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Variability in iron, zinc, phytic acid and protein content in pre-breeding wheat germplasm under different water regimes

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Abstract

More than one-third of the global population suffers from iron and zinc deficiency, developing anaemia like diseases mostly in the developing countries. Therefore, the current study focuses on the investigation of variability and bioavailability of micronutrients such as iron and zinc in bread wheat mediated by lower levels of phytic acid which is often categorised as an antinutritional compound. Phytic acid in cereals acts as an chelator of major micronutrients such as iron and zinc, thus lowering their bioavailability both in humans and animals. In addition, drought is a major component affecting the micronutrient accumulation in wheat kernels. Therefore, a pre-breeding wheat germplasm set comprising 137 genotypes was grown under irrigated and restricted irrigated conditions for 2 years. This germplasm set was used to assess the variability for iron, zinc and phytic acid content in the wheat kernels. Mean iron and zinc content was 45.83 and 49.43 ppm under irrigated conditions, whereas it was 40.53 and 49.62 ppm under restricted irrigated conditions. Afterward, the molar ratios of phytate with iron and zinc were calculated to predict their bioavailability. Based on the daily recommended values, promising genotypes were shortlisted with low phytic acid combined with high iron and zinc content. These promising genotypes will be further used in wheat breeding programme to breed biofortified wheat cultivars with higher micronutrient and reduced phytic acid concentration combined with enchanted abiotic stress tolerance which can potentially help in alleviating the hidden hunger under changing climatic conditions.

Keywords Wheat · Iron · Zinc · Phytic acid · Nutritional security · Drought · Biofortification

Introduction

A major proportion of global population is suffering from micronutrient malnutrition predominantly in developing countries (Manjeru et al. 2019; Galani et al. 2022). A large section of this population belongs to the low socioeconomic groups with primarily cereal-based diets, since they do not have access to variable foods or food supplements (Grote et al. 2021; Padhy et al. 2022a, b). Wheat (*Triticum aestivum* L.), is the staple diet in addition to rice and is consumed as bread, *chapat*i, semolina, pasta, macaroni, noodles, biscuits etc. (Shewry and Hey, 2015; Grote et al. 2021; Padhy et al. 2022b). It meets the requirement of starch, protein, minerals and dietary fibres for the masses (Sharma et al. 2018) and is consumed by approximately 2.5 billion people providing 20% of calories out of total calorie intake across the globe (Gautam et al. 2020). Enhancing the micronutrient concentration, specifically protein, iron and zinc content in wheat via genetic fortification using modern plant breeding approaches has been considered as most effective and safe approach to alleviate micronutrient malnutrition (Mahawar et al. 2022; Philipo et al. 2021; Kaur et al. 2022). World Health Organization (WHO) has stated that Zinc (Zn) deficiency ranks in top five most important disease contributing deficiencies in the world while iron deficiency ranks sixth in the same list. Zinc is involved in the cellular growth and differentiation (Sharma et al. 2013; Stahl-Gugger et al. 2022) and its deficiency causes growth impairment, immune dysfunction with increased morbidity, mortality with adverse

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pregnancy outcomes (Philipo et al. 2021) and abnormal neurobehavioral development (Petry et al. 2016). More than one third of global population is considered to be affected by the iron deficiency or anaemia (Natekar et al. 2022). Anaemia affects approximately 1.6 billion people with pre-school children and pregnant women at greatest risk across the globe (Stahl-Gugger et al. 2022; Li et al. 2019). It is considered to adversely affect the cognitive development, immunity development productivity during pregnancy (Philipo et al. 2021; Natekar et al. 2022; Stahl-Gugger et al. 2022).

A number of issues concerning the nutritional quality of staple grains and their biofortification revolve around the seed phosphorus (P) storage compound called phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate). Phytic acid (PA) is deposited in seeds as mixed phytate or phytin salts of potassium and magnesium, and usually represents 65% to 80% of total P in seed (Raboy, 2020). Phytic acid is an effective chelator of positively charged cations and binds to nutritionally important minerals such as calcium, iron, zinc, and to proteins both in seeds as well when fed to humans or animals (Brouns and Shewry, 2022). This chelating process by phytic acids leads to mineral deficiency in humans, which makes it an antinutritional component and hence, the recent focus in wheat biofortification in all leading breeding programs focuses on developing germplasm with lower phytic acid content (Govindan et al. 2022).

Besides high yield and disease resistance, enhanced nutritional quality including biofortification of wheat has been the major mandate of the wheat breeding program at Punjab Agricultural University (PAU), Ludhiana. Following the same, ‘PBW1Zinc’, the first genetically biofortified cultivar having enhanced zinc content was released by PAU in 2017 having 12% more zinc content in the grains (ISGPB, 2021). Efforts are on the way to develop and release more wheat cultivars with enhanced iron, zinc and grain protein content. However, keeping in view the antinutritional properties of the phytic acid in the grains, the biofortification or increment in mineral content may not lead to increased bioavailability of minerals (Raboy 2020). Since the lower concentration of phytic acid is associated with increased micronutrient bioavailability, therefore, breeding for low phytate content seems to be a reasonable objective to enhance nutritional quality of wheat (Wang and Guo, 2021). Brouns and Shewry (2022) reported that the cereals with lower phytic acid concentration and higher phytate degradation during digestion increases the micronutrient bioavailability in the gut. Even though, phytic acid is detrimental among the grain quality traits, there have been limited reports on phytic acid variability (Kutman et al. 2010) and its relation to micronutrient bioavailability in bread wheat (Erdal et al. 2002; Liu et al. 2014; Wang and Guo 2021). Genetic variability is required in plant breeding to utilize it to produce desired permanent gain in the performance with respect to lowering the phytic acid content. Therefore, it necessitates the evaluation of the natural variation of phytic acid levels in diverse bread wheat cultivars along with its impact on bioavailability of various micronutrients such as iron and zinc.

The biochemical characteristics and published reports are limited regarding the wheat germplasm with low phytate. Further, drought, an important abiotic stress anticipated to be even harsher owing to fast climate change over the future years (dos Santos et al. 2022) has been reported to affect phytate and micronutrient content in wheat kernels. It significantly reduces sustainable crop production affecting not only yield but also the nutritional properties of the wheat grain (Dugasa et al., 2021, Seleiman and Battaglia 2021). Wheat plants respond to drought stress through certain morphological, physiological, biochemical or molecular mechanisms which finally translate into reduced yields, shrivelled grains and low grain quality (Pandey et al. 2022; Vukovic et al. 2022). Also, little information is available on the effect of drought like conditions (reduced water availability) on the phytic acid content in wheat grain. This study was undertaken to understand the genetic variability for mineral and phytate content in wheat, with the following objectives:- (1) describe the variability in grain protein, Fe, Zn and phytic acid concentration in a pre-breeding set of wheat; (2) estimate the phytic acid, protein, Zn and Fe in whole-meal wheat flours; and (3) examine the effect of reduced irrigation and genotype by environment (G×E) interaction on these nutritional quality traits. These objectives will help to study the variation in the micronutrients (Fe, Zn) and protein concentration with respect to changing phytic acid content in wheat during irrigated and restricted irrigated conditions. The analysis will help to identify lines with better yield and biofortified with micronutrients having lower phytic acid content. The selected lines will further be used in wheat improvement programmes.

Materials and methods

Plant materials

The plant material consisted of pre-breeding germplasm set of 137 accessions procured from the International Maize and Wheat Improvement Centre (CIMMYT), Mexico (S-Table 1). For comparative performance, commercial wheat varieties recommended for cultivation in the region (PBW725, HD2967 and HD3086) were included in the study. The tall, traditional Indian wheat varieties from pre green revolution era (C591 and C306) and an Australian cultivar (Gladius) that are known for having components of drought tolerance were also used as trait specific check/controls in the present study.

Experiment location and climatic conditions

The study was carried out in the experimental area of Plant Breeding Department, Punjab Agricultural University, Ludhiana, India (30° 54′ N latitude, 75° 48′ E longitude, and 247 m above sea level), where the soil is classified as the loamy sand having neutral pH 6–8. The conventional rice–wheat rotation has been followed on the field since last 10 years. The climatic conditions of the area are characterized as subtropical and semi-arid with winters from November to January and mild climate during February and March, and very hot and dry during summer from April to June, later followed by hot and humid conditions from July to September. The daily minimum temperature ranges from 0 to 4 °C in January, and the maximum temperature during May ranges from 40 to 45 °C.

Experimental design and phenotypic data

The experiment was laid out in alpha lattice design with two replications during the cropping season 2020–2021 (S1) and 2021–2022 (S2) under irrigated (IR) and restricted irrigated/drought conditions with a plot size of 2.5 m × 2.4 m with a row spacing of 20 cm. The experiment was planted under timely sown irrigated conditions and timely sown restricted irrigation conditions (rainfed conditions/withholding irrigation and allowing only pre-irrigation before sowing). Standard agronomic and crop protection practices were used to raise the healthy crop.

Observations were recorded for morphological traits viz. days to heading (DTH), plant height (PH), grains per spike (GPS), spikelet per spike (SPS), yield and yield related traits. PH, GPS, SPS were recorded at maturity whereas DTH was recorded at early anthesis stage (70–100 days after sowing for all the genotypes). Chlorophyll meter (SPAD) was used to measure the relative chlorophyll content of the leaves from irrigated and restricted irrigated conditions at anthesis stage (70–100 days after sowing for all the genotypes). Five readings were taken from single plant leaves and their average was considered for determination of chlorophyll content from the central position of leaves.

Grain parameters and protein content estimation

Whole plots were harvested mechanically and grain yield was determined (t/ha). Grain samples were kept for analysis of test weight (kg/hL). 1000 wheat kernels were weighed to determine the test weight of each sample. The percent protein content was determined from wheat grains using Infratec 1241 whole grain analyser M/S FOSS by non-destructive method previously standardized for high throughput screening of whole wheat grains for protein content (Kaur et al. 2020).

Iron, Zinc and phytic acid determination with molar ratios

Iron and zinc concentrations of grain were determined by using a bench top, non-destructive energy dispersive X-ray fluorescent spectrometry (EDXRF) instrument (X-Supreme, Oxford Instruments plc, Abingdon, UK). Phytic acid concentration was determined by modified method as suggested by Megazyme (2016). A 5 g grain sample was milled into whole meal flour. One gram wheat flour was digested with 20 ml of 0.6 M HCl placed in mixer with occasional shaking for 14 h at room temperature. 1 ml of extract was transferred to Eppendorf tube and centrifuged at 4000 r.p.m. for 20 min. Supernatant was transferred to new tube and solution was immediately neutralized by adding 0.3 ml of 2:1 (NaOH:HCl) mixture. Trichloroacetic acid was used to prepare the solution as suggested by (Megazyme 2016). Finally reading for reaction solution was observed at 655 nm using spectrophotometer. Molar ratios and phytic acid content was calculated as suggested by (Megazyme 2016).

Statistical analysis

Analysis of variance (ANOVA) was performed using Statistical Analysis Software (SAS) v 9.4, 2016. Pearson correlation coefficients (r), multivariate analysis and statistical significance for each comparison in the entire study were obtained using SAS and R software (R Core Team 2020).

Results

Effect of genotype, environment and genotype × environment (G × E) interaction on grain traits

A germplasm set comprising 137 wheat genotypes along with check cultivars grown under two environments was analysed to determine variation in grain quality, yield and yield related traits. The combined analysis of variance revealed significant difference among genotypes for all the traits under study pooled over two years (Table 1). Genotype and environment were the most important factors explaining the variation found followed by G × E. Genotype and environment interaction had shown significant effect on the grain quality traits but was non-significant for most of the ancillary traits except grain yield. Variation in environmental conditions including air temperature, relative humidity rainfall and vapour pressure, during cropping seasons of 2020–2021 (S1) and 2021–2022 (S2) have been shown in Fig. 1.

Table 1 Effects of genotypes, environment, years and their interaction (G×E) expressed as the mean sum of squares from ANOVA analysis for yield and component traits

	Yield	TW	SPS	GPS	PH	DTH	CC
Genotype	14,829.26**	234.97**	16.25*	825.82**	344.49**	160.73**	421.89**
Environment	30,503.21**	5485.67**	69.50*	2436.09**	12,504.38**	20,378.71**	749.59**
Block (Rep)	162.82^{ns}	19.33^{ns}	4.96^{ns}	11.18^{ns}	31.29^{ns}	11.21^{ns}	14.01^{ns}
G×E	24.71*	6.57^{ns}	3.01^{ns}	7.95^{ns}	25.67^{ns}	9.40^{ns}	10.37^{s}
Year	159.42^{ns}	37.97^{ns}	335.51**	6.13^{ns}	0.17^{ns}	130.36^{ns}	518.16**
Error	239.75	52.27	12.27	37.75	44.12	69.58	12.48

All values were significant at 0.05, “*”, < 0.01, “**”, and ^{ns} non-significant

TW test weight, *SPS* Spikelet per spike, *GPS* Grains per spike, *PH* Plant height, *DTH* Days to heading, *CC* Chlorophyll content/SPAD

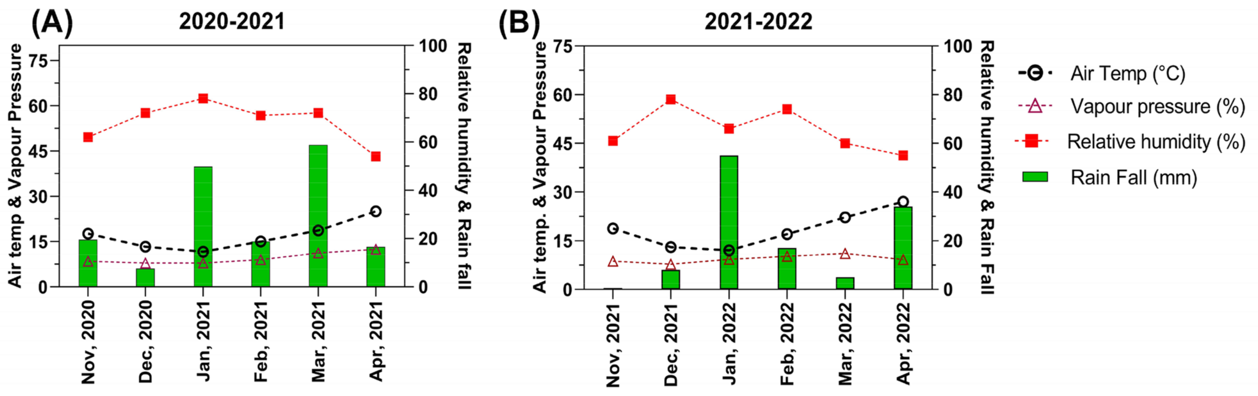

Fig. 1 Represents air temperature (°C), vapour pressure (%), relative humidity (%) and rain fall (mm) during Rabi season **A** 2020–2021 and **B** 2021–2022

Phytic acid, micronutrient content and other traits

Distribution of phytic acid and micronutrient content is shown in Fig. 2. A total of 7 lines had phytic acid content less than 0.7% (Fig. 2A) whereas 18 lines had iron concentration higher than 55 ppm. Zinc concentration was higher than 65 ppm in 6 lines which is 5% of the complete set of lines. 42% lines (58 lines) had protein content higher than 12%. These figures have shown the variation in lines based on different parameters and potential lines with higher micronutrient content and lower phytic acid concentration that can be selected from set of pre-breeding lines for micronutrient analysis and agronomic studies under restricted irrigated conditions.

Phytic acid content varied from 0.40 to 2.10% with a mean value of 1.11% under irrigated (IR) conditions whereas under restricted irrigation (RI) it showed slight decrease that varied from 0.16 to 1.70% with a mean value of 0.91% (Table 2, Fig. 3A). Similarly, grain Fe content also showed reduction under restricted irrigation conditions with a mean value of 45.83 ppm and 40.53 ppm in irrigation and restricted irrigation conditions respectively (Table 2, Fig. 3B). Under irrigated conditions the mean for Zn concentration in the grains was 49.43 ppm which was comparable with mean value of 49.62 in restricted irrigation conditions. Zn content ranged from 28.10 to 72.10 ppm and 18.60 to 73.60 ppm under irrigated and restricted irrigation conditions respectively. Mean value for thousand grain weight (TGW) also decreased under restricted irrigated conditions from 41.00 to 36.50 gm in comparison to irrigated environment and varied from 31.80 to 50.00 gm plus 25.80 to 47.50 gm under two environment conditions (Table 2, Fig. 3E). Though most of traits under investigation showed reduction in mean value for a trait under RI yet protein content in the grains displayed increase in the trait value under this environment (Table 2). Moreover, some lines from a germplasm set were also performing better than the check cultivars for traits of economic importance including nutritional factors, showing their potential to use in the wheat breeding programme. In case of RI conditions, the minimum and maximum values for protein content were 4.35% and 20.40% respectively with a mean of 12.13% whereas it varied from 7.00 to 15.10% under irrigated environment with mean value of 11.14% (Table 2, Fig. 3D).

Under RI conditions the dry spell also affected the spikelet per spike, grains per spike, days to heading and

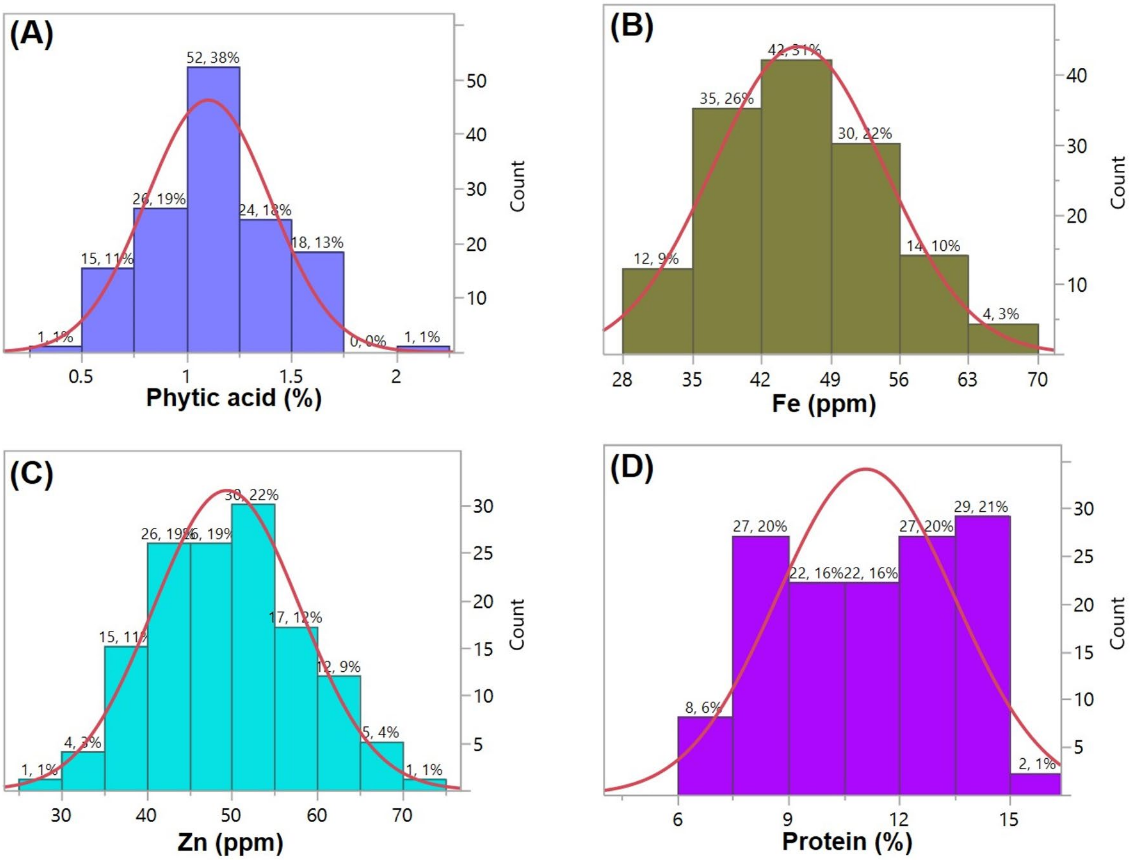

Fig. 2 Distribution of **A** Phytic acid, **B** iron, **C** zinc and **D** protein content in pre-breeding germplasm set under irrigated conditions

Table 2 Comparison between traits means and ranges for full irrigation and restricted irrigation environments

	Irrigated (IR)		Restricted irrigated (RI)	
	Mean	Range	Mean	Range
Phytic acid (%)	01.11	00.40–02.10	00.91	00.16–1.70
Fe (ppm)	45.83	30.10–66.80	40.53	20.20–65.40
Zn (ppm)	49.43	28.10–72.10	49.62	18.60–73.60
Protein (%)	11.14	07.00–15.10	12.13	04.35–20.40
Yield (t/ha)	04.51	02.13–7.75	03.44	01.13–6.81
Thousand grain weight (gms)	41.00	31.80–50.00	36.50	25.80–47.50
Spikelet per spike	18.65	14.90–22.50	18.14	13.55–22.13
Grains per spike	48.75	33.00–66.00	45.77	29.50–63.00
Plant height (cm)	93.47	76.80–110.70	86.71	65.30–105.70
Days to heading (no.)	90.00	80.50–100.50	81.42	69.00–92.50
Chlorophyll Content (SPAD values)	46.79	32.90–58.70	45.11	27.30–59.30

plant height (Table 2, Figs. 3F, 3G, 3H). Under irrigated conditions the mean SPAD value for chlorophyll content (CC) was 46.79 and it ranged from 32.90 to 58.70 in comparison to mean of 45.11 under RI environment with a range of 27.30 to 59.30 across all the genotypes (Table 2, Fig. 3I). Grain yield also displayed considerable decrease under RI environment in comparison to irrigated conditions with mean of 3.44 t/ha and 4.51 t/ha respectively (Table 2). All the genotypes had significant effects on molar ratios of Fe and Zn with respect to phytic acid (Table 3). G × E interactions had significant effects on molar ratio of Fe and Zn.

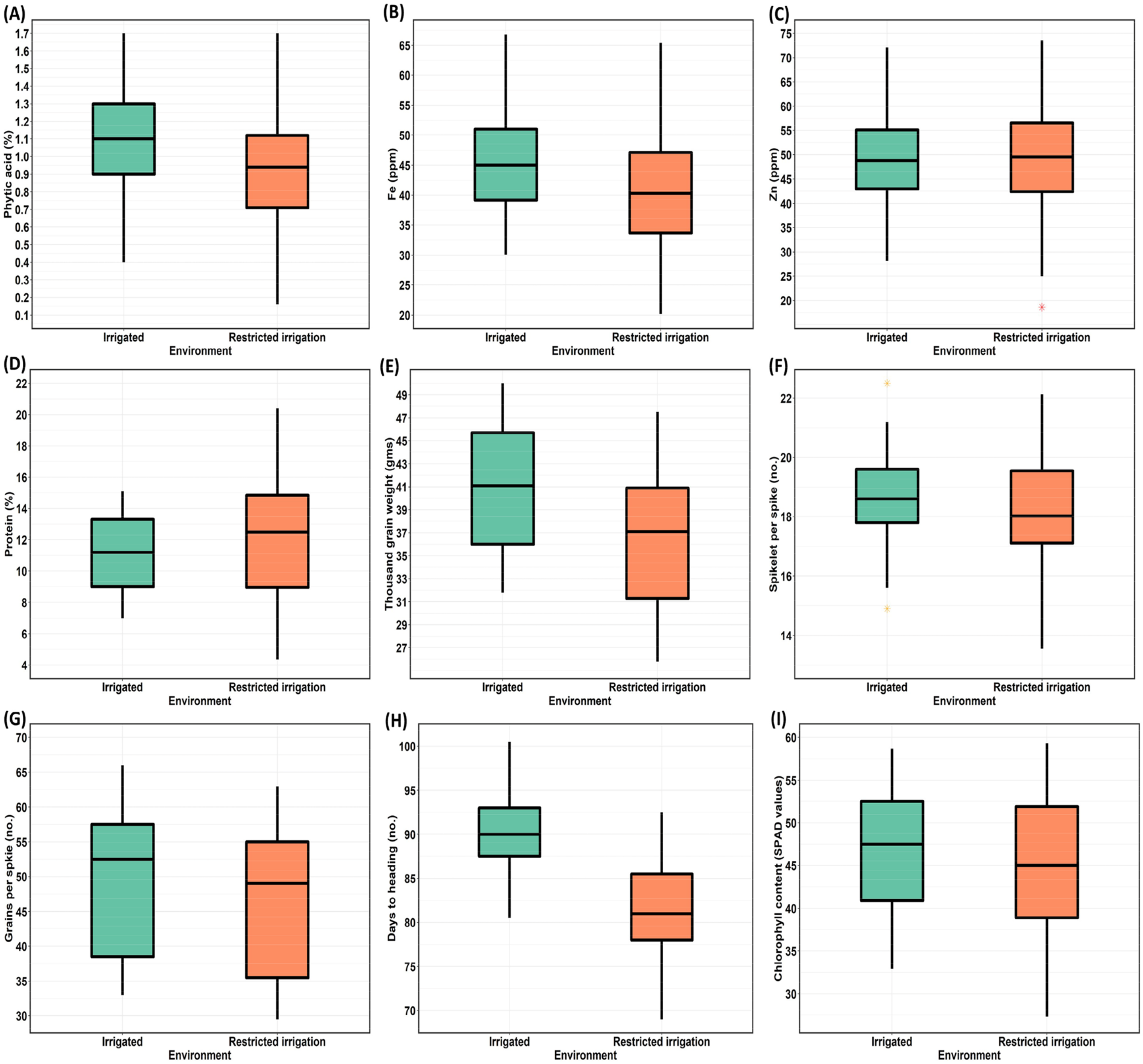

Fig. 3 Box plots showing variation for **A** phytic acid, **B** Fe content, **C** Zn content, **D** protein content, **E** thousand grain weight, **F** spikelet per spike, **G** grains per spike, **H** days to heading, and **I** chlorophyll content under irrigated conditions

Table 3 Effects of genotypes, environment, years and their interaction (G×E) expressed as the mean sum of squares from ANOVA analysis for grain quality traits

	Phytic acid	Fe	Zn	Protein	PA: Fe	PA: Zn
Genotype	0.39**	601.80**	719.06**	40.78**	0.034**	0.042**
Environment	9.96**	7632.1**	12.31^{ns}	292.71**	0.001^{ns}	0.107**
Block (Rep)	0.03^{ns}	9.14^{ns}	6.49ns	6.78 ns	0.00	0.00
G×E	0.17**	40.24**	81.67**	34.54**	0.005**	0.006**
Year	0.99**	856.71**	1584.48**	9.34	0.01*	0.184**
Error	0.03	8.76	9.71	6.85	0.002	0.003

All values were significant at 0.05, "*", < 0.01, "**", and ^{ns} non-significant

PA Phytic acid, *Fe* Iron content, *Zn* Zinc content

Variation in micronutrients (Fe, Zn), phytic acid and other traits under restricted/reduced irrigation conditions

Restricted irrigated conditions have shown impact on all the traits and these changes were expressed as variation percentage in comparison with irrigated conditions (Fig. 4). Phytic acid content in grains was reduced by 16% (S1) to 17% (S2) under restricted irrigated conditions (Fig. 4A), whereas, Fe content was reduced by 2% and 17.8% in S1 and S2 respectively (Fig. 4B). Furthermore, Zn content has shown opposite trend where it decreased by 2.9% in S1 and increased by 4.1% in S2 (Fig. 4C), which is in relation with phytic acid content. Reduction in phytic acid content in S2 has positive impact on the Zn accumulation in wheat grains. Protein content in grains has followed the positive trend where it displayed a positive change of 7.9% (S) and 10.6% (S2) under restricted irrigated conditions in comparison to irrigated environment (Fig. 4D).

RI conditions had negative impact on all the physiological and agronomic characters of pre-breeding germplasm set (Fig. 4). RI conditions has reduced the yield content

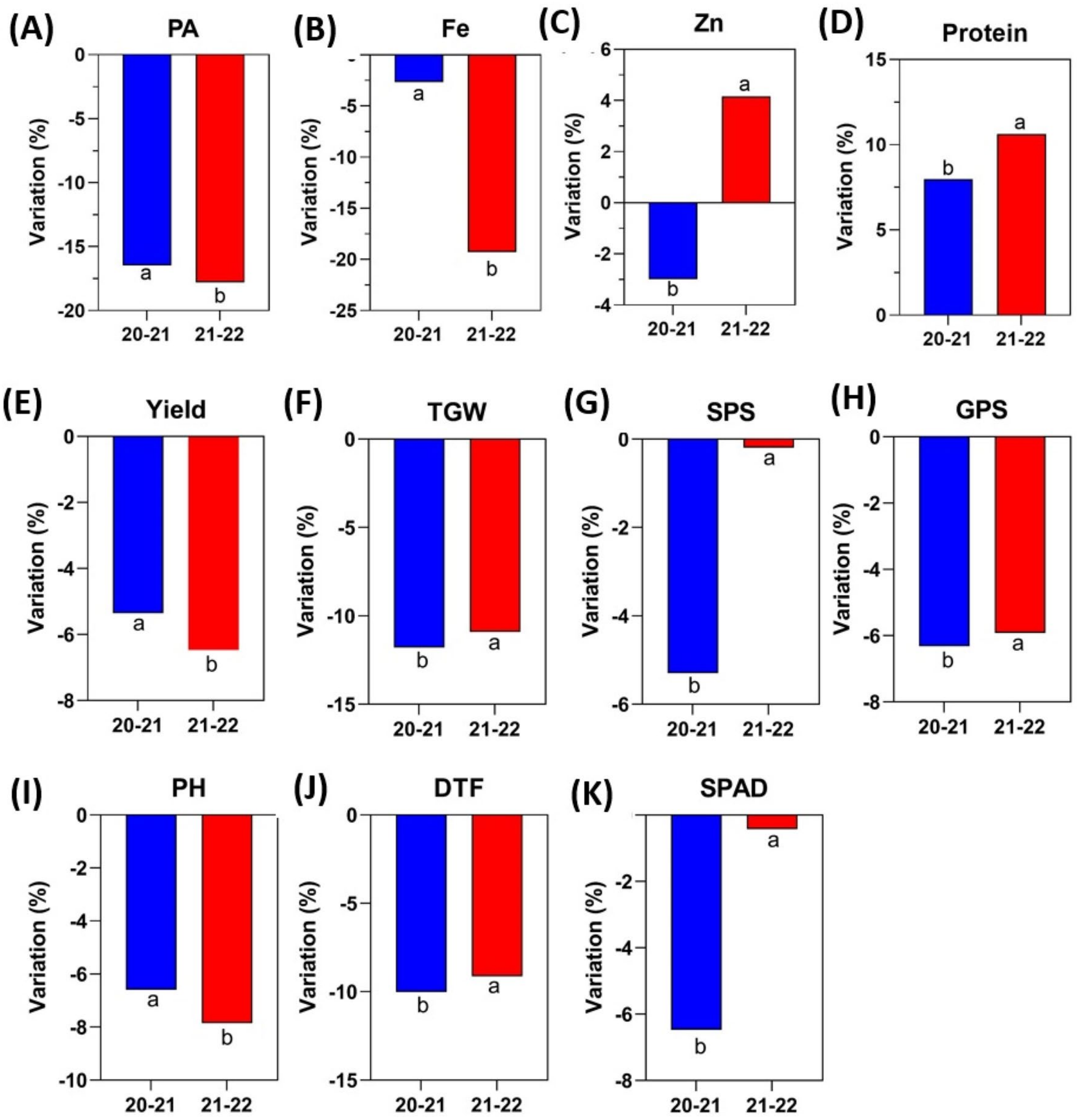

Fig. 4 Variation in phytic acid (PA), iron (Fe), zinc (Zn) and protein content under restricted irrigated conditions for two cropping seasons (2020–2021 and 2021–2022) in comparison with irrigated conditions. (*a, b: Different letters indicating significant difference between cropping season). *PA* Phytic acid, *TGW* thousand grain weight; *SPS* spikelet per spike, *GPS* grains per spike, *PH* plant height, *DTF* days to flowering, *SPAD* Chlorophyll content/SPAD

(Fig. 4E) by 5.3% (S1) and 6.4% (S2) which is directly associated with reduction in thousand grain weight in both the seasons S1 (11.7%) and S2 (10.9%) and grains per spike 6.3% (S1) and 5.9% (S1) under RI environment in contrast to irrigated environment.

Correlation and multivariate analysis for micronutrients (Fe, Zn), phytic acid and agronomic characters

To analyse the relationship among micronutrients content, phytic acid and agronomic traits, the Pearson correlation coefficients were estimated (Fig. 5). SPS showed positive correlation with GPS with Pearson coefficient (r) values of 0.33 and 0.21 under irrigated and RI conditions respectively. Plant height has shown some positive correlation with grain yield in the germplasm set with r values of 0.26 and 0.20 in both the environments (IR and RI respectively).

Molar ratios for the iron concentration in irrigated conditions has negative correlation with agronomic and physiological parameters (Table 4), whereas molar ratio of zinc had negative correlation with SPS, plant height and chlorophyll content under IR conditions. RI conditions follow the similar trend for the molar ratio of iron grain yield, spikelet per spike, grain per spike and chlorophyll content. Molar ratios for both the micronutrients (Fe and Zn) had negative correlation with grain yield in irrigated and restricted irrigated conditions (Table 4). Interestingly, order of correlation for molar ratio of zinc content with spikelet per spike and plant height get reversed in restricted irrigated conditions as compared to the irrigated conditions. Phytic acid content had negative or weakly positive correlation with the micronutrient concentration in mature wheat grain.

Multivariate analysis has been expressed as principal component analysis for micronutrient contents, phytic acid content and related field traits has shown the variation under irrigated and restricted irrigated conditions. Information generated from the experiment is expressed in the form of orthogonal variables. It is displayed in a map to simplify the high dimensional complex data using a smaller set that can be easily analysed and visualized. Figure 6 is showing the PCA in biplot for Fe, Zn and phytic acid content with other field traits under irrigated (A) and restricted irrigated (B) conditions. Comparison under different treatments has shown the major changes in all the traits more specifically significant change in Fe, Zn concentration, phytic acid content and related field traits. Yield has shown the change under RI conditions. Plant height, days to flowering are the traits which are affected significantly under RI as shown in Fig. 6A, B.

Discussion

Biofortification of bread wheat for micronutrients is target of wheat breeders throughout the world (Gupta et al. 2022). Different genetic resources have been utilized for this purpose depending on the locations and availability. Biofortified wheat developed through the conventional breeding has been utilized by various research institutes (Jaiswal et al. 2022; Velu et al. 2019). To carry out breeding processes, known commercial cultivars with micronutrient and phytic acid levels need to be taken into account (Gupta et al. 2015). Furthermore, under changing climatic conditions with increase in global mean temperature and erratic rainfall pattern, understanding the agronomic and bio-chemical properties of plant can play a crucial role for the development of climate resilient cultivars (Raza et al. 2019). Therefore, the present study was planned under the two environmental conditions (IR and RI) to tackle the climatic constraints so that wheat breeding programme can be strengthen with targeted development of new wheat cultivars tolerant to multiple stresses with enhanced micronutrient concentration. High throughput and standardized methodologies are available for the micronutrient and phytic acid detection that help to screen the large germplasm set for nutritional factors. The EDXRF (energy-dispersive X-ray fluorescence spectrometry) equipment as described by Paltridge et al. (2012) has been found an extremely useful tool for analysing micronutrient (Fe and Zn) concentrations in this regard. These standardised methodologies assisted detection of micronutrient and phytic acid concentrations in a germplasm set comprising 137 genotypes.

Effect of water deficit stress on micronutrient content and other traits

Variation was found in all the lines for micronutrient concentration with respect to local (PBW 725) and national check cultivars (HD 3086). Variation for grain Fe content under IR conditions ranged from 30.10 to 66.80 ppm. These results were in concordance with the study by Ficco et al. (2009), where they found a slight reduction in the grain Fe concentration under RI conditions. Decrease in iron and zinc concentration of grains in most of the cultivars was also suggested by Guzmán et al. 2016; Velu et al. 2016) in durum and bread wheat. Reason for this decrease is associated with the reduction in grain filling period under RI conditions, which in response causes small reduction in micronutrient content. RI conditions affect the micronutrient content by altering the mobilization of micronutrients in different parts of wheat plant

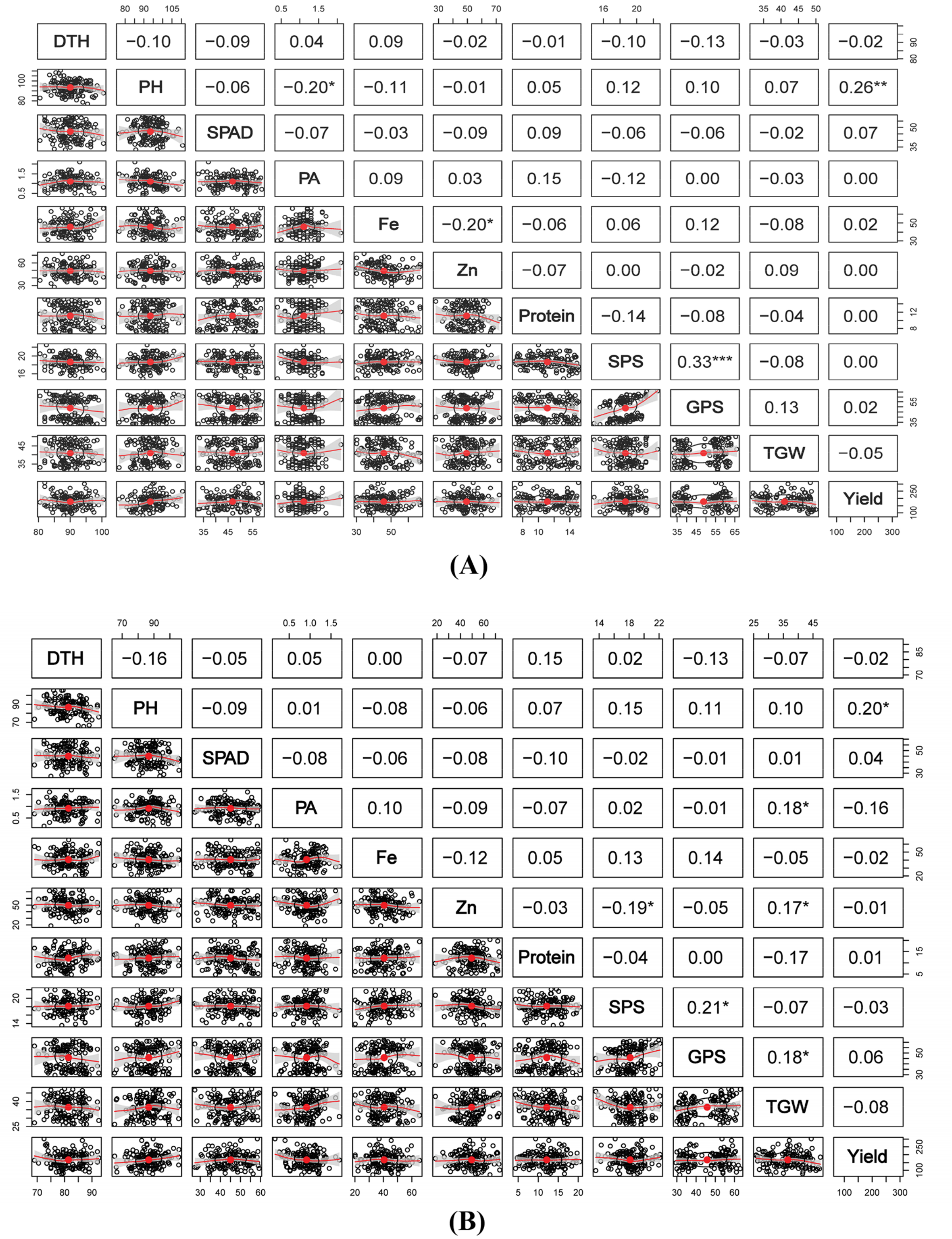

Fig. 5 Pearson correlation coefficients for the estimated traits under **A** Irrigated and **B** restricted conditions (*, ** and ***: Significant at 0.05%, 0.01% and 0.001%)

(Broberg et al. 2021). Similarly, a good diversity was observed in the investigated germplasm set for all the estimated traits that can act as a boon for the wheat breeding programme. Similar variations were also reported in studies conducted on different genotypic sets tested under controlled as well as field conditions (Ficco et al. 2009; Zhao et al. 2009; Cakmak et al. 2004; Rachoń et al. 2012; Liu et al. 2014). El Haddad et al. 2022, has explained the

Table 4 Association between Molar ratios, Yield, TW, SPS, GPS, PH, DTF, SPAD

	Full irrigation		Restricted irrigated	
	Phy:Fe	Phy:Zn	Phy:Fe	Phy:Zn
Yield	− 0.041	− 0.035	− 0.074	− 0.087
TW	0.115	0.008	0.176*	0.061
SPS	− 0.119	− 0.025	− 0.082	0.103
GPS	− 0.069	0.052	− 0.104	0.047
PH	− 0.013	− 0.092	0.032	0.051
DTF	− 0.035	0.013	0.09	0.025
SPAD	− 0.042	− 0.034	− 0.022	− 0.035

TW Test weight, *SPS* Spikelet per spike, *GPS* Grains per spike, *PH* Plant height, *DTF* Days to flowering, *CC* Chlorophyll content/SPAD

variation of micronutrients with drought stress in lentils. This study has explained the reduction in protein content by 53% and Fe and Zn content by 20% and 18% respectively under drought conditions. Maintaining large genetic diversity in the modern wheat genetic pool and their use in the breeding programme is mandatory to meet the global food demand. Due to this, pre-breeding germplasm sets of wheat were also studied for the micronutrient concentrations in the earlier studies (Cakmak et al. 2004; Gomez-Becerra et al. 2010).

Potential bioavailability of micronutrients in relation to phytic acid content

Increasing the bioavailable micronutrient concentration in wheat kernels is a challenging task (Frontela et al. 2009). Phytic acid is an abundant component of wheat grains that acts as a phosphorus reservoir. It is considered as an anti-nutrient factor as it chelates the Fe and Zn during digestion and avoid their absorption in the animals including humans. Molar ratios of phytic acid: micronutrient concentration was estimated to detect the potential bioavailability of Fe and Zn. Based on evidences, micronutrient bioavailability is inversely proportional to the molar ratio. Molar ratio < 1 (PA:Fe) is considered an excellent material with improved Fe bioavailability as suggested by Gomez-Becerra et al. 2010. Similarly, molar ratio < 5 is considered best for bioavailability of Zn and it corresponds to approximately 50% bioavailability of Zn (Gibson 2006). Relation of phytic acid content with bioavailable micronutrients was also explained in rice. According to this study phytic acid content may be manipulated by breeding approaches. Presence of phytic acid and its correlation with micronutrient concentration was the main reason for its involvement in the present study.

For this purpose, a modified methodology of phytic acid qualification was validated. Different methods for the determination of phytic acid has been described in the earlier studies (Dost and Tokul 2006; Hauga and Lantzschb 1983). Though to reduce the phytic acid detection cost, biochemical

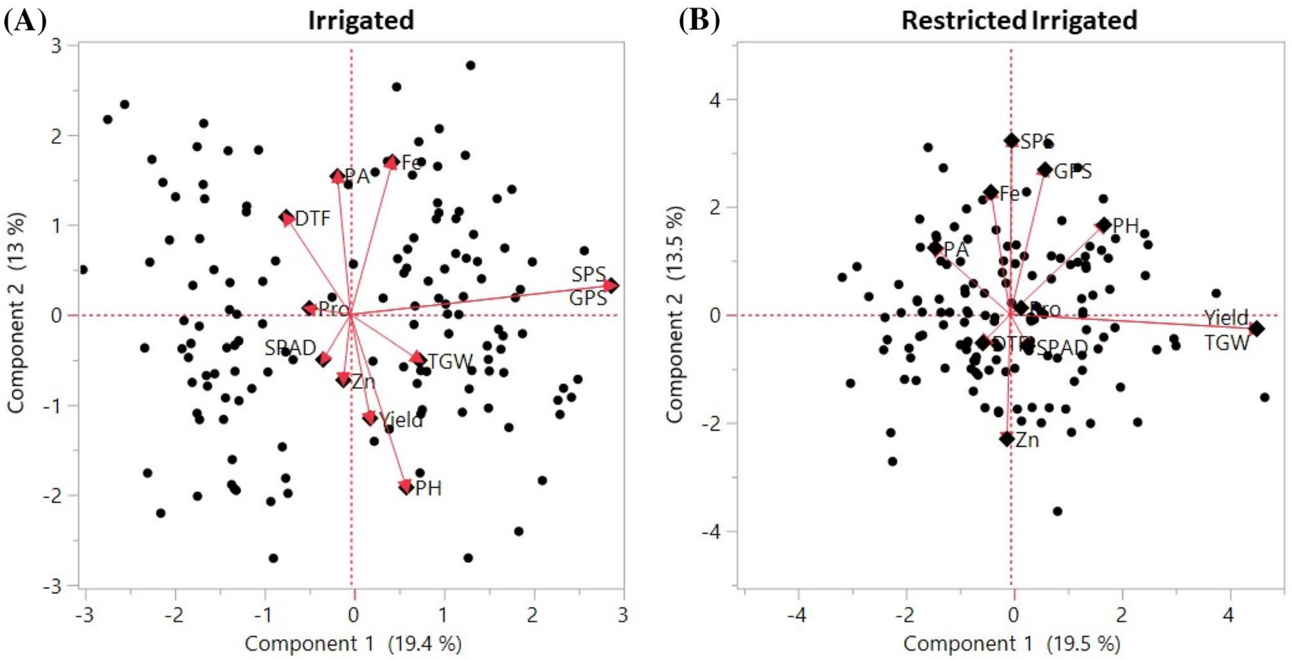

Fig. 6 Principal component analysis (PCA) for micronutrients (Fe, Zn), phytic acid content and related field traits under irrigated and restricted irrigated conditions

method was validated as number of samples were high. The phytic acid in the investigated lines ranged from 0.40 to 2.10% and 0.16 to 1.70% under irrigated and restricted irrigated conditions respectively. Results suggested large variations for phytic acid with lower mean value of 0.91 under RI environment in the tested set that was also in accordance with the study by Branković et al. 2015 in wheat cultivars of different origins. Reduced phytic acid under RI environment has implications for improving the nutritional properties of wheat under drought stress as the severity of drought on crop can increase in the future owing to climate change. Similar results to the ones of the present study in two Iranian cultivars as well as in 65 bread wheat varieties (Hussain et al. 2012) grown at different locations. Wheat breeding programme across the globe is in process to combat the hidden hunger (micronutrient deficiency) which is more prominent in developing areas of the world. So, reducing phytic acid concentration in the cereal grains can help to improve the micronutrient concentration and their bioavailability whereby producing nutritionally enriched cultivars. Ahmed et al. (2014) has explained the different ways to reduce the phytic acid content in wheat flour to increase the bioavailable micronutrients. Tran et al. (2021) has studied the geographically diverse 101 durum wheat genotypes for bioavailability of Fe and Zn. Their study has suggested the role of fungal inoculums for soil to increase the bioavailability of micronutrients.

Grain protein content under different water regimes

Grain protein content is also an important trait that significantly modify the wheat quality and is affected by the water scarcity or drought stress. Significant variation for grain protein was found for all the investigated genotypes in the present study. Maximum value of protein content was 20.40% under RI conditions in contrast to 15.10% under irrigated environment with a mean value of 12.13% and 11.14% respectively. These results are in concordance with the earlier studies (Flagella et al. 2010; Krisztina et al. 2011) where they report increase in grain protein content under drought stress. This increase in grain protein content under drought stress is attributed to lower starch accumulation in the grains owing to drought conditions. Rakszegi et al. 2019 has studied the stability of grain composition during drought stress. Protein content was significantly affected under drought stress in different cultivars in comparison with wild relatives of wheat (Rakszegi et al. 2019).

This study provides useful information regarding the wheat breeding strategy with a prime focus on improving the nutritional quality. Overall, the result suggested significant variation in the wheat pre-breeding germplasm set for different traits of economic importance along with the important nutritional component traits. Based on the results, promising lines were identified having low concentration of phytic acid and enhanced levels of micronutrient especially Fe and Zn. These lines will be exploited in the mainstream wheat breeding programme to bred nutritionally enriched cultivars with stable yield and enhanced stress tolerance.

Conclusion

The data generated in present study has shown the variation for micronutrient content (Fe and Zn) in relation to their quencher phytic acid under irrigated and restricted irrigated conditions in wheat pre-breeding germplasm set. The lines have been identified from set with top notch results for all parameters under restricted irrigated conditions. Selected lines had high Fe and Zn content (65 and 73 ppm respectively) with low phytic acid (0.16–0.14%) concentration. The lines with high micronutrient contents also had high grain protein content (11–12%). These lines will be used in the breeding programmes to develop drought tolerant varieties with improved micronutrient concentration and reduced phytic acid content. These identified lines will assist the development of wheat varieties with enhanced water use efficiency and nutritional quality to eradicate the hidden hunger in affected population.

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Data availability The datasets and materials generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interest We declare that all the authors have read and understood the manuscript thoroughly and declare no conflict of interest. The authors have no relevant financial or non-financial interests to disclose.

Ethical approval Authors declare that they are research work carried out for the article along with drafting of the manuscript are in compliance with research ethics.

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