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Research Article

Identification of Superior Genotypes with Distinct Quality Traits For Biofortification

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Abstract

Breeding programmes for wheat biofortification often evaluate grain micronutrients and processing-related quality traits separately, leaving limited evidence on their simultaneous improvement within routine breeding material. The present study assessed wheat material to identify genotypes combining favourable grain quality with elevated grain iron (Fe) and zinc (Zn) concentrations. A total of 164 segregating entries (57 F4 and 107 F6) were screened at Karnal during crop season 2023-24 for different quality traits *viz.* grain protein content, sedimentation volume, grain Fe and Zn concentrations. Wide variation was observed in both generations: in F4, protein ranged from 7.69 to 12.00%, sedimentation volume from 29.42 to 52.95 mL, Fe from 31.60 to 47.60 ppm, and Zn from 23.50 to 48.10 ppm; corresponding ranges in F6 were 7.36-11.53%, 25.50-48.59 mL, 30.60-43.80 ppm, and 23.10-43.20 ppm, respectively. Positive and statistically significant correlations among protein, Fe, and Zn were observed in both generations, with a repeatable Fe-Zn correlation of $r = 0.65$ ($P < 0.001$). On the basis of overall performance, four entries were subsequently selected and assessed across four locations (Hisar, Ludhiana, Dharwad, and Karnal) during crop season 2024-25 along with two check cultivars (DBW 187 and DBW 327). Based on overall superior quality, QCHIIWBR-01 expressed the highest across-location mean Fe concentration (47.3 ppm) and thousand-kernel weight (51.3 g), whereas QCHIIWBR-04 recorded the highest mean hectoliter weight (77.0 kg hL^{-1}) and protein content (15.3%). These results indicate that CRP Biofortification-derived material can provide useful donor lines for combining processing-related quality traits with micronutrient enrichment; however, inference for multi-location performance is limited to descriptive across-location means because replication-level data required for formal stability analysis were unavailable.

Keywords: Biofortification, bread wheat, grain iron, grain zinc, multi-location comparison, processing quality

1. Introduction

Wheat is central to global food and nutritional security, and future breeding must address not only grain yield but also the quality attributes demanded by millers, processors, and consumers. At the same time, iron (Fe) and zinc (Zn) deficiencies remain major components of hidden hunger in cereal-dependent populations, making wheat biofortification an important breeding objective (Mottaleb *et al.*, 2023; Gupta *et al.*, 2023; Sheera *et al.*, 2025). For early-generation screening, breeders require quality traits that can be measured rapidly on limited grain samples. Hectoliter weight and thousand-kernel weight indicate grain filling and physical grain soundness; grain protein content is a major nutritional and bread-making determinant, and sodium dodecyl sulfate (SDS) sedimentation volume provides a rapid proxy for gluten strength. These measures though do not represent the full rheological profile of wheat, but they are practical breeding indicators for preliminary selection when large-scale end-use testing is not feasible (Ram *et al.,* 2018; Khalid *et al.,* 2023; Subedi *et al.,* 2023). In parallel, increasing grain Fe and Zn concentration has become a priority because useful genetic variation exists for both minerals and simultaneous improvement is feasible, although strong environmental effects complicate selection and interpretation. Recent reviews and multi-environment studies have emphasized the importance of integrating micronutrient targets into mainstream wheat breeding while maintaining acceptable quality profiles (Govindan *et al.,* 2022; Wani *et al.,* 2022; Gupta *et al.,* 2023; Tanin *et al.,* 2024; Sheera *et al.,* 2025). Despite this progress, fewer reports clearly document whether breeding material derived within CRP Biofortification pipelines can simultaneously provide practical quality traits and micronutrient enrichment at the stage where advancement decisions are made. This question is particularly relevant for material involving high-phytase mutant derivatives in the PBW502 background, where combined selection for nutritional and processing attributes may identify donor lines of immediate pre-breeding value. Accordingly, the present study evaluated segregating and advanced bread wheat material developed under the CRP Biofortification programme to: (i) quantify phenotypic variation for grain protein, SDS sedimentation volume, Fe, and Zn in F4 and F6 populations at Karnal; (ii) examine associations among these traits; and (iii) compare selected advanced lines across four test locations using location-wise observations and across-location descriptive means. The emphasis was on identifying donor lines with favourable multi-trait combinations rather than claiming formal stability. In the present study, a set of selected wheat lines advanced under

