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Biofortification of wheat (*Triticum aestivum* L.) genotypes with zinc and manganese lead to improve the grain yield and quality in sandy loam soil

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Introduction: Micronutrients particularly Zn and Mn play a vital role in agricultural plants as well as human beings. Categorization of existing genotypes in wheat toward Zn and Mn application is extremely important to select the most efficient Zn as well as Mn genotype with higher yield and quality.

Methods: In this study, the differentiation of 14 genotypes was done for nutrient use efficiency characteristics through two methods to identify the most efficient Zn and Mn wheat genotypes. The categorization of genotypes was done based on efficiency and responsiveness under control and Zn + Mn treated conditions.

Results and discussion: The mean values of plant growth parameters, crop yield, nutrient concentration (Zn, Mn, N, P and К), quality parameters (crude fiber and protein content) and nutrient utilization efficiency were considerably greater in Zn + Mn treated plots signifying the higher response of traits toward Zn and Mn application. The interaction between genotype and Zn + Mn indicated that the application of Zn + Mn significantly affected the studied genotypes for different characteristics. The results of the study indicated that genotypes PBW-824, BWL-8855, PBW-872, PBW-869 and PBW Zn1 were efficient in terms of crop yield. Based on the quality parameters, genotypes PBW-824 and PBW-869 were the highest ranked among the studied genotypes. The identified Zn + Mn efficient genotypes would be valuable resources for higher crop production along with improved wheat grain quality.

KEYWORDS

biofortification, wheat genotypes, Zn and Mn nutrition, grain quality, micronutrients
efficient genotypes

1. Introduction

Greater production of staple food for the increasing population has reduced the nutritional quality of food and thus resulted in hidden hunger in living beings. Hidden hunger/micronutrient malnutrition refers to the insufficient intake of micronutrients which has severely affected human health as well as socio-economic growth. Thus, ending malnutrition by 2030 has been a set forward goal under the UN-Sustainable Development Goal 2 (UN-SDG2), which is insurmountable in the limited timeline. In this context, staple crops biofortification through scientific strategies such as agronomic approaches, conventional breeding and metabolic engineering, would gain great importance in the upcoming time. The biofortification process enables the augmentation of the nutrient levels in the edible part of crops, thus advocated as an efficient way to reduce micronutrient malnutrition by providing nutritionally enhanced crops. Economic efficiency along with the feasibility of using agronomically bio-fortified interventions have evolved as a valuable technique to reduce micronutrient deficiencies in developing countries as some studies have reported higher levels of micronutrients through biofortification. The other quality parameters of the crops have been found to increase in parallel with the nutrient content. However, the effectiveness of biofortification was primarily studied to increase the content of only one nutrient per crop.

Micronutrient deficiency in plants and soils, such as Zn (zinc) and Mn (manganese) has resulted in severe effects such as decreased yield and low micronutrient concentration in crops, leading to micronutrient malnutrition in humans and animals (Dhaliwal et al., 2020). Zinc is an important micronutrient in plants since it participates in a variety of physiological functions and activities of enzymes, including protein and auxin production, glucose metabolism, immune system development, cellular membrane maintenance, and pollen generation. Unfortunately, zinc deficiency affects nearly half of Indian soils (Chattha et al., 2017), causing visible anomalies in plants including stunted growth, chlorosis, reduced leaves, fungal infections and increased sensitivity to extreme light or temperature harm. Zinc has been listed as an imperative micronutrient for humans and is part of approximately 300 metalloenzymes involved in a series of metabolic reactions (Livingstone, 2015). In addition, it is known for its role in mitigating reactive oxygen species by elevating antioxidant metabolism in the body (Ram et al., 2016). Zinc intake has advocated the reduction in cancer cases, neurological disorders and autoimmune diseases (Chasapis et al., 2012). Besides Zn, Mn acts as an important micronutrient for the growth of humans and plants (Foy et al., 1978). Its crucial role in reproduction, carbohydrate/lipid metabolism, brain functioning and neurotransmitter synthesis is well-known (Greger, 1999; Golub et al., 2005). Manganese deficiency has shown adverse effects on plant carbohydrates, which further reduces crop yield and quality due to low pollen fertility and low carbohydrates during grain filling (Dhaliwal et al., 2023). The Mn deficiency significantly decreases the photosynthesis efficiency in plants, reducing plant productivity and dry matter yield (Rashed et al., 2021). The deficiency of Mn may result in severe health consequences in humans such as anorexia, weakness and apathy (Huang et al., 1989).

Micronutrient deficiencies in human are prevalent in regions with low plant-available concentrations of micronutrients (Cakmak, 2008; Cakmak et al., 2017). The existence of a strong relationship between soil and crop nutrient status along with human health has been already postulated (Welch et al., 2013; Cakmak and Kutman, 2018). Wheat contains lower nutrient content to satisfy human nutritional needs (Cakmak, 2008; Cakmak and Kutman, 2018). Therefore, the extreme consumption of monotonous wheat leads to micronutrient malnutrition. Hence, there is a dire need to increase the micronutrient content of wheat grain, particularly in developing countries where there is a lack of diet diversity. In recent years, work has been done to increase the micronutrient concentration in grain in particular wheat genotypes, but this study focuses on the comparative evaluation of 14 wheat genotypes through Zn and Mn fertilization for various growth parameters, proximate composition analysis, micro- and macro-nutrient concentrations.

2. Materials and methods

2.1. Field specification

The present experiment was conducted in split plot design (SPD) involving three replications, throughout both the *Rabi* seasons (November – April) of 2020–21 and 2021–22 at Research Farm, Department of Soil Science, PAU, Ludhiana (30° 56' N, 75° 52' E and 247 m above mean sea level). The texture of the soil under investigation was sandy loam exhibiting pH, EC and soil organic carbon values of 7.30, 0.35 dS m^{-1} and 0.38%, respectively (Jackson, 1973; Dhaliwal et al., 2021a). The original content of micronutrients, i.e., Zn, Cu, Fe and Mn in soil was calculated using AAS (Varian AAS-FS 240 model) (Lindsay and Norvell, 1978) and the values were 1.20, 0.88, 5.32 and 4.17 mg kg^{-1}, respectively. Whereas, N, P and K values were estimated to be 0.084%, 26.52 Kg ha^{-1} and 7.95 Kg ha^{-1} using alkaline KMnO$_4$, the Olsen extractable P and neutral ammonium acetate methods, respectively (Merwin and Peech, 1950; Jackson, 1973). The climatic condition of the area was subtropical as well as hot and rainy summer followed by a drier winter. Annually the rainfall ranged between 400 and 600 mm where the highest level of rain, i.e., 70% was observed from July to September (Dhaliwal et al., 2021b).

