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ORIGINAL PAPER

Morpho-nutritional status of micronutrient efficient wheat (*Triticum aestivum* L.) genotypes under changing environments

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Abstract In India, where cereal-based meals make up the majority of the daily diet, bread wheat (*Triticum aestivum* L.) is a key grain crop. Micronutrient deficiencies are a result of the lack of a diverse food culture in the nation. Genotypes of bread wheat that have been biofortified might be introduced to address this. It is anticipated that more information on the genotype x year interaction of these nutrients in grain will help us better understand the size of this interaction and perhaps even identify more stable genotypes for this attribute. Year revealed divergent responses to grain iron and zinc. Compared to zinc, iron showed lowest variation across year. Maximum temperature was the major determinant for the four traits. Iron is also significant correlation with zinc. Among the total fifty-two genotype, HP-06, HP-22, HP-24, HP-25, HP-33, HP-44, and HP-45 were found superior for zinc and iron content. These genotypes with high levels of zinc and iron can be used in a hybridization programme to further crop improvement. Wide-scale cultivation of the chosen genotype with high zinc and iron content in the agro-climatic conditions of Jammu will work with the region's current cropping systems.

Keywords Biofortification · Malnutrition · Wheat · Genetic variability · G × E interactions · Correlation

Introduction

Micronutrient malnutrition, sometimes known as "hidden hunger," is a global problem that results in low birth weight, anaemia, learning difficulties, higher rates of morbidity and mortality, low job productivity, and high healthcare expenditures (Batra & Seth, 2002; Welch & Graham, 2002). Lack of iron (Fe) and zinc (Zn) affects a number of metabolic processes in humans, including oxygen transport, cell development and differentiation, DNA replication, protein synthesis, reduction of oxidative stress, and defence against brain cancers (Thavarajah et al., 2009). The importance of micronutrients in the formation of a well-functioning immune system is well established during the COVID-19 pandemic (WHO, 2020), and the efforts to increase Zn and Fe bioavailability through dietary supplementation, food

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fortification, and dietary diversification have been made to reduce micronutrient malnutrition, but the potential methods for reducing global micronutrient deficiency has been anticipated through the production of staple food crop cultivars fortified with micronutrients (Singh, 2017). The HarvestPlus programme of the CGIAR was conceptualized at mitigating the nutritional deficit targeting high-value crops across globe. Wheat being a major food crop grown across the world was focused for biofortification (HarvestPlus Brief, 2006), and project's major goal is to generate nutritionally enhanced cultivars of common wheat (*Triticum aestivum* L.) to increase people's intake of zinc (Zn) and iron (Fe), two micronutrients that are considered to be vital for human health (Pfeiffer & McClafferty, 2007). Advanced elite lines developed through this programme are being utilized by the location-specific breeding programmes in wheat. The genetic improvement towards biofortification necessitates the studies on genetic variability for Fe and Zn content in the seed, their inheritance in the plant, and their sinking into the progeny seed. The International Maize and Wheat Improvement Center (CIMMYT, International), with funding from the HarvestPlus Challenge Program and the CGIAR Research Program on Agriculture for Nutrition and Health, are in charge of a global initiative to create and spread to partners in South Asia high-yielding wheat varieties that contain high levels of grain Zn and Iron (Guzman et al. 2014). According to Singh et al. (2017) and Johnson et al. (2013), there is substantial genetic variation across wheat germplasm for a number of micronutrients, and genetic (G) x environmental (E) interactions play a key role in the inheritance of Fe and Zn content in wheat and other crops. Breeding cultivars with high Fe and Zn content in their seeds is complicated by environmental factors such soil fertility, soil type, seed characteristics, seed composition, and climate influences (Thavarajah et al., 2009). G × E interactions have been viewed as a barrier to crop development for nutritional features (Kumar et al., 2018) because it reduces trait heritability estimates, which may lead to less genetic gain through selection (Ceccarelli, 1989).

Thus, it is essential to study the impact of environment on Fe and Zn content in order to utilize them to make genetic improvement towards biofortification effective. With this background, preliminary investigation on the study was to determine the genetic variability for Fe and Zn content among exotic micronutrient enriched elite genotypes and adapted commercial varieties was undertaken and the impact of seasons/years with varying environment on the Fe and Zn content in the wheat, thus identifying stable genotypes for utilization in hybridization.

Material and methods

Experimental material for the present study comprised of forty-nine zinc and/or iron-enriched HarvestPlus genotypes and along with three adapted wheat varieties, viz. HD 3086, JAUW 683, and RSP 561 (Table 1).

These genotypes were evaluated during two consecutive *Rabi* seasons (November to April), viz. 2019–20 and 2020–21, in randomized complete block design with three replications having a plot size 1.0 m^2. The description of experimental site along with weather conditions are presented in Table 2 and Fig. 1. All the agronomic and plant protection measures were followed as per the packages and practices of SKUAST-Jammu to raise healthy crop. Five plants per genotype per replication were randomly chosen for recording morpho-metric and yield attributing traits during both the crop seasons viz., plant height, number of tillers per plant, days to 50 per cent flowering, flag leaf area (cm^2), spikelets per spike, days to maturity, 1000 grain weight (g), and grain yield per plant (g).

Micronutrients profiling for zinc and iron

A combination of tap water, diluted HCl (0.01 *N*), and distilled water was used to wash the grain samples. Samples were dried in a hot air oven at 60 °C for five minutes. Grain was processed and used for future chemical analysis after reaching consistent weight. Grain samples were microwave-digested with HNO_3 by placing 0.5 g of the sample into a PTFE-TFM jar, adding 7 ml of suprapure HNO_3, and predigesting the mixture overnight. The vessel was then sealed and placed in microwave digestion. The heating programme (Multiwave ECO, Anton Paar) was configured with operational parameters of a ramp period of 25 min to reach 180 to 190 °C and a hold time of 25 min at 180 to 190 °C (Datta et al., 2017). To ensure the full transfer of content, the samples were

Table 1 The details of 52 germplasm lines acquired from HarvestPlus and adapted variety

S.No	Coded Name	Pedigree
1	HP-2	KACHU#1
2	HP-3	MAYIL
3	HP-4	ZINCSHAKTHI
4	HP-5	DANPHE#1*2/SOLALA//BORL14
5	HP-6	DANPHE#1*2/SOLALA//BORL14
6	HP-7	VALI//KACHU/KIRITATI
7	HP-8	MANKU//MUTUS*2?TECUE#1
8	HP-9	VILLA JUAREZ F2009/3/T.DICOCCON PI94625/…
9	HP-10	FRANCOLIN#1/3/IWA8600211//2*PBW343*2/KUKUNA/7/TRAP#1/
10	HP-11	C80.1/3*BATAVIA//2*WBLL1/3/ATTILA/3*BCN*2/BAV92/4/…
11	HP-12	C80.1/3*BATAVIA//2*WBLL1/3/ATTILA/3*BCN*2/BAV92/4/…
12	HP-13	C80.1/3*BATAVIA//2*WBLL1/3/ATTILA/3*BCN*2/BAV92/4/…
13	HP-14	TRAP#1/BOW/3/VEE/PJN/2*TVI/4/BAV92/RAYON15/KACHU#1/6/
14	HP-15	ROLF07*2/KIRITATI/3/IWA8600211//2*PBW343*2/KUKUNA/4/…
15	HP-16	SHAKTI/2*BORL14
16	HP-17	SHAKTI/2*BORL14
17	HP-18	SHAKTI/2*BORL14
18	HP-19	SHAKTI/2*MUCUY
19	HP-20	SHAKTI/6/KAUZ/ALTAR84/AOS/3/PASTOR/4/873.97/5/…
20	HP-21	SHAKTI/7/SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/KRONSTAD F2004/…
21	HP-22	SHAKTI/5/WHEAR/KIRITATI/3/C8001/3*BATAVIA//2*WBLL1*2/4/…
22	HP-23	KATERE/MUCUY/7/TRAP#1/BOW/3/VEE/PJN//2*TVI/4/BAV92/…
23	HP-24	KATERE/MUCUY/7/TRAP#1/BOW/3/VEE/PJN//2*TVI/4/BAV92/…
24	HP-25	KATERE//ONIX/KBIRD/6/C80.1/3*BATAVIA//2*WBLL1/3/ATTILA/…
25	HP-26	ZINCOL//BECARD/QUAIU#1/7/INQALAB91*2/TUKVRV//WHEAR/6/.
26	HP-27	DANPHE#1*2/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//…
27	HP-28	HG094.7.1.12//WBLL1*2/KUKUNA/3/WBLL1*2/KURUKU/4/…
28	HP-29	VALI/3/MUTUS*2//ND643/2*WBLL1/6/C80.1/3*BATAVIA//…
29	HP-30	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4/…
30	HP-31	QUAIU#1/SOLALA//QUAIU#2/3/MANKU/4/KACHU#1/KIRITATI//…
31	HP-32	KOKILA/3/MUTUS*2//ND643/2*WBLL1/8/PSN/BOW//SERI/3/…
32	HP-33	KIRITATI/4/2*SERI.IB*2/3/KAUZ*2/BOW//KAU2/5/CMH81.530/…
33	HP-34	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLLI/4/CMH75A.66/…
34	HP-35	DANPHE#1*2/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//…
35	HP-36	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4/…
36	HP-37	MANKU/6/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/5/PRL/…
37	HP-38	VILLA JUAREZ F2009/3/T.DICOCCON PI94625/…
38	HP-39	VALI/5/2*VILLA JUAREZ F2009/3/T.DICOCCON PI94625/…
39	HP-40	QUAIU#1/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//…
40	HP-41	MAYIL/2*VALI
41	HP-42	MAYIL/2*VALI
42	HP-43	MAYIL*2//SUP152*2/TELUE#1
43	HP-44	VILLA JUAREZ F2009/3/T.DICOCCON PI94625/…
44	HP-45	KOKILA/2*VALI
45	HP-46	KOKILA/2*KUTZ
46	HP-47	ZINCOL/5/28QUAIV#1/3/T.DICOCCON PI94625/…

Table 1 (continued)

S.No	Coded Name	Pedigree
47	HP-48	WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLL1/4/CMH75A.66/…
48	HP-49	PAURAQ//RL6043/4*NAC/3/2*QUAIU#1/SOLALA//QUAIU#2
49	HP-50	PAURAQ//AG/5*NAC/3/2*QUAIU#1/SOLALA//QUAIU#2
50	HD3086	Adapted variety /commercial variety (Timely sown, irrigated)
51	JAUW 683	Adapted variety / advanced line (Timely sown, irrigated)
52	RSP 561	Adapted commercial variety (Timely sown and late sown irrigated)

Table 2 The site of experiments conditions at SKUAST-Jammu, India

S. No	Parameters	Units
1	Altitude	239 m AMSL
2	Longitudes	74°48E
3	Latitudes	32°40N
4	Climate	Subtropical with cold winters and dry summers
5	Soil texture	Sandy loam soil
6	pH	7.0
7	Temperature Regime 2019–20	22–10.6 °C
8	Min Temperature 2019–20	10.60 °C
9	Max Temperature 2019–20	24.03 °C
10	Min Temperature 2019–20	9.78 °C
11	Relative humidity (Morning) 2019–20	89.37
12	Relative humidity (Evening) 2019–20	58.01
13	Morning Relative humidity 2020–21	89.21
14	Evening Relative humidity 2020–21	53.27
15	Average rainfall 2019–20	16.32 mm
16	Average rainfall 2020–21	7.20 mm

thoroughly shaken after being cooled to room temperature and added Milli-Q water. The resulting mixture was passed through a Whatman No. 42 filter before being diluted to a final amount of 100 ml in a volumetric flask using Milli-Q water that contained 1% suprapure HNO3. A atomic absorption spectrophotometer (AAS) was used to measure the total Zn and Fe in the digest. To prepare a reagent blank, a similar process was used but without a sample.

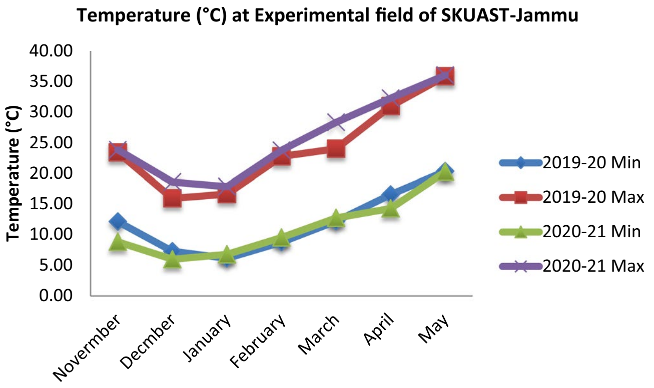
Statistical analysis

Pooled and year-wise analysis of variance (ANOVA) along with mean performance of genotypes during two consecutive years (2019–20 and 2020–21) were considered, and relationship among genotypes was assessed employing R ver. 4.1 and Windostat 9.3 software.

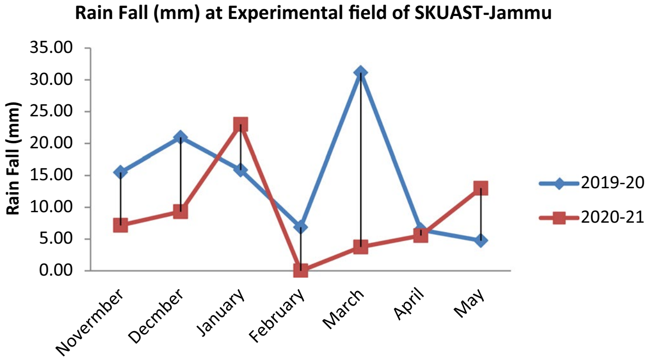
Results and discussion

Analysis of variance revealed mean sum of squares due to genotype to be highly significant for all traits during both years (Tables 3a and 4). The pooled ANOVA for genotype and year was found significant ($p < 0.01$) for all the traits, while interactions were found significant ($p < 0.01$) for only 1000-grain weight (g) and at $p < 0.05$ for grain yield per plant (Table 5). Table 6 summarizes the results on environmental variations for yield and yield contributing traits and micronutrient content (Zn and Fe) in fifty-two wheat genotypes grown under Jammu

Fig. 1 **a** Temperature (°C) at Experimental field of SKUAST-Jammu from the month of November to May of 2019–20 and 2020–21. **b** Rainfall (mm) at Experimental field of SKUAST-Jammu from the month of October to March of 2019–20 and 2020–21



(**a**) Temperature (°C) at Experimental field of SKUAST-Jammu from the month of November to May of 2019-20 and 2020-21.



(b) Rainfall (mm) at Experimental field of SKUAST-Jammu from the month of November to May of 2019-20 and 2020-21.

agro-climatic conditions. Significant genetic variation among genotypes is a prerequisite to increase the concentration of Zn and Fe content in wheat grain through conventional breeding.

Significant differences between treatments at the genotypic level may result from genetic variation of genotypes, whereas significant variations at the annual level and genotype x year interactions may result from changes in humidity, precipitation, climate, soil conditions, or other cultivation practises used throughout the cropping season (Joshi et al., 2010). The environment has a big impact on yield and the qualities that make up that yield. Table 6 summarises the mean data of the morpho-metric characteristics and micronutrients (Zn and Fe) content of 52 accessions for the two following years, 2019–2020 and 2020–2021.

It was prominently elucidated the significant variation among the genotype, year, and genotype x year interactions studied. The most stable plant height was recorded in the genotype RSP-561 (100 cm in 2019–20 and 99.67 cm in 2020–21) followed by HP-02 (93.33 cm in 2019–20 and 92.67 cm in 2020–21), HP-29 (87.00 cm in 2019–20 and 86.33 cm in 2020–21), and HP-39 (86.33 cm in 2019–20 and 87.00 cm in 2020–21), whereas the highest plant height was recorded for JAUW-683 (105 cm in 2020–21). For number of tillers per plant, the most superior and stable performance was attained by the genotype HP-02 (9.33 in 2019–2020 and 10.00 in 2020–21) and HP-48 (8.00 in 2019–2020 and 9.33 in 2020–21). Plant height and number of tillers per plant are the most sensitive to environmental fluctuations. It is indicated that the

Table 3 Analysis of variance (ANOVA) for different morpho-micronutrient traits (Zn and Fe) in fifty-two genotypes of wheat grown for Ist year (2019–20) at SKUAST-Jammu

		Mean sum of Squares									
Source of Variations	DF	Plant height	No. of tillers per plant	Days to 50 per cent flowering	Flag leaf area (cm^2)	Spikelets per spike	Days to maturity	1000 grain weight (g)	Grain yield per plant (g)	Zinc (ppm)	Iron (ppm)
Replication	2	5.31	0.31	10.95	14.55	3.87	8.33	0.95	0.26	0.72	1.87
Treatments	51	156.98**	3.42*	19.93**	66.93**	6.08**	3.96**	45.04**	26.79**	76.06**	421.89**
Error	102	23.19	2.05	3.29	31.01	3.13	2.12	0.7	0.63	0.9	1.12

*Significant at $p \leq 0.05$ and **Significant at $p \leq 0.01$

Source of Variations

Table 4 Analysis of variance (ANOVA) for different morpho-micronutrient traits (Zn and Fe) on 52 genotypes of wheat grown for Ist year (2020–21) at SKUAST-Jammu

	DF	Mean sum of Squares									
		Plant height	No. of tillers per plant	Days to 50 per cent flowering	Flag leaf area (cm^2)	Spikelets per spike	Days to maturity	1000 grain weight (g)	Grain yield per plant (g)	Zinc (ppm)	Iron (ppm)
Replicate	2	21.42	2	5.87	74.57	0.94	13.77	26.55	55.66	4.64	6.25
Treatments	51	108.20**	3.98**	24.96**	58.37**	6.32**	9.59**	49.00**	31.48**	223.65**	36.72**
Error	102	14.35	2.18	3.88	32.38	2.89	4.18	4.36	7.21	5.20312	3.1585

*Significant at $p \leq 0.05$ and **Significant at $p \leq 0.01$

Table 5 Pooled Analysis of variance (ANOVA) of genotype x year interaction for different morpho-micronutrient traits (Zn and Fe) on fifty-two genotypes of wheat during two consecutive years (2019–20 and 2020–21) at SKUAST-Jammu

Source of Variations	DF	Mean sum of Squares									
		Plant height	No. of tillers per plant	Days to 50 per cent flowering	Flag leaf area (cm^2)	Spikelets per spike	Days to maturity	1000 grain weight (g)	Grain yield per plant (g)	Zinc (ppm)	Iron (ppm)
Replicate	2	23.58	0.87	12.67	70.63	4.08	21.16**	10.76*	31.56**	1.17	1.37
Environments	1	2077.0**	103.84**	53.33**	351.36**	16.15**	136.01**	182.00**	1544.15**	4253.53**	5475.15**
G x Y Interactions	2	3.17	1.45	4.15	18.49	0.73	0.94	16.73**	24.35*	4.19	6.75
Treatments	51	210.91**	6.45**	37.67**	75.63**	7.21**	9.46**	87.93**	42.99**	198.05**	276.11**
Error	255	25.88	1.88	4.31	35.29	3.44	3.34	3.25	6.19	22.77	38.21

*Significant at $p \leq 0.05$ and **Significant at $p \leq 0.01$

relative inconsistent performance of other genotypes was marked due to genotype and environment interaction. For days to 50 per cent of flowering, the genotype HP-04 (94.33 in 2019–2020 and 95.67 in 2020–21) and HD 3086 (94.00 in 2019–2020 and 96.00 in 2020–21) projected the lowest days to 50 per cent of flowering, which could be directly correlated with early maturity. While, the stable genotype was achieved by HP-03 (95.67) and HP-08 (97.33). Plant heights, flag leaf, days to 50 per cent of flowering, and seed morphological variation are the primary descriptor for characterization of germplasm. The leaf morphological traits of the wheat germplasm (9HPYT) under study showed a wide range of variability. Regarding the flag leaf, it was found that RSP-561 (30.23 cm) showed the most consistent trait during the two years investigated, followed by HP-22 (26.23 cm for 2019–20 and 26.57 cm for 2020–21), HP–09 (27.70 cm for 2019–20 and 28.23 cm for 2020–21), and HD 3086. (27.73 cm for 2019–20 and 28.37 cm for 2020–21). While in reference to spikelets per spike, the stable performance was presented by HP-39 (18.67) followed by HP-15 (18.00) and HP-03 (16.33), in case of days to maturity, genotype HP-14 (137.67) and lowest days to maturity was recorded in genotype, HP-04 (133.67 in 2019–20) and RSP-561 (133.67 in 2020–21). One thousand grain weight (g) and grain yield per plant (g) are the major economic traits and are important for successful agronomic practices and global demand. Thus, exploiting the highest 1000-grain weight (g) was observed for both the years in genotype HP-47 (46.40 g in 2019–20 and 43.63 g in 2020–21) followed by HP-21 (43.67 in 2019-20 g and 8.87 g in 2019–20), whereas for grain yield per plant (g), genotype HP-09 (24.40 g in 2020–21) followed by HP-02 (24.33 g in 2020–21) and HP-40 (23.30 g in 2020–21) was recorded highest. While, the most stable genotype for 1000-grain weight (g) was recorded in HP-06 (37.40) followed by HP-19 (42.33 gm in 2019–20 and 42.50 gm in 2020–21), HP-13 (39.00 gm in 2019–20 and 38.83 gm in 2020–21), and grain yield per plant (g), were revealed by genotype HP-08 (14.13) followed by HP-26 (14.37 gm), HP-27 (10.47 gm), and HP-28 (16.03) in 2019–20 and 2020–21, respectively. Highly significant differences among the genotype were observed for both grain Fe and Zn concentration, indicating the presence of sufficient amount

Table 6 Mean morphological and micronutrient (Zn and Fe) comparison of wheat accessions for two consecutive years (E1 = 2019–20 and E2 = 2020–21) under agro-climatic conditions of Jammu

S. No.	Accession name	Plant height		No. of tillers per plant		Days to 50 per cent flowering		Flag leaf area (cm^2)		Spikelets per spike		Days to maturity		1000 grain weight (g)		Grain yield per plant (g)		Zinc (ppm)		Iron (ppm)	
		E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
1	HP-02	93.33	92.67	9.33	10.00	95.67	95.00	28.50	25.83	17.33	18.33	134.33	136.00	38.27	37.37	19.43	24.33	35.33	40.33	36.00	34.00
2	HP-03	93.33	91.00	5.67	7.67	95.67	95.67	40.50	33.93	21.00	19.67	134.00	134.33	37.47	37.70	16.10	18.50	30.33	29.00	34.33	34.33
3	HP-04	90.00	92.00	5.33	7.33	94.33	95.67	31.47	22.90	16.33	16.33	133.67	135.00	39.83	38.93	13.03	16.03	35.33	33.33	43.33	42.67
4	HP-05	95.67	101.67	6.33	7.67	98.67	99.33	26.73	30.47	19.67	20.00	135.33	137.00	41.97	42.23	15.67	19.43	32.67	31.67	34.33	33.67
5	HP-06	90.33	92.67	4.67	5.33	96.00	100.00	36.90	30.23	17.33	17.67	138.00	140.00	37.40	37.40	11.00	13.87	29.33	29.00	56.00	52.33
6	HP-07	87.00	98.00	5.67	8.33	97.67	95.67	30.97	30.23	18.00	17.67	135.67	139.33	38.50	39.93	15.63	17.97	32.00	34.67	49.33	47.00
7	HP-08	94.00	98.00	7.00	8.67	97.33	97.33	42.80	35.57	20.67	20.00	136.33	137.67	38.87	37.87	14.13	14.13	34.67	34.67	43.00	43.00
8	HP-09	88.33	94.00	5.67	6.00	100.67	101.67	27.70	28.23	21.00	19.33	136.33	139.00	35.27	36.63	18.00	24.40	29.67	30.00	49.67	48.00
9	HP-10	81.00	88.67	7.67	9.00	101.33	105.33	23.90	30.10	20.67	20.33	136.67	140.00	33.43	33.80	13.03	15.97	35.33	34.00	48.33	47.33
10	HP-11	74.33	96.00	5.67	6.33	100.33	103.33	38.90	35.10	18.67	19.67	137.33	139.00	42.87	42.67	15.00	17.40	37.67	35.67	43.33	41.67
11	HP-12	89.67	97.33	7.67	8.00	105.00	102.33	38.50	34.60	18.67	22.00	135.00	133.67	42.80	43.57	10.90	14.67	36.67	39.00	30.00	29.00
12	HP-13	82.33	95.67	6.67	7.33	105.00	105.67	37.20	30.40	23.00	19.33	137.67	136.67	39.00	38.83	11.57	16.03	8.00	28.00	9.67	30.00
13	HP-14	67.67	85.33	6.67	6.33	102.33	99.00	29.30	30.83	18.67	20.00	137.67	137.67	31.97	33.27	11.63	18.77	36.00	35.00	12.00	40.67
14	HP-15	79.00	86.33	6.33	6.67	105.33	106.33	27.63	28.87	18.00	18.00	135.67	139.33	36.07	35.20	11.57	19.10	35.67	34.00	36.00	35.33
15	HP-16	77.67	82.00	5.00	5.00	100.67	102.00	23.10	25.47	19.67	18.33	137.67	140.33	36.80	34.00	15.67	11.93	36.00	34.00	41.33	40.00
16	HP-17	87.67	84.00	4.33	5.67	100.67	101.00	27.00	30.23	19.33	20.33	135.67	139.00	38.07	34.73	14.37	19.33	39.33	37.67	39.33	38.33
17	HP-18	89.33	86.33	5.00	6.33	99.33	101.00	23.77	28.33	18.00	17.67	137.67	138.00	38.23	33.97	13.63	20.03	37.67	36.00	37.00	35.00
18	HP-19	77.33	78.33	5.67	6.33	97.67	102.67	22.93	27.30	21.00	16.67	136.33	141.33	42.33	42.50	16.23	22.23	34.33	33.00	38.00	38.33
19	HP-20	81.33	86.33	5.33	7.33	99.00	97.00	26.73	24.43	18.00	18.33	138.33	141.33	43.67	40.77	12.37	23.20	27.67	27.33	40.33	37.33
20	HP-21	77.67	83.67	3.67	5.33	100.33	101.67	20.53	23.33	19.67	19.33	136.33	139.33	29.63	25.63	8.67	13.97	34.67	33.33	46.00	44.00
21	HP-22	86.33	87.33	6.33	7.00	98.33	99.33	26.23	26.57	18.00	18.67	137.33	139.33	34.13	30.60	7.50	14.30	37.00	35.00	53.67	51.33
22	HP-23	86.67	88.33	4.33	5.67	103.00	98.33	33.77	26.27	20.33	16.67	136.00	140.67	40.70	38.80	8.60	16.60	37.67	36.00	42.00	41.67
23	HP-24	85.00	82.00	4.00	5.33	102.33	104.67	28.93	20.73	18.67	16.33	137.00	137.67	38.93	36.00	13.37	18.17	39.67	37.67	48.67	45.67
24	HP-25	80.67	82.00	5.67	4.33	99.00	99.67	27.63	22.80	20.00	17.33	137.33	138.67	38.37	33.90	16.70	20.70	39.67	39.33	46.00	45.33
25	HP-26	84.67	82.33	6.33	7.33	98.67	101.00	30.50	23.20	19.33	18.67	137.33	136.33	40.50	37.83	14.37	14.37	37.00	35.00	44.67	43.00
26	HP-27	82.00	84.33	5.33	5.67	97.33	97.00	26.17	23.27	19.67	18.00	137.33	137.00	33.57	30.80	10.47	10.47	36.33	36.00	41.00	38.33
27	HP-28	76.67	83.00	4.67	7.00	100.67	102.67	33.53	22.80	18.00	17.67	138.00	138.67	43.37	42.00	16.03	16.03	39.33	38.00	41.00	38.00
28	HP-29	87.00	86.33	4.33	5.00	99.67	98.00	33.80	21.40	19.00	18.67	138.00	136.67	33.67	34.40	12.27	21.43	34.67	33.67	45.00	41.67
29	HP-30	82.33	87.33	4.67	6.33	96.00	97.67	33.27	23.50	20.33	16.67	137.00	136.33	43.57	42.10	16.70	21.27	29.33	28.00	38.00	37.67
30	HP-31	83.67	86.67	4.67	6.33	101.00	101.67	34.20	20.90	20.00	18.00	137.33	135.33	38.17	35.00	15.67	19.60	30.00	28.67	36.67	34.00

Table 6 (continued)

S. No.	Accession name	Plant height		No. of tillers per plant		Days to 50 per cent flowering		Flag leaf area (cm^2)		Spikelets per spike		Days to maturity		1000 grain weight (g)		Grain yield per plant (g)		Zinc (ppm)		Iron (ppm)	
		E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
31	HP-32	77.67	90.00	5.33	7.00	99.00	99.67	24.67	40.70	18.67	18.33	136.00	137.67	33.73	33.00	13.70	18.47	39.00	37.33	61.00	59.00
32	HP-33	76.33	88.67	5.00	6.67	99.67	97.33	25.63	28.50	19.33	20.00	137.33	138.00	29.03	28.23	12.60	19.83	40.33	40.00	50.67	49.00
33	HP-34	72.67	90.67	5.67	8.00	99.67	99.00	29.10	27.47	20.67	16.67	136.00	137.33	41.03	40.50	9.63	19.53	38.00	36.67	49.67	44.33
34	HP-35	74.33	93.67	5.67	7.00	99.67	102.00	30.17	21.20	17.33	21.33	137.33	139.67	37.03	35.50	13.10	19.30	33.00	31.00	41.00	39.67
35	HP-36	74.33	90.33	6.00	7.00	103.67	104.00	29.17	28.50	18.00	17.33	136.67	139.00	34.67	33.13	14.63	17.37	36.00	35.00	37.33	35.00
36	HP-37	79.67	86.67	5.00	6.67	103.67	103.00	31.40	25.17	19.00	18.00	136.67	136.00	35.23	33.60	7.17	14.03	36.00	34.67	41.67	41.00
37	HP-38	84.33	90.67	4.33	6.33	96.33	99.33	27.57	31.50	17.33	19.33	136.33	139.33	33.53	32.17	9.50	17.23	36.67	35.00	42.33	39.00
38	HP-39	86.33	87.00	6.67	8.00	99.67	98.67	28.63	24.83	18.67	18.67	137.00	135.67	35.27	32.57	14.53	20.23	35.33	34.00	40.00	36.67
39	HP-40	83.67	92.67	6.33	7.33	99.00	102.33	25.87	22.37	18.67	17.33	138.00	138.67	40.13	37.83	12.33	23.23	32.67	30.00	44.33	44.00
40	HP-41	78.33	81.00	5.00	4.67	99.33	102.67	25.63	31.07	17.33	19.33	138.00	140.00	38.53	35.27	13.47	17.90	40.67	37.33	49.67	50.00
41	HP-42	86.67	90.33	5.00	7.33	97.00	99.33	33.33	31.80	18.67	18.00	135.67	138.67	42.10	40.50	8.87	16.43	35.33	38.33	49.33	45.67
42	HP-43	92.00	94.33	5.33	7.00	99.33	97.67	37.07	31.17	18.00	17.33	135.67	137.33	40.47	38.43	16.53	19.93	33.33	36.00	42.67	43.00
43	HP-44	82.67	94.00	6.33	6.67	98.33	98.00	28.13	31.00	17.33	20.67	137.67	138.00	41.57	33.07	17.40	18.63	34.67	35.33	71.00	68.67
44	HP-45	85.00	88.67	6.00	6.33	97.33	101.33	29.03	31.53	18.33	19.33	136.67	139.00	43.37	42.80	7.53	15.63	40.00	41.67	55.00	51.67
45	HP-46	91.00	89.67	6.33	6.67	99.00	103.00	31.13	24.23	17.33	16.00	137.67	138.67	43.60	39.27	11.17	14.10	32.00	33.33	47.33	42.67
46	HP-47	86.33	85.00	5.33	6.67	98.33	104.00	28.90	27.70	18.33	17.33	136.67	138.00	46.40	43.63	12.97	18.30	35.00	35.67	39.00	42.33
47	HP-48	76.67	80.33	8.00	9.33	96.67	94.67	27.27	33.23	17.33	16.67	137.33	137.67	42.30	40.87	10.50	15.97	35.00	34.67	38.00	39.67
48	HP-49	84.33	87.67	4.67	6.67	98.33	98.33	29.43	24.33	19.33	16.33	136.33	137.67	43.37	39.13	13.93	17.33	38.33	39.33	88.33	72.33
49	HP-50	88.33	93.33	5.67	7.00	98.67	100.00	32.97	21.70	18.00	17.33	136.67	139.67	39.63	38.73	11.87	17.73	37.67	35.00	40.00	39.33
50	JAUW-683	102.33	105.00	6.33	7.00	97.67	99.67	26.30	27.67	23.00	20.67	138.67	137.33	39.50	40.90	20.10	20.33	30.00	27.67	28.00	30.00
51	RSP 561	100.00	99.67	5.67	7.33	101.00	99.67	30.23	30.23	19.67	20.00	134.33	133.67	34.57	38.23	12.53	11.73	28.67	31.00	31.33	28.67
52	HD3086	98.33	102.67	6.33	8.33	94.00	96.00	27.73	28.37	17.33	20.33	135.00	137.00	38.97	36.20	14.40	11.63	29.67	33.00	33.00	30.00
Mean		84.49	89.65	5.69	6.84	99.31	100.14	29.86	27.73	18.96	18.50	136.65	137.97	38.49	36.96	13.23	17.68	34.54	34.40	42.78	41.93
C.V.		5.70	4.23	25.16	21.57	1.83	1.97	18.65	20.52	9.33	9.19	1.07	1.48	2.18	5.65	6.02	15.20	2.75	5.17	2.47	5.44
S.E.		2.78	2.19	0.83	0.85	1.05	1.14	3.22	3.29	1.02	0.98	0.84	1.18	0.48	1.21	0.46	1.55	0.55	1.03	0.61	1.32

of genetic variability for grain Fe and Zn concentration among the genotypes studied. The pooled mean percentage of Zn and Fe in grain of the fifty-two accessions ranged from 8.00 ppm to 40.67 ppm and 27.33 ppm to 41.67 ppm in Zn and from 9.67 ppm to 88.33 ppm and 28.67 ppm to 72.33 in Fe for 2019–20 and 2020–21, respectively (Fig. 2). The accession HP-45 (41.67 ppm in 2020–21) was recorded to establish the highest Zn content followed by accession HP-41 (40.67 ppm in 2019–20) and HP-02 (40.33 ppm in 2020–21), while the highest Fe content was depicted by HP-49 (88.33 ppm in 2019–20 and 72.33 ppm 2020–21) followed by HP-44 (71 ppm in 2019–20 68.67 ppm in 2019–20) and HP-45 (55.00 ppm in 2019–20 and 51.67 in 2020–21). Since, grain contains higher amounts of Zn and Fe, it is possible that harvesting plant will assist in the sustainable exploitation of natural conservation. As a result of polygenic control, environmental or nongenetic factors, and their interaction, few accessions, on the other hand, showed inconsistent Zn and Fe content over the course of two years (G × E interaction). When the performance of the traits was compared between the two years, it was evident that the accessions performed better in the first year for many morpho-metric and yield-attributing variables, including plant height, days to 50% of flowering, flag leaf, spikelets per plants, and Zinc (ppm) (2019–20). The accessions performed superior for number of tillers per plant, 1000-grain weight (g), and grain yield per plant (g) and iron (ppm) characters in the second consecutive year (2020–21). Overall based on the ten morphomatric attributes, Zn and Fe content, the accessions HP-08, HP-26, HP-27, HP-28, HP-33, HP-41, and HP-49 displayed a comparable consistent performances pattern in the agro-climatic conditions of the North Western Himalayan region for the two consecutive years studied. HP-33, HP-41, HP-45, and HP-49 had higher Zn and Fe content.

Regarding qualities that contribute to yield, other genotypes behaved differently in both years. The findings showed that genotype-by-environment interactions complicate crop variety development and decrease the efficacy of breeding programmes aimed at improving yield (Ahmad et al., 2011). When the experimental materials for the current study were evaluated during the two years (2019–20 and 2020–21), different patterns of minimum and maximum temperature and rainfall were observed (Fig. 1). This gave researchers the chance to examine how genetic make-up and/or environmental factors affect the level of Fe and Zn (Kumar et al., 2018). The development of breeding techniques for creating biofortified wheat cultivars is aided by knowledge of the interplay between genotype and environment. Given that genotype x year interactions were significant in the current study for both Fe and Zn content, it is likely that a sizeable amount of the Fe and Zn content in wheat seed depends on soil conditions, crop management techniques, temperature, precipitation, and these factors (moisture, aeration, and soil pH). In contrast, substantial genotype x location interactions for Zn and Fe concentrations in both wild and

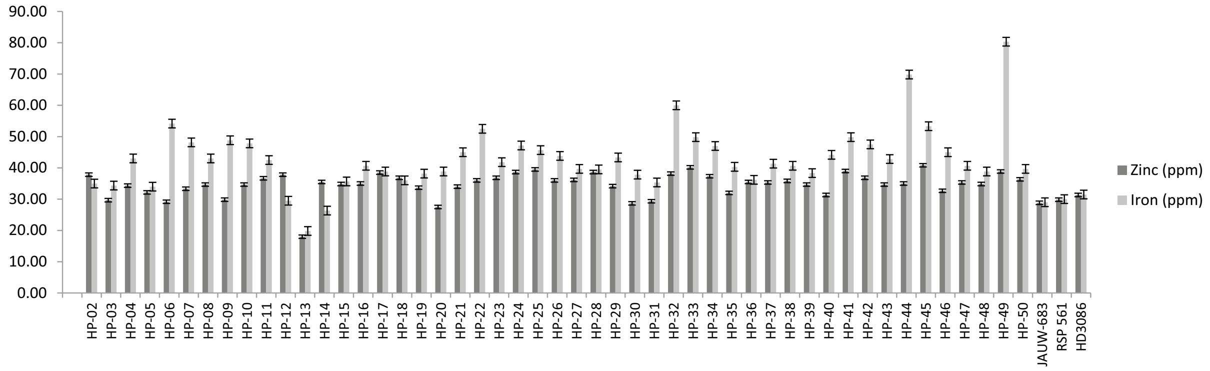
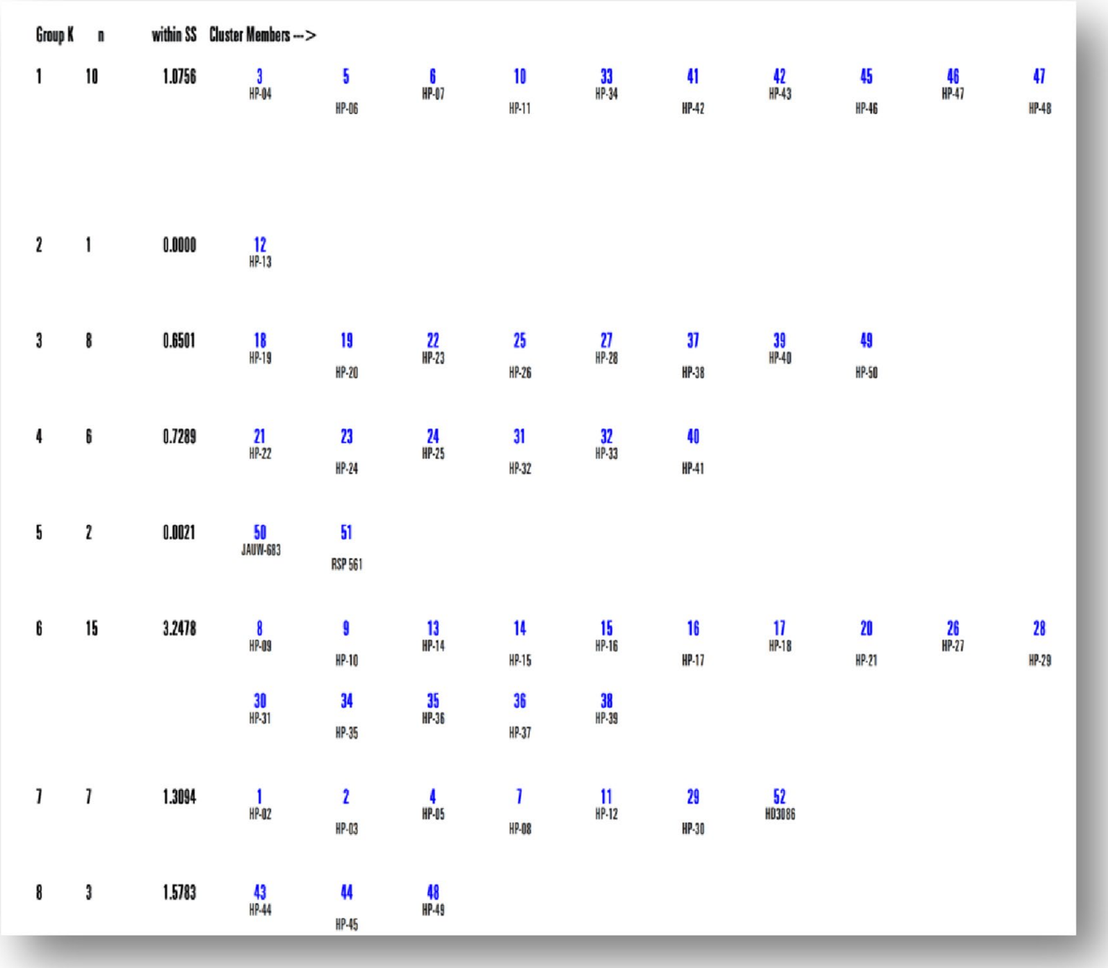


Fig. 2 Pooled grain Zn and Fe content during (2019–20 and 2020–21)

cultivated cultivars of wheat were found (Gomez-Becerra et al., 2010; Ortiz-Monasterio et al., 2007; Trethowan, 2007). Year-to-year changes in Fe and Zn content were likewise extremely significant in the current investigation, showing that the environmental conditions depicted in Fig. 1 are present. Fifty-two genotypes were divided into eight clusters based on the K-means cluster. The distribution pattern of genotypes in the different cluster is presented in Fig. 3. The cluster VI was the largest cluster consisting of 15 genotypes followed by cluster I (10 genotypes), cluster III having 8 genotype, cluster VII having 7 genotypes, cluster IV (6 genotypes), cluster VIII (3 genotypes), cluster V (2 genotype), and cluster II having 1 genotype. It is pertinent to mention that all the zinc- and iron-enrich genotypes were obtained from the HarvestPlus breeding programme and likely to have some part of common ancestry and thus fall in the same cluster. Similar results have been reported by Ajmal et al. (2013); Shahryari et al. (2011). The clustering of genotypes from different ecogeographic regions into one cluster could be due to the exchange of breeding material among global partners. Dendrogram was achieved from cluster analysis of fifty-two genotypes on the basis of two micronutrient (Zn and Fe) content (Fig. 4). According to this grouping



Group K	n	within SS	Cluster Members -->									
1	10	1.0756	3 HP-04	5 HP-06	6 HP-07	10 HP-11	33 HP-34	41 HP-42	42 HP-43	45 HP-46	46 HP-47	47 HP-48
2	1	0.0000	12 HP-13									
3	8	0.6501	18 HP-19	19 HP-20	22 HP-23	25 HP-26	27 HP-28	37 HP-38	39 HP-40	49 HP-50		
4	6	0.7289	21 HP-22	23 HP-24	24 HP-25	31 HP-32	32 HP-33	40 HP-41				
5	2	0.0021	50 JAUW-683	51 RSP 561								
6	15	3.2478	8 HP-09	9 HP-10	13 HP-14	14 HP-15	15 HP-16	16 HP-17	17 HP-18	20 HP-21	26 HP-27	28 HP-29
			30 HP-31	34 HP-35	35 HP-36	36 HP-37	38 HP-39					
7	7	1.3094	1 HP-02	2 HP-03	4 HP-05	7 HP-08	11 HP-12	29 HP-30	52 HD3086			
8	3	1.5783	43 HP-44	44 HP-45	48 HP-49							

Fig. 3 Clustering pattern of fifty-two wheat genotypes on the basis of K-means cluster analysis

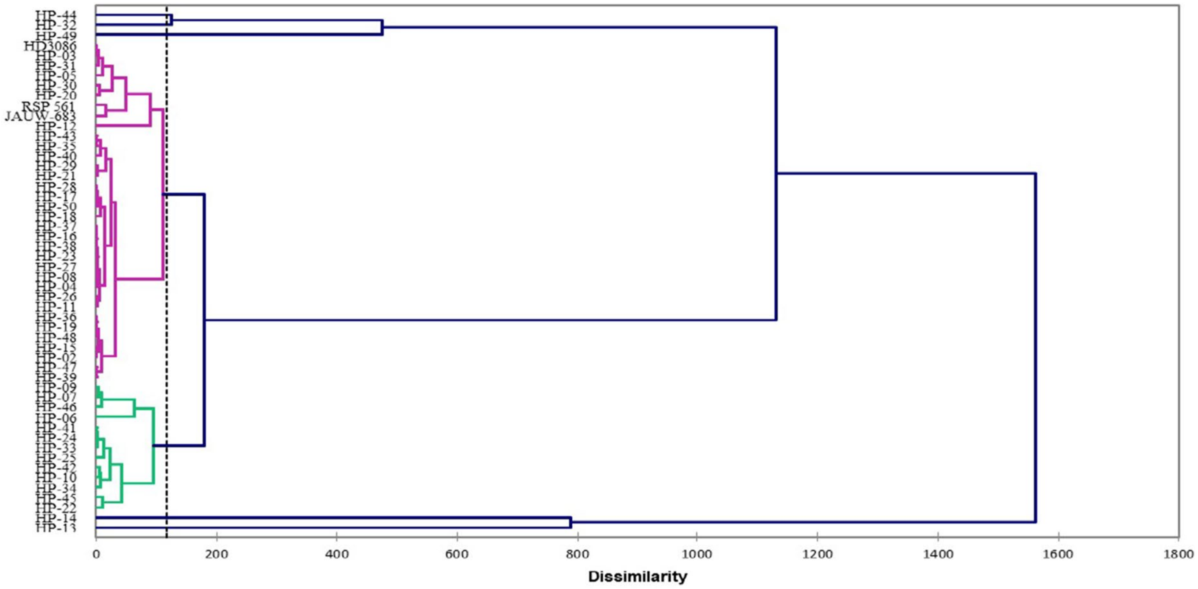


Fig. 4 Dendrogram showing genetic relationship among wheat genotypes based on Euclidean distance for grain zinc and iron content

under-study wheat genotypes divided into seven clusters. Cluster II and VI was considered most desirable cluster for selecting the genotype for use in hybridization.

It is possible to simultaneously increase the concentration of zinc and iron in grains by selection because the genotypic and phenotypic correlations between zinc and iron were highly positive (Table 7). By Velu et al. (2012) and Chatrath et al. (2018), similar associations between grain iron and zinc concentration were reported. The genotypic connection between the iron and days to maturity was highly positive, and Velu et al. (2012) observed a similar finding. Similar results were found by Velu et al. (2012) and the zinc yield had a significantly favourable phenotypic connection with days to maturity. Spikelets per spike and grain weight revealed a substantial positive genotypic connection with grain yield (Table 7). According to Joshi et al. (2007), the number of effective tiller and grains per spike are the most important traits for grain yield in wheat. From this research, genotypic correlation between grain yield with days to 50 per cent flowering and flag leaf area was found significant negative (−0.158**) and (−0.170*), respectively.

Conclusion

Wide adaptation and consistent performance in a range of circumstances are admirable objectives for resource conservation. In order to have more success, scientists have concentrated on the phenomenon of genotype environment (G × E) interaction, which enables them to distinguish between genotype performance in various environments and to selectively target suitable genotypes for commercial cultivation to specific environmental niches. Using 10 morpho-nutrient (Zn and Fe content) properties, it was determined that HP-03, HP-06, HP-08, and HP-27 were the consistently performing biofortified wheat accessions. These genotypes might be used for hybridization under the Jammu agro-climatic of the Northwestern Himalayan region, and HP-06, HP-22, HP-33, HP-34, HP-41, HP-44, HP-45 and HP-49, which have high Zn and Fe concentration. This study showed that environment and its interaction with genotypes have significant and higher effects than the effects due to the genotypes per se on the grain Zn and Fe concentration in wheat. It is, therefore, concluded that improvement of grain Zn and Fe concentration is possible but potentially slow due to the substantial influence by the environment.

Table 7 Phenotypic (rp) and genotypic (rg) correlation grain yield, yield attributes and micronutrients

Traits	r	No. of tillers per plant	Days to 50 per cent flowering	Flag leaf area (cm^2)	Spikelets per spike	Days to maturity	1000 grain weight (g)	Zinc (ppm)	Iron (ppm)	Grain yield per plant
Plant height	rp	0.173*	−0.213*	0.197*	0.180*	−0.177*	0.184*	−0.198*	−0.0950	0.0595
	rg	0.420**	−0.357**	0.478**	0.380**	−0.638”	0.238*	−0.421**	−0.215*	0.1021
No. of tillers per plant	rp		−0.1419	0.0786	0.0414	−0.166*	0.1428	−0.1294	−0.1212	0.0844
	rg		−0.243*	0.462**	0.181*	−0.510**	0.265**	−0.336”	−0.255*	0.1282
Days to 50 per cent flowering	rp			−0.0080	0.0794	0.159*	−0.0842	−0.0629	−0.157*	−0.0666
	rg			−0.1043	0.316**	0.292**	−0.0918	−0.1488	−0.192*	−0.158*
Flag leaf area (cm^2)	rp				0.1077	−0.0234	0.158*	−0.0270	−0.0521	−0.0186
	rg				0.398**	−0.674**	0.411**	−0.194*	−0.1525	−0.170*
Spikelets per spike	rp					−0.0119	−0.1107	−0.178*	−0.1340	0.0082
	rg					−0.253*	−0.223*	−0.483**	−0.558**	0.194*
Days to maturity	rp						−0.0367	0.168*	0.0251	0.0376
	rg						−0.0692	0.362”	0.270**	0.0099
1000 grain weight (g)	rp							−0.1337	0.0428	0.1454
	rg							−0.1288	−0.0519	0.189*
Zinc (ppm)	rp								0.304**	−0.0221
	rg								1.1461	−0.0823
Iron (ppm)	rp									−0.0535
	rg									−0.0905
Grain yield per plant (g)	rp									
	rg									

*Significant at $p \leq 0.05$ and **Significant at $p \leq 0.01$

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Author contributions TD and AS designed the study; AS and NA wrote the first draft; TS and ZD participated in the data collection; BK and MKP elaborated and analysed the data and critically revised the manuscript.

Declarations

Conflict of interests The authors declare no competing interests.

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