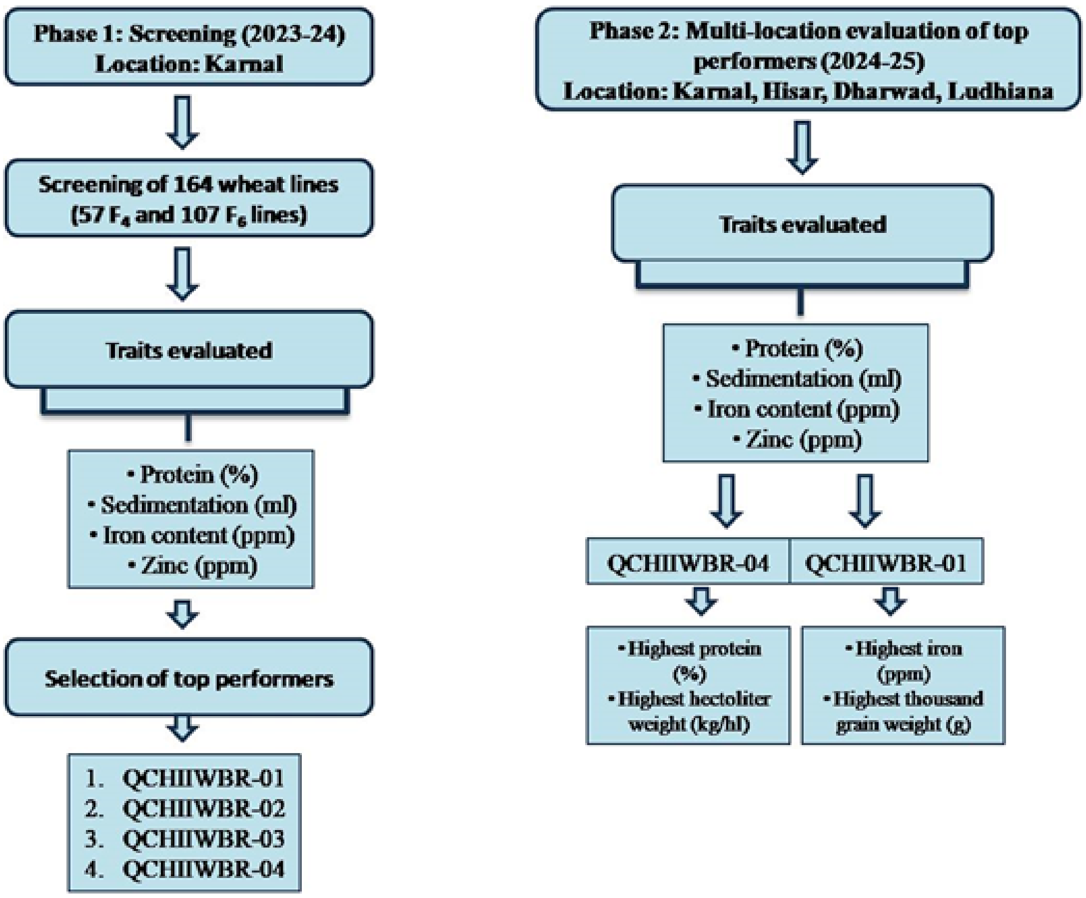


Fig. 1 Graphical representation of the experiment

CRP Biofortification breeding program was evaluated across multiple locations to assess variation in various processing and nutritional quality traits. The objective was to identify wheat genotypes possessing distinct and superior quality characteristics that could be utilized in future wheat improvement programs targeting both processing quality and nutritional enhancement.

