

Nutritional assessment of bread wheat genotypes in relation to processing quality and yield related attributes

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Abstract

Wheat is an important constituent of cereal-based diet that contributes to human health. However, existing wheat varieties are low in protein and micronutrients that lead to malnutrition. Biofortification is an emerging, successful sustainable approach to alleviate the problem. However, for production of different type of food products these varieties must fulfill the minimum quality criteria. In this study, 1520 genotypes were analyzed for their iron (Fe), zinc (Zn), and phytic acid content. Additionally, eleven selected genotypes were evaluated for various physiological and yield parameters. The study identified significant variability in Fe and Zn concentrations in mature grain, with concentrations ranging from 21.8 to 71.4 ppm for Fe and 20.4 to 84.3 ppm for Zn. A positive correlation between Fe and Zn suggests the potential for concurrent nutrient enhancement through breeding. Phytic acid levels varied from 0.11% to 0.48%, impacting nutrient bioavailability. Hierarchical clustering of genotypes revealed eight clusters, with Cluster III standing out for high Zn (>60 ppm) and low phytic acid (<0.20%). The eleven selected genotypes demonstrated high protein content, excellent milling quality, and competitive yields. Notably, they also showed favorable canopy temperatures, indicating heat tolerance. Overall, these genotypes offer promising prospects for improving both nutritional value and agronomic performance in future breeding programs.

Keywords: Biofortification, Micronutrients, Phytic acid, Physiological and Quality traits

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1. Introduction

Nutrient malnutrition is a critical global challenge, affecting over 30% of individuals in underdeveloped nations and causing child mortality (Kenzhebayeva *et al.* 2019). Nearly 43% of children and 29% of women in their reproductive years suffer from anemia, with 50% due to iron deficiency (WHO, 2021). Iron (Fe) is vital for electron transport, oxygen transport, and nucleic acid synthesis (Abbaspour *et al.* 2014). Zinc (Zn), another essential micronutrient, is crucial for enzyme function, carbohydrate and nucleic acids metabolism, with deficiencies leading to a weaker immune system, neurological disorders, and growth issues (Wuehler *et al.* 2007). Cereal and legume crops supply 56-88% of zinc, 78% of dietary iron, and over 60% of daily

calories in underdeveloped nations (Ritchie and Roser, 2018). Two-thirds of the world's population relies on wheat, one of the primary cereal grains, as a staple food (Myers *et al.*, 2014). However, wheat contains low levels of essential micronutrients like iron and zinc, and traditional processing techniques further reduce their nutritional content (Mahomed *et al.*, 2012). Enhancing the nutritional value of crops is thus necessary to meet the nutrient demands in most countries. Developing nutritionally superior, high-yielding genotypes with enhanced levels of micronutrients and desirable processing and quality characteristics can help address this issue (Velu *et al.*, 2019).



Several initiatives have been undertaken to enhance the nutritional value of food, such as mineral supplementation, dietary diversity and food fortification using post-harvest technologies. However, current fortification methods are inadequate and these strategies require continuous investment and infrastructure (Hurrell *et al.* 2010). A key strategy for nutrient addition is biofortification, which can be accomplished through conventional plant breeding or biotechnological approaches (Cakmak, 2008). The latter might involve genetic engineering to insert novel gene constructs, which has significant ramifications such as public approval (Akhtar *et al.* 2020). Biofortification not only improves the nutritional quality of staple crops but also offers a cost-effective and environmentally friendly solution to malnutrition. By focusing on enhancing the micronutrient content of widely consumed crops like wheat, these initiatives can significantly impact global health, particularly in underdeveloped regions where nutrient deficiencies are most prevalent. The Consultative Group on International Agricultural Research (CGIAR) established HarvestPlus, Biofortification Challenge Program, to increase the iron, zinc, and β -carotene content in major cereal crops. This program aims to develop biofortified crops through conventional plant breeding and biotechnological approaches, addressing micronutrient deficiencies in a sustainable manner.

Studies indicate that biofortification by selective breeding is a long-term, sustainable method for addressing human nutritional insufficiency. It is also reliable, cost-effective, and environmentally safe (Bouis and Saltzman, 2017). The bioavailability of these micronutrients is a key consideration in biofortified crops with enhanced micronutrient concentrations. Wheat is naturally low in micronutrients and high in the antinutritional substance phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate), which strongly chelates micronutrients and limits their bioavailability. Therefore, any breeding program should address the micronutrient's bioavailability in addition to raising their concentration (Akfirat and Uncuoglu, 2013). To keep up with the world's rising population, plant breeders regularly create high-yielding wheat varieties. However, the absence of sufficient information on quality traits necessitates systematic studies on wheat varieties suitable for end-users. Therefore, the present study aims to characterize high-yielding wheat cultivars with enhanced

micronutrients, low phytic acid content, and good-quality traits to improve processing efficiency and product quality.

2. Material and Methods

2.1 Plant Materials

The study included 1,520 bread wheat genotypes from various sources, including in-house material developed at PAU, Ludhiana and material received from CIMMYT, Mexico under different projects. The genotypes were sown in an augmented design with two replications at the experimental fields of the Department of Plant Breeding and Genetics, PAU, Ludhiana, during the cropping seasons of 2017-18 and 2018-19. This location is situated at 30° 54' north latitude and 75° 48' east longitude. The soil of the experimental field was sandy loam with 0.38% organic carbon, 32 kg/ha P₂O₅, 225.8 kg/ha K₂O, 3.66 kg/ha Fe, and 0.70 kg/ha Zn. During the Rabi seasons of 2017-2018 and 2018-19, the average air temperature recorded was 18.33°C and 17.6°C, respectively. Relative humidity was 62.7% and 65.9%, and rainfall was 14.16 mm and 28.8 mm, respectively. After harvesting, mature grains were collected and evaluated for the following parameters:

2.1 Estimation of Fe and Zn

Grain Fe (ppm) and Zn (ppm) concentrations were determined using a bench-top Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF) instrument (model X-supreme 8000 by Oxford Instruments, Shanghai, UK), standardized for Fe and Zn in whole wheat grains using glass calibration beads (FLUXANA).

2.2 Phytic Acid

Phytic acid content was estimated by the modified method of Fruhbeck *et al.* (1995). Whole wheat grains (200 mg) were homogenized in 2 ml of 2.4% hydrochloric acid. The samples were shaken for 2 hours on a mechanical shaker and centrifuged at 10,000 rpm for 30 minutes at 4°C. To 0.2 ml of the supernatant, 2.8 ml of double-distilled water (DDW) and 1 ml of Wade reagent (30 mg FeCl₃·6H₂O and 300 mg sulfosalicylic acid in 100 ml DDW) were added. The test tubes were incubated at room temperature for 10 minutes, and the absorbance of the samples was recorded at 500 nm. The amount of phytic acid was calculated from a standard curve prepared using sodium phytate as the standard in the range of 10-100 µg.



2.3 Protein Content (%)

Total protein content was estimated using a whole grain analyzer (Infratec 1241 by M/S Foss Analytical AB, Sweden) standardized for high throughput screening of whole wheat grains. The instrument uses near-infrared light that transmits through the grains and scans the samples in the range of 850-1050 nm. The results were displayed as percent protein content.

2.4 Quality Characteristics

2.4.1 Test Weight (TW)

Test weight was analyzed using an instrument developed by the Indian Institute of Wheat and Barley Research, Karnal, India. It employs a standard container with a 100 cc capacity (Misra *et al.* 1998), and the test weight is expressed in kg/hl.

2.4.2 Grain Appearance Score (GAS)

Grain appearance score was subjectively assigned based on grain size, shape, color, and luster, with a maximum score of ten.

2.4.3 Phenol Reaction Score (PRS)

One hundred wheat grains, presoaked overnight in distilled water, were treated with a 1% phenol solution for 4 hours. After draining the phenol solution, the grains were dried for half an hour and evaluated subjectively for darkness, receiving a score out of five. The darker the color of the grains, the higher the score assigned.

2.4.4 SDS (Sodium Dodecyl Sulfate) Sedimentation Value

Wholemeal samples were evaluated for SDS sedimentation value using the method of Axford *et al.* (1979). Whole wheat flour was made from mature grains of each genotype using a lab grinder, and a 6 g sample was used for the test, which included a resting period of 20 minutes.

2.4.5 Grain Hardness (GH)

Grain hardness was measured using a grain hardness tester (M/S Ogawa Seiki Co. Ltd., Japan). Ten randomly selected wheat grains were crushed one by one, and the mean force (kg) required to crush the grains was recorded.

2.5 Physiological Traits

2.5.1 Canopy Temperature (CT)

Canopy temperature (°C) was measured using an infrared thermometer (LT 300 Sixth Sense) at anthesis and 15 days after the anthesis (DAA) stage.

2.5.2 Days to Flowering (DTF)

Observations for days to flowering were recorded two months after sowing.

2.5.3 Number of Tillers (NT)/m²

The number of productive tillers was recorded from a 6-meter length of each row for all genotypes.

2.5.4 Grain Number per Spike (GN/Sp)

To determine the grain number per spike, ten mature spikes from each genotype were selected and hand-threshed. The grains obtained from these spikes were counted and divided by the number of spikes (10).

2.5.5 Thousand Grain Weight (TGW)

Thousand grains were weighed separately in three replications to determine the thousand grain weight of each genotype.

2.6 Yield (t/ha)

Grain yield was calculated per net plot and then converted to the grain yield t/ha using CPCS1 software.

2.7 Statistical analysis

Frequency histograms and radial tree plot were created using the “ape” library in R Studio (version 3.4.2; 2017-09-28). These visualizations helped in understanding the distribution and relationships within the data. The data presented in the tables represent the average values from three replicates, ensuring accuracy and reliability. For statistical analysis, a Randomized Block Design (RBD) was employed, which is suitable for controlling variability in the experimental data. This analysis was conducted using CPCS-1 software, with significance tested at the 5% level.

3 Results

3.1 Variability in Fe and Zn Concentration

Significant variability in Fe and Zn concentrations was observed in mature grains. Frequency histograms (Fig. 1a, 1b) revealed that Fe concentrations ranged from 21.8 to 71.4 ppm, and Zn concentrations ranged from 20.4 to 84.3 ppm, with average values of 38.22 ± 0.19 ppm and 46.71 ± 0.28 ppm, respectively. Most genotypes (1,055) had Fe concentrations between 30 and 45 ppm (Fig. 1a). Genotype HP 8068 recorded the highest Fe concentration at 71.4 ppm, followed by HP 1077 and HP 1119 at 65.6 ppm. For Zn concentration, forty genotypes exceeded 70 ppm (Fig. 1b), with HP 924 (84.3 ppm), HP 964 (81.5 ppm), HP 1208 (82.2



ppm), and HP 1153 (80.2 ppm) showing the highest levels. A significant positive correlation ($R^2 = 0.292$) between grain

Fe and Zn at $P < 0.01$ indicates the potential to breed for both nutrients concurrently (Fig. 2).

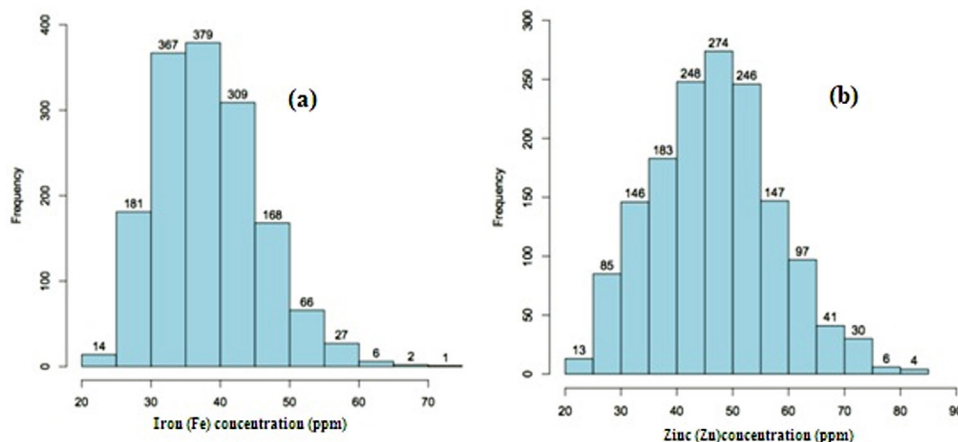


Fig 1: Frequency histogram depicting variability for (a) Fe and (b) Zn concentration

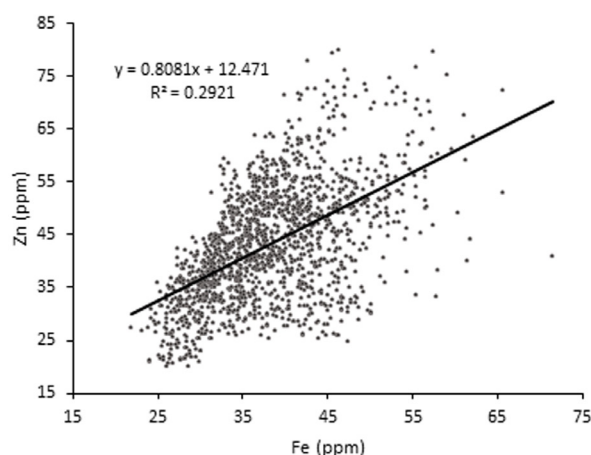


Fig 2: Correlation analysis between grain Fe and Zn concentration

3.2 Phytic Acid

Based on grain Fe and Zn concentration, 234 genotypes were selected for phytic acid estimation: 26 with high Fe, 149 with high Zn, and 59 with high levels of both Fe and Zn. The phytic acid content ranged from 0.11% to 0.48%, with an average of $0.30 \pm 0.003\%$.

3.3 Clustering of Genotypes

Hierarchical clustering based on Fe, Zn, and phytic acid concentrations grouped the genotypes into eight clusters (Fig. 3). Clusters I, II, and III contained genotypes with Fe concentrations ranging from 30 to 45 ppm and Zn concentrations greater than 60 ppm, but differed in phytic acid content. Clusters I and II had phytic acid content

Table 1: List of elite genotypes with pedigree detail

Sr. No.	Genotype	Pedigree
1	HP 1154	KATERE//ONIX/KBIRD/6/C80.1/3*BATAVIA//2*WBL1/3/ATILIA/3*BCN*2//BAV92/4/WBL1*2/KURUKU/5/IWA8600211//2*PBW343*2/KUKUNA
2	HP 1195	SAMNYT 409 (KCB-26) (<i>T.dicoccum</i> PI 94625 / <i>Ae.squarrosa</i> 372 // 3*PASTOR) / PBW 550
3	HP 901	PBW 698*3/BF22
4	HP 910	BF 13/2* PBW 703
5	HP 912	BF 21/2* PBW 621
6	HP 940	PBW 703*3/BF14
7	HP 956	PBW 698*3/ BF 10
8	HP 968	BF20/2*PBW703
9	HP 1213	MUNAL/HEILO//MUNAL/3/2*BORL14
10	BWL 5228	WL 711 <i>Ae.Triuncialis</i> IL/4*PBW550/4/WL711 <i>Ae.ovata</i> / CS(S)// WL 711NN/3/4*PBW 550
11	BWL 7126	PRL/2*PASTOR*2/5/CROC_1/ <i>Ae.squarrosa</i> (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2
12	HD 3086 (C)	DBW14/HD2733//HUW468
13	PBW 1Zn (C)	T.DICOCCON,CI9309/ <i>Ae.squarrosa</i> (409)//MUTUS/3/2*MUTUS



Table 2: Characterization of elite genotypes on basis of quality traits

Sr. No.	Genotype	Protein content (%)		Test Weight (kg/hl)		Grain Appearance Score		Phenol Reaction Score		Sodium Dodecyl Sulphate (cc)		Grain Hardness (kg)	
		2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19
1	HP-1154	13.1	13.3	80.0	78.0	6.0	6.2	3.2	3.4	48.0	45.0	10.0	12.0
2	HP-1195	13.3	13.4	80.0	78.0	5.4	5.6	3.0	3.0	50.0	45.0	12.0	11.0
3	HP-901	10.2	9.9	77.0	75.0	5.8	6.0	4.0	4.0	42.0	40.0	12.0	11.0
4	HP-910	12.9	12.9	78.0	80.0	6.5	6.4	2.5	2.2	45.0	47.0	12.0	10.0
5	HP-912	12.9	12.5	78.0	80.0	5.8	6.0	3.1	2.9	45.0	43.0	10.0	12.0
6	HP-940	13.1	13.8	80.0	78.0	6.6	6.8	3.0	2.7	32.0	35.0	11.0	12.0
7	HP-956	8.5	9.4	75.0	80.0	6.2	6.4	3.0	3.2	34.0	32.0	11.0	11.0
8	HP-968	10.4	11.2	77.5	75.0	6.5	6.7	2.5	2.7	34.0	36.0	12.0	11.0
9	HP-1213	12.7	12.9	75.0	74.0	5.8	6.0	2.8	3.0	40.0	42.0	11.0	12.0
10	BWL-5228	11.1	10.9	78.0	80.0	6.0	5.8	2.5	2.8	36.0	38.0	10.0	11.0
11	BWL-7126	12.4	13.1	78.0	80.0	5.9	6.1	2.9	3.1	38.0	40.0	11.0	10.0
12	HD-3086	11.0	10.1	74.0	76.0	5.7	5.8	3.2	3.8	47.0	48.0	9.6	9.7
13	PBW-1-Zn	12.1	11.8	75.0	76.0	5.9	6.0	4.0	4.4	45.0	46.0	11.1	10.9
Mean±SE		11.8±0.47	12.1±0.45	77.9±0.53	78.0±0.70	6.1±0.11	6.2±0.11	2.9±0.13	3.0±0.14	40.4±1.84	40.3±1.41	11.2±0.22	11.1±0.17
CD (5%)		1.14	0.88	NS	2.97	NS	NS	0.6	NS	3.11	3.87	NS	NS





Table 3: Characterization of elite genotypes on basis of yield parameters

Sr. No.	Genotype	Days to Flowering		Number of Tillers/m ³		Grain Number per Spike		Thousand Grain Weight (g)		Yield (t/ha)		Canopy Temperature (°C)	
		2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19
1	HP-1154	98.0	95.0	227.0	207.0	67.0	65.0	40.0	41.0	7.36	7.04	20.3	21.6
2	HP-1195	100.0	98.0	221.0	238.0	70.0	64.0	40.0	39.0	7.14	7.11	19.6	22.4
3	HP-901	102.0	100.0	135.0	189.0	65.0	67.0	42.0	40.0	4.03	4.30	19.3	20.4
4	HP-910	104.0	102.0	276.0	256.0	77.0	69.0	42.0	39.0	6.42	7.04	18.4	19.2
5	HP-912	102.0	100.0	245.0	234.0	71.0	68.0	44.0	42.0	7.19	7.18	15.4	19.8
6	HP-940	100.0	98.0	214.0	156.0	60.0	55.0	41.0	38.0	6.99	7.15	16.9	18.8
7	HP-956	102.0	100.0	178.0	195.0	67.0	70.0	38.0	35.0	5.41	5.24	15.6	14.9
8	HP-968	98.0	100.0	245.0	234.0	55.0	58.0	35.0	37.0	5.28	6.72	19.2	16.3
9	HP-1213	95.0	97.0	178.0	189.0	63.0	65.0	39.0	41.0	4.52	6.00	15.8	17.7
10	BWL-5228	98.0	100.0	228.0	207.0	68.0	69.0	42.0	40.0	6.92	7.32	14.5	15.4
11	BWL-7126	97.0	95.0	225.0	234.0	70.0	77.0	42.0	39.0	7.29	7.16	15.1	15.9
12	HD-3086	87.0	89.0	235.0	241.0	64.0	70.0	38.0	39.0	7.03	6.59	16.1	15.9
13	PBW-1-Zn	100.0	102.0	230.0	237.0	65.0	67.0	40.0	39.0	6.72	6.39	15.7	15.4
Mean±SE		99.6±0.81	98.6±0.68	215.6±11.7	212.6±8.84	66.6±1.77	66.1±1.79	40.5±0.74	39.2±0.60	6.23±0.36	6.57±0.30	17.3±0.64	18.4±0.77
CD (5%)		4.19	3.98	16.72	16.7	NS	NS	NS	NS	1.17	0.99	1.05	0.82

greater than 0.2%, while Cluster III genotypes had phytic acid less than 0.2%. Cluster IV included genotypes with Fe and Zn concentrations ranging from 50 to 60 ppm and phytic acid greater than 0.3%. Cluster V contained genotypes with Fe concentrations greater than 45 ppm and Zn concentrations greater than 65 ppm, with phytic acid content above 0.3%. Cluster VI had genotypes with Fe concentrations from 45 to 55 ppm, Zn from 55 to 70 ppm, and phytic acid between 0.2% and 0.3%. Cluster VII included genotypes with Fe concentrations above 55 ppm, Zn between 20 and 50 ppm, and phytic acid from 0.2% to 0.3%. The largest number of genotypes (78) was

in Cluster VIII, with Fe concentrations from 25 to 45 ppm, Zn concentrations above 60 ppm, and phytic acid between 0.25% and 0.40%. Cluster III is of particular importance, which contains 11 genotypes with high Zn concentrations (>60 ppm) and low phytic acid (<0.20%). These 11 elite genotypes were selected for further evaluation of physiological and quality traits. The pedigree details of these genotypes, along with two checks high yielding check HD-3086 and biofortified high zinc containing PBW-1-Zn, are shown in Table 1. These selected genotypes were further evaluated during 2018-19 crop season and compared with data obtained from 2017-18 crop season (Tables 2 and 3).

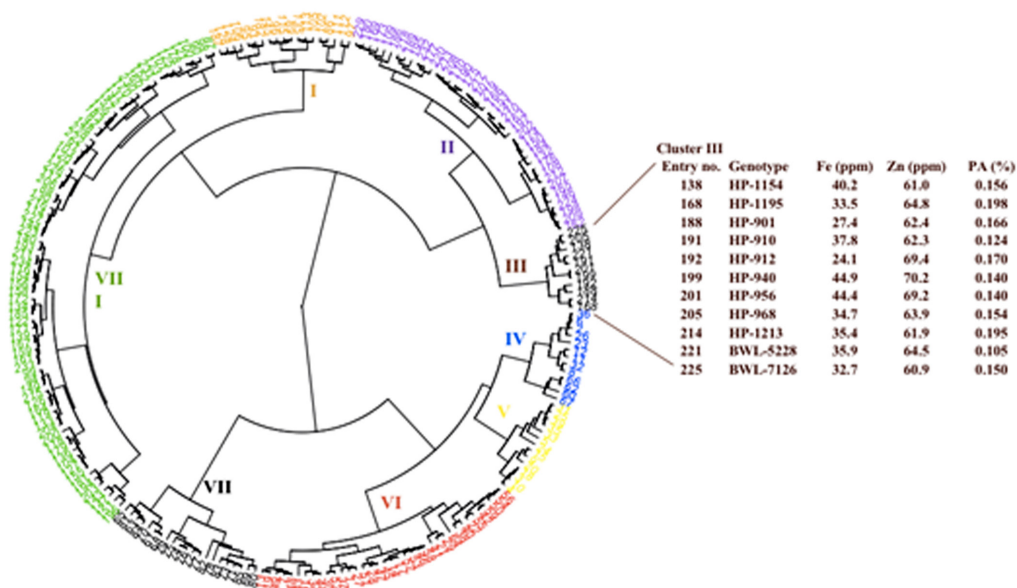


Fig 3: Hierarchical clustering of genotypes based on Fe (ppm), Zn (ppm) and phytic acid (%)

3.4 Physiological and quality characteristics

Eleven genotypes, selected for their iron, zinc, and phytic acid content, demonstrated superior processing qualities compared to the high-yielding check HD-3086 and the high-zinc containing PBW-1-Zn, in terms of end-use quality. Protein content in the selected genotypes ranged from 8.5% to 13.3% in 2017-18 and 9.4% to 13.8% in 2018-19. Three genotypes, HP-1154, HP-1195, and HP-940, recorded protein content above 13.0% during both crop seasons (Table 2). Test weight ranged from 75.0 to 80.0 kg/hl in 2017-18 and from 74.0 to 80.0 kg/hl in 2018-19, with an average of approximately 78 kg/hl, indicating good milling quality (Table 2). Genotype HP-940 had the highest grain appearance score (GAS) of 6.6, followed by HP-910 and HP-968 with scores of 6.5, and HP-956, HP-

1154, and BWL-5228 with scores of 6.0. In the 2018-19 crop seasons, all genotypes except HP-1195 and BWL-5228 had GAS greater than 6.0 due to their lustrous grains and attractive color. Phenol reaction score (PRS) ranged from 2.5 to 4.0 in 2017-18 and from 2.7 to 4.0 in 2018-19. All selected genotypes, except HP-901, exhibited lower PRS scores compared to both checks (Table 2), indicative of good flour quality. The average sodium dodecyl sulfate (SDS) sedimentation value was 40.4 cc in 2017-18 and 40.3 cc in 2018-19. Genotypes HP-940, HP-956, HP-968, and BWL-5228 had sedimentation values below 40.0 cc, while HP-1195 recorded the highest value of 50.0 cc in 2017-18, surpassing both checks. Grain hardness ranged from 9.6 to 12.0 kg in 2017-18 and 9.7 to 12.0 kg in 2018-19, with all selected genotypes exhibiting values greater than HD-3086 and equal to or higher than PBW-1-Zn (Table 2).



Data on various yield parameters is presented in Table 3. The average number of days to flowering was 99.6 in 2017-18 and 98.6 in 2018-19. Genotype HP-910 took the maximum number of days to flower, with 104 days in 2017-18 and 102 days in 2018-19. Elite genotypes generally took more days to flower compared to the high-yielding check HD-3086. However, HP-1154, HP-1213, and BWL-7126 took fewer days to flower than PBW-1-Zn during both crop seasons (Table 3). Genotype HP-910 produced the highest number of productive tillers per m³, with 276 tillers per m³ in 2017-18 and 256 tillers per m³ in 2018-19. The average number of productive tillers per m³ in elite genotypes was 215 in 2017-18 and 212 in 2018-19. The average number of grains per spike was 66 in both seasons. Genotype HP-910 had the highest number of grains per spike (77) in 2017-18, and BWL-7126 had the highest (77) in 2018-19. Genotype HP-968 had fewer than 60 grains per spike in both seasons (Table 3). The thousand-grain weight ranged from 35.0 g (HP-968) to 44.0 g (HP-912) in 2017-18, and from 35.0 g (HP-956) to 42.0 g (HP-912) in 2018-19. Genotype HP-968 had a thousand-grain weight lower than both checks in both seasons. Grain yield ranged from 4.03 to 7.36 t/ha (average 6.23 t/ha) in 2017-18, and from 4.30 to 7.32 t/ha (average 6.57 t/ha) in 2018-19. Four genotypes, HP-1154, HP-1195, HP-912, and BWL-7126, achieved grain yields equivalent to the high-yielding check HD-3086 in 2017-18 (Table 3). In 2018-19, all selected genotypes, except HP-901, HP-956, and HP-1213, showed higher grain yields compared to HD-3086. Canopy temperature at the reproductive stage was lowest in BWL-5228 (14.5°C) in 2017-18 and in HP-956 (14.9°C) in 2018-19. The average canopy temperature in selected genotypes was 17.3°C in 2017-18 and 18.4°C in 2018-19.

4. Discussions

Significant variability in iron (Fe) and zinc (Zn) concentrations was observed in mature grains, highlighting the potential for selective breeding to enhance crop nutritional quality. A total of 1520 genotypes were evaluated using EDXRF to identify those with high iron (Fe) and zinc (Zn) content. The wide range of Fe concentrations (21.8 to 71.4 ppm) and Zn concentrations (20.4 to 84.3 ppm) indicates substantial genetic diversity among the evaluated genotypes. Genotypes such as HP 8068, with the highest Fe concentration (71.4 ppm), and HP 924, with the highest Zn concentration (84.3 ppm), demonstrate the

feasibility of developing nutrient-dense varieties. These findings align with recent research emphasizing the genetic variation in micronutrient content and the importance of biofortification to address malnutrition (Niyigaba *et al.*, 2022; Velu *et al.*, 2022; Kenzhebayeva *et al.*, 2022). The significant positive correlation ($R^2 = 0.292$) between grain Fe and Zn concentrations ($P < 0.01$) suggests that breeding for higher concentrations of both nutrients simultaneously is possible. This correlation is consistent with previous studies indicating that Fe and Zn accumulation can be co-inherited, facilitating the development of biofortified crops (Gomez-Becerra *et al.* 2010, Cakmak and Kutman 2022).

To enhance the bioavailability of micronutrients in wheat-based diets, it is crucial to reduce the antinutrient phytic acid, which binds micronutrients and limits their absorption. The phytic acid content among the 234 selected genotypes ranged from 0.11% to 0.48%, with an average of 0.30%. Several genotypes demonstrated a favorable combination of high Fe and Zn concentrations with low phytic acid content, which is crucial for improving the nutritional quality of grains. These results are supported by recent studies emphasizing the need to balance micronutrient density with lower antinutrient levels to improve bioavailability (Ram *et al.*, 2018; Kenzhebayeva *et al.*, 2022, Raboy, 2022). Hierarchical clustering based on Fe, Zn, and phytic acid concentrations grouped the genotypes into eight distinct clusters. The clustering pattern revealed genotypes with specific nutrient profiles and varying phytic acid levels, providing a basis for targeted breeding programs. Notably, Cluster III, which included 11 genotypes with high Zn concentrations (>60 ppm) and low phytic acid (<0.20%), is of particular interest for further evaluation. These genotypes present an optimal balance of high nutrient density and lower antinutrient content, making them prime candidates for biofortification initiatives (Cakmak and Kutman, 2022).

The selected genotypes (Cluster III) demonstrated superior processing qualities compared to the high-yielding check HD-3086 and the high-Zn variety PBW-1-Zn. Protein content ranged from 8.5% to 13.8%, with three genotypes (HP-1154, HP-1195, and HP-940) consistently recording protein content above 13.0% over two crop seasons. High protein content is essential for improving the nutritional value and functional properties of wheat flour, as supported by recent findings (Shewry and Hey, 2021).



Wheat grains with protein content in the range of 8-10% are suitable for biscuits, 10.0-11.0% for crackers, 11.5-13.0% for pan bread (Chapati), and above 13.0% for macaroni products (Kumar *et al.*, 2021). The average protein content in the eleven selected genotypes was 11.8% in 2017-18 and 12.1% in 2018-19, indicating their suitability for bread making. Among these, three genotypes (HP-1154, HP-1195, and HP-940) with protein content >13.0% can be used for macaroni products. Only HP-956, with protein content <10.0%, is suitable for biscuit and pie making. The remaining seven genotypes, with protein content between 10.0-12.9%, are suitable for pan bread (Chapati). Similar ranges for protein content in bread wheat genotypes have been reported, varying from 10.21% to 17.57% (Kumar *et al.*, 2021; Velu *et al.*, 2022; Niyigaba *et al.*, 2022).

Test weight and grain appearance scores further highlighted the superior milling and end-use qualities of the genotype. Test weight indicates grain density and flour yield. An average test weight of approximately 78 kg/hl indicates good milling quality, which is crucial for commercial wheat production (Peterson *et al.*, 2018). In the eleven elite genotypes test weight ranged from 75.0 to 80.0 kg/hl, suggesting good flour yield. Test weight values of 69.67-83.0 kg/hl have been reported by Kumar *et al.* (2021) and 73.8-83.3 kg/hl by Velu *et al.* (2022). Grain Appearance Score (GAS), reflecting consumer preference and processing quality ranged from 5.4 to 6.6 in 2017-18 and 6.0 to 6.8 in 2018-19, which is higher than or comparable to checks HD-3086 and PBW-1-Zn. Kumar *et al.* (2021) and Salh and Kaur (2021) reported a GAS range of 3.57-6.60 in various bread wheat cultivars. Phenol Reaction Score (PRS), indicating dough darkening during storage and processing, ranged from 2.9 to 3.0 during 2017-18 and 2018-19, which is lower than the checks. A lower PRS value is preferable as it suggests reduced darkening and nutritional losses during long-term storage. Kumar *et al.* (2021) reported PRS values of 3.3-4.0 in wheat varieties, while Minz *et al.* (2018) found a range of 1.2-6.4 in 32 high-yielding advanced breeding lines. The high grain appearance scores (GAS) of genotypes such as HP-940 (6.6) and the favorable phenol reaction scores (PRS) demonstrate their potential for producing high-quality flour with desirable sensory attributes. SDS-sedimentation value, reflects gluten content and bread making quality of the flour. The average sodium dodecyl sulfate (SDS) sedimentation values and grain hardness measurements

also indicated that the selected genotypes possess desirable baking qualities. SDS-sedimentation values ranged from 32 to 50 cc during 2017-18 and 32 to 48 cc during 2018-19, indicating good chapatti making qualities. These values align with results reported by Kaur *et al.* (2020). Grain hardness, which influences chapatti making quality, ranged from 10.0 to 12.0 kg during both crop seasons. This range is higher than the high-yielding check HD-3086 but comparable to the high Zn check PBW-1-Zn, which demonstrate their good chapatti qualities. Kaur *et al.* (2020) reported a grain hardness range of 9.95-11.42 kg, and Panghal *et al.* (2017) found a positive correlation between grain hardness and chapatti quality.

In addition to micronutrients and quality parameters, the eleven selected genotypes were also evaluated for yield and yield-related attributes. The yield parameters of the selected genotypes underscore their agronomic superiority. Phenotypic traits such as the number of productive tillers, grains per spike, and thousand grain weights significantly contribute to yield. The average number of days to flowering, productive tillers per m³, grains per spike, and thousand-grain weight all demonstrate the competitive performance of these genotypes. Notably, the higher grain yields of the selected genotypes compared to the checks, particularly in the 2018-19 crop seasons, highlight their potential for both high yield and nutritional quality. Similar ranges of these yield related parameters have been reported by Niyigaba *et al.* (2022), Kenzhebayeva *et al.* (2022), and Velu *et al.* (2022). Canopy temperature, related to transpiration and cooling, ranged from 14.1 to 20.3°C (average=17.3°C) during 2017-18 and 14.9 to 22.4°C (average=18.4°C) during 2018-19, indicating potential performance under heat/drought stress. The lower canopy temperatures observed in certain genotypes at the reproductive stage suggest improved heat tolerance, which is critical for maintaining yield stability under climate change conditions. Recent studies emphasize the importance of heat tolerance in wheat breeding programs to ensure food security in the face of global warming (Asseng *et al.*, 2018 Kumari *et al.* (2018)).

Conclusions

The results obtained in the present study showed significant variation for Fe, Zn and phytic acid among the genotypes. The data of physiological and yield parameters of selected eleven genotypes with high Zn concentration



and low phytic acid content depicts their good processing characteristics and high grain yield. These genotypes can be useful to develop variety-based products rich in micronutrients to overcome malnutrition. The variability in Fe and Zn concentrations, along with the favorable physiological and quality traits of the selected genotypes, provides a strong foundation for developing nutrient-dense, high-yielding wheat varieties. These findings support ongoing efforts to enhance the nutritional quality of staple crops through biofortification and targeted breeding strategies.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contribution

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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