICAL NITROGEN FIXATION (BNF)

In SSA, low productivity is a common issue facing agriculture, and low yield is often linked with poor soil fertility. Most of the farmers are smallholders who cannot afford a sufficient use of fertilizers, including minerals (Yanggen et al., 1998). Most fertilizers and minerals are imported (Mugabe, 1994; Chianu et al., 2012). BNF in indigenous orphan legumes could be a key source of nitrogen for farmers to sustaining yield in marginal soil. Legume crops often develop root nodules with the compatible rhizobia population in soil, which change atmospheric nitrogen to ammonia for plant use. In the field, the reported BNF values range from cowpea (17–198 kg N ha^{-1}) (Belane and Dakora, 2010), followed by groundnut (22–124 kg N ha^{-1}) (Rebafka et al., 1993; Katayama et al., 1995; Ncube et al., 2007), soybean (3–112 kg N ha^{-1}) (Sanginga et al., 1997; Chikowo et al., 2004; Mapope and Dakora, 2016), and common bean (8–31 kg N ha^{-1}) (Ojiem et al., 2007). Pigeon pea also reported a very high BNF contribution (37–164 kg N ha^{-1}), which is generally associated with a longer crop cycle (Adu-Gyamfi et al., 2007; Egbe et al., 2007). In SSA, the BNF values of BG have been reported of 4–200 kg N ha^{-1} (Mohale et al., 2014) and 32–82 kg N ha^{-1} under the Malaysian acidic soil condition (Musa et al., 2016). In an intercropping system, BG can fix 11.27–39.47 kg N ha^{-1} in the BG/maize and BG/cowpea intercrop system (Egbe et al., 2013), respectively.

In AYB, Adegboyega (2019) studied the nitrogen fixation values of 25 accessions by using the carbon isotope method and found BNF ranged from 12.76 to 22.47 kg N ha^{-1}. Kermah et al. (2017) also reported similar results where legumes BNF ranged from 11–31 kg N ha^{-1} in two different geographical regions, namely, southern Guinea savanna and northern Guinea savanna of Ghana. In KG, the nitrogen fixation ranged between 12 and 50 kg N ha^{-1} (Mohammed et al., 2018). These reports indicate that the amount of BNF added in soil by the AYB and KG is lower than other legumes such as BG, cowpea, groundnut, and soybean (Sanginga et al., 1997; Chikowo et al., 2004; Belane and Dakora, 2010; Mohale et al., 2014). The total BNF fixed over time differs mainly due to several factors like the legume species, cropping system, variety, location/site, climate, and crop management practices. The identification of locally well-adapted rhizobia strains is also important because they could be utilized as inoculants to competitively increase BNF and crop yield in these indigenous African orphan legumes. The knowledge of taxonomy and phylogenetic relationship among rhizobia and its population is an essential step to move forward for improving the productivity of these legumes and soil health using BNF (Chidebe et al., 2018). Therefore, there is an urgent need for more studies to enhance the understanding of the phylogenetic and functional diversity of rhizobia populations that modulates these indigenous orphan African legumes.

CONSERVATION AND DISTRIBUTION OF ORPHAN LEGUMES

The conservation and availability of genetic resources for distribution are an important pillar for the sustainable utilization of crop diversity for global food security and a prerequisite for the improvement of any crop species. GRC-IITA is one of the CGIAR gene banks at Ibadan, Nigeria that conserves over 6,747 accessions of orphan legumes of SSA, including BG, AYB, KG, and other minor legumes. Digital object identifiers (DOIs) have been generated, for all these accessions and are available online in Genesys, which is an open-access data portal (https://www.genesys-pgr.org/). DOI is a unique alphanumeric string which gives a permanent digital reference to each accession in gene bank. DOIs are also linked with their existing identifiers so curators do not need to modify their current process. DOIs provide a promising method to standardize the identification and tracking of accessions within or across the gene banks, publications, breeding programs, and research institutes. For sustainable conservation, these collections are also backed up for safety in other CGIAR gene banks outside Africa and in the Svalbard Global Seed Vault in Norway. GRC has distributed 9,598 accessions of these orphan legumes to different parts of the world for various purposes including national and international research centers in the last 20 years (**Figure 6**). Out of 9,598 distributed accessions, 6,528 accessions were distributed only for BG (3,599 accessions), AYB (2,878 accessions), and KG (51 accessions) in last 10 years. The distribution pattern of orphan legumes in 10 years indicate that a higher proportion of BG (average 360 accession/year), followed by the AYB (average 288 accessions/year), was distributed to 11 different African countries and European (Germany, Spain, and the United Kingdom), and Asian (India and Indonesia) regions.

CONCLUSION

In this review, the potential of indigenous African orphan legumes for food and nutrition security to SSA and the need to intensify modern breeding research and fill the existing gaps in the germplasm collections and conservation were highlighted. The available genetic resources of these legumes can be utilized to ameliorate food shortage and nutrition deficiencies among the rural population; it could also serve the purpose of income generation for women and rural farmers. The available conserved collection of indigenous African orphan legumes at GRC is still insufficient, particularly AYB and KG collections, as compared to the genetic resources available for major legume crops worldwide. The availability of a large and diverse collection of genetic

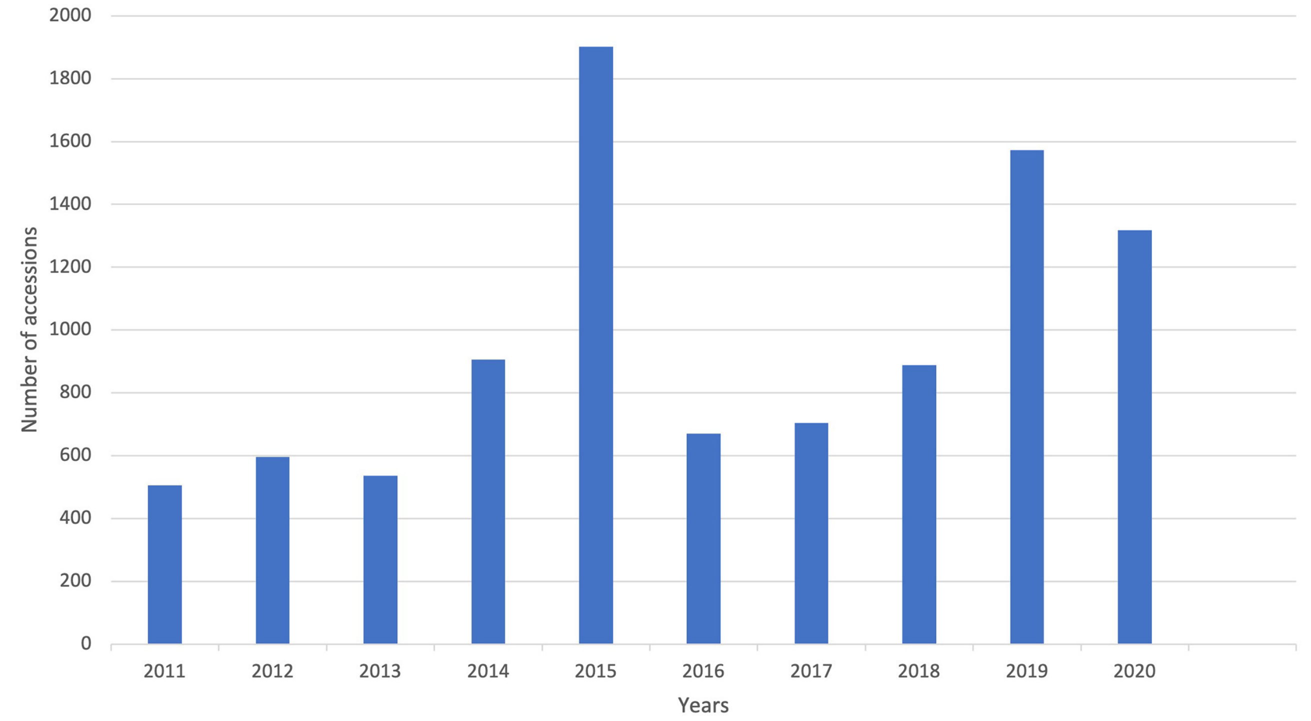

FIGURE 6 | The number of distributed germplasm samples of orphan legumes in IITA genebank in last 10 years.

resources is essential for the improvement of any crop species. Most of the farmers growing these indigenous African legumes are aged women in rural areas in many parts of SSA. Genetic erosion is a clear threat, and there is a need for a strategic approach to capture the existing genetic diversity of these legumes by expanding the existing *ex situ* collection for both the wild and landraces of these legumes, particularly the KG and AYB, by filling the gaps in the collection in both the national and international gene banks. Adequate documentation of the traditional knowledge about the cultivation and uses of the legumes is also necessary as it is currently in the hands of the aged farmers in the rural areas where the crops are predominantly being cultivated.