Materials and Methods

2.1 Plant Material and Experimental Sites

The study was conducted in two stages over two crop seasons as depicted in Fig. 1. In phase I, 57 F4 and 107 F6 segregating entries derived from crosses involving high-phytase mutant derivatives in the PBW502 background and CRP Biofortification donor material were evaluated at Karnal during 2023-24 using the recommended package of practices. The F4 material was grown in 2 m two-row plots and the F6 material in 2 m four-row plots, each with 25 cm row spacing. Grain samples harvested in 2024 were analysed for quality and micronutrient traits. Based on combined superiority for grain protein, Fe, Zn, and overall grain quality profile, four promising entries were advanced to multi-location evaluation during 2024-25. The original archived breeder-selection notes did not include a formal selection index or fixed numerical thresholds, so advancement is reported here as phenotype-based selection for combined trait superiority. These lines were coded as QCHIIWBR-01, QCHIIWBR-02, QCHIIWBR-03, and QCHIIWBR-04. Two released varieties, DBW 187 and DBW 327, served as check cultivars and were coded as QCHIIWBR-05 and QCHIIWBR-06, respectively. The multi-location comparison was conducted at Hisar, Ludhiana, Dharwad, and Karnal. The pedigrees of the advanced entries were as follows: QCHIIWBR-01 (HD2967/PBW502M134//GLUPRO/3*PBW 568), QCHIIWBR-02 (HD3086/GLUPRO/3*PBW 568//PBW502M242), QCHIIWBR-03 (HD3086/GLUPRO/3*PBW 568//PBW502M242), and QCHIIWBR-04 HD3086/GLUPRO/3*PBW 568). The harvested grains were cleaned and used for the analysis of grain quality traits including protein content, micronutrient concentration (iron and zinc) and other parameters as per standard procedures.

2.2 Trait measurement

2.2.1 Hectoliter/ Test Weight

Test weight was determined using a low-cost rapid test-weight measuring device developed at ICAR-Indian Institute of Wheat and Barley Research, Karnal. Approximately 90-100 g of grain sample was used per observation, and values were expressed as kg hL^{-1}.

2.2.2 Grain Protein Content

Grain protein content was estimated using a near-infrared transmittance-based whole grain analyzer (Infratec Nova Grain Analyzer, FOSS, Denmark). Protein values were expressed on a 12% moisture basis.

2.2.3 Sedimentation Volume (SDS Test)

Sedimentation volume (SV), an indicator of gluten strength and protein quality, was determined using the 1.0 g SDS sedimentation test (Ram *et al.,* 2018). One g whole-meal flour was placed in a clean test tube and mixed with 4.0 mL distilled water. The mixture was vortexed and allowed to stand for 5 min, followed by another vortexing and resting period of 5 min. Subsequently, 12 mL SDS-lactic acid solution was added and the tube was inverted 10 times for proper mixing. After standing for 10 min, the sedimentation volume was recorded in millilitres (mL).

2.2.4 Determination of Grain Iron and Zinc

Grain iron (Fe) and zinc (Zn) concentrations were determined using energy dispersive X-ray fluorescence (ED-XRF) spectroscopy (Make: Oxford). Approximately 3.0-5.0 g of clean grain sample was placed in aluminium cups fitted with plastic inserts and analysed after selecting the appropriate elemental programme. The instrument was allowed to warm up for 15 min before analysis. To maintain unit consistency throughout the manuscript, Fe and Zn concentrations are reported as ppm grain.

2.3 Statistical analysis

For the F4 and F6 populations, descriptive statistics including mean, standard error, median, standard deviation, sample variance, skewness, kurtosis, minimum, maximum, and range were summarized for protein content, sedimentation value, Fe, and Zn. Pearson correlation coefficients among the quality traits were used to examine associations within each generation. For the multi-location set, location-wise values and grand means across Hisar, Ludhiana, Dharwad, and Karnal were compared to identify entries with consistently superior performance.