2.2. Experimental details

In the present study, the wheat genotypes were screened through the foliar application with four treatments, i.e., control, ZnSO$_4$.7H$_2$O (0.5%), MnSO$_4$.7H$_2$O (0.5%) and ZnSO$_4$.7H$_2$O (0.5%) + MnSO$_4$.7H$_2$O (0.5%), respectively as main plots. The 14 wheat genotypes, i.e., WH-1105, HD-3086, PBW-550 (U), PBW-725, PBW-343 (U), PBW Zn 1, PBW-826, PBW-824, PDW-291, WHD-943, BWL-8855, PBW-872, PDW-274, PBW-869, procured from Punjab Agricultural University, Ludhiana, India were taken as subplots. As per treatments total of four foliar sprays were given, i.e., the first spray was applied 2–3 days before first irrigation (30 days after sowing; for effective absorption of zinc and manganese through plant foliage), the second spray at 15 days after first spray, the third spray before flowering and the fourth spray at 10 days after flowering, respectively. The land was prepared by sowing wheat with a seed-*cum*-fertilizer drill method at a depth of 4–6 cm. The sowing of genotypes was done in November with a row-to-row spacing of 15–20 cm. The recommended doses of fertilizers (RDF) in wheat crop, while the whole nitrogen (urea fertilizer) @ N at 125 kg ha^{-1} was applied as three splits and whole

phosphorus (diammonium phosphate fertilizer) was applied @ P_2O_5 at $62.5 \, kg \, ha^{-1}$ as a basal dose. At sowing, half and the full dose of N and P were used, respectively, whereas the remaining half dose N was divided into two equal doses applied at the first as well as second irrigations.

2.3. Growth attributes

At the stage of physiological maturity, growth parameters including plant height, number of tillers and chlorophyll content were noted as the average value of five plants in each plot. The height of the plant from base to tip was noted by using a meter scale and the mean height was expressed in cm. The total tillers at maturity were calculated manually. The chlorophyll content in plant samples was measured using a chlorophyll meter (SPAD 502). It is a device developed by the soil and plant analysis department (SPAD) of Minolta Camera Co., Japan for computing chlorophyll content in leaves. The readings of the SPAD meter were recorded for the second or third fully opened leaves from the top of the main stem. The mean value of the 10 plants per plot was reported as chlorophyll meter (SPAD 502) value.

2.4. Yield study

Net plot area was used to calculate the wheat yield where the border rows were ignored and this was further written in t ha^{-1}. The samples (grain and straw), were made to dry in a hot air oven for 3 days at 65°C and were grounded in a Lab Willey Grinder (Model Arthur H. Thomas type) manufactured by Laboratory Equipments, Pune, Maharashtra, India. The powdered samples of straw (1.0 g) and grain (0.5 g) were digested using a mixture of diacids (HNO_3 and $HClO_4$ in a ratio of 3:1; Kumar and Dhaliwal, 2021). After digestion, the samples were analyzed for Zn and Mn content using an atomic absorption spectrophotometer (Model AAS 240 FS, Varian, Germany Limited).

2.5. Estimation of certain quality parameters

Total ash, crude fiber, total soluble solids (TSS) and protein content in wheat grain were determined in the present study where the results were given as mean of triplicates.

2.5.1. Moisture content

The moisture content was calculated through the method given by AOAC (1995) where 2.0 g sample was weighed in an aluminum dish and was allowed to dry properly in an oven at $130 \pm 3°C$ for 1 h until a constant weight was achieved. Loss in weight after cooling the sample was determined using the following formula.

$$\text{Moisture} \left(\% \right) = \frac{M_2 - M_3}{M_2 - M_1} \times 100.$$

M_1 is mass of empty dish; M_2 is mass of dish + sample (before drying); M_3 is mass of dish + sample (after drying)

2.5.2. Ash content

Total ash in the sample was recorded by AOAC (1995) method where 5.0 g sample in a dish was burnt on a hot plate until blackened. The sample was allowed to cool at room temperature followed by heating in a muffle furnace at 550°C to get gray ash which was further allowed to cool and its weight was noted. The remaining weight was noted, and ash content was calculated as follows:

$$\text{Ash} \left(\% \right) = \frac{M_{2-M_1}}{M} \times 100$$

M_1 is mass of dish before burning; M_2 is mass of dish + sample after burning; M is the sample mass

2.5.3. Crude fiber

The sample (2.0 g) was added to the beaker having 200 mL of H_2SO_4 (1.25%) which was then heated for 30 min (AOAC, 1995). After heating, filtration of the solution was done using Whatman filter paper No. 54 and the remains were washed with hot distilled water. The residue was transferred to sodium hydroxide solution (1.25%) and heated for another 30 min which was then filtered and again washed with distilled water. The contents on filter paper were allowed to dry in an oven at 105°C until a constant weight was obtained. The contents were cooled in a desiccator and weighed. The drop in weight was attributed to the crude fiber present in the sample which was calculated using the following formula:

$$\text{Crude fiber} \left(\% \right) = \frac{M_2 - M_1}{\text{Mass of sample}} \times 100$$

M_1 is mass of filter paper; M_2 is mass of residue + filter paper.

2.5.4. Total soluble solids

Abbe's refractometer was used to calculate the total soluble solids in wheat grain by the index of refraction which was referred to as the degrees brix. A drop of extract from wheat grain prepared in ethyl acetate through ultra-sonication was placed on a clean and dry prism and calibration was done at zero line on the scale. Then the samples of wheat grain were analyzed for their TSS value by reading the line of demarcation on the scale of a refractometer (AOAC, 1995).