The research on most of these African indigenous legumes is still far behind compared to other legumes such as cowpea, groundnut, and soybean; therefore, an investment from national and international funding agencies for the research of these orphan legumes will be necessary to develop their full potential with modern crop breeding. The available genetic resource could be utilized to identify the accession for important agronomical traits, including nutrition, biotic, and abiotic stress traits using high-throughput phenotyping and genotyping and could further be used as parental material in different breeding programs using modern breeding tools. In BG, the artificial crossing method has been developed to develop successful breeding progenies, but the development of the easy crossing method is a prerequisite to initiate large-scale breeding programs in these crops. The development of improved short duration and low anti-nutrition factors and synchronized pod maturity lines should be prioritized in AYB crop breeding programs. The government and private sector can help enhance the market demand of these crops by producing more value-added food products of these legumes for the African population using food technology.

These legumes are a combination of high-nutrition and high-calorie food, making them nutritional food security crops of poor livelihoods in SSA; reducing the ANF and long cooking time could be a significant advantage to promote their use in the food plate of the African population. Several reports indicate the significant negative effect of climate change on crop production in SSA. It can reduce up to 20–40% of crop production by 2050 in humid and West African countries. To mitigate the climate change effect, indigenous African legumes could be promoted as an alternative or substitute crops to diversify the African cropping system as well as food diets. The application of GAB tools such as high-throughput molecular characterization and modern genomic tools could play a major role to unleash the potential of these legumes and speed up the breeding programs of African indigenous orphan legumes to develop an improved cultivar for nutrition and high yielding under harsh climate. Enhancing food security in SSA countries requires improved climate-resilient and high-nutrition-content varieties of these orphan legumes to help with the SSA region's sustainable food and nutrition security. These leguminous crops will also improve soil fertility and play an essential role in integrated soil fertility management. Most of the African indigenous legumes possess a great potential for climate resilience, which could pave the way for food and nutrition security in the face of climate change, but they are still being underexploited in terms of the solution to hunger and poverty they could offer. There is an urgent need to explore more of this potential.

AUTHOR CONTRIBUTIONS

MA and RP reviewed and edited the manuscript. All authors contributed to the writing of the original draft of the manuscript, read, and approved the final manuscript.

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EDITED BY
Raju Datla,
Global Institute for Food Security
(GIFS), Canada

REVIEWED BY
Albert Thembinkosi Modi,
University of KwaZulu-Natal,
South Africa
Fouad Maalouf,
International Center for Agricultural
Research in the Dry Areas, Lebanon

*CORRESPONDENCE
Babasola Daniel Adewale
daniel.adewale@fuoye.edu.ng

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Introduction to food, feed, and health wealth in African yam bean, a locked-in African indigenous tuberous legume

Babasola Daniel Adewale[1]* and Catherine Veronica Nnamani[2]

[1]Department of Crop Science and Horticulture, Federal University Oye-Ekiti, Ikole-Ekiti Campus, Ikole, Nigeria, [2]Plant Systematics and Conservation Biology Research Unit, Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria

The African yam bean, *Sphenostylis stenocarpa* Hochst Ex. A. Richmond, Harms, is an indigenous tuberous legume of the humid tropics of Africa. Its edible pulse and tuber host significant promises for food, nutrition, and health security. It was identified as a counterpart of cowpea in the 1970s and rated to be highly nutritious, but notable constraints have denied it research and funding attention. "Cowpea revolution" further deprived focus on the African yam bean. However, some research updated and promoted its significant food, feed, and nutritional-pharmaceutical values between 1973 and 2000. The global trend for food diversification has further improved awareness and research on the African yam bean this past decade, but research focus on the tuber is incomparably small. The abundant minerals, vitamins, and bioactive compounds in the two economic products unveiled in the present review assure food, health, and nutritional security. The analytical comparison of nutritional values of the African yam beans and other grain legumes demonstrated the significant place of the crop among its counterparts. Furthermore, investigative research identified the grain as a good substitute for soybean for livestock feed formulations. Although no clinical study has been reported, some *in vivo*, *in vitro*, and *ex vivo* biological activities and human studies of the two economic products revealed their efficacy in the management of anti-natal lactation induction, anemia, diabetes, arthritis, etc. However, African yam bean still suffer displacement in rank, utilization, and popularity compared to the "favored" legumes. While the present review adds to its advocacy, awareness, and utilization, a coordinated research program that will boost its value chain is most necessary for progress.

KEYWORDS

African yam bean, cowpea revolution, food, health, locked-in, nutrition

Introduction

By 2050, the world's population is set to increase by 40%, and the expected agricultural production to match the population is estimated to be 70% (Ebert, 2014). Wheat, rice, and maize, which provide more than 50% of human calorie intake, occupy 555 million ha (i.e., 40%) of the global arable land (Keyzer et al., 2005; Tilman et al., 2011; Stamp et al., 2012). The continued promotion of these few crops at the expense of other useful crops reveals the locked-in concept in crop production agriculture. According to Pahl-Wostl (2009), locked-in is "a situation when institutions and role players in decision-making continue with a conventional approach, despite that it is no longer leading to desired outcomes." Other crops with fewer references have inherent great potential when incorporated into diversified and sustainable food and feed production systems.

Globally, about 80,000 plant species are used for food, feed, and fiber as well as for industrial and medical purposes; 10,000 seems to be a realistic number of edible plant species (Mayes et al., 2012). In Nigeria alone, according to Ogunwusi and Ibrahim (2016), <20 of the identified 5,000 plant species are currently developed and used for food and industrial raw materials. To put it in perspective, about 99% of these useful plants exist as minor crops, many have declined to uncultivated status, others with resilience exist as wild plants, and many are now out of human reach. Per capita availability of pulses in Nigeria is over 10 times less than cereals and roots/tubers (Akah et al., 2021). By inference, the contribution of pulses to daily calorie and protein intake is poor; this is the reason for malnutrition in various communities. This situation is further compounded by a decline in agricultural productivity (especially underutilized legumes), monotonous diets of starchy staples, and/or dietary shifts to exotic high-caloric foods. Legumes are cheap and rich sources of protein, calories, vitamin B, minerals, and dietary fiber. Furthermore, they are low in fat, contain low/no cholesterol, possess a cholesterol-lowering effect, and regulate blood glucose due to their low glycemic index (Ene-Obong et al., 2013; Akah et al., 2021). Hunger, malnutrition, and food insecurity still stare at our faces because of the great and appreciated efforts of improvement on a few crops (Williams and Haq, 2002; Ikhajiagbe et al., 2021) which have not overturned the complex food challenges, especially in sub-Saharan Africa and other developing world. To address this, according to Abberton et al. (2022), crop diversification, cultivation, promotion, and utilization of climate-resilient crops are among the choicest strategies.

The domestication, cultivation, and distribution of the African yam bean (*Sphenostylis stenocarpa* Hochst Ex. A. Richmond, Harms) is very evident in tropical Africa (Potter, 1992; Okpara and Omaliko, 1997), especially central and western Africa, where it had been reported to exhibit very high diversity. Edible pulse and tuber are the two major economic products of the African yam bean (see Figure 1). There is a cultural and regional preference for each in the meals of the Africans; while west Africans mostly eat the seeds (with almost zero consumption of the tubers), the tuber is the major food product for the east and central Africans (Potter, 1992; Nwokolo, 1996). The economic potentials of the African yam bean are immense; the protein in tuber and seed is comparatively higher than most tuberous legumes (Dakora, 2013), the consumption of both seed and tuber by humans and livestock is safe with immense nutritional benefits, the grains are an equal or better substitute for common legumes in livestock feed formulation, and its medicinal properties to address some terminal diseases is worth examining. The beneficial possibilities of African yam bean for humans and livestock are well-documented in this review. The present review seeks to advance the unrealized inherent food, feed, nutrition, and health benefits in the economic products of African yam bean. Information are scattered in literature on the food, feed, and health wealth of this indigenous tuberous legume; this review, therefore, seeks to consolidate them. It is believed that an outcome of this review would be the repositioning of the locked-in crop, leading to its better utility and investment in its research for improved food, feed, health, and wellbeing.

African yam bean in research history from 1973 to 2000

African yam bean has been featuring for long in the traditional, cultural, and farming systems of Africans. The first notable documentation on its botany and taxonomy was by Milne-Redhead and Polhill (1971). African yam bean (*Sphenostylis stenocarpa*) (Hochst ex. A. Rich. Harms) came to the limelight during the First International Institute of Tropical Agriculture (IITA) Grain Legume Improvement Workshop held from 29 October to 2 November 1973 at the International Conference Center, IITA, Ibadan, Nigeria. In that conference, Okigbo (1973) introduced the crop, and Rachie (1973) on Highlights on Grain Legume Improvement at IITA 1970–1973 remarked *Sphenostylis stenocarpa* as an exceptionally nutritious crop. Many research attempts followed the introduction of the crop at the conference; a list of some of the major scientific reports and research outcomes on African yam bean between the first notable mention in 1973 to 2000 is presented in Table 1. This time frame was the quiescent period in the research on African yam bean.

We had access to just 51 published documents on African yam bean from 1973 to 2000; regardless, these numbers seem major. Most (77.5%) of the published works appeared in journals, quite a few (18.4%) were in handbooks and books, and 4.1% were in online archives (Table 1). The largest percentage (43%) of the publications were on food science and nutrition, followed by agronomy (20.4%) and general information on the crop (12.2%), most of which were in books (Table 2). Pre-breeding studies on African yam bean were 10.2%, ethnobotanical studies were 4%, and pharmacology, metabolomics, and diversity area were each 2% (Table 1).

Except for the food and nutrition studies, research on other areas or disciplines was very limited within this period. This greatly informs why knowledge of the crop and awareness was very low, even among scholars. Sadly, from Table 1, it is evident that there were no reports on the agricultural extension of African yam bean. Moreover, most of the publications

FIGURE 1
The two economic products of African yam bean pulses with testa morphs and clusters of tubers with irregular shapes from different accessions. Photo by Adewale Daniel.

were in the academic domain, on the shelves as an archive where they may not be available to potential users. More than 90% of the publications during the reviewed time frame were from Nigeria; research contribution from other countries where this crop is also cultivated was very limited. Poor attraction within agronomic research could be due to the long gestation period of the crop compared to other crops with short durations. Furthermore, there was no designated genetic resources conservation unit or center during the period for the conservation of accessions. During this period, most genetic resources of African yam bean for research were obtained from rural endemic sources in the southeastern and western regions of Nigeria. In addition, most of such research used $<$10 cultivars which were delineated for uniqueness based on seed coat color and color patterns.

However, African yam bean has since witnessed a huge research boost in various disciplines in the past two decades (Ojuederie et al., 2020; Adewale et al., 2021; Nnamani et al., 2021). However, literature on the agricultural extension of African yam beans is still very scanty, and this seems to have a negative correspondence to the general awareness of the crop. The effort of the Genetic Resources Center (GRC), International

TABLE 1 African yam bean in literature within 1973 to 2000 with remarkable research features and outcomes.

General information	Sources	References
i. Introduction of African yam bean as an underutilized tropical African legume. ii. Nutritional constituent in the seed and tuber. iii. Cultivation, distribution and production of the crop.	Five books and one journal article	Okigbo, 1973; Rachie and Roberts, 1974; National Research Council, 1979; Dukes, 1981, 1992; Anochili, 1984; Kay, 1987
Food science and nutrition		
i. Comparative study of proximate, mineral, amino acid, physico-functional characteristics of starches in seeds of African yam bean with some other grain legumes. ii. Identification and evaluation of probable toxic effect in the seed extract, antinutritional factors, nutritive value of sprouted seeds, chemical and sensory properties of its vegetable milks. iii. Processing methods including: presoaking, presoaking with salt, roasting, soaking and heating, fermentation with *Rhizopus microsporus* var.oligosporus to improved physico-chemical properties of the cooked bean, cooking time, protein digestibility, reduction in antinutritional factors and flatus producing oligosaccharides in African yam bean seed. iv. Increase consumption of African yam bean was encouraged for: very high Nitrogen free extract polysacharides, amylose, increased all growth indices, food conversion efficiency, protein efficiency, digestibility, biological values, net protein utilization and retention efficiency in rat. v. The African yam bean whole seed and hull is rich in protein, K and Ca, low in crude fat, cellulose, fatty acid composition, non-cellulose, lignin and phytate.	Two books and 20 journal articles	Evans and Boulter, 1974; Ihekoronye and Ngoddy, 1985; Azuzu and Undie, 1986; Nwokolo, 1987, 1996; Abbey and Berezi, 1988; Njoku et al., 1989, 1991; Edem et al., 1990; Ene-Obong and Carnoville, 1992; Ene-Obong and Okoye, 1992; Obizoba and Nnam, 1992; Oshodi et al., 1995; Agunbiade and Longe, 1996, 1998, 1999a,b; Apata and Ologhobo, 1997; Nnam, 1997; Nwinuka et al., 1997; Agunbiade, 1998; Oboh et al., 1998; Adeyeye et al., 1999
Botany, ethnobotany and pharmacological studies		
i. Basic botany and floral description of *Sphenostylis and Sphenostylis stenocarpa,* synonyms and some health-related folklores about of the crop in Africa ii. Linguistics evidences to ascertain African yam bean origin	Two online documents as at 2009, one book and two journal articles	Allen and Allen, 1981; Azuzu, 1986; Terrell et al., 1986; Potter, 1992; Potter and Doyle, 1992; Rehm, 1994
Pre-breeding studies including intra-specific diversity, metabolomics and seed protein sciences		
i. Species characterization based on: morphology, isozymes, chloroplast DNA ii. Phylogenetic and systematics studies of *Sphenostylis* and *Nesphostylis* iii. Investigation of seed coat hardness through water imbibition rate in seeds of African yam bean iv. Isolation lectin extracts and seed protein from African yam bean seeds v. Review of African yam bean resources in Ghana vi. Floral development and seed yield variability studies in landraces	Eight journal articles	Ene-Obong and Okoye, 1992, 1993; Okoye and Ene-Obong, 1992; Potter and Doyle, 1992, 1994; Togun and Olatunde, 1997; Omitogun et al., 1999; Amoatey et al., 2000; Machuka and Okeola, 2000
Agronomy: utility as fodder, nodulation capacity, fungicide and fertilizer test, grain yield evaluation, etc.		
i. African yam bean is suitable for fodder, food legume and cover crop ii. African yam bean naturally harbor seed borne fungi, e.g. *Fusarium* and *Aspergillus spp.*; pre-planting seed treatment with fungicides significantly improved germination percentage, seedling emergence, overall seed and tuber yield. iii. Staking, sowing date and plant density in significantly affects grain yield of African yam bean iv. African yam bean nodulates profusely with some bacteria species v. Fertilizer response trial on African yam bean and recommendation dosage of NPK for grain production	One book and nine journal articles	Asare et al., 1984; Nwachukwu and Umechuruba, 1991; Umechuruba and Nwachukwu, 1994; Okpara and Omaliko, 1995, 1997; Assefa and Kleiner, 1997; Obiagwu, 1997; Togun and Olatunde, 1998; Schippers, 2000

Institute for Tropical Agriculture (IITA), Ibadan, Nigeria has been very positive. The Center has freely availed researchers with African yam bean accessions, provided training, and supported postgraduate research on the crop. Actors and stakeholders are increasing and the future appears promising for African yam bean research. The increased research concentration on the

TABLE 2 Constraints, gaps, insights and way-forward in African yam bean research.

	Constraints	References	Current research status and some gaps	Insights and way-forward
S/N	**(a). Agronomic**			
1	Longer time to reach maturity (6–8 months)	Okigbo, 1973	Many individuals, groups, institutional and self-funded researches are ongoing in different disciplines on African yam bean in Africa.	Partnership for collaborations and initiation of coordinated researches on African yam bean among all stake holders.
2	Photoperiodic sensitivity	Okpara and Omaliko, 1995	Its nodulation proficiency which cultural cultivation has attested is yet to be identified, investigated and discovered for eco-friendly utilization in soil fertility managements and crop production.	Formalized genetic resources rescue programs and characterizations to unravel germplasm potentials leading to significant agronomic, genetic and nutritional improvement.
3	Climbing growth habit that necessitates staking	Okigbo, 1973		
4	Shattering tendencies of pods at maturity	Adewale et al., 2012		
5	Inconsistencies in tuber production in space and time	Adewale et al., 2012; Ojuederie et al., 2015		
6	Crop cultivation and germplasm is declining	Klu et al., 2001		
	(b). Genetics			
1	Lowering of genetic resources quantity	Akande, 2008	There had been some substantial diversity studies including: genomics, biochemicals, phenotypic etc., but utilization of the discovered potentials in breeding and improvement seem to be most necessary now.	Wider exploration and ethnobotanical survey to harness cultural and useful tool to assist research direction.
2	Available genetic resources are landraces and accessions	Adewale and Odoh, 2013		
3	No improved cultivars	Akande, 2008	Where needed variation for improvement is not available, creation of variation program need to start.	Cultivar development should embrace participatory approach.
4	Chromosome number still needing validation (n =18, 20, 22, 24)	Adesoye and Nnadi, 2011; Popoola et al., 2011		
5	No record of gene action on phenotypic traits	Adewale et al., 2021		
6	Population development and breeding program still invisible	Adewale et al., 2021		
	(c). Utilization			
1	"Beany flavor" due to anti-nutritional factors	Azeke et al., 2005	Uniquely rich tubers of *Sphenostylis stenocarpa* do not have relevance beside other tuberous crops of Africa, moreover, its nutritional richness are hardly known outside Africa. Informative cultural rudiments on the culinary of the crop is fading and would need to be captured in all eco-cultural niches.	Increased rural socio-economic and nutritional awareness and development of market valued products from African yam bean. Increasing research focus on its nutritional novelties, adaptive qualities and links with nutrition, health and livelihoods.

(Continued)

TABLE 2 (Continued)

	Constraints	References	Current research status and some gaps	Insights and way-forward
2	Hard-to-cook	Agunbiade and Longe, 1996; Nwokolo, 1996		
3	Grains availability only in rural markets	Nnamani et al., 2017		
4	Grossly poor knowledge about the crop and its economic products	Olanipekun et al., 2017		
5	Tubers are not displayed commodity even in rural markets			

African yam bean and the number of requests by researchers for its genetic resources prompted GRC to organize a symposium to harness the stakeholders in African yam bean research. The meeting, which was held on 18–19 October 2016 at IITA provided an opportunity for review, updates, and future research. Moreover, the launch of the Society for Underutilized Legume (SUL–www.sulegumes.org) was another outcome of the symposium. The Center currently conserves a diverse collection of over 450 accessions of the African yam bean landraces from Ghana, Nigeria, and the Democratic Republic of Congo; in addition, over 50% of these accessions are from Nigeria (Abberton et al., 2022).

Cowpea revolution: A setback for other indigenous tropical African grain legumes

The launch of the Grain Legume Improvement Program (GLIP) in IITA in 1973 initiated the beginning of the "cowpea revolution." Years following the 1973 workshop greatly promoted cowpea [*Vigna unguiculata* (L.) Walp]. In many respects, cowpea research efforts and improvement programs aided its attraction, wide acceptability, cultivation, and production. Enhanced research concentration on cowpea appears to have been encouraged by the short gestation period (reaching pod production within 3–4 months after planting). Moreover, its three notable growth habits (climbing, bushy, and decumbent/spreading) make cowpea more attractive to subsistence farmers than African yam bean to be grown in sole and/or companion crops. Furthermore, the ability of cowpea to sustainably grow in wider ecologies, especially drier regions, equally supported its competitive advantage over African yam bean both in research and production. Among the various grain legumes, cowpea production experienced the highest growth (4.7% per annum which expanded by 27% between 1994–1996 and 2008–2010, leading to a 90% increase in production (Nedumaran et al., 2015). Since 1970, IITA in Nigeria has worked on breeding and distributing improved cowpea materials and new germplasm lines to over 60 countries (Singh et al., 1997).

Cowpea [*Vigna unguiculata* (L.) Walp.] is still the most widely cultivated and utilized food legume and research material in sub-Saharan Africa and most countries of the world. It is currently considered the single most important pulse in the dry areas of tropical Africa (Snapp et al., 2018). Its consumption has grown at the rate of 3.2% per annum between 1980 and 2009, and the average level of consumption is 4.5 kg/person/year in sub-Saharan Africa (Nedumaran et al., 2015). Between 1971 and 1996, cowpea production and grain yield increased in Nigeria by ∼441 and 410%, respectively (Ortiz, 1998). Cowpea production received steady and tremendous positive growth during the period from 1970 to 2018; its production covered 96% arable land area of the total grain legumes and 98% of the total pulse production (Ortiz, 1998; Nedumaran et al., 2015; Akah et al., 2021). About ten other food legumes with promising values are known in Nigeria, but their production is low and declining, their cultivation, geographical distribution, and production statistics are not available, and their food, feed, nutrition, and health benefits are fading as their utilization declines. The Food and Agriculture Organization Corporate Statistical Database categorized them as pulses that could not be individually differentiated in their compilation due to their limited international and/or local importance.

Moreover, where legumes are concerned, to enhance the availability of cheap protein, very few (soybean, groundnut, and cowpea) are mostly supported by research and policy promoters (Foyer et al., 2016). The study by Saka et al. (2004) identified the Lima bean (*Phaseolus lunatus*), pigeon pea (*Cajanus caja*), African yam bean (*Sphenostylis stenocarpa*), and Bambara groundnut (*Voandzea subtteranean*) as prominent minor grain legumes that are grown in mixture with major crops on an average range land area of 0.2–0.4 ha. In total, these four grain legumes occupy <10% of the total cultivated land area in southwestern Nigeria. It is characteristic that their production is mostly among the older farmers (50 years and above) who produce an average of 80% of the total production. Their declining cultivation status may have prompted Klu et al. (2001), Ikhajiagbe (2003), Saka et al. (2004), and Adewale and Odoh (2013) to remark that these traditional grain legumes are moving toward extinction.

Dakora (2013) argued that the process of displacement of indigenous food crops by the introduction of new crop species continues to occur to date through the disproportionate promotion in the cultivation of newly introduced commercial crops by national and international research centers and local departments of Agriculture. The increasing promotion of non-indigenous crops in many African countries has consequentially resulted in the marginalization of many domesticated and undomesticated food plants. In his report, Dakora (2013) specifically cited the decline in the use of the tubers *Sphenostylis* and other indigenous food genera.

The promising food, feed, and health resources in African yam bean are unambiguously clear. Okpara and Omaliko (1995) found it to be a very good substitute for cowpea in forest agro-ecologies. The agro-zone is the endemic region for its genetic resources, cultivation, and production. Since the grain productivity of cowpea is not well-supported in rainforest and southern Guinea savanna regions of Nigeria and west Africa, a focused research development on this humid tropical legume could be enhanced given its potential for improved livelihood, which is now well-documented. Summarily, the African yam bean is well-adapted to vast environmental conditions, especially the humid tropics where it has been part of the food, economic, and medicinal resources with remarkable sustainability. It has supported household subsistence in central and west Africa.

Constraints to wide utilization of African yam bean and prospects

As reported in the literature (Machuka and Okeola, 2000; Adewale and Odoh, 2013; Okoye and Nkemakonam, 2018; Oshomoh and Ilodigwe, 2018), poor or low acceptability, adoption, and utilization of African yam bean have been due to classified constraints highlighted in Table 2. These are:

- **Agronomic**—demanding and challenging cultural practices coupled with poor demand and marketing which reduces farmers' enthusiasm for increased production.
- **Genetics**—no improved cultivar yet, available genetic resources are accessions and landraces, and poor knowledge of gene action of traits.
- **Utilization**—hard-to-cook nature of the bean which demands high fuel, cost, and time, the presence of anti-nutritional factors (ANF), "beany" flavor, unpleasant smell and taste, and food value of tuber unknown and neglected in utilization.

There is no record of the geographical distribution and production statistics of African yam bean by the Food and Agricultural Organization (FAO). This makes projection for strategy development difficult. Regardless, Table 2 offers some insights, gaps, and way forward on African yam bean research. According to Eneh et al. (2016), the list of constraints in African yam bean is inconsequential owing to the positive significant food, nutrient, and medicinal loads it hosts; the major constraint is inappropriate awareness. This is because knowledge of the nutrition and other potentials of crop species reflects their preference in human menu lists and considerations in other forms of utilization (Saka et al., 2004).

The significant impediment to African yam beans' acceptability is the prolonged cooking time to get the paste by the boiling process. This problem has prevented the utilization of its invaluable food security potential as long cooking period affects consumers' choices, nutrient contents, anti-nutrient condition, demands much energy usage—a factor of higher cost, long waiting time for meal readiness, etc. (Shitta et al., 2021). One of the findings in the report by Shitta et al. (2021) is that the cooking hours were dependent on the cooking methods used, the energy source, and the germplasm considered. This suggests that cooking time in African yam bean is genetically influenced. Seed hardness is heritable but can also be influenced by environmental factors at production and storage stages (Argel and Paton, 1999; Sandhu et al., 2018). Therefore, understanding the genetic basis of cooking time is necessary for improving the trait of the bean. A pre-breeding program involving the testing a large quantity of germplasm for its cooking time can suggest the selection of genotypes with a lower cooking time for subsequent advancement.

African yam bean: A safe, medicinal, and nutritious food

Consumption of African yam bean as food and drug is safe. Christopher et al. (2013) and Nwankwo et al. (2018) observed that the extract from the seed was non-toxic and did not show behavioral changes for humans and animals at a concentration of 5,000 mg/kg. Igbo folklore medicine had long identified the crop to contain ingredients for the treatment of anemia, and Nwaoguikpe (2008) wrote a follow-up remark highlighting that African yam bean has an anti-sickling effect on sickled hemoglobin due to its high content of hydrophobic amino acids. Esan and Fasasi (2013) reported the content of hydrophobic amino acids in African yam bean seed to be 33.99 g/100 g. The

TABLE 3 Anti-nutritional factors, amino acid and bioactive compound in the seeds and tubers of African yam bean as revealed in literature.

Anti-nutritional factors	Seed/Grain										Tuber	
Saponin (%)	0.111 ± 0.007	Ajibola and Olapade, 2016			0.07	Anya and Ozung, 2019					0.000302	Konyeme, 2021
Oxalate (%)	0.005 ± 0.0007		0.45	Shitta et al., 2022	0.01		0.00963	Abioye et al., 2015			0.000517	
Phytate (%)	0.013 ± 0.0004		5.16		0.72		0.259		0.00408	Adegboyega et al., 2020	0.000314	
Alkaloids (%)	0.227 ± 0.015				0.78						37.07	
Tannin (%)	0.003 ± 0.0008		0.0047	Shitta et al., 2022	0.34		0.01315	Abioye et al., 2015	2.38	Adegboyega et al., 2020	0.000251	
Trypsin (TIU/mg)	2.782 ± 0.205				0.21		0.32 ± 0.04				0.000223	
HCN (mg/kg)	7.184 ± 0.228				0.03							
Steroids (%)					0.02							
Total Glucosides (g/100g)											10.02	Konyeme, 2021
Total organic acids (ppm)											0.001726	
Amino acids and bioactive compounds												
Total Phenolics (g/100g)											23.52	Konyeme, 2021
Total Flavonoids (g/100g)											10	
Ascorbic Acid (mg/100g)					4.99 ± 0.15	Abioye et al., 2015						
β-Carotene (μg/100g)					70.19 ± 4.07							
Haemagglutinins (mg/100g)					0.35 ± 0.05							
Total Amino acids (g/100g)											13.25	Esan and Fasasi, 2013
Total Essential Amino Acid (TEAA) (g/100g)							53.69	Esan and Fasasi, 2013				
Total Non-Essential amino acid (TNEAA) (g/100g)							46.07					
TEAA/Total Amino Acid (%)							53.82					
Total Sulfur Amino Acid (Methionine+Cystine) (g/100g)							10.88					
Aromatic Essential Amino Acid (Phenylalanine+Tyrosine) (g/100g)							9.93					
TEAA/TNEAA (%)							116.54					
Hydrophobic Amino Acid (g/100g)							33.99					

oral administration of the extract to anemic Wistar albino rat at 100, 200, and 400 mg/kg body weight as investigated by Christopher et al. (2013) showed a significant increase ($p < 0.05$) in the packed cell volume (PCV), red blood cells (RBC) and hemoglobin concentration in the rat after 4 days of treatment. The observation justified the correctness of the long-held folklore as the observed increase in PCV, RBC, and hemoglobin concentration was speedy and comparable with Ranferon (a standard blood tonic drug) often used in the treatment of anemia (Christopher et al., 2013). The extract administration restored anemic conditions in rats, thus giving credence to the use of African yam bean in the management of anemia in humans and animals. Among the many lists of deficiencies and attending health challenges especially in Africa according to Baldermann et al. (2016) are iron deficiency and anemia, iodine deficiency and mental impairments, vitamin A deficiency, and blindness. The use of some of the neglected species could alleviate most of these health issues. The consumption of African yam bean grains experimentally enhanced recovery from anemia (Christopher et al., 2013). Ajayi et al. (2009) remarked that the seeds of African yam bean hold a high quantity of Iron. Konyeme et al. (2020) equally reported that the same mineral was highest in the tuber of African yam bean.

The seeds of African yam bean are potential sources of supplements in human and ruminant livestock (Ajayi et al., 2009). The whole seed is rich in potassium and phosphorus (Oshodi et al., 1995). The seeds are also rich in magnesium, calcium, and zinc but low in sodium and copper (Edem et al., 1990). Since anemia has been implicated in the etiology of many diseases, Christopher et al. (2013) strongly recommended the consumption of this plant for the full benefit of its nutritional and medicinal properties. The nutritional qualities of these seeds gave the seeds novel properties that made the legume a potential but latent nutraceutical (Nwankwo et al., 2018). Ndidi et al. (2014) reported that African yam bean grains have low caloric value, which makes it an interesting healthy food because high-fat diets lead to increased blood cholesterol levels and cardiovascular-related health issues. Details of anti-nutritional factors, amino acids, and bioactive compounds in both seeds and tuber of African yam bean based on literature is presented in Table 3. Anti-nutritional factors, some bioactive compounds, and amino acids were higher in the seed compared to the tuber.

Moreover, in the seed, the proportion of the essential amino acid in the total amino acid was higher than 50% (Table 3).

Nutritional profile of African yam bean grain

Awareness of the nutritional value of African yam bean seems to be improving in recent times. A survey among 500 respondents in the five Igbo-speaking states in southeastern Nigeria by Nnamani et al. (2017) revealed that proportional quantities of the population had a good awareness of the food and nutritional values inherent in African yam bean seeds. They further reported that the grain is sold at a significantly higher price than cowpea and other legumes. African yam bean is a rare legume that offers rich food, medicine, and nutrient-dense value for humans and livestock (Onyenekwe et al., 2000; Azeke et al., 2005; Akinmutimi et al., 2006). That it is exceptionally nutritious (Rachie, 1973) has long been the assertion of older adults in villages where they are highly relished and preferred over other contemporary legumes. The grain of the African yam bean has high metabolic energy, true protein digestibility (62.9%), moderate mineral content, and high-water absorption capacity (Ukom et al., 2019). The amino and fatty acid contents are comparable to those of most edible pulses. It also carries over 32% essential amino acids, with lysine and leucine being predominant (Onyenekwe et al., 2000) with very low-fat content. African yam bean is a nicely rounded non-fat food, containing 50–75% carbohydrate, 20–25% protein, about 1% oil, and about 6% fiber; all these provide nearly 400 calories per 100 g dry-weight (National Research Council, 2006). George et al. (2020) recently aggregated and presented the range of proximate composition in African yam bean grains from different authors as carbohydrate (49.88–63.51%), protein (19.53–29.53%), ash (1.86–5.35%), fat (1.39–7.53%), and fiber (2.47–9.57%). The nutrient density of the crop makes it a viable food crop for ameliorating food security and malnutrition challenges being faced in many developing countries, *via* direct consumption or fortification and enrichment of less nutritious staples (George et al., 2020). It provides a complete meal with significant mineral and vitamin loads. Table 4 shows that the contents of crude fiber, carbohydrates, and lipids were highest in African yam bean in comparison with soybean, cowpea, and bambara groundnut. Furthermore, the sodium and zinc contents were higher in quantity than the other legumes (Table 4). This makes it a qualified meal for food, nutrition, and health.

African yam bean tubers—A neglected product of the species

African yam bean is an underutilized crop (Potter and Doyle, 1992; Nwokolo, 1996; Azeke et al., 2005). Comparing the consumption, utilization, and research focus on the two economic products of African yam bean, the tuber has suffered more neglect. Predominantly in west Africa, the crop grows as an intercrop with other crops such as cassava, maize, yam, sorghum, and cocoyam (Saka et al., 2004) and primarily for the aerial pods which produce the pulse. Most farmers have denied ever seeing the tubers of African yam bean; the few who have seen it noted it as insignificant compared to the major carbohydrate providers (i.e., root and tuber crops), and hence, it is not cherished. Moreover, since the African yam bean is usually intercropped mainly for the aerial pulse, an intuitive probe to the subterranean part of the plant to get tubers is usually a rare venture (if ever attempted) among farmers whose carbohydrate needs are made available from major tuberous crops. This may answer why the African yam bean tuber has been a neglected product within the neglected species, especially in west Africa. Therefore, to most farmers in southeastern and western Nigeria, African yam bean tuber has poor economic relevance beside yam (*Dioscorea rotundata*), cassava (*Manihot esculenta*), sweet potatoes (*Ipomea batatas*), Irish potato (*Solanum tuberosum*), cormels from Tania (*Xanthosoma sagitifolium*), and Taro (*Colocasia esculenta*).

Nutritional profile of African yam bean tuber

The awareness that the crop produces tuber is visited with the feeling that the underground root would be toxic. Ojuederie et al. (2020), in their study, established that consumption of the tubers of African yam beans was harmless to Wistar rats and human beings. They found that it contained fewer antinutrients; for example, the proportion of the phytate in the tuber to the seed was in the general ratio of 4:10; trypsin inhibitors and lectin were not identified in tubers of some genotypes and the content of cyanogenic glycoside was very negligible. Specifically, its consumption by Wistar rats did not trigger any toxic effect in the tissues and organs of the specimen. Therefore, the investigation by Konyeme et al. (2020) updated the rich nutritional value of the tubers, while the research of Ojuederie et al. (2020) supported their safe consumption by human beings and livestock.

Table 5 provides the comparative ranges in values for proximate and mineral contents in the tuber of African yam bean, *Pachyrhizus*, cassava, sweet potato, and Irish potato drawn from various studies. African yam bean had the lead in crude protein with 13.25% amino acid (Table 3), total ash, and fat. Moreover, the comparison of its content with the nine minerals revealed that it holds the top position (Table 5). This comparison confers significance on the crop above the other contemporary roots and tubers, further confirming the earlier assertion of the National Research Council (1979) and Amoatey et al. (2000).

TABLE 4 Ranges in proximate, mineral and vitamin contents on dry matter basis in the seed of African yam bean, cowpea, soybean and bambara ground nut as revealed in literature.

S/N	Items	Range of values in literature for AYB	Ref.	Range of values in literature for Cowpea	Ref.	Range of values in literature for Soybean	Ref.	Range of values in literature for BGN	Ref.
Proximate composition									
1	Moisture content (%)	7.9–13.3	a, b, e	7.23–12.82	g, h, i, j	3.86–8.07	k, l, m	9.20–10.2	n, o, p, q
2	Crude ash (%)	1.86–5.35	a, b, c, d, e	3.24–4.2	g, h, i, j	4.29–4.92	k, l, m	3.40–4.36	n, o, p, q
3	Crude fat (%)	1.32–1.88	a, b	0.44–2.98	g, h, i	18.5–28.2	k, l, m	5.57–7.2	n, q
4	Crude protein (%)	17.66–24.31	a–e	23.01–26.44	g, h, i, j	31.19–37.69	k, l, m	16.44–22.40	n, o, p, q
5	Crude fiber (%)	2.47–10.5	a–e	1.86–4.4	g, h, i, j	4.99–6.27	k, l, m	1.45–4.80	n, o, p, q
6	Carbohydrate (%)	57.65–70.46	a, b, c, e	53.87–59.34	g, h, j	16.31–30.47	k, l, m	44.10–62.87	n, o, p, q
7	Dry matter (%)	87.55–91.09	c, d	NA		96.12 ± 0.23	m	NA	
8	Total lipid (%)	7.68 ± 0.21	c	4.03 ± 0.19	j	NA		5.17–7.60	o, p
9	Ether extract (%)	1.02–5.12	d, e	NA		NA		NA	
10	NFE (%)	48.47–59.94	d, e	53.51 ± 4.49	j	NA		NA	
Minerals									
1	Calcium (ppm)	240–4,360	e, f	202.86–2,420	g, h, j	231.6–10,800	k, l, m	350–38,740	o, p, q
2	Magnesium (ppm)	4,320–5,810	e, f	77.56–1,157	g, h, j	249.8–25,824	k, l, m	2,090–19,240	o, q
3	Potassium (ppm)	3,610–11,640	e, f	244.83–111,922.5	g, h, j	16,300	m	3,150–5,070	o, p, q
4	Sodium (ppm)	42,130	e	14.52–21,405.8	g, h, j	300–3,300	l, m	1,170–2,390	o, q
5	Phosphorus (ppm)	2,340–2,740	e, f	86.53–154.62	g, h	5,300–69,520	l, m	1,740–3,960	o, p
6	Manganese (ppm)	NA		NA		0.615	k	390	p
7	Iron (ppm)	100–1,260	e, f	7.5–140	h, j	579–1,640	k, l	180–425	o, q
8	Copper (ppm)	2,300	e	NA		NA		3,000	p
9	Zinc (ppm)	5,000	e	5.0–101.93	g, h, j	241.4–270	k, l	2,560–13,900	o, p
Vitamins									
1	Vit. C (mg/100g)	12.94 ± 0.26	c	NA				1.79	q
2	Vit. B1 (mg/100g)	0.12 ± 0.03	c	NA				0.4	q
3	Vit. B2 (mg/100g)	0.19 ± 0.03	c	NA				0.15	q
4	Vit. B3 (mg/100g)	0.53 ± 0.02	c	NA				2.34	q
5	B-carotene (mcg/100g)	NA		NA				8.83	q
6	Vit. E (mg/100g)	NA		NA				0.85	q

Range of values is the mean of means of presented values for the different items in the results sections of every publication cited, n–number of accessions/cultivars/genotypes considered in the various studies. Significant variations existed among the cultivars employed in the different studies.

AYB, African yam bean; BGN, Bambara ground nut; NA, Not applicable; NFE, Nitrogen free extract; Vit., Vitamins; Ref., References.

a, Adegboyega et al. (2020), n = 25; b, Ajibola and Olapade (2016), n = 5; c, Nnamani et al. (2018), n = 34; d, Anya and Ozung (2019), n = 2; e, Ameh (2007); f, Ojuederie and Balogun (2019), n = 4; g, Biama et al. (2020), n = 15; h, Antova et al. (2014), n = 4; i, Aletan (2018), n = 2; j, Owolabi et al. (2012), n = 5; k, Uwem et al. (2017), n = 3; l, Ogbemudia et al. (2017); m, Nwosu et al. (2019), n = 20; n, Igbabul et al. (2013); o, Olaleye et al. (2013); p, Musah et al. (2021); q, Okudu and Ojinnaka (2017).

TABLE 5 Ranges in proximate and mineral contents in the tuber of African yam bean, *Pachyrhizus*, cassava, sweet and irish potato as revealed in literature.

S/N	Items	Range of values in literature for AYB	Ref.	Range of values in literature for *Pachyrhizus spp*	Ref.	Range of values in literature for Cassava	Ref.	Range of values in literature for Sweet potato	Ref.	Range of values in literature for Irish potato	Ref.
Proximate component											
1	Crude protein (%)	8.32–14.45	a, b, c	6.03–6.1	d, e	1.29–2.62	f, g	3.15–3.36	j, k	1.69–8.26	l, m
2	Moisture Content (%)	39.4–55.5	a, b, c	77.75	d	6.55	f	67.28–71.70	j, k	69.35–78.42	l, m
3	Total ash (%)	2.44–7.69	a, b, c	1.94–2.27	d, e	1.39–4.48	f, g	1.24–3.32	j, k	0.96–2.5	l, m
4	Crude fiber (%)	2.4–9.8	a, b, c	6.64	e	1.28	g	0.43–2.38	j, k	0.57–0.88	l, m
5	Crude fat (%)	1.28–2.77	a, c	0.57	e	0.44–2.82	f, g	0.28–2.29	j, k	0.07–2.3	l, m
6	Carbohydrate (%)	54.47–79.24	a, b, c	13.34–91.12	d, e	90.34	f	23.18–24.98	j, k	16.57–18.06	l, m
7	Calorific value (Kjg-1)	1,451	a	388.79	e	NA		134.369	j		
8	NFE (%)	71.18	b	NA		88.88	g	NA			
9	Ether extract (%)	0.6	b	0.37	d	NA		NA			
Minerals											
1	Calcium (ppm)	500–2,680	a, b			783.33	h	2,560	i		
2	Magnesium (ppm)	1,670–4,670	a, b			850	h	2,352	i		
3	Potassium (ppm)	7,400–48,790	a, b			3,166.67	h	31,650	i		
4	Sodium (ppm)	21,440	b			54.15	h	3,184	i		
5	Manganese (ppm)	114.06	c			28.87	h	NA			
6	Iron (ppm)	80–3,160	a, b, c			156.76	h	63	i		
7	Zinc (ppm)	78.56–3,750	b, c			7.33	h	1,540	i		
8	Copper (ppm)	31.02–1,600	b, c			7.08	h	NA			
9	Phosphorus (ppm)	20–66,010	a, b			1,051	h	4,380	i		

*AYB, African yam bean; Ref., References; NFE, Nitrogen free extract, quantitative estimates were on dry matter basis.

a, Ojuederie and Balogun (2019), n = 4; b, Ameh (2007); c, Konyeme et al. (2020), n = 17; d, Ascheri et al. (2014), n = 5; e, Buckman et al. (2018); f, Nilusha et al. (2021), n = 5; g, Fakir et al. (2012), n = 7; h, Adeniji et al. (2007), n = 5; i, Sanoussi et al. (2016), n = 10; j, Aweke and Roba (2016), n = 10; k, Alam et al. (2016), n = 9; l, Gikundi et al. (2021), n = 3; m, Ezekiel et al. (2020).

Promotional initiatives to improve African yam bean utilization

Poor monotonous diets, with low calorific quantity and quality, void of nutritional and food diversity, are the primary cause of poor human health in many developing countries (Nnamani et al., 2021). Nutrient-dense diets based on diverse crops deliver better nutrition and greater health with additional benefits for human productivity (Chivenge et al., 2015). This is why food and nutrition security are a foundation for human livelihood. Agunwah et al. (2019) emphasized that the African yam bean represents a source of alternative protein supplements and its protein isolates possess certain characteristics that aid in protein enrichment for some food products.

The identification of the potential nutritional value inherent in African yam bean and the poor acceptability-adoption complexes have led to the development of some initiatives to bring the crop in part or whole to the urban menus. Consumption of processed food and light snacks is a common feature in urban centers. With the growing consumer awareness of the need to consume healthy foods, the presence of fiber, some useful starch, and essential fatty acids, African yam bean is a good candidate for the development of new functional foods for consumer health (George et al., 2020). Since African yam bean is available, affordable, and inexpensive, its incorporation in food products and continual utilization promise nutrition and health benefit to consumers across all socio-economic statuses. Some initiatives employed the grain legume as a fortifier in biscuits (Idowu, 2014) and cookies (Okoye and Obi, 2017). They noted that this readily available nutritionally-dense grain legume satisfactorily fortified biscuits and cookies, improving calcium, potassium, phosphorus, magnesium, and iron contents and widely improving the physicochemical properties of the two confectioneries including their color. Elusoji (2015) reported that all the cookies made from wheat flour supplemented with African yam bean flour compared favorably well with the control (100% wheat flour). Therefore, cookies production from wheat flour blended with African yam bean may be a promising initiative to increase the consumption and utilization of the crop. Positively, the utilization of African yam bean in the production of cookies would improve the nutritional value of cookies and hence the acceptability of the crop, and the consumption of such composited cookies and biscuits could increase the utilization of African yam bean, thereby increasing the economic power of local farmers. This could also reduce the importation of wheat, thus saving foreign exchange and enhancing food security (Igbabul et al., 2015; Ikhajiagbe et al., 2021). African yam bean utilization initiative research by Igbabul et al. (2015) led to the successful production of acceptable cookies from composite flours of wheat, cocoyam, and African yam bean; the product was remarked to have increased protein, ash, and crude fiber.

Health benefits of African yam bean

The report by Azuzu (1986) seemed to be the first study on the medicinal qualities of the crop in curing stomach aches and acute drunkenness. There is no record of clinical studies on the usefulness of both the seed and tuber of the crop. However, successful *in vitro* and human studies revealed the efficacy of the crop in the management of anemia, diabetes, and arthritis.

