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Research Article

Assessment of Grain Iron and Zinc Content in Diverse Wheat Accessions Under Drought and Heat Stress

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Abstract

Wheat is an important staple foodgrain that acts as a primary source of dietary energy, and essential micronutrients. Thus, it is crucial to detect potential wheat genotypes that can act as donors for wheat biofortification to enhance nutritional values. The goal of current study was to evaluate a set of 155 diverse wheat genotypes, including wild relatives, synthetic hexaploid wheats (SHWs) and cultivated varieties for grain iron (Fe) and zinc (Zn) contents under normal as well as stress (drought, heat, and combined stressed) conditions. The findings revealed significant genotypic variation for grain Fe and Zn contents, indicating the presence of exploitable genetic diversity under drought and heat stressed conditions. The wild species such as *Aegilops peregrina* and *Ae. tauschii* exhibited comparatively higher Fe and Zn content than cultivated and SHWs signifying their potential as valuable genetic resources in breeding programs. Correlation analysis revealed significant positive association of Fe and Zn under all the conditions, suggesting the potential for concurrent breeding improvement. The genotypes *viz.*, PI 603931, PI 604176, and accession 14336 were higher in grain Fe and Zn content under both drought and heat stress, while PI 603931 was identified as most stable genotype across the environments. The identified genotypes could be used as potential donor parents for Fe and Zn in future wheat biofortification programs. This study highlights the possibility of using wheat genetic variation for Fe and Zn enrichment, promoting nutritional security, and preventing hidden hunger.

Keywords: *Aegilops*, Drought, Heat stress, Wheat biofortification, Wild relatives

1. Introduction

Wheat is the most important foodgrain in the world, contributing around 20% of the global calories (Sharma and Sharma, 2025). It is the most essential staple cereal for 30% of the population globally, thus, the concentration of micronutrients such as iron (Fe) and zinc (Zn) in wheat grains is crucial for human health (Devate *et al.*, 2022). According to the World Health Organization estimates, 815 million people worldwide suffer from micronutrient deficiencies, while an estimated 2 billion people experience "hidden hunger" including 45% of all child fatalities under the age of five each year (Das *et al.*, 2019). About one-third of people in developing countries suffer from Fe and Zn deficiencies, which is a greater risk factor for human health (Rathan *et al.*, 2022). The main cause of the prevalent Zn and Fe deficiencies in developing nations has been attributed to the repetitive intake of cereal-based meals with low concentrations of vitamins and minerals in addition to decreased bioavailability of these micronutrients (Hasheminasab *et al*., 2023). Common cereals like wheat and rice have below-optimal quantities of micronutrients, particularly Fe and Zn (Kumar *et al*., 2016). Globally, Zn deficiency is the most common micronutrient shortage in agricultural lands, which lowers the nutritious value of agricultural products (Jalal *et al.,* 2024). Although micronutrient deficiencies can be addressed by food fortification, supplementation, and dietary diversity but these tactics are expensive and short-term, particularly for the impoverished in rural areas (Bechoff *et al.,* 2023). Therefore, biofortification or increasing the nutritional concentrations of crops using both conventional and molecular techniques is a viable and efficient way to address the problems associated with micronutrient deficiencies (Krishnappa *et al*., 2022). However, the complexity of the genetic and metabolic networks governing Fe and Zn homeostasis makes breeding for these micronutrients extremely difficult (Hanikenne and Bouché, 2023). Grain's ability to accumulate mineral nutrients is a complicated process that involves multiple minor genes and is profoundly influenced by environment. Thus, expanding the genetic base through utilizing wheat landraces and wild relatives, as well as the identification of genetic loci controlling these traits should be the focus of breeding programs.

Further due to climate change, food and nutritional security will be impacted by increased frequency and severity of drought and heat waves in most wheat producing regions (Velu *et al*., 2016). It is estimated that wheat production will reduce by 6.4% for each 1°C increase in temperature, while the frequent droughts will loss wheat production by about 12% (Helman and Bonfil, 2022). The terminal drought and heat stress reduced the spike weight and yield severely, overall causing nearly 30-50% yield loss (Kandić *et al.,* 2023). Heat and drought stress result in smaller seeds, shorter grain filling duration, and less starch accumulation eventually reducing grain sink capacity (Zahra *et al*., 2021). In addition, these stresses will probably alter the nutritional makeup of wheat grain. A meta-analysis found that combined heat and drought stress reduces grain Fe and Zn by 15–30% in wheat, disproportionately affecting arid regions (Asseng *et al*., 2019). Breeding for micronutrient rich varieties under drought and heat stress requires a deep understanding of the genetic regulation of nutritional traits and their link with grain yield and its attributing traits under these stresses (Samineni *et al*., 2022). Therefore, to identify the genetic material higher in grain Fe and Zn concentration is crucial for future wheat biofortification programs. Numerous studies have been undertaken to assess the grain Fe and Zn in wheat genotypes (Thapa *et al.,* 2022; Hasheminasab *et al.,* 2023; Petrovi *et al.,* 2024), but there is a dearth of quality research to evaluate grain Fe and Zn contents in diverse wheat accessions particularly in wild relatives, under climatic stresses. Assessing the genetic variability under different stressed conditions is essential to found stable, and nutrient-dense wheat genotypes suitable to utilize in breeding programs. Moreover, the effect of drought and heat stress on micronutrient accumulation is poorly understood. The bioavailability of Fe and Zn and other anti-nutritional factors associated with these micronutrients are often ignored during stress conditions. The effects of these stresses on grain Fe and Zn concentration need to be thoroughly investigated since they have a direct impact on plant physiology, photosynthesis, and nutrient translocation. Furthermore, stress-calibrated high-throughput phenotyping technologies are not well-developed for large screens. The development of stress-adapted, micronutrient-dense wheat will be accelerated by bridging these gaps through the combination of physiological and molecular studies, calibrated high-throughput assays, controlled and multi-environment stress screening, and pre-breeding techniques. Hence, the current study was carried with the objective to evaluate the grain Fe and Zn content under drought and heat stressed conditions in diverse wheat accessions that will be helpful to select the potential donors for future wheat biofortification programs.

2. Material and Methods

2.1. Plant material

A panel of 155 diverse wheat genotypes were evaluated for grain Fe, Zn and hundred grain weight (HGW) under four conditions, *viz*, timely sown irrigated (TSIR) as control, timely sown drought in rainout shelter (ROS), late-sown irrigated to induce heat stress (LSIR) and late sown rainfed (LSRF) for combined drought and heat stress. The diverse set of wheat genotypes included wild and cultivated wheat germplasm from *Ae. peregrina* (4x), *Ae. tauschii* (2x), *Triticum dicoccoides* (4x), SHWs (6x) and bread wheat (*T. aestivum)* (6x). The wheat accessions were evaluated during two *Rabi* seasons 2022-23 and 2023-24 at ICAR-Indian Institute of Wheat and Barley Research, Karnal. The experiment for drought and control conditions was sown in the first fortnight of November, whereas heat and combined heat and drought stressed trials were sown in the second fortnight of December to induce heat stress in wheat. Recommended irrigations were given in TSIR and LSIR trials while no irrigation was applied to ROS and LSRF to induce drought stress. The experiment was conducted in an alpha lattice design with two replications and each genotype was sown in two rows of 2 cm with a spacing of 30 cm between rows and 10 cm between plants.

2.2. Data recording

For each genotype, the spikes from three plants of each replication were collected in clean polyethylene bags at maturity and were manually threshed. Grain Fe and Zn contents (mg/kg) were estimated with 20gm seeds of each line through a high-throughput energy-dispersive X-ray fluorescence (ED-XRF) spectrometer (model X-Supreme 8000; Oxford Instruments plc, Abingdon, United Kingdom) calibrated with glass bead-based values. It is preferred for its rapid, non-destructive nature and suitability for large-scale screening. The grain Fe and Zn concentrations were measured following the protocol outlined by Paltridge *et al.* (2012). Hundred grains were manually counted and weighted for all the genotypes to record HGW.

2.3 Statistical analysis

Analysis of variance (ANOVA) for the studied traits was performed to determine significant differences among genotypes, environments, years, and their interactions using Agricolae and lattice packages in R. To summarize and interpret the data, the descriptive statistics was analyzed including the mean, standard deviation (SD), range (minimum and maximum), and coefficient of variation (CV) for each trait under four conditions for two-year datasets. The Pearson correlation coefficient was also computed among studied traits under four conditions using R packages. The "corrplot" package was used to create a visual representation of the correlation matrix, and significance levels were also displayed (Wei and Simko, 2021).

3. Results and Discussion

3.1 Genetic variation estimates

The analysis of variance (ANOVA) showed significant ($p<0.001$) effects of genotype, environment, year, and their interactions for all the studied traits (Table 1). The high significance of genotype effects underscores the substantial genetic variability present in diverse germplasm panel, suggesting excellent potential for genetic improvement through breeding. In addition, significant GXE interactions were observed for Zn and HGW only indicting that genotypes responded differently across environment. While, for grain Fe content the genotypes showed consistent performance. These findings align with previous studies showing strong G×E interactions for grain micronutrients in wheat (Velu *et al.*, 2018; Murphy *et al.,* 2008).