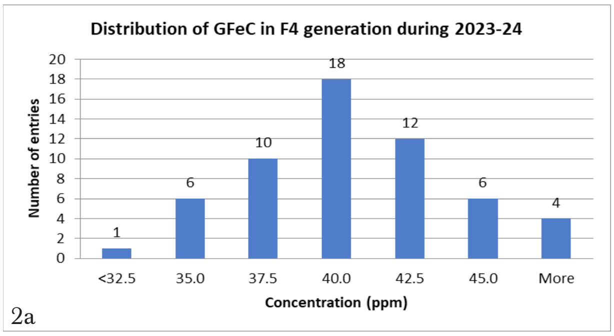
3. Results

3.1 Variability among segregating entries for processing quality and micronutrients

During 2023-24, 164 potential biofortification lines were screened at Karnal for grain protein, SDS sedimentation volume, and grain Fe and Zn concentrations. The screening material showed substantial variability for all measured traits, indicating exploitable diversity for both technological and nutritional quality. In the F4 generation (n = 57), mean protein content was 9.59% (range 7.69-12.00%), sedimentation volume 40.29 mL (29.42-52.95 mL), Fe 39.31 ppm (31.60-47.60 ppm), and Zn 32.85 ppm (23.50-48.10 ppm) (Table 1). Figure 2a,b shows the distributions of grain Fe and Zn among the F4 entries. Among the 57 entries, 22 exceeded 40 ppm Fe and three exceeded 40 ppm Zn, indicating the presence of promising recombinants for micronutrient enrichment.

Table 1. Descriptive statistics and Pearson correlation coefficients for F4 entries (Karnal, 2023-24; n = 57). Correlation coefficients marked *, ** and *** are significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Parameters	PC @ 12% MC	SV (mL)	Fe (ppm)	Zn (ppm)
Mean	9.59	40.29	39.31	32.85
Standard Error	0.13	0.73	0.47	0.61
Median	9.59	40.75	39.30	32.00
Standard Deviation	1.02	5.51	3.53	4.60
Sample Variance	1.03	30.41	12.43	21.13
Kurtosis	-0.57	-0.26	-0.20	1.14
Skewness	0.15	0.03	0.19	0.67
Range	4.31	23.53	16.00	24.60
Minimum	7.69	29.42	31.60	23.50
Maximum	12.00	52.95	47.60	48.10
Sum	546.40	2296.58	2240.80	1872.60
Count	57	57	57	57
Pearson correlation between various parameters				
Traits	**PC @ 12% MC**	**SV (mL)**	**Fe (ppm)**	**Zn (ppm)**
PC @ 12% MC	1			
SV (mL)	0.57***	1		
Fe (ppm)	0.49***	0.34**	1	
Zn (ppm)	0.70***	0.31*	0.65***	1



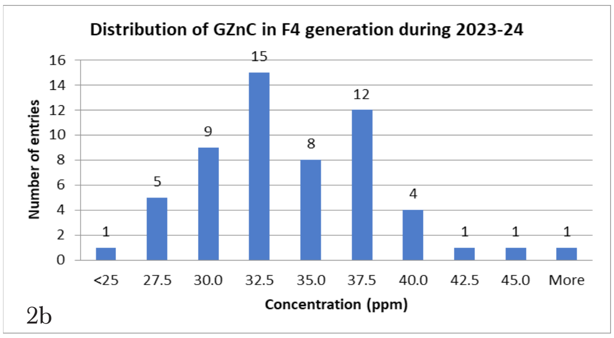


Fig. 2 Distribution of grain Fe (2a) and grain Zn (2b) among F4 entries (Karnal, 2023-24; n = 57). Units: ppm.

Similarly, the descriptive statistics for the F6 generation (n = 107) are summarized in Table 2. Protein content averaged 8.83% and ranged from 7.36 to 11.53%, while sedimentation volume averaged 37.63 mL and ranged from 25.50 to 48.59 mL. Mean Fe and Zn concentrations were 36.76 and 30.84 ppm, respectively, with ranges of 30.60-43.80 ppm for Fe and 23.10-43.20 ppm for Zn. Among the 107 entries, 16 exceeded 40 ppm Fe and one exceeded 40 ppm Zn (Figure 3a,b).