Traditional culinary and health

A report of its medicinal value in traditional Igbo settings in Enugu State, Nigeria, informed as follows: the seed is an important ingredient utilized in the topical treatment of stroke, insomnia, diabetes, measles, and stress. In addition, the liquid extract of mashed African yam bean cooked seed is often served to lactating mothers to induce and improve lactation after delivery. The fried seed coat are grounded and used in the treatment of stroke (Nnamani et al., 2018). Moreover, the roasted bean is traditionally prepared to entertain guests during marriage ceremonies. The legume is also reported to be of importance in the management of chronic diseases like diabetes, hypertension, and cardiovascular diseases because of its high dietary fiber content (Enwere, 1998). Nwankwo et al. (2018) reported the anti-diabetic activity of the seed extract of African yam bean. The seed milk extract of *S. Stenocarpa* possessed anti-diabetic activity like the reference drug glibenclamide. Graded doses of the seed milk extract were reported to have a blood glucose-lowering effect in a time and concentration-dependent manner (Nwankwo et al., 2018).

Management of gouty arthritis

African yam bean has a long history of pharmacological potential, especially in the management of gout and arthritis in addition to the already known nutritional properties (Eneh et al., 2016). There are a lot of other health benefits that can be derived from this plant. According to Soetan and Adeola (2018), the bioactive compounds in the grains of African yam beans have pharmacological and beneficial effects on disease management. Phenolic extracts from African yam bean seed (See the range of values in Table 3) have antioxidant power and the ability to scavenge free radicals (Enujiugha et al., 2012). The pathology of gout involves the deposition of urate crystals at the joints and tissues (Nsirim, 1999). Consumption of African yam bean was observed to reduce uric acid levels in the serum thus mopping up urate crystals (Eneh et al., 2016). This research unveiled the potential of African yam bean to contain potent bioactive ingredients for gouty arthritis management. The study by Eneh et al. (2016) equally identified the use of the crop as a local analgesic. Their findings revealed the African yam bean as a valuable and indispensable leguminous crop whose nutritional and pharmacological properties need to be commercially harnessed.

African yam bean grain as alternative crude protein for livestock feeds

Protein components in livestock feeds are the costliest. Soybean has been mostly used but their availability and cost are often challenging. This has made the consideration of alternative grain legumes a quest. African yam bean has been suggested as a very good substitute because its crude protein content falls within the range of values required for the substitution of soybean (Akinmutimi et al., 2006). In their research on weaner rabbit, Akinmutimi et al. (2006) observed that the feed-to-gain ratio increased as the quantity of raw African yam bean meal increased in the diet and became significantly different from the control (not fed on African yam bean). In another study on broiler chicken, Akinmutimi et al. (2011) reported that African yam bean supported better growth performance, low mortality, improved carcass characteristics, and cost per kg weight gain of meat, especially when the beans were boiled before usage as feed materials. This was a development over the initial research on weaner rabbits in which raw African yam bean was used.

Feed consumption by the rabbits was low when the content of African yam beans was much higher in the feeds of the rabbits. The presence of saponin and tannin was implicated (Akinmutimi et al., 2006) for the low feed intake. Processing by boiling reduced trypsin inhibitor and hydrocyanic acid and increased feed intake by the broiler chickens (Akinmutimi et al., 2011; Ndidi et al., 2014). In another study by Okereke et al. (2011) on finisher feeds for broiler chickens, toasting the grains enhanced feed intake, and supported optimum growth. Processing of the seed before utilization as a feed component yielded very good and highly comparable results (Anya and Ozung, 2019). Moreover, fermentation with lime subdued the beany flavor of the meals from African yam bean grains (Ngwu et al., 2014); for instance, Ajayi (2011) noted that fermentation grossly reduced phytate content in African yam bean meals. Okereke et al. (2011) further found that the replacement of the more expensive conventional feed ingredients with alternative cheap and affordable grain legumes was a possible solution to the escalating cost of livestock feeds. Fermentation and roasting increased the nutrient content and acceptability of its meal by livestock.

Conclusion and future research

African yam bean, like many other legume species, is an excellent food, low in fat, and rich in protein, fiber, minerals, and vitamins. Identification of the adoption driver for any species is an appropriate primary process; sometimes, genetic improvement may be secondary while markets or food politics could be primary. Some of the constraints to underutilization may be addressed in the laboratory and field plots, while others may need advocacy and market development strategy; sometimes in practice, many will need both. So, identifying crops that stand out as having the trait (and associated market) show higher potential than other common crops and are capable of improving food security is the first task (Mayes et al., 2012). This review unveils the food, feed, and health benefits of African yam bean. What do orphan crops have to offer that is not in the mainstream of the most used crops? Identification and realization of such potential according to Mayes et al. (2012) could justifiably lead to consideration of its/their uniqueness.

Most cultural systems or indigenous communities have cultivated and utilized diverse traditional crop varieties/landraces. The knowledge from seed to the table of such crops remains with the diverse indigenous cultures. They are not documented but are socially embedded in the cultures, traditions, and beliefs, they were locally derived and have for many years co-evolved with the ecosystem and lifestyle of each cultural domain (Berkes et al., 2000; Chivenge et al., 2015). The information in them has always been transferred through generations, observations and narrations. The present world of science which lacks knowledge of these crops and cannot find archival documentaries has one option—to directly link to the past through consultations with the indigenous cultures. Nnamani et al. (2018) did that for African yam bean among the five Igbo community states of Nigeria with very significant discoveries.

On Olanipekun's et al. (2017) concerns on the lack of knowledge about underutilized crops in the intellectual domain, future research on rescuing underutilized crops including African yam bean could be difficult if basic information (some of which are still intact with the indigenous communities) are not duly and promptly harnessed. The gap between the past and the present has made the quest for research on the underutilized crop unappealing because the link to past culture is often undocumented, and the benefits they provide are unknown because uses and possibilities from them died with the history. Important uses and functions of these species in the past as food or drug would have to be traced. Information from such search would lead to the realization of the forgotten or unknown importance of the crops. Therefore, since African yam bean is cultivated and utilized in different cultures in most parts of Nigeria and west and central Africa, a national survey to rescue fading important information and germplasm collection is urgently needed.

It is clear from the literature that locked-in crops such as the African yam bean have suitable and promising place in addressing human food, livestock feeds, and nutritional problems. In the future, harnessing its utility includes: significant awareness creation on the crop, its products and end products, policy support of its productivity/availability, research to address the inherent hard-to-cook and anti-nutritional problems, and innovative valorization along its value chain. This will enhance its attraction and drive the demand for is consumption and utilization. According to Akah et al. (2021), tireless efforts toward achieving some of these should, therefore, be pursued by all stakeholders to curb the prevalent nutritional problems.

Author contributions

BA and CN: conceptualization, literature search, outline planning, and review and editing. BA: original draft. Both 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.

Publisher's note

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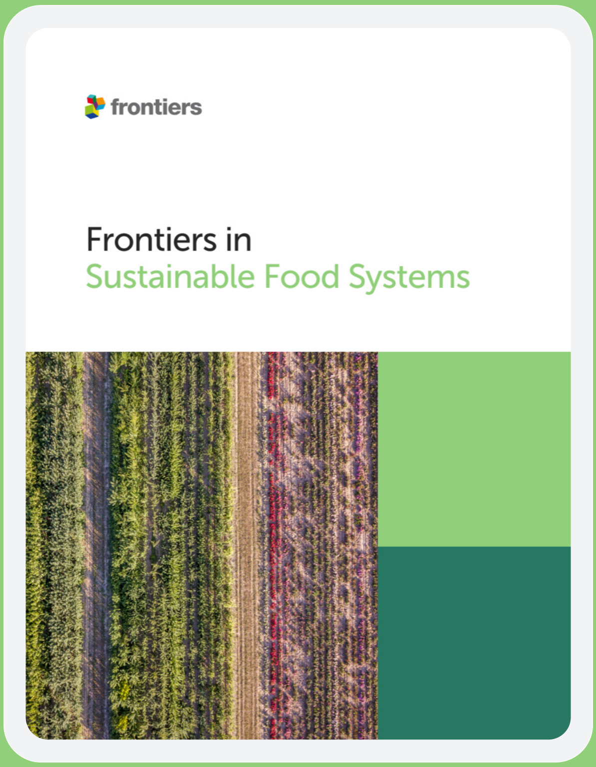

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