The genotypes showed huge variability for Fe content with CV ranging from 3.9% to 50.8% across the environments. The wild tetraploid *Ae. peregrina* showed maximum variability (50.8%), while cultivated *T. dicoccoides* was most stable (6.5%). The extreme variability in *Ae. peregrina* suggests this species possesses valuable trait specific genomic regions that could be exploited for biofortification under stress condition, consistent with reports of wild wheat containing valuable micronutrient genes (Cakmak *et al.,* 2004). For Zn content, the CV ranged from 6.3 to 30.2% across the years. Whereas, for HGW, the CV ranged from 11.2 to 47.2 % (Table 2). Overall, higher CV was observed under stress conditions, especially for wild species across both years, indicating greater genotypic variability in response to drought and heat stresses. In contrast, control condition exhibited lower CV, suggesting more stable performance of genotypes under optimal growth conditions. Heritability estimates for grain Fe and Zn contents varied across environments. Both the micronutrients exhibited moderate to high heritability ranging from 47.8 to 82.5% across all conditions, indicating that selection for these traits would be effective. Previous research in wheat have revealed similar patterns of moderate to high heritability for Zn and Fe contents (Velu *et al.,* 2019; Hasheminasab *et al.,* 2023).

Table 1: Analysis of variance (ANOVA) for studied traits evaluated across four environmental conditions

Source of variation	DF	Fe	Zn	HGW
Genotype	154	1162***	964***	25.59***
Environment	3	5227***	1802***	60.04***
Year	1	577***	8221***	07.13***
Genotype: Environment	448	70***	60***	0.51***
Genotype: Year	154	115***	60***	0.47***
Environment: Year	3	2539***	8434***	28.07***
Environment: Replication	4	69	340***	0.11
Genotype: Environment: Year	448	78***	52***	0.35***
Residuals	959	40	34	0.22

***indicate the difference at p ≤ 0.001 levels of significance, DF- Degree of freedom, Fe- Iron, Zn-Zinc, HGW- Hundred grain weight

Table 2: Descriptive statistics for iron (Fe) and zinc (Zn) (mg/kg) estimates in wheat grains and hundred grain weight (HGW) (g) under four conditions

			2022-23			2023-24			Pooled		
Species	**Condition**	**Trait**	**Mean±SD**	**Range**	**CV**	**Mean±SD**	**Range**	**CV**	**Mean±SD**	**Range**	**CV**
Ae. peregrina	TSIR	Fe	70.3±14.8	22.0–91.1	21.0	59.7±9.8	46.3–87.2	16.5	65.0±7.8	40.9–78.3	18.7
		Zn	57.0±9.8	36.3–75.1	17.2	63.5±12.3	45.7–86.8	19.3	60.2±6.8	48.2–73.5	18.3
		HGW	1.3±0.3	1.0–1.9	19.9	1.0±0.3	0.7–1.9	26.4	1.2±0.3	0.9–1.8	23.1
	ROS	Fe	51.5±5.1	38.6–61.3	9.9	60.7±10.3	48.1–81.8	17.0	56.1±5.6	43.4–68.2	13.5
		Zn	68.2±10.3	45.8–91.3	15.1	55.8±14.1	40.1–97.4	25.3	62.0±8.7	43.0–79.3	20.2
		HGW	1.1±0.3	0.6–1.7	22.7	1.0±0.2	0.4–1.4	22.6	1.0±0.2	0.7–1.4	22.7
	LSIR	Fe	48.9±7.9	43.5–77.3	16.3	58.4±11.2	48.9–92.4	19.1	53.6±6.6	46.2–71.8	17.7
		Zn	38.5±11.0	28.5–79.3	28.6	62.9±14.9	48.0–91.0	23.6	50.7±8.6	38.3–73.4	26.1
		HGW	0.8±0.2	0.1–1.1	28.2	1.0±0.1	0.7–1.2	13.6	0.9±0.2	0.6–1.1	20.9
	LSRF	Fe	67.6±19.8	43.1–98.1	29.2	57.2±15.6	45.3–84.3	27.2	62.4±12.3	44.2–86.6	28.2
		Zn	48.4±9.7	38.5–73.4	20.1	53.6±15.1	41.9–83.0	28.2	51.0±9.4	40.2–73.4	24.1
		HGW	0.9±0.2	0.5–1.1	16.9	0.6±0.3	0.3–1.0	42.3	0.8±0.2	0.4–1.1	29.6
Ae. tauschii	TSIR	Fe	65.5±9.2	49.9–91.3	14.0	58.8±8.0	46.3–84.2	13.6	62.2±6.1	49.5–79.9	9.7
		Zn	41.3±4.6	33.2–56.2	11.1	49.1±8.4	38.1–90.5	17.0	45.2±4.9	37.2–64.1	10.7
		HGW	1.3±0.3	0.3–1.9	23.7	1.1±0.3	0.6–2.2	22.9	1.2±0.2	0.5–1.7	19.4
	ROS	Fe	45.3±5.8	37.4–69.2	12.7	58.4±8.5	48.1–76.6	14.5	51.9±5.2	43.1–65.8	10.1
		Zn	50.6±5.9	38.1–68.3	11.7	48.1±7.2	40.1–69.0	14.9	49.4±4.7	39.1–61.1	9.5
		HGW	1.1±0.2	0.7–1.8	16.2	1.0±0.1	0.8–1.3	12.4	1.1±0.1	0.8–1.4	11.0
	LSIR	Fe	46.5±3.2	46.1–69.7	6.8	56.7±8.4	47.2–78.6	14.9	51.6±4.3	46.6–62.3	8.3
		Zn	35.1±2.8	34.7–55.3	7.9	55.2±7.7	48.0–75.4	13.9	45.0±4.1	34.7–55.1	9.2
		HGW	0.9±0.2	0.3–1.8	26.0	1.0±0.2	0.4–1.2	16.5	0.9±0.1	0.5–1.4	15.9
	LSRF	Fe	46.0±9.8	43.1–91.6	21.2	55.4±14.4	45.3–94.7	25.9	50.7±9.1	44.2–76.8	18.0
		Zn	39.2±2.5	38.5–50.3	6.3	49.7±11.4	41.9–83.2	23.0	44.5±6.0	40.2–60.9	13.6
		HGW	0.9±0.2	0.3–1.1	21.0	0.9±0.3	0.3–1.5	39.6	0.9±0.2	0.3–1.1	21.8
T. aestivum	TSIR	Fe	37.5±5.1	28.6–61.1	13.7	39.7±3.6	30.6–46.3	9.0	38.6±3.5	32.2–53.0	9.0
		Zn	28.5±4.3	21.2–39.8	15.1	38.3±6.2	24.8–53.0	16.1	33.4±4.3	23.5–44.0	12.8
		HGW	4.7±0.6	3.3–7.2	12.4	3.7±0.7	2.2–5.2	17.9	4.2±0.5	3.2–5.3	11.9
	ROS	Fe	36.5±3.6	28.8–46.6	9.9	37.2±3.4	30.2–50.2	9.1	36.9±2.7	30.6–43.5	7.5
		Zn	43.0±5.3	33.6–56.4	12.3	30.7±4.0	23.6–40.3	13.0	36.8±3.5	30.3–47.6	9.6
		HGW	3.1±0.5	2.2–4.5	15.5	3.6±0.5	2.6–4.8	14.8	3.3±0.4	2.7–4.3	12.3
	LSIR	Fe	41.4±5.4	32.5–58.2	13.2	45.4±4.2	37.0–57.1	9.2	43.4±3.5	35.7–52.5	8.1
		Zn	29.1±5.1	20.1–50.7	17.4	40.1±5.2	26.1–57.5	12.8	34.6±4.0	27.2–45.7	11.4
		HGW	3.3±0.5	2.3–4.5	15.3	3.7±0.5	2.3–5.1	14.7	3.5±0.4	2.8–4.4	10.7

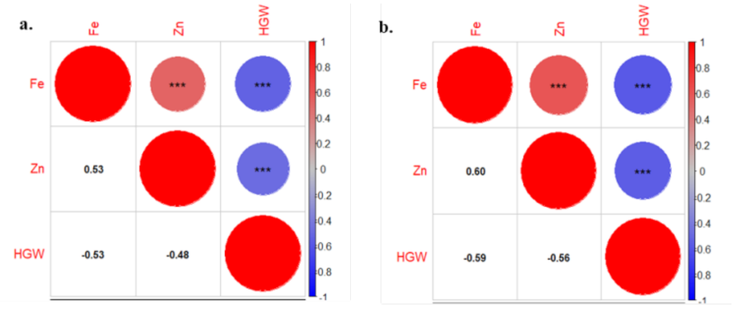
			2022-23			2023-24			Pooled		
Species	**Condition**	**Trait**	**Mean±SD**	**Range**	**CV**	**Mean±SD**	**Range**	**CV**	**Mean±SD**	**Range**	**CV**
	LSRF	Fe	36.2±4.2	28.0–56.3	11.5	38.9±3.3	33.2–45.6	8.4	37.6±2.9	31.4–47.4	7.7
		Zn	32.7±4.3	25.3–42.7	13.1	35.1±6.5	25.1–50.9	18.5	33.9±4.2	26.6–46.5	12.4
		HGW	3.3±0.6	2.0–4.7	18.3	3.4±0.6	2.1–4.5	16.5	3.3±0.6	2.0–4.6	16.9
Synthetic hexaploid wheats	TSIR	Fe	45.7±5.7	31.4–62.8	12.4	43.0±5.2	4.9–56.2	12.2	44.3±3.8	24.8–53.4	8.6
		Zn	34.7±4.4	20.0–49.3	12.8	44.8±5.9	29.8–64.2	13.2	39.7±4.0	29.8–51.4	10.0
		HGW	5.1±0.9	2.7–8.6	18.4	4.2±0.7	2.5–5.5	17.4	4.7±0.6	2.6–6.3	13.9
	ROS	Fe	38.2±4.2	27.1–52.0	11.0	42.3±4.7	30.0–52.1	11.0	40.2±3.0	30.2–47.3	7.5
		Zn	45.1±5.5	33.2–70.3	12.3	39.1±9.6	21.1–95.2	24.6	42.1±5.4	30.4–66.5	12.9
		HGW	3.8±0.6	1.6–5.2	16.6	3.7±0.6	2.4–5.2	15.9	3.7±0.5	2.4–4.8	12.1
	LSIR	Fe	45.7±5.4	33.1–73.0	11.7	46.5±4.8	34.8–58.8	10.2	46.1±3.7	37.1–60.4	8.1
		Zn	37.1±8.8	6.0–63.7	23.6	46.4±7.2	32.9–70.9	15.5	41.8±6.0	23.0–62.1	14.3
		HGW	3.9±0.6	1.7–5.1	14.8	3.8±0.6	2.1–5.1	14.6	3.9±0.4	2.5–4.7	11.3
	LSRF	Fe	40.5±4.5	31.9–68.1	11.1	42.9±3.7	33.5–51.5	8.7	41.7±3.1	35.9–54.0	7.4
		Zn	39.2±7.7	27.3–80.3	19.7	40.0±7.6	25.3–61.1	19.0	39.6±5.5	29.7–62.2	13.8
		HGW	3.9±0.7	1.3–5.9	17.0	4.1±0.6	2.5–5.1	14.4	3.9±0.6	2.1–5.9	14.0
T. dicoccoides	TSIR	Fe	44.5±6.8	34.1–56.4	15.3	39.3±3.8	33.1–46.3	9.7	41.9±3.8	37.6–48.7	9.2
		Zn	37.0±6.0	28.7–47.6	16.2	48.3±6.8	42.2–62.0	14.0	42.6±5.4	36.3–54.8	12.7
		HGW	3.6±0.9	2.4–4.7	24.6	2.8±0.7	1.6–3.7	23.6	3.2±0.4	2.5–3.9	13.3
	ROS	Fe	34.1±2.7	30.1–37.6	7.9	39.5±5.5	28.5–48.2	13.9	36.8±2.6	32.3–40.8	7.0
		Zn	43.5±4.9	34.7–51.7	11.2	42.0±12.7	23.6–68.3	30.2	42.8±6.0	33.9–52.5	14.0
		HGW	2.9±0.7	2.1–4.2	22.4	3.2±0.6	1.7–4.0	20.1	3.1±0.5	2.5–4.0	15.0
	LSIR	Fe	44.9±2.9	39.1–50.1	6.5	40.9±3.5	36.0–46.7	8.5	42.9±2.5	39.2–46.4	5.8
		Zn	36.7±5.2	32.4–51.4	14.1	47.4±7.1	38.5–61.2	15.0	42.0±5.6	36.0–56.3	13.4
		HGW	2.9±1.2	1.0–4.5	41.2	3.2±0.7	2.0–4.2	21.0	3.1±0.6	2.0–4.0	20.1
	LSRF	Fe	41.0±2.7	36.1–45.7	6.5	41.6±1.6	39.4–43.4	3.9	41.3±1.9	37.8–44.6	4.7
		Zn	45.1±9.0	35.2–63.4	19.9	47.4±10.8	35.9–67.2	22.8	46.2±6.3	37.4–55.6	13.7
		HGW	2.5±1.2	1.0–3.9	47.2	3.2±0.4	2.8–3.8	11.8	2.7±0.9	1.0–3.9	31.8

3.2. Genotypic performance for studied traits

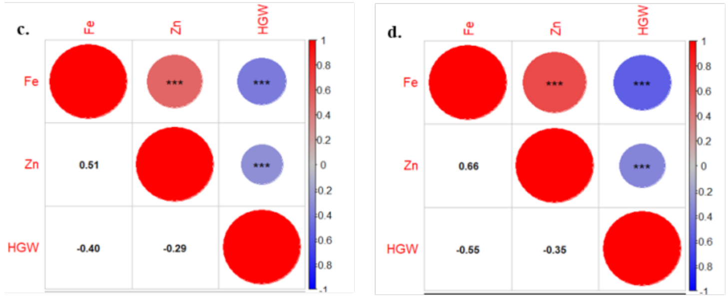
For *Ae. peregrina*, the average grain Fe concentration was recorded highest in non-stressed condition, followed by LSRF, LSIR and ROS during 2022-23, whereas, in 2023-24, the highest mean grain Fe concentration was observed under ROS condition, followed by TSIR, LSIR and LSRF (Table 2). The overall grain Fe concentration ranged 22-98.1mg/kg and 45.3-92.4 mg/kg during 2022-23 and 2023-24, respectively, across all four conditions. The broader range of Fe concentrations in TSIR compared to stressed conditions suggest greater variability under non-stress conditions, possibly due to genotypic differences in nutrient utilization efficiency (Cakmak *et al.*, 2004). The highest average Zn concentration was recorded under ROS and TSIR condition during 2022-23 and 2023-24, respectively. Across all the conditions, Zn content ranged from 28.5 to 97.4 mg/kg during both the years. The observed variability in micronutrient concentrations highlights the potential of *Ae. peregrina* as a genetic resource for biofortification programs to improve Fe and Zn content in cultivated wheat as earlier reported by Velu *et al.* (2014). Similarly, for *Ae. tauschii*, the highest average Fe concentration was observed for non-stress condition in both years dataset, and ranged from 37.4 to 91.3 mg/kg. Whereas, the average Zn content lied between 33.2 and 90.5 mg/kg with highest value under ROS condition in 2022-23, and LSIR condition during 2023-24. Accessions from *T. dicoccoides* exhibited high value of grain Fe and Zn content in late sown trials as compared to timely sown. The findings show the importance of genetic variability present in *T. dicoccoides* for these traits, can be incorporated in future heat and drought breeding schemes. Cultivars from *T. aestivum* species also performed better in stressed conditions (LSIR and ROS) as compared to normal and LSRF condition for both grain Fe and Zn concentration with average range of 28.0-61.1 mg/kg and 20.1-57.5 mg/kg, respectively across the years.

Based on pooled analysis, it was observed that wild species *Ae. peregrina* accessions exhibited highest grain Fe and Zn content across the years under all environmental conditions followed by *Ae. tauschii*, SHWs, *T. dicoccoides* and *T. aestivum* genotypes. It indicates the potential of wild species as valuable genetic resources for biofortification. *Ae. peregrina* exhibited superior performance under LSRF condition for Fe, and ROS condition for Zn accumulation. Similarly, accessions from *Ae. tauschii* outperformed under TSIR and ROS for Fe and Zn, respectively, out of the all studied conditions. *T. dicoccoides* and SHWs were stable over the years with best performance under LSRF condition. *T. aestivum* exhibited non-significant differences for both micronutrients across the conditions pooled over the years, except some genotypes which exhibited lower value in stress as compare to normal sowing conditions. These results align with previous studies indicating that wild wheat relatives possess greater genetic potential for micronutrient enrichment (Kumar *et al.*, 2016). Velu *et al.* (2016) observed reduced grain Zn and Fe in CIMMYT wheat lines under drought and elevated temperatures with some genotypes maintaining stable micronutrient levels which corroborated with current findings. In contrary, *T. aestivum* and SHWs, has higher HGW than wild species under all conditions with maximum HGW under non-stress condition. This inverse relationship suggests the possibility of a dilution effect, in which larger grains may accumulate more carbohydrates than minerals, lowering the concentration of micronutrients.

The identified genotypes with higher Fe and Zn content are presented in Table 3. Thirteen genotypes were found to be higher in both grain Fe and Zn content (>55 mg/kg). Out of which, ten genotypes were from *Ae. peregrina*, two of *Ae. tauschii* and one SHWs. Accessions PI 603931, PI 603247, PI 604145, and PI 604176 of *Ae. peregrina* depicted the higher values in more than one environment, indicating the stability of the traits and their probable use as potential donor in breeding for biofortification. In addition to these genotypes, a total of 15 genotypes has Fe content >55 mg/kg (Table 3). Nine of them were *Ae. tauschii* accessions and rest were *Ae. peregrina.* Accession 3761 of *Ae. tauschii* and accessions PI 604169, PI 604147, and PI 604172 of *Ae. peregrina* had higher grain Fe and Zn values in multi-environments. Similarly, high Zn (> 55 mg/kg) was recorded in another 15 accessions; which included one accession of *T. dicoccoides* and three from SHWs. However, higher grain Zn content was recorded again in *Ae. peregrina* (accessions PI 604192, PI 604162, PI 603247, and PI 604185) across different sowing conditions. Previous study carried out by Kumar *et al.* (2016) also detected the wild wheat genotypes outperforming the cultivated wheat in grain Fe and Zn concentrations in optimal conditions. These findings highlight the importance of exploiting species diversity for improving grain Fe and Zn content in wheat through



ROS_Drought TSIR_Control



LSIR_Heat LSRF_Drought and Heat

Fig. 1: Correlation among Iron (Fe), Zinc (Zn) and Hundred Grain Weight (HGW) under (a) ROS-Drought, (b) TSIR-Control, (c) LSIR-Heat and (d) LSRF-Drought and Heat stress combined conditions

Table 3: List of superior genotypes with high grain iron (Fe) and zinc (Zn) concentrations under different studied conditions

Condition	Genotype	Species	Grain Fe (mg/ kg)	Genotype	Species	Grain Zn (mg/kg)
ROS	EC-57331	*Ae. peregrina*	68.2	EC-573331	*Ae. peregrina*	79.3
	Accession 13764	*Ae. tauschii*	65.8	PI 603247	*Ae. peregrina*	76.9
	Accession 13780	*Ae. tauschii*	65.7	PI 604186	*Ae. peregrina*	72.9
	PI 604169	*Ae. peregrina*	63.9	PI 604192	*Ae. peregrina*	72
	PI604147	*Ae. peregrina*	63.5	PI 604162	*Ae. peregrina*	70.2
	PI603931	*Ae. peregrina*	63	PI 603931	*Ae. peregrina*	69.8
	Accession 3761	*Ae. tauschii*	62.6	PI 604185	*Ae. peregrina*	67.9
	PI 604186	*Ae. peregrina*	62.4	PI 604169	*Ae. peregrina*	67.8
	PI 604145	*Ae. peregrina*	62	SYN 78	SHW	66.5
	PI 487275	*Ae. peregrina*	60.6	PI 604189	*Ae. peregrina*	66.5
LSIR	PI 603931	*Ae. peregrina*	71.8	PI 603931	*Ae. peregrina*	73.4
	PI 604169	*Ae. peregrina*	63.6	PI 604145	*Ae. peregrina*	63.7
	PI 604145	*Ae. peregrina*	63.1	PI 604176	*Ae. peregrina*	62.9
	Accession 9788	*Ae. tauschii*	62.3	SYN 79	SHW	62.1
	PI 604172	*Ae. peregrina*	61.2	PI 603247	*Ae. peregrina*	61.1
	SYN55	SHW	60.4	PI 604162	*Ae. peregrina*	59.9
	Accession 3761	*Ae. tauschii*	60.1	PI 604189	*Ae. peregrina*	58.1
	PI 604189	*Ae. peregrina*	59.9	Accession 7121	*T. dicoccoides*	56.3
	Accession 3753	*Ae. tauschii*	59.4	PI 554324	*Ae. peregrina*	55.1
	Accession 3805	*Ae. tauschii*	59.3	SYN55	Synthetic	55
LSRF	PI 603931	*Ae. peregrina*	86.6	PI 603931	*Ae. peregrina*	73.4
	PI 604169	*Ae. peregrina*	82.4	PI 604176	*Ae. peregrina*	68.2
	PI 604186	*Ae. peregrina*	82.1	PI 604167	*Ae. peregrina*	65
	PI 604176	*Ae. peregrina*	81.5	SYN34	SHW	62.2
	PI 603931	*Ae. peregrina*	77.4	PI 603247	*Ae. peregrina*	61.5
	Accession 14336	*Ae. tauschii*	76.8	Accession 14578	*Ae. tauschii*	60.9
	Accession 13763	*Ae. tauschii*	72.7	Accession 14336	*Ae. tauschii*	58.1
	PI 604167	*Ae. peregrina*	70.6	PI 604162	*Ae. peregrina*	58.7
	Accession 9807	*Ae. tauschii*	70.3	PI 604192	*Ae. peregrina*	58.7
	PI 603247	*Ae. peregrina*	69.7	SYN 47	SHW	57.6
TSIR	Accession 13764	*Ae. tauschii*	79.9	PI 604145	*Ae. peregrina*	73.5
	PI 604145	*Ae. peregrina*	78.3	PI 604167	*Ae. peregrina*	71.4
	PI 604147	*Ae. peregrina*	76.8	PI 603247	*Ae. peregrina*	70.4
	PI 604162	*Ae. peregrina*	76.7	PI 604162	*Ae. peregrina*	68.8
	PI 603247	*Ae. peregrina*	74.2	PI 604192	*Ae. peregrina*	66
	PI 487275	*Ae. peregrina*	73.9	PI 604176	*Ae. peregrina*	65.3
	PI 604172	*Ae. peregrina*	73.1	Accession 13764	*Ae. tauschii*	64.1
	Accession 14325	*Ae. tauschii*	72.3	PI 604185	*Ae. peregrina*	64.1
	Accession 3761	*Ae. tauschii*	71.6	PI 603931	*Ae. peregrina*	63.9
	PI604176	*Ae. peregrina*	70.6	PI 487275	*Ae. peregrina*	62.6

selection and breeding programs. Earlier research on the Fe and Zn content of wheat grain has been restricted to a few genotypes and was mostly carried out in non-stressed environments (Velu *et al.,* 2016; Kumar *et al.,* 2016). The current study assessed a large genetic basis in a variety of stress situations and offers fresh perspectives on how stress tolerance and micronutrient enrichment interact. This study advances our knowledge of the genetic potential for Fe and Zn biofortification in the context of climate change in addition to identifying genotypes resilient for both stress and nutritional traits.

3.3. Correlation coefficient analysis

The correlation coefficient was estimated between three traits under four conditions (Figure 1). Grain Fe and Zn were significantly and positively associated under all four studied conditions. The positive correlation between Fe and Zn concentrations showed that these two traits can be improved simultaneously in a breeding program as there is possibility that a common transport mechanism or the genes for Fe and Zn are possibly co-segregating (Velu *et al.*, 2016). These findings are in corroboration with the various earlier studies carried in wheat (Thapa *et al.*, 2022; Liu *et al.*, 2014; Chatrath *et al.*, 2018, and Ghimire *et al.*, 2019; Krishnappa *et al.*, 2022). Hundred grain weight (HGW) had negative and significant correlation with grain Zn and Fe concentrations, whereas earlier studies indicated the non-significant association of thousand grain weight with grain Zn and Fe concentrations (Velu *et al.*, 2018; Li-Na *et al.*, 2022). Based on correlation analysis, it could be concluded that from grain Fe and Zn, the selection for either trait would be beneficial to improve another trait simultaneously. It suggests a possible involvement of shared genetic factors or transport pathways; however, this remains a hypothesis and requires validation through QTL mapping or functional studies.

Conclusion

Diverse ploidy wheat genotypes including wild species, synthetic hexaploidy wheats (SHW) and cultivated wheat were evaluated for grain Fe and Zn contents under drought and heat stressed conditions. Significant genotypic variability in micronutrient accumulation was observed, with wild species such as *Ae. peregrina* and *T. dicoccoides* exhibiting higher Fe and Zn concentrations compared to cultivated wheat (*T. aestivum*) and SHW. The wild accessions with higher Fe and Zn contents across years and stressed conditions highlights their potential for genetic biofortification in wheat breeding programs, particularly PI 603931, PI 603247, PI 604145, and PI 604176 of *Ae. peregrina.* The strong positive correlation between Fe and Zn across the conditions implies that selection for one micronutrient may concurrently enhance the other. The study identified several genotypes with high Fe and Zn concentrations under stress conditions, which could be further utilized for development of nutritionally superior wheat cultivars.

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

Authors contribution

Seema Sheoran: Data compilation and analysis, interpretation of results, manuscript drafting; Sawan Kumar Mehla: Structuring and drafting the manuscript; Sonu: Data compilation; CN Mishra: Laboratory analysis and manuscript editing, BS Tyagi: manuscript editing; Sindhu Sareen: Planning, interpretation and manuscript editing. All the authors read and finalized the manuscript.

Ethical Approval

The article doesn’t contain any study involving ethical approval.

Declaration on the use of Generative AI or AI-assisted technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript. All text, data interpretation, and analysis were conducted entirely by the authors.

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