2.5.5. Protein content

Protein percentage was recorded by multiplying N content (noted by carrying out the digestion of samples using H_2SO_4 where total N was calculated through micro Kjeldahl's method as reported by Jackson, 1967) by multiplying it with a factor 6.25.

Total Protein (%) = N content in the sample x 6.25

2.6. Categorization of genotypes

Categorization of efficient genotypes was done based on the background of each wheat genotype along with combined foliage application of Zn + Mn in terms of yield, quality attributes and nutrient content. Thus, the categorization of 14 genotypes showing variation in characteristics was done under Zn + Mn treatment as E (Efficient), M (Moderately efficient) and I (Inefficient).

2.6.1. Method 1

The wheat genotypes were categorized (μ) and the standard deviation (SD) of every factor under control and fertilizer application was noted (Osborne and Rengel, 2002; Aziz et al., 2017). Based on the response to Zn and Mn, genotypes showing E (Efficient), M (Moderately efficient) and I (Inefficient) characteristics had values>μ + SD (Efficient), ranging between μ + SD to μ-SD (Moderately efficient), and <μ - SD (Efficient), respectively. Like the scores 3 (E), 2 (ME), and 1(I) were assigned to E, M and I denote response to genotypes, respectively and were further ranked based on the cumulative scores.

2.6.2. Method 2

The genotypes were also categorized for efficiency and responsiveness to Zn and Mn application based on the crop yield (grain and straw) and nutrient use efficiency (NUE) (Rawal et al., 2022). Nutrient use efficiency was computed through the formula given by Manske et al. (2002):

$$NUE = \frac{Total\ dry\ mass\,(Zn + Mn) - Total\ dry\ mass\,(control)}{Nutrient\ applied}$$

The genotypes having higher crop yield as compared to the average yield of studied genotypes have been classified as efficient and those exhibiting higher NUE in comparison to average NUE were classified as responsive genotypes. Thus, the classification of genotypes was done under four categories, i.e., (i) efficient and responsive (ER), (ii) efficient and non-responsive (ENR) (iii) inefficient and responsive (IR) and (iv) inefficient and non-responsive (INR) as decribed by Kosar et al. (2003).

2.7. Statistical analysis

Statistical analysis of the results was done by using the SPSS package, version 16.0 (SPSS Inc., Chicago, United States of America). All attributes were calculated using two ways analysis of variance (ANOVA) for obtaining minima, maxima, mean, standard deviation (SD), standard error (SE), the mean sum of squares, *F*-value, P-significance along with the coefficient of variation (CV).

3. Results and discussion

3.1. Statistical analysis and study of variance for measured parameters

3.1.1. Effect of fertilizers on some growth parameters of different wheat genotypes

The descriptive statistics of plant growth parameters of 14 studied genotypes under different fertilizer applications, i.e., control, Zn application, Mn application and Zn + Mn application are presented in Table 1.

The average data of plant height, tillers per plant along with chlorophyll content were higher with the Zn + Mn application compared to the control, Zn application and Mn application. The highest percentage increase was noticed for the number of tillers/plant

(25.82%) followed by chlorophyll content (7.38) and plant height (2.86) under Zn + Mn application. Among the 14genotypes, the plant height ranged from 58.50(PDW291) to 80.57 cm (BWL8855) and 60.46(PDW274) to 81.07 (BWL8855) under control and Zn + Mn application, respectively, whereas the number of tillers/plant ranged from 86.50 (PBW869) to 120.50(HD3086) and 115.75 (BWL8855) to 160.75 (HD3086) under control and Zn + Mn application, respectively among the tested genotypes. Likewise, chlorophyll content varied from 37.41 (PDW274) to 43.53 (PBW725) and 39.85 (PBWZn1) to 46.82 (WHD941) under control and Zn + Mn application, respectively. The study of variance for measured parameters suggested substantial differences among various genotypes (Table 2) where the interaction between genotypes and fertilizer type was significant.

The beneficial effects of Zn fertilization on wheat have been recorded by significant improvement in plant growth attributes such as increased plant height, number of tillers per plant as well as chlorophyll content. The results were corroborated through a study by Singh et al. (2019) on wheat. The trend could be due to the higher Zn availability which favors the metabolic functions of the crop during different growth stages including chlorophyll synthesis, carbohydrate metabolism and ribosomal functioning (Kumar et al., 2019; Zulfidar et al., 2020). A significant enhancement in green gram height under Zn treatment has been observed by Muindi et al. (2020). Jatav and Singh (2018) recorded a greater improvement in plant height, chlorophyll content and number of tillers per plant of hybrid rice with Zn application.

Also, the results of the present support the hypothesis that Mn is also an essential nutrient for plant growth. The improvement in the height of plant, the number of tillers and chlorophyll content reflected the crucial role of Mn in various metabolic processes. The probable reason for the enhanced height of the plant with Mn application was because of its role in stem elongation (Marschner, 1995). Similar results with Mn application on plant height of cotton have been observed by Dordas (2009). Further, the promoting effects of Mn in chlorophyll synthesis might be due to its role in photosynthesis and as an activator of many enzymatic reactions (Kabata-Pendias and Pendias, 1999). The present study results were in agreement with the results of Shahi and Srivastava (2018) where it was stated that foliar spray of Mn enhanced the chlorophyll content in the crop. Thus, the integrated application of Zn + Mn showed a maximum increase in plant growth parameters due to their combined beneficial effects.

3.1.2. Effect of fertilizers on some quality parameters of different wheat genotypes

Different wheat genotypes were studied for their proximate composition. The descriptive statistics of five measured traits, i.e., moisture content, ash content, crude fiber, total soluble solids (TSS) and protein content among 14 studied genotypes of wheat through Zn, Mn and Zn + Mn fertilization are presented in Table 3.

The average data of moisture (%), ash (%), crude fiber (%), TSS (%) and protein content (%) were higher in the combined application of Zn and Mn with maximum values of 7.53% in PBW-824, 7.44% in PDW-274, 1.53 in PBW-824, 21.90 in PBW-824 and 7.88 in PBW-824 genotypes, respectively. The highest percentage increase of 14.4% was observed in protein content for the collective use of Zn as well as Mn in comparison to control. Further, crude fiber and TSS showed an increase of 13.07 and 10.7%, respectively. Overall, moisture, ash, crude fiber, TSS and protein contents varied from 5.12 to 7.53%, 1.21 to

TABLE 1 Statistical analysis of growth parameters in wheat genotypes with different fertilizer applications (Mean data of both seasons).

Parameters	Treatment	Minimum	Maximum	Mean	SD	SE	Increase (%)
Plant height (cm)	Control	58.50 PDW291	80.57 BWL8855	72.71	6.93	1.85	-
	Zn	60.83 PDW291	81.46 PBW869	74.51	6.97	1.86	2.48
	Mn	61.49 PDW274	81.48 BWL8855	74.48	6.51	1.74	2.43
	Zn + Mn	60.46 PDW274	81.07 BWL8855	74.79	6.91	1.85	2.86
Number of tillers per m^2	Control	86.50 PBW869	120.50 HD3086	104.61	8.65	2.31	-
	Zn	98.00 PBW869	137.50 HD3086	111.48	10.30	2.75	6.57
	Mn	107.25 BWL8855	146.75 HD3086	120.89	9.63	2.57	15.56
	Zn + Mn	115.75 BWL8855	160.75 HD3086	131.62	11.08	2.96	25.82
Chlorophyll content	Control	37.41 PDW274	43.53 PBW725	40.26	1.69	0.45	-
	Zn	39.17 PDW274	44.67 PBW725	42.58	1.79	0.48	5.76
	Mn	40.08 PBWZn1	45.48 PBW869	42.49	1.67	0.45	5.54
	Zn + Mn	39.85 PBWZn1	46.82 WHD943	43.23	2.02	0.54	7.38

Zn-0.5% ZnSO$_4$.7H$_2$O, Mn- 0.5% MnSO$_4$.7H$_2$O, Zn + Mn-0.5% ZnSO$_4$.7H$_2$O + 0.5% MnSO$_4$.7H$_2$O, SD, Standard deviation; SE, Standard error.

TABLE 2 Study of variance for measured parameters with different fertilizer applications.

Variables	Mean sum of squares			F-value			p-value			CV (%)
	G	F	G × F	G	F	G × F	G	F	G × F	
Df	13	3	39	13	3	39	13	3	39	
Plant height	193.63	145.12	2.88	277.34	207.85	4.13	***	***	***	9.22
Number of tillers per plant	1,153	5,778	42.32	113.54	568.8	4.17	***	***	***	8.46
Chlorophyll content	44.41	99.40	2.95	88.11	197.19	5.86	***	***	***	4.25

G-Genotype, F-Fertilizer, df-degree of freedom, CV (Coefficient of variation). ***, significant at 1% level of probability.

1.74%, 0.87 to 1.53%, 15.50 to 21.90% and 5.69 to 7.88%, respectively with minimum values observed in HD-3086 genotype. The analysis of variance (ANOVA) for measured parameters showed substantial differences among various genotypes (Table 4) where the interaction between genotypes and fertilizer type was significant.

The coefficient of variation varied from 0.36% (moisture content) to 7.20% (protein content) among the traits investigated. The results pertaining to the moisture content in 14 genotypes of wheat undertaken in the present study revealed the superiority of combined Zn and Mn application over other treatments. The outcomes were in agreement with the results obtained by Punia et al. (2019) where the moisture content ranged from 7.79 to 9.45% among different genotypes. These variations in moisture contents among various wheat genotypes with collective use of Zn and Mn were major because of variations in levels of sun drying for crops when harvested. Moreover, the moisture content could also be determined through the genetic composition of grain along with variations in climate (Maity et al., 2016). Two aspects are mainly associated with grain moisture content. Primarily, the increased moisture may result in enhanced kernel weight. Furthermore, it may reduce the total storage period of grain (Abramson et al., 2005). Similar results were observed by Asim et al. (2018) for a percentage of moisture in different wheat genotypes. Whereas, the percentage of ash in the present experiment was in accordance with the results observed by Amir et al. (2013). Various environmental conditions like fertility of soil, temperature and type of fertilizer used majorly contribute toward the variation in ash content

TABLE 3 Descriptive statistics of the moisture, ash, crude fiber, TSS and protein contents in wheat genotypes under different fertilizer applications.

Trait (%)	Fertilizer application	Minimum	Maximum	Mean	SD	SE	Increase (%)
Moisture	Control	5.12 PDW-291	7.37 PBW-824	6.37	0.070	0.019	-
	Zn	5.45 PBW-550 (U)	7.44 PBW-824	6.46	0.035	0.034	0.94
	Mn	5.52 PBW-550 (U)	7.55 PBW-824	6.48	0.026	0.026	2.38
	Zn + Mn	5.54 PBW-550 (U)	7.53 PBW-824	6.56	0.029	0.029	2.12
Ash	Control	1.21 PDW-291	1.56 PBW-824	1.39	0.020	0.005	-
	Zn	1.26 PBW-550 (U)	1.61 PBW-824	1.46	0.018	0.004	3.10
	Mn	1.27 PBW-550 (U)	1.63 BWL-824	1.49	0.022	0.006	4.29
	Zn + Mn	1.33 PBW-550 (U)	1.74 PDW-274	1.56	0.019	0.005	10.34
Crude fiber	Control	0.87 PDW-291	1.33 PBW-824	1.13	0.021	0.006	-
	Zn	0.92 PDW-291	1.42 PBW-824	1.18	0.016	0.004	6.30
	Mn	0.93 PDW-291	1.45 PBW-824	1.21	0.026	0.007	8.27
	Zn + Mn	1.00 PDW-291	1.53 PBW-824	1.15	0.012	0.003	13.07
Total soluble solids	Control	15.50 PDW-291	19.55 PBW-824	17.43	0.16	0.043	-
	Zn	15.63 PBW-550 (U)	20.65 PBW-824	17.88	0.27	0.071	5.30
	Mn	16.10 PDW-291	20.80 PBW-824	18.29	0.18	0.047	6.00
	Zn + Mn	17.15 PDW-291	21.90 PBW-824	25.35	8.91	2.38	10.7
Protein	Control	5.69 PDW-291	6.74 PBW-824	7.12	0.43	0.12	-
	Zn	5.78 PDW-291	7.35 PBW-824	6.66	0.57	0.15	8.20
	Mn	5.75 PDW-291	7.11 PBW-824	6.29	0.39	0.10	5.20
	Zn + Mn	5.95 PDW-291	7.88 PBW-824	6.21	0.50	0.13	14.4

Zn-0.5% $ZnSO_4.7H_2O$, Mn- 0.5% $MnSO_4.7H_2O$, Zn + Mn-0.5% $ZnSO_4.7H_2O$ + 0.5% $MnSO_4.7H_2O$, SD, Standard deviation; SE, Standard error.

for different genotypes. The percentage increase in crude fiber with Zn and Mn application was because of the important role of Zn in metabolic reactions and synthesis of protein that ultimately affected the properties of fiber (Nofal et al., 2011). In one study, the percentage of fiber varied between 0.79 to 0.93% in wheat which was lesser in comparison to the present study (Punia et al., 2019).

Additionally, the TSS level increased with Zn and Mn application as compared to the control. An increase in chlorophyll levels in leaves through Zn application resulted in improved rates of photosynthesis which further improved the synthesis of sugars as well as carbohydrates (Hussain et al., 2020). Increased TSS was also related to the higher photosynthate translocation to wheat grain over other sections of the plant (Borah et al., 2021). Also, the increased content of protein in wheat grain under Zn and Mn treatment was because of enhanced nitrogen fixation along with greater levels of available nitrogen in soil as well as improved uptake of nitrogen by plants. Zinc is responsible

TABLE 4 Analysis of variance for moisture, ash, crude fiber, TSS and protein content under different fertilizer applications.

Variables	Mean sum of squares			F-value			P-value			CV (%)
	G	F	G × F	G	F	G × F	G	F	G × F	
df	13	3	39	13	3	39	13	3	39	
Moisture	6.736	0.267	0.010	8687.3	343.85	12.775	***	***	***	0.36
Ash	0.168	0.221	0.002	746.1	981.66	10.01	***	***	***	1.45
Crude fiber	0.372	0.103	0.001	1650.3	455.05	6.609	***	***	***	1.46
TSS	425.9	592.7	401.3	3.193	4.443	3.008	0.0004	0.005	***	1.09
Protein	2.496	7.24	0.085	17.97	52.15	0.615	***	***	0.957	7.20

G-Genotype, F-Fertilizer, df-degree of freedom, CV (Coefficient of variation). ***, Significant at 1% level of probability.

for protein metabolism involving various enzyme systems and translocating protein to wheat grain enhancing the overall percentage of protein in wheat (Amir et al., 2020). Different factors affecting protein content include drought stress, fertility of the soil, thermal stress and practices related to agriculture (Li et al., 2013; Mutwali et al., 2016). Wheat genotypes exhibited different levels of protein content which ultimately resulted in a significant enhancement in its quality which added nutritional value to various genotypes.

3.1.3. Impact of fertilizers on grain and straw yield in different wheat genotypes

Among 14 wheat genotypes, PBW-824 produced significantly highest grain yield (5.14 t ha^{-1} for control, 5.25 t ha^{-1} for Zn treatment, 5.48 t ha^{-1} under Mn treatment and 5.42 t ha^{-1} under Zn + Mn treatment, respectively) and the lowest grain yield was recorded in HD 3086 genotype (Table 5). Whereas, the maximum values of straw yield for PBW-824 were observed to be 8.60 t ha^{-1} for control, 8.87 t ha^{-1} for Zn treatment, 8.83 t ha^{-1} under Mn treatment and 9.11 t ha^{-1} under Zn + Mn treatment, respectively (Table 6). For the control treatment, the minimum value of straw yield was observed in HD 3086 genotype, whereas, under Zn, Mn and Zn + Mn treatments minimum straw yield was obtained in PBW-550 (U) genotype.

The enhanced grain, as well as straw yield observed through combined fertilization of Zn, was due to their favorable effect on wheat productivity and the role of Zn as a catalyst or stimulant in many physiological as well as metabolic processes. Also, Zn acts as a metal activator of enzymes which help in the synthesis of carbohydrates and proteins. Further, the improved levels of growth attributes like chlorophyll, leaf area index, height of plant and better root system of wheat were observed through Zn supplementation (Hussain et al., 2020). The role of Zn in the synthesis of carbohydrates, and lipids along with nucleic acid also improved the wheat yield due to their importance in growth and plant development (Khan et al., 2002). Indeed, Zn also plays a significant role in the functioning of pollen, fertilization as well as germination (Aravind and Prasad, 2003; Pandey et al., 2006; Cakmak, 2008) that automatically leads to enhanced crop performance (Shojaei and Makarian, 2015). Comparable results were reported in the literature where ZnSO$_4$ foliar application improved seed and straw yield in field pea (Shankar et al., 2004; Verma et al., 2018; Borah et al., 2021). On the other hand, Mn holds the central position in the oxygen-evolving complex during photosystem II which confirms its importance as compared to other nutrients in photosynthesis. Improved yield parameters observed in the present investigation might be related to enhanced Zn and Mn

supply which resulted in proper growth and development in wheat (Ullah et al., 2017).

3.1.4. Effect of fertilizers on micro−and macro-nutrient concentrations in different wheat genotypes

The statistical analysis of the nutrient content of 14 studied genotypes under different fertilizer applications showed that nutrient content significantly increased with the application of fertilizers (Table 7).

The highest increase in Zn and Mn content was observed under the sole application of Zn (12.90%) and Mn (19.04%), respectively in comparison to the combined Zn + Mn application. The concentration of Zn ranged from 21.47 (WH-1105) to 36.76 (PBW-872) mg kg^{-1} in control and 25.71 (PBW-872) to 38.32 (PBW-Zn1) mg kg^{-1} under Zn application, whereas Mn content ranged from 13.24 (PDW-291) to 19.56 (PDW-274) mg kg^{-1} in control and 15.41 (PDW-274) to 23.59 (PBW-872) mg kg^{-1} under Mn application. Whereas, the results pertaining to the concentrations of N, P and K showed that their mean values were higher in the combined application of Zn and Mn with maximum values of 1.29, 0.40 and 0.48% for N, P and K in PBW-824 cultivar. The highest percentage increase of 17.50% was observed in P content for the collective use of Zn and Mn in comparison to control followed by K content of 16.70%. Overall, N, P and K content ranged between 0.91 to 1.29%, 0.26 to 0.40% and 0.33 to 0.48%, respectively, with minimum values observed in PDW-291 wheat cultivar. The study of variance for measured parameters suggested substantial differences among various genotypes (Table 8) where the interaction between genotypes and fertilizer type was significant. Whereas, the coefficient of variation between Zn, Mn, N, P and K ranged from 7.20% (N content) to 15.23% (Zn content) among the investigated traits.

The results pertaining to the macronutrient contents in 14 genotypes of wheat undertaken in the present study predicted the superiority of combined Zn and Mn application through sprays on plant foliage as compared with other treatments. Utilization of Zn and Mn mineral elements by a plant varies with the hereditability of each genotype (Sadeghi et al., 2021). Among different varieties, the significant differences in Zn and Mn concentrations and their variable response toward nutrient application have also been reported in previous studies (Mathpal et al., 2015). This difference might be due to variations in accumulation, translocation and utilization mechanisms because of variable genetic makeup (Fageria and Baligar 2003). Remarkable improvement in grain Zn and Mn concentration with Zn and Mn application, respectively, was due to the effective

TABLE 5 Impact of Zn and Mn use on grain yield of 14 wheat genotypes (Mean data of both seasons).

Varieties	Grain yield (t ha^{-1})											
	Control			Zn			Mn			Zn + Mn		
	2020–21	2021–22	Mean	2020–21	2021–22	Mean	2020–21	2021–22	Mean	2020–21	2021–22	Mean
PBW-826	3.70	3.78	3.74	3.72	4.09	3.90	3.78	4.15	3.97	4.97	4.14	4.55
PBW-824	4.79	5.49	5.14	4.85	5.65	5.25	4.49	6.48	5.48	6.22	4.61	5.42
WHD-943	3.06	3.89	3.48	3.27	4.27	3.77	3.57	4.21	3.89	4.56	4.40	4.48
BWL-8855	4.43	4.31	4.37	4.59	4.48	4.54	4.65	4.54	4.59	5.79	4.03	4.91
PBW-872	3.44	3.86	3.65	3.60	3.89	3.75	3.75	4.38	4.07	4.87	3.98	4.43
WH-1105	4.12	4.05	4.09	4.21	4.40	4.30	4.25	4.78	4.52	5.30	4.23	4.76
PBW-550 (U)	4.05	3.98	4.01	4.61	4.00	4.30	4.64	4.26	4.45	5.88	4.36	5.12
PBW-869	4.61	4.43	4.52	4.68	4.42	4.55	4.82	4.32	4.57	6.06	5.64	5.85
PBW-725	3.80	3.93	3.86	3.94	4.36	4.15	4.16	4.52	4.34	5.25	3.90	4.58
PBW-343 (U)	4.15	4.51	4.33	4.46	4.11	4.28	4.48	4.26	4.37	5.56	4.54	5.05
PBW ZN 1	3.87	3.81	3.84	3.95	3.95	3.95	4.10	4.23	4.17	5.17	4.11	4.64
PDW-291	2.99	3.80	3.40	3.06	4.46	3.76	3.17	4.79	3.98	4.17	3.95	4.06
PDW-274	3.26	3.99	3.63	3.62	3.88	3.75	3.76	4.19	3.97	4.79	4.26	4.52
HD-3086	4.00	3.91	3.96	4.04	4.20	4.12	4.27	4.42	4.34	5.46	4.02	4.74
LSD $_{0.05}$	0.56	0.85	0.74	0.62	0.75	0.82	0.51	0.85	0.76	0.75	0.36	0.80

Zn-0.5% ZnSO$_4$.7H$_2$O, Mn- 0.5% MnSO$_4$.7H$_2$O, Zn + Mn-0.5% ZnSO$_4$.7H$_2$O + 0.5% MnSO$_4$.7H$_2$O.

TABLE 6 Impact of Zn and Mn use on straw yield of 14 wheat genotypes (Mean data of both seasons).

Varieties	Straw yield (t ha^{-1})											
	Control			Zn			Mn			Zn + Mn		
	2020–21	2021–22	Mean	2020–21	2021–22	Mean	2020–21	2021–22	Mean	2020–21	2021–22	Mean
PBW-826	7.89	7.04	7.46	7.70	7.30	7.50	8.00	7.46	7.73	8.00	7.66	7.83
PBW-824	9.09	8.11	8.60	9.27	8.48	8.87	9.54	8.13	8.83	9.70	8.52	9.11
WHD-943	8.05	7.19	7.62	8.34	7.13	7.73	8.51	7.05	7.78	8.79	7.48	8.13
BWL-8855	8.46	8.08	8.27	8.98	7.61	8.29	9.04	7.80	8.42	9.25	7.90	8.58
PBW-872	8.59	8.16	8.38	8.61	8.21	8.41	8.78	8.34	8.56	11.95	9.74	10.84
WH-1105	8.33	7.05	7.69	8.45	7.08	7.77	8.47	7.49	7.98	8.87	7.38	8.12
PBW-550 (U)	7.44	7.10	7.27	7.57	7.14	7.35	7.71	7.21	7.46	7.92	7.64	7.78
PBW-869	8.78	7.54	8.16	8.85	7.64	8.24	9.06	8.58	8.82	9.22	7.98	8.60
PBW-725	8.32	7.51	7.92	8.70	7.45	8.08	8.32	7.88	8.10	8.67	7.93	8.29
PBW-343 (U)	7.37	7.66	7.52	8.48	7.27	7.88	8.65	7.43	8.04	9.15	7.17	8.16
PBW ZN 1	8.44	7.78	8.11	8.55	8.04	8.29	8.76	8.12	8.43	9.02	8.29	8.66
PDW-291	8.41	7.55	7.98	8.68	7.79	8.23	8.89	7.88	8.38	9.12	7.83	8.47
PDW-274	8.33	8.11	8.22	8.39	8.16	8.27	8.57	8.23	8.39	8.68	8.33	8.50
HD-3086	7.25	7.12	7.19	7.60	7.49	7.55	7.88	7.88	7.88	8.03	8.21	8.12
LSD$_{0.05}$	0.75	0.68	0.67	0.78	0.53	0.70	0.72	0.52	0.68	1.22	0.39	1.07

Zn-0.5% ZnSO$_4$.7H$_2$O, Mn- 0.5% MnSO$_4$.7H$_2$O, Zn + Mn-0.5% ZnSO$_4$.7H$_2$O + 0.5% MnSO$_4$.7H$_2$O.

TABLE 7 Descriptive statistics of the Zn, Mn, N, P and K concentrations in wheat genotypes under different fertilizer applications.

Nutrient content	Fertilizer application	Minimum	Maximum	Mean	SD	SE	Increase (%)
Zn (mg Kg⁻¹)	Control	21.47 WH-1105	36.76 PBW-872	28.53	4.27	1.14	-
	Zn	25.71 PBW-872	38.32 PBW-Zn1	32.21	4.87	1.30	12.90
	Mn	21.40 WH-1105	36.90 PBW-Zn1	27.29	4.70	1.26	−4.35
	Zn + Mn	22.44 PBW-872	37.90 WHD-943	29.93	4.44	1.19	4.91
Mn (mg Kg⁻¹)	Control	13.24 PDW-291	19.56 PDW-274	16.17	1.76	0.47	-
	Zn	12.99 PDW-291	21.08 PDW-274	16.24	2.05	0.55	0.43
	Mn	15.41 PDW-274	16.69 PBW-872	19.25	2.67	0.71	19.04
	Zn + Mn	14.64 PDW-274	21.95 PBW-343 U	17.77	2.32	0.62	9.89
N (%)	Control	0.91 PDW-291	1.08 PBW-824	0.99	0.07	0.02	-
	Zn	0.92 PDW-291	1.17 PBW-824	1.07	0.09	0.02	7.6
	Mn	0.92 PDW-291	1.14 PBW-824	1.01	0.06	0.01	5.2
	Zn + Mn	0.95 PDW-291	1.29 PBW-824	1.14	0.08	0.02	16.2
P(%)	Control	0.26 PDW-291	0.33 PBW-824	0.31	0.27	0.07	-
	Zn	0.29 PDW-291	0.34 PBW-824	0.36	0.17	0.05	2.94
	Mn	0.22 PDW-291	0.36 PBW-824	0.29	0.22	0.06	8.33
	Zn + Mn	0.28 PDW-291	0.40 PBW-824	0.31	0.28	0.08	17.5
K (%)	Control	0.33 PDW-291	0.40 PBW-824	0.40	0.09	0.03	-
	Zn	0.36 PDW-291	0.41 PBW-824	0.37	0.03	0.01	2.43
	Mn	0.34 PDW-291	0.41 PBW-824	0.36	0.02	0.01	2.43
	Zn + Mn	0.26 PDW-291	0.48 PBW-824	0.38	0.02	0.01	16.7

Zn-0.5% ZnSO₄.7H₂O, Mn- 0.5% MnSO₄.7H₂O, Zn + Mn-0.5% ZnSO₄.7H₂O + 0.5% MnSO₄.7H₂O, SD, Standard deviation; SE, Standard error.

absorption of nutrients on plant leaves and its mobility in wheat through crease phloem, and transported to endosperm (Cakmak et al., 2010). A similar trend of increased Zn content on Zn applications has been reported in previous studies. In line with the present study, Dhaliwal et al. (2021a) reported a significant upsurge in Zn concentration in chickpeas under the Zn application. Similar improvement in Zn concentration in linseed has also been recorded with Zn application (Dhaliwal et al., 2022). The lower Zn and Mn

concentration in Zn + Mn treated plants as compared to the sole application of nutrients might be due to the dilution effect.

In the present experiment, the Zn and Mn application significantly increased N, P and K concentrations in wheat grains as compared to the control. Increased consumption, as well as absorption of Zn and Mn in plants, positively affected their concentrations in wheat. Zn and Mn not only enable their own absorption, but they also help in improving the absorption of nutrients such as N, P and K in the plant.

TABLE 8 Study of variance for Zn, Mn, N, P and K concentrations under different fertilizer applications.

Variables	Mean sum of squares			F-value			P-value			CV (%)
	G	F	G×F	G	F	G×F	G	F	G×F	
Df	13	3	39	13	3	39	13	3	39	
Zn	248.48	167.55	5.62	362.13	244.19	8.19	***	***	***	15.23
Mn	54.28	89.65	3.20	125.74	207.68	7.41	***	***	***	12.66
N	0.06	0.19	0.002	17.98	52.18	0.61	***	***	0.958	7.20
P	0.008	0.040	0.001	5.668	28.349	0.430	***	***	0.998	8.43
K	0.009	0.019	0.001	9.363	19.083	1.158	***	***	0.273	8.36

G-Genotypes, F-Fertilizers, df-degree of freedom, CV (Coefficient of variation). ***Denotes no values.

TABLE 9 Genotype classifications into efficient (E), medium (M) and inefficient (I) based on various traits measured under control treatment.

Genotypes	Control								Zn + Mn								Rank
	Protein	Crude fiber	TSS	Zn	Mn	N	P	K	Protein	Crude fiber	TSS	Zn	Mn	N	P	K	
PBW826	M	M	M	M	I	M	M	M	M	M	M	M	M	M	M	M	6
PBW824	E	E	E	M	M	E	E	E	E	E	E	M	M	E	E	E	1
WHD943	M	M	E	M	M	M	M	M	M	M	E	M	M	M	M	M	4
BWL8855	M	I	I	M	I	M	M	M	M	I	I	M	I	M	M	M	3
PBW872	M	M	M	E	M	M	M	M	M	M	I	E	M	M	M	M	5
WH1105	M	E	E	I	M	M	E	M	M	M	E	I	M	M	M	M	4
PBW550(U)	M	M	M	I	M	M	M	M	M	M	I	I	M	M	M	M	7
PBW869	E	E	E	M	M	E	E	M	E	E	E	M	M	E	E	E	2
PBW725	M	I	I	M	M	I	I	I	I	M	I	M	M	I	I	M	9
PBW343(U)	M	M	M	M	M	M	M	M	M	M	M	M	E	M	M	M	5
PBWZn1	M	M	M	M	M	M	M	M	M	M	M	M	E	M	M	M	5
PDW291	I	I	I	M	I	I	I	I	I	I	I	M	I	I	I	I	10
PDW274	I	I	I	E	E	I	M	I	I	I	I	E	E	I	M	M	8
HD3086	M	M	E	M	M	M	M	M	M	M	E	M	M	M	M	M	4

The leaf epidermis absorbs fertilizer-applied Zn and Mn through stomata as well as trichomes (DomõÂnguez et al., 2011). This accelerates the macronutrient content and their uptake especially N as compared to P and K in wheat genotypes by increasing the level of amino acids needed for the synthesis of protein (Mohammad and Naseem, 2006). Similarly, the increase in Zn and N content through Zn fertilization was reported by Ghasal et al. (2017). Also, Pooniya and Shivay (2011, 2013) noted a significant enhancement in N, P and K concentrations with Zn application.

3.1.5. Categorization of wheat genotypes for nutrient use efficiency

Under method 1, the different genotypes displayed significant variations in quality attributes like protein, crude fiber, TSS and nutrient concentration considered for ranking in both control and Zn + Mn treatment (Table 9).

The genotypes PBW-824 recorded the highest rank overall followed by PBW-869. On the other hand, PDW-274, PDW-725 and PDW-291 recorded the lowest ranks among the studied genotypes. Further, the studied genotypes under method 2 were classified into four groups based on total dry mass as well as nutrient use efficiency

in Zn + Mn treatment (Figure 1). The genotypes PBW 872, PDW-869 and PBW-Zn1 were classified into ER group. Further, the genotypes WHD 943, WH 1105, PBW 725, PBW 343 and PDW-291 were classified in INR group. Additionally, the genotypes PBW 824 and BWL 8855 were categorized in ENR group with the fertilizer treatment of Zn + Mn. both P conditions. Whereas, genotypes PBW-826, PBW 550(U), PDW 274 and HD 3086 were categorized in the IR group under the Zn + Mn treatment.

4. Conclusion

The present study concluded that the studied 14 wheat genotypes differ significantly toward yield, nutrient content and other quality parameters. The genotypes were categorized for yield and quality parameters using two different techniques under control and Zn + Mn treatment. The plant growth parameters, crop yield, nutrient content and quality parameters significantly increased under Zn + Mn treated conditions. The genotypes PBW-824 and BWL-8855 produced a higher yield under control conditions, whereas PBW-872, PBW-869 and PBW Zn1 showed a higher yield under Zn + Mn treatment. Further, PBW-824
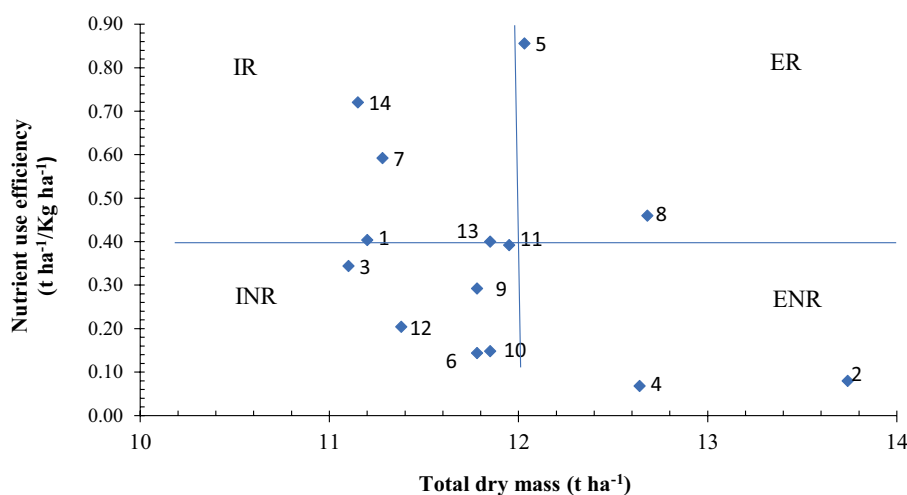
FIGURE 1

Genotype classifications based on nutrient use efficiency and total dry mass. This divides genotypes into four groups such as efficient with responsive (ER), inefficient with responsive (IR), efficient with non-responsive (ENR) and inefficient with non-responsive (INR).(1) PBW-826, (2) PBW-824, (3) WHD-943. (4) BWL-8855, (5) PBW-872, (6) WH-1105, (7) PBW-550 (U), (8) PBW-869, (9) PBW-725, (10) PBW-343 (U), (11) PBW Zn1, (12) PDW-291, (13) PDW-274 and (14) HD-3086.

and PBW-869 were ranked higher in terms of quality parameters. Thus, these genotypes could be further studied for higher crop production to reduce micronutrient malnutrition in humans.

Data availability statement

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

Author contributions

SD, VS, AS, VV, MK, and PS: conceptualization, investigation, methodology, resources, validation, and writing–original draft. SD, SB, SA, MS, and AH: data curation and writing–review and editing. SD, SB, SA, MS, and AH: formal analysis and software. SD, SB, and AS: supervision. SD, SB, SA, AS, and AH: funding acquisition. SB: resources. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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