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Research Article

Multi-Environment Analysis of Biofortification Traits in Pre-bred Bread Wheat (*Triticum aestivum* L.)

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Abstract

A set of 14 exotic-based wheat genotypes along with two check varieties were evaluated in four environments. The experimentation was conducted for two consecutive years during 2018–19 and 2019–20 at Karnal and Hisar locations. The variability observed in exotic-based genotypes is much higher than check varieties. Simultaneous improvement of GFeC, GZnC, and GPC is possible due to strong and positive correlation among the studied traits. Two genotypes i.e., G7 (BFKW-7) and G3 (BFKW-3) are suitable candidates to be utilized as potential donors for simultaneous improvement of all three traits, as these are found to be both stable and have high trait value. G4 (BFKW-4) for GFeC and GPC, and G11 (BFKW-11) for GFeC and GZnC, and G10 (BFKW-10) for GZnC and GPC were also potential donors. These stable genotypes would be a potential source for high GFeC, GZnC, and GPC to develop biofortified wheat varieties.

Key words: Wheat, Biofortification, Grain iron, Zinc, Protein, GGE

1. Introduction

Malnutrition caused by micronutrient and protein deficiency is considered to be one of the major public health issues in many parts of the world, particularly in low and middle-income countries. Micronutrient deficiency (hidden hunger) is caused due to inadequate intake or absorption of essential minerals and vitamins to support normal growth and development in children and normal physical and mental functions in adults. Currently, more than 2 billion people are suffering from micronutrient deficiency across the globe (Gillespie *et al.*, 2016). Three micronutrients i.e., iron, zinc, and vitamin A are recognized as limiting in the global diet (Ortiz-Monasterio *et al.*, 2007). Micronutrient deficiencies is an important global health issue, affecting both physical and mental development, disease vulnerability, mental retardation, blindness, reduced cognitive ability, and general losses in productivity and potential. Iron deficiency is the primary cause of anaemia or low haemoglobin content, which affects around 40% of children under the age of 5 years and nearly 30% of pregnant women worldwide (WHO, 2021). The risk of maternal death and reduced birth weight of the infants increases particularly if anaemia occurs during the gestation period. Worldwide, approximately 2.5–3.4 million maternal and neonatal deaths are reported annually (Stevens *et al.*, 2013) due to iron deficiency.

Zinc is another key micronutrient, which stimulates the immune system and thereby increases the resistance against infectious diseases such as diarrhoea, pneumonia, and malaria. Also, zinc plays an important role in healthy gestation (Ackland and Michalczyk, 2016). Zinc deficiency affects approximately 17.0% of the global population (Wessells and Brown, 2012), causing the death of 0.09 million people and the loss of 9.1 million disabilityadjusted life year (DALY's) in the year 2010 (Lim et al., 2012). Wheat quality i.e., nutritional and end product quality is mainly determined by both grain protein content and protein quality. Reduced secondary immunity due to protein energy malnutrition (PEM) is one of the most common causes of various infections. Acute PEM in children is clinically referred as marasmus (chronic wasting) or kwashiorkor (edema and anemia) (Schaible and Kaufmann, 2007). Chronic PEM results in altered cognitive development in young children (Kar*et al*., 2008). Micronutrient deficiency coupled with PEM are the major risk factors for losing health in developing nations, further, young children and pregnant women constitute the major risk groups (Muller and Krawinkel, 2005).

The various interventions to alleviate micronutrient malnutrition are dietary diversification, pharmaceutical supplementation, industrial fortification, and biofortification. The consumption of a diversified diet rich in micronutrients is the simplest and most effective strategy, the majority, especially economically weaker sections from low and middle-income countries may not afford it. The supplementation and fortification are not sustainable over the long term. Additionally, fortified food is unavailable and unaffordable to needy people, particularly for the rural poor. Hence, a practice of increasing the nutrient status of agricultural produce through plant breeding, agronomic, and transgenic approaches, known as "biofortification", emerged as a cost-effective and sustainable solution to the problem of micronutrient deficiencies. Micronutrient deficiencies are most common in South Asia, Latin America, and developing countries such as Sub-Saharan Africa. Thus, effective policies to address micronutrient deficiencies in food systems such as the use of micronutrient fertilizers and plant breeding to develop nutrient-rich crops for human consumption are being considered (Gregory *et al*., 2017).

The improvement of grain micronutrient and grain protein content depends on the availability of sufficient genetic diversity in the gene pool. Ficco *eta l*., (2009) reported variability in the range of 28.5 to 46.3mg/kg for grain zinc concentration (GZnC) and 33.6 to 65.6 mg/kg for grain iron concentration (GFeC). Similarly, Zhao *et al*.,(2009) observed a variation between 13.5 and 34.5 mg/kg for GZnC and 28.8 and 50.8 mg/kg for GFeC among the studied 150 bread wheat genotypes. Velu *et al*., (2012) evaluated a set of 600 core collection accessions comprising of both durum and bread wheat and found that the GZnC and GFeC ranged from 16.85 to 60.77 mg/kg and 26.26 to 68.78 mg/kg, respectively. Gopalareddy *et al*., 2015) observed variability for GFeC ranging from 27.85–54.60 mg/kg and for GZnC was 19.30–70.55 mg/kg. Wheat breeding programs must be re-orient to broaden the genetic base using landraces and crop wild relatives to effectively dissect the genetic basis of nutritional qualitytraits, and to develop wheat varieties with enhanced micronutrients and protein content Cakmak *et al*., (2010). Landraces are considered to be one of the important sources of wheat biofortification (Rasheed *et. al*., 2019). Higher grain zinc content has been successfully incorporated into elite breeding materials through the conventional breeding approach by the crossing of improved and adapted high-yielding wheat cultivars with *Aegilops tauschii*-derived SHWs or *Triticum spelta* accessions (Velu *et al*., 2018). The iron and zinc status of modern cultivated wheat can be enhanced throughthe effective utilization of *Triticum dicoccoides* (wild emmer) in crop breeding programs (Çakmak *et al*., 2004). *Triticum dicoccoides* derived *Gpc-B1* locus which was identified on the short arm of the 6B hasa pleiotropic effect on grain protein, zinc, and iron content (Distelfeld *et al*., 2007). A NAC transcription factor *(NAM-B1)* encoded by an ancestral wild wheat locus *Gpc-B1* enhances nutrients including iron, zinc, and protein, probably by accelerating the senescence and thereby mobilization from leaves to developing grains (Uauy *et al*., 2006). Synthetic wheat developed from *Aegilops tauschii* has high grain zinc content and can serve as a valuable genetic resource to enhance grainzinc concentration in cultivated wheat (Calderini *et al*., 2003). The present study aimed to estimate the magnitude of environment and GEI effects in the expression of GFeC, GZnC, and GPC in a set of exotic wheat genotypes and to identify promising and stable genotypes by the GGE model to be used as donors in the quality breeding program to develop high yielding wheat varieties with improved wheat quality.

2. Materials and Methods

Genetic materials consisted of 14 exotic wheat genotypes along with two high-yielding local check varieties

(DBW187 and HI8498). The exotic wheat genotypes were imported from the University of Nottingham, United Kingdom under the Biotechnology and Biological Sciences Research Council (BBSRC) project. Most of the exotic genotypes were backcrossed to widely adapted Indian wheat varieties through pre-breeding. The set of 16 genotypes was evaluated at two locations belonging to the North Western Plains Zone (NWPZ) during the winter (rabi) season of the years 2018–2019 and 2019–2020. The locations include Karnal i.e., ICAR-Indian Institute of Wheat and Barley, Karnal (29° 41' 8.2644" N, 76° 59' 25.9692" E, 250 m AMSL) and Hisar i.e., ICAR-Indian Institute of Wheat and Barley, Regional Station, Hisar (29° 5'0 5" N, 75° 45'0 55"E, 215.2 m AMSL). The crop was sown in the first fortnight of the November Rabi (winter) season during both years at both locations under irrigated conditions. The genotypes were planted in a randomized complete block design (RCBD) with two replications per genotype and two rows (5 m length) per replication with a plant-to-plant distance of 10 cm and row-to-row distance of 25 cm. Standard agronomic practices were followed for growing the crop.

After physiological maturity, a random sample of 20–35 spikes from each genotype was harvested manually. Approximately 20 g grains were sampled for micronutrient analysis and proper care was taken to avoid dust and metal contamination. A new cost-effective, non-disruptive, high throughput method called Energy Dispersive X-ray Fluorescence (ED–XRF) instrument ("Bench-top" X-Supreme 8000; Oxford Instruments plc, Abingdon, UK) available at ICAR-Indian Institute of Wheat and Barley Research (ICAR-IIWBR), Karnal, India was used for the estimation of GFeC and GZnC, which was expressed in milligrams per kilogram (mg/kg). The GPC was estimated by Infra-red transmittance-based instrument Infra-tec 1125 at (ICAR-IIWBR) and the values were expressed at 12% moisture basis. Stability analysis was done by following the GGE model using GenStat 18th Edition (VSN International Ltd, Hemel Hempstead, UK). Pearson's correlations of the means were determined for all four environments and three traits.

3. Results and Discussion

The pedigree details of genotypes used in the study along with pooled mean for GFeC, GZnC, and GPC are presented in Table 1. The exotic-based genotypes had a wide variability for GFeC ranging from 41.5 mg/kg (BFKW-8) to 53.8 mg/kg (BFKW-10) compared to check varieties DBW187 (37.3 mg/kg) and HI8498 (44.8 mg/kg). Similarly, exotic-based genotypes exhibited a wide range of variability for GZnC ranging from 43.4 mg/kg (BFKW-8) to 54.3 mg/kg (BFKW-3) compared to check varieties DBW187 (35.8 mg/kg) and HI8498 (40.6 mg/kg). The exotic-based genotypes also exhibited very high grain protein levels compared to both the check varieties. The exotic-based genotypes of GPC ranged from 15.2% (BFKW-8) to 18.9% (BFKW-4) compared to check varieties DBW187 (13.7%) and HI8498 (12.8%). Most of the exotic-based wheat genotypes exhibited much higher genetic variability in all three studied traits (GFeC, GZnC, and GPC) compared to check varieties. Location-wise GFeC, GZnC, and GPC in wheat genotypes aregiven in Table 2.

Table 1. List of genotypes used in the study and pooled mean of grain iron, zinc, and protein content.

Name	Cross / Pedigree details	GFeC (mg/kg)	GZnC (mg/kg)	GPC (%)
BFKW-1	Chinese. Spring / *Ae. mutica* (P 213004)	42.4	45.6	17.4
BFKW-2	Macoun / *Th. bessarabicum* (EC787008)	45.7	47.8	16.7
BFKW-3	Pavon 76 /*Ae. Mutica* (P 2130012)	52.1	54.3	16.8
BFKW-4	Highbury / *S.anatolicum* (P 208/142)	52.3	50.7	18.9
BFKW-5	*Th. Bessarabicum* (EC787708)/ cv Margarita	42.8	45.6	18.1
BFKW-6	SYN207//PBW502/16/621-50	45.1	48.0	15.6
BFKW-7	Chinese spring / *Th. bessarabicum* EC787015/HD3086	53.3	54.2	17.1
BFKW-8	PDW 314 / *CvIcarasha*	41.5	43.4	15.2
BFKW-9	PARAGON/WH1105/GW322	52.1	53.1	18.0

BFKW-10	PARAGON/WH1105//DBW88	53.8	52.0	18.3
BFKW-11	Chinese spring/ cv Margarita	48.3	51.0	16.0
BFKW-12	Pavon 76 / *Ae.mutica* (2130012)/ HD 3086	50.8	51.2	17.8
BFKW-13	*T. sphaerococcum* (EC10511) / DBW 90	42.3	44.8	15.4
BFKW-14	*T. sphaerococcum* / Pavon 76	45.7	47.8	16.9
Check	DBW187 (A)	37.3	35.8	13.7
Check	HI8498 (D)	44.8	40.6	12.8

GFeC: grain iron concentration; GZnC: grain zinc concentration; GPC: grain protein content

Table 2. Location-wise grain iron, zinc, and protein content in wheat genotypes were evaluated during 2018–19 and 2019–20 at Karnal and Hisar locations.

Name	GFeC (mg/kg)				GZnC (mg/kg)				GPC (%)			
	L1		L2		L1		L2		L1		L2	
	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
BFKW-1	40.8	41.2	42.8	44.8	45.6	46.4	44.0	46.4	15.8	17.2	18.6	18.0
BFKW-2	45.6	44.8	45.2	47.2	45.9	48.3	45.8	51.0	15.9	16.7	16.9	17.3
BFKW-3	49.3	53.3	51.8	54.2	54.1	55.9	54.8	52.4	16.9	16.1	16.4	17.8
BFKW-4	50.9	54.6	50.8	52.8	53.9	48.7	49.6	50.6	18.5	17.9	19.7	19.3
BFKW-5	42.6	44.2	41.6	42.6	42.8	46.0	47.3	46.1	17.1	17.7	18.4	19.0
BFKW-6	44.9	43.6	44.7	46.9	49.8	47.4	46.2	48.6	14.7	15.7	16.4	15.6
BFKW-7	50.8	54.2	53.1	55.1	54.2	56.0	54.6	51.8	15.9	17.1	17.9	17.5
BFKW-8	41.2	43.0	39.7	41.9	42.8	43.8	42.8	44.0	14.8	15.6	15.6	15.0
BFKW-9	54.1	50.2	51.4	52.6	55.2	52.0	51.8	53.4	19.3	17.7	17.8	17.2
BFKW-10	57.2	53.7	51.5	52.9	51.2	53.4	50.7	52.7	17.9	18.9	17.9	18.5
BFKW-11	48.4	50.8	45.6	48.3	49.7	50.9	50.8	52.4	15.7	17.3	15.2	15.8
BFKW-12	48.9	51.6	49.8	52.6	53.6	49.8	51.6	49.8	18.6	17.6	17.7	17.3
BFKW-13	40.8	42.0	41.9	44.3	44.7	46.6	43.2	44.6	14.2	15.4	16.4	15.6
BFKW-14	43.1	46.8	45.2	47.8	48.4	45.9	47.6	49.5	16.9	15.9	17.6	17.2
DBW187 (A)	35.5	38.4	36.7	38.5	34.6	36.6	35.1	36.9	13.2	14.0	14.2	13.4
HI8498 (D)	43.5	44.8	44.5	46.3	40.8	40.0	40.1	41.5	12.1	12.9	12.8	13.2

GFeC: grain iron concentration; GZnC: grain zinc concentration; GPC: grain protein content; L1: Karnal; L2: Hisar; Y1: 2018–19; Y2: 2019–20

The cultivated wheat varieties have relatively narrow genetic variation for many quality traits in wheat including GFeC, GZnC, and GPC. Hence, limiting the genetic gain for higher grain micronutrients and protein. Hence, landraces and synthetic hexaploidy wheat and other pre-breeding material may act as an important genetic resource for improving grain micronutrient concentrations in cultivated wheat. Cakmak *et al.*, (2004) screened 825 accessions of *Triticum turgidum* ssp. *dicoccoides* from Fertile Crescent and observed the extensive variation and the highest concentrations of micronutrients ranged from 14.0 to 190.0 mg kg^{-1} for GZnC and from 15.0 to 109.0 mg/kg for GFeC, significantly higher than those of cultivated wheat. Rawat *et al.*, (2009) analyzed 80 germplasm accessions of nine species of wild *Triticum* and *Aegilops* along with 15 semi-dwarf cultivars of bread and durum wheat. Around 2–3 folds higher GFeC and GZnC was observed in *Aegilops kotschyi* and *Aegilops longissima* as compared to widely adapted high yielding wheat cultivar WL 711. They induced homoeologous chromosome pairing between *Aegilops* and wheat genomes using Chinese Spring *(Ph1)* and observed that most inter-specific hybrids had higher GZnC and GFeC than parents. Hence, they concluded that the parental *Aegilops* donors possess a more efficient system for uptake and translocation of the micronutrients which could ultimately be utilized for wheat grain biofortification. Thus, they suggested that the parental *Aegilops* donor genotypes possess a more efficient uptake and translocation system for the micronutrients, which could ultimately use for enhancing the nutrient status of wheat grain through biofortification.

Arora *et al.*, (2019) evaluated 167 diverse *Aegilops tauschii* accessions and observed almost two-fold (30.3–69.4 mg/kg) and 2.8-fold (17.5–49.8 mg/kg) genetic variation for iron and zinc respectively. The wild wheat species with increased iron and zinc concentration are useful to provide an efficient genetic system for enhanced grain micronutrients in modern cultivars. But this is hampered due to crossability barriers and sterility associated with interspecific and intergeneric hybridization. Hence, Synthetic hexaploids developed from crossing tetraploid wheat (AB genome) with *Aegilops tauschii* (D genome) generates extensive variation for the trait of interest and can efficiently avoid problems related to gene introgression. The biofortified varieties like Zinc-Shakti and WB-02 are developed using synthetic hexaploids nested in their pedigree. A total of 47 synthetic hexaploid wheat (SHW) lines developed from crosses between tetraploid wheat cultivar 'Langdon' and 47 *Aegilops tauschii* lines, which were collected from various geographical locations (Gorafi *et al.*, 2018). The GFeC ranged from 22.2 – 78.5 mg/kg with a mean of 45.4 and 20.6–65.8 mg/kg for GZnC with an average of 39.9 mg/kg. Therefore, it is important to utilize natural variability that existed in both cultivated and wild germplasm to develop biofortified cultivars.

The environment-wise GFeC, GZnC, and GPC are graphically illustrates as box plots in Fig. 1A. Both year and location effects were observed for all the studied traits. For GFeC, the highest mean was recorded in Env4 followed by Env2, Env1, and Env3. Similarly, for GZnC, the highest pooled mean was recorded in Env4. Grain protein content was recorded relatively lower in Env1 compared to the other three environments, which had recorded comparable mean values. Correlation studies of 16 wheat genotypes for different quality traits are depicted in Fig 1B. Generally, correlations among GFeC, GZnC, and GPC were found to be positive and significant. Similar kind of significant and positive associations was also reported in previous studies (Rathan *et al.*, 2022, Krihsnappa *et al.*, 2022; Krishnappa *et al.*, 2017). Therefore, all three associated traits (GFeC, GZnC, and GPC) can simultaneously be improved in the breeding programmes. Through conventional breeding approach, high grain zinc content has already been successfully transferred to elite breeding material from *Aegilops* tauschii-based synthetic hexaploid wheats (SHWs) or *Triticum spelta* accessions. Previously, *Triticum dicoccoides* derived *Gpc-B1* locus on chromosome 6B has been foundto have a pleiotropic effect on GFeC, GZnC, and GPC (Distelfeld *et al.*, 2007).

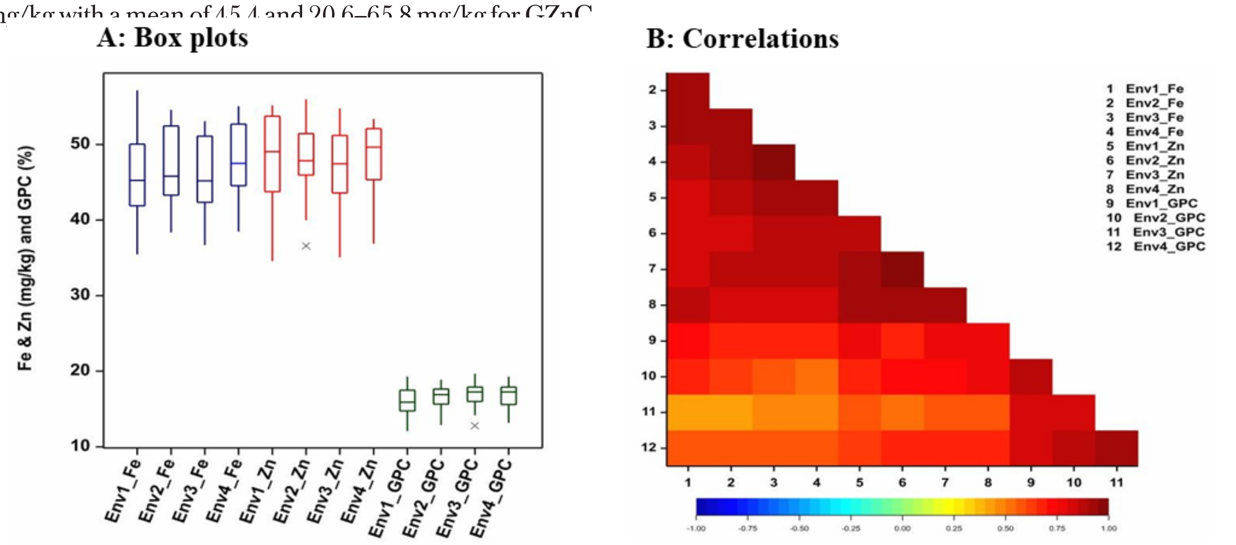

Fig 1. Graphical illustration of gain iron, zinc, and protein content in wheat genotypes. (A) Box plots, (B) Correlations. Env1: Karnal 2018–19, Env2: Karnal 2019–20, Env3: Hisar 2018–19, Env4: Hisar 2019–20. Fe: grain iron concentration; GZnC: grain zinc concentration; GPC: grain protein content

GGE biplot analysis provides a graphical representation to identify stable genotypes across the environments as well as genotypes adapted to specific environments. Graphical presentations of GFeC, GZnC, and GPC are given in Fig 2 as GGE biplots. For all three studied traits, the first two principal components represent more than 95.0% of the trait's variability. For GFeC, G4 (BFKW-4) was relatively more stable and higher mean value followed by G11 (BFKW-11), G12 (BFKW-12), G7 (BFKW-7), and G3 (BFKW-3). Whereas, G11 (BFKW-11) was both stable and had relatively higher mean values followed by G10 (BFKW-10), G7 (BFKW-7), and G3 (BFKW-3) for GZnC. Stable genotypes were also identified for GPC, G14 (BFKW-14) was relatively more stable along with higher mean values followed by G2 (BFKW-2), G3 (BFKW-3), G10 (BFKW-10), G4 (BFKW-4), G5 (BFKW-5), G7 (BFKW-7). Previously, stable genotypes for various quality traits were also reported (Krishnappa *et al.* 2019).

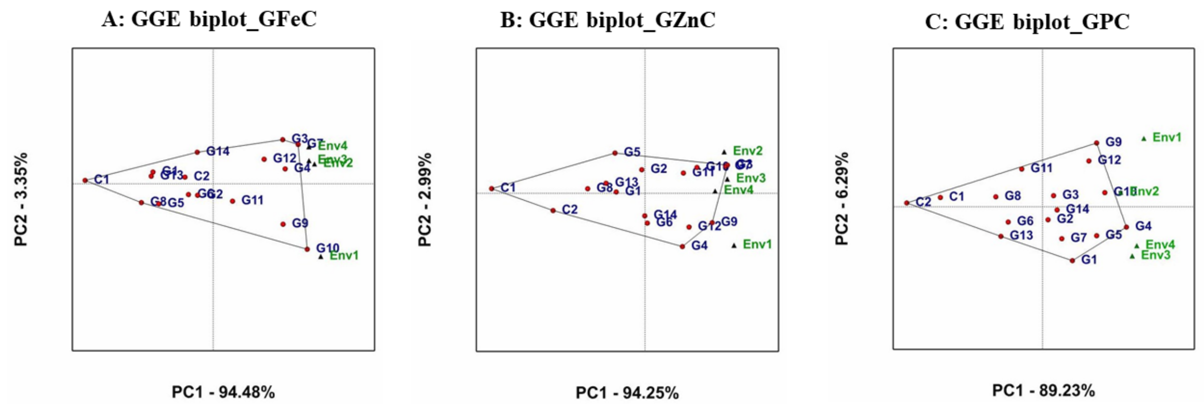

Fig 2. GGE biplots for grain iron, zinc and protein content in wheat genotypes tested in four environments. G1: BFKW-1, G2: BFKW-2, G3: BFKW-3, G4: BFKW-4, G5: BFKW-5, G6: BFKW-6, G7: BFKW-7, G8: BFKW-8, G9: BFKW-9, G10: BFKW-10, G11: BFKW-11, G12: BFKW-12, G13: BFKW-13, G14: BFKW-14, C1: DBW187 (A), C2: HI8498 (D)

Conclusion

The study with 14 exotic-based wheat genotypes along with two check varieties exhibited wide range of variability for GFeC, GZnC, and GPC. The *per se* trait value for all three traits was much higher for exotic-based genotypes than for check varieties. The strong positive correlation among grain micronutrients and GPC suggested the possibility of improving these traits simultaneously. Two genotypes i.e., G7 (BFKW-7) and G3 (BFKW-3) were found to be stable for all the three studied traits. Similarly, G4 (BFKW-4) was found to be stable for GFeC and GPC; G11 (BFKW-11) was stable for GFeC and GZnC; G10 (BFKW-10) for GZnC and GPC. Also, G14 (BFKW-14), G2 (BFKW-2), and G5 (BFKW-5) were found to be stable only GPC, whereas, G12 (BFKW-12) was found to be stable for only GFeC. Therefore, these stable and high trait vale genotypes would be a potential source as high grain protein, iron, and zinc donor parents. This valuable germplasm may be utilized in breeding programs to develop bread wheat varieties with high protein and micronutrient content.

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Author contributions

Conceptualization of experimentation (BST&SSa); Designing of the experiments (BST,GPS & GY); Experimental materials (AG); Execution of field experiments and data collection (VKG, CS, CNM, SKu, AO, GPS and GS); Analysis of data and interpretation (GK, VG); Preparation of the manuscript (all authors).

Conflict of interest

The authors declare no conflict of interest.

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