Table 2. Descriptive statistics and Pearson correlation coefficients for F6 entries (Karnal, 2023-24; n = 107). Correlation coefficients marked *, ** and *** are significant at P < 0.05, P < 0.01 and P < 0.001, respectively.

Parameters	PC @ 12% MC	SV (mL)	Fe (ppm)	Zn (ppm)
Mean	8.83	37.63	36.76	30.84
Standard Error	0.09	0.47	0.28	0.35
Median	8.72	37.70	36.40	31.00
Standard Deviation	0.93	4.85	2.92	3.66
Sample Variance	0.86	23.53	8.51	13.37
Kurtosis	-0.47	-0.20	-0.25	0.33
Skewness	0.47	0.11	0.34	0.16
Range	4.18	23.10	13.20	20.10
Minimum	7.36	25.50	30.60	23.10
Maximum	11.53	48.59	43.80	43.20
Sum	944.44	4026.62	3933.50	3300.30
Count	107	107	107	107
Pearson correlation between various parameters				
Traits	**PC @ 12% MC**	**SV (mL)**	**Fe (ppm)**	**Zn (ppm)**
PC @ 12% MC	1			
SV (mL)	0.58***	1		
Fe (ppm)	0.42***	0.42***	1	
Zn (ppm)	0.56***	0.29**	0.65***	1

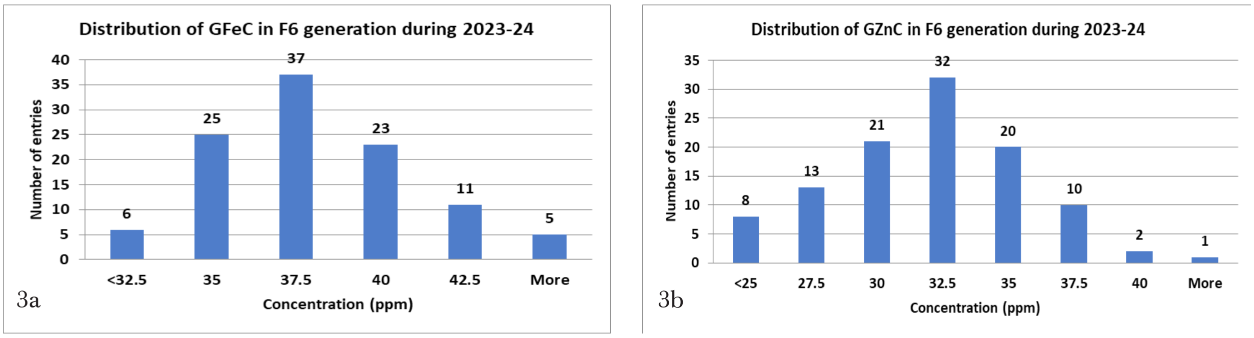


Fig. 3 Distribution of grain Fe (3a) and grain Zn (3b) among F6 entries (Karnal, 2023-24; n = 107). Units: ppm.

3.2 Trait associations in F4 and F6 populations (Cropping season 2023-24):

Trait relationships were favourable for simultaneous improvement of processing quality and micronutrient content. In the F4 population, protein content was positively correlated with sedimentation volume (r = 0.57, P < 0.001), Fe (r = 0.49, P < 0.001), and Zn (r = 0.70, P < 0.001). Iron and zinc were also positively associated (r = 0.65, P < 0.001), indicating that selection for one micronutrient is likely to improve the other (Table 1). Similar patterns were observed in the F6 generation, where protein content correlated with sedimentation volume (r = 0.58, P < 0.001), Fe (r = 0.42, P < 0.001), and Zn (r = 0.56, P < 0.001), while Fe and Zn again showed a positive association of r = 0.65 (P < 0.001) (Table 2). These repeatable positive relationships across generations support the feasibility of combined selection for grain quality and mineral density within the available breeding material.

3.3 Multi-location performance of selected advanced entries (Cropping season 2024-25):

Four superior entries selected from the initial screening were tested across Hisar, Ludhiana, Dharwad, and Karnal during 2024-25 together with the checks DBW

187 and DBW 327. The advanced material showed clear differentiation for physical grain traits, protein content, and Fe concentration (Table 3). We also evaluated the zinc content in control and test entries, but one of the locations showed very high zinc content and that is why the data of Zn for all locations have not been taken into consideration during this analysis (data not reported). Multi-location evaluation enabled the identification of two promising genotypes possessing superior grain quality traits compared to the standard checks. QCHIIWBR-04 emerged as the strongest line for processing quality-oriented traits, recording the highest mean hectoliter weight (77.0 kg hL^{-1}) and the highest mean protein content (15.3%). It was also competitive for TKW (50.8 g) and Fe concentration (45.5 ppm). QCHIIWBR-01 performed best for grain Fe concentration, with a mean of 47.3 ppm, and also recorded the highest TKW (51.3 g). Its mean protein content (14.7%) and hectoliter weight (74.4 kg hL^{-1}) were also superior or comparable to the checks. Relative to the checks, QCHIIWBR-01 and QCHIIWBR-04 consistently displayed more desirable multi-trait combinations. QCHIIWBR-04 exceeded DBW 187 and DBW 327 for both protein and test weight, while QCHIIWBR-01 surpassed both checks for Fe concentration and TKW.

Table 3. Location-wise performance and across-location descriptive means ± SE of selected advanced entries and checks during 2024-25. TKW, thousand-kernel weight; Fe, ppm grain.

Parameter	Code	Pedigree	Hisar	Ludhiana	Dharwad	Karnal	Mean ± SEM
TKW (g)	QCHIIWBR-01	HD2967/PBW502M134//GLUPRO/3*PBW 568	54.3	46.1	50.9	53.7	51.3±1.87
	QCHIIWBR-02	HD3086/GLUPRO/3*PBW 568//PBW502M242	59.8	29.3	46.7	44.7	45.1±6.25
	QCHIIWBR-03	HD3086/GLUPRO/3*PBW 568//PBW502M242	48.2	37.3	53.8	42.7	45.5±3.55
	QCHIIWBR-04	HD3086/GLUPRO/3*PBW 568	58.2	43.2	50.0	52.1	50.8±3.09
	QCHIIWBR-05	DBW 187	60.6	42.2	47.2	51.3	50.3±3.90
	QCHIIWBR-06	DBW 327	53.2	33.0	47.3	55.0	47.1±4.99
Hectoliter weight (kg hL^{-1})	QCHIIWBR-01	HD2967/PBW502M134//GLUPRO/3*PBW 568	68.6	76.7	76.4	75.8	74.4±1.93
	QCHIIWBR-02	HD3086/GLUPRO/3*PBW 568//PBW502M242	73.4	75.1	77.1	72.8	74.6±0.97
	QCHIIWBR-03	HD3086/GLUPRO/3*PBW 568//PBW502M242	70.8	72.5	78.0	70.9	73.1±1.70
	QCHIIWBR-04	HD 3086/CRP1663 HD3086/GLUPRO/3*PBW 568	74.2	77.6	77.1	78.9	77.0±0.99
	QCHIIWBR-05	DBW 187	72.1	74.2	74.9	76.8	74.5±0.97
	QCHIIWBR-06	DBW 327	73.9	69.2	74.9	78.0	74.0±1.82
Protein (% @ 12% moisture)	QCHIIWBR-01	HD2967/PBW502M134//GLUPRO/3*PBW 568	13.3	15.9	14.5	15.3	14.7±0.56
	QCHIIWBR-02	HD3086/GLUPRO/3*PBW 568//PBW502M242	10.9	13.5	15.7	13.2	13.3±0.98
	QCHIIWBR-03	HD3086/GLUPRO/3*PBW 568//PBW502M242	14.1	12.9	12.8	13.4	13.3±0.30

	QCHIIWBR-04	HD3086/ GLUPRO/3*PBW 568	16.5	15.6	15.5	13.5	15.3±0.63
	QCHIIWBR-05	DBW 187	12.3	15.0	16.2	11.6	13.8±1.09
	QCHIIWBR-06	DBW 327	14.4	15.0	16.8	10.5	14.2±1.33
Fe (ppm)	QCHIIWBR-01	HD2967/PBW502M134// GLUPRO/3*PBW 568	53.4	46.3	47.2	42.3	47.3±2.30
	QCHIIWBR-02	HD3086/ GLUPRO/3*PBW 568// PBW502M242	35.5	51.3	49.2	40.4	44.1±3.71
	QCHIIWBR-03	HD3086/ GLUPRO/3*PBW 568// PBW502M242	39.3	38.3	42.0	45.0	41.2±1.50
	QCHIIWBR-04	HD3086/ GLUPRO/3*PBW 568	42.6	50.3	47.5	41.5	45.5±2.07
	QCHIIWBR-05	DBW 187	45.2	43.3	46.1	36.3	42.7±2.22
	QCHIIWBR-06	DBW 327	37.8	40.2	48.5	39.5	41.5±2.39

4. Discussion

The present study demonstrates that segregating and advanced wheat breeding material can contain useful combinations of processing quality and micronutrient density; two target groups that are often handled separately in breeding pipelines. The broad ranges observed for protein, sedimentation volume, Fe, and Zn confirms the presence of substantial phenotypic variability, which is the foundation of effective selection. From a wheat quality perspective, the positive relationship between grain protein and sedimentation volume is particularly relevant. Grain protein content plays a crucial role in breeding wheat varieties with enhanced nutritional and processing quality. Improvement in grain protein content is a major objective in quality wheat breeding because it contributes to better dough properties, baking performance, and protein nutrition for consumers. The observed variability among genotypes may be attributed to genetic differences as well as environmental influences affecting nitrogen assimilation and protein accumulation in wheat grains. Protein concentration alone does not fully define bread-making quality, but it remains a major practical criterion in breeding and trade. Sedimentation tests complement protein estimates by capturing gluten swelling behaviour and, indirectly, dough strength. Reviews of wheat quality have consistently shown that protein content and sedimentation-related tests are valuable for assessing end-use potential, because they reflect the underlying balance of storage proteins and gluten functionality (Sharma *et al.,* 2020). In this context, the superior performance of QCHIIWBR-04 for both protein content and hectoliter weight makes it attractive as a donor for quality-oriented breeding, especially where stronger flour and better grain density are desired. The comparatively higher hectoliter weight observed in these genotypes may be attributed to the presence of well-filled, bold, and dense grains with superior packing efficiency. Hectoliter weight is an important commercial quality parameter in wheat as it directly influences grain grading and market value. Schuler *et al.* (1995) reported the importance of test weight for wheat growers due to its strong association with market grade and milling performance. Since hectoliter weight reflects grain soundness and plumpness, genotypes possessing higher values are generally preferred for trade and processing purposes. (Ram *et al.,* 2018; Khalid *et al.,* 2023; Subedi *et al.,* 2023). These proxies do not represent the full end-use quality profile, but they are useful for identifying lines worth advancing when seed quantity is limited.

The positive associations among protein, Fe, and Zn observed in both generations are encouraging for simultaneous improvement. Such relationships may arise because mineral deposition and protein accumulation can be co-influenced by genotype, grain development, and source-sink processes, and because a substantial proportion of Fe, Zn, and nitrogen-rich compounds accumulate in overlapping grain tissues. However, the present study measured phenotypic associations only; it did not test underlying physiological mechanisms or shared genetic control directly. Even so, the repeated

Fe-Zn correlation (r = 0.65 in both generations) agrees with recent biofortification literature indicating that joint improvement of both minerals is feasible in wheat breeding populations (Govindan *et al.,* 2022; Gupta *et al.,* 2023; Tanin *et al.,* 2024; Sheera *et al.,* 2025).

The multi-location phase of the study adds considerable value because environment strongly influences wheat quality and mineral accumulation. Published evaluations of biofortified wheat across target environments have shown that genotype, environment, and their interaction can all affect grain Zn and Fe, yet well-adapted lines can still be identified if the genetic rankings are reasonably stable across sites (Govindan *et al.,* 2022). In the present work, QCHIIWBR-01 and QCHIIWBR-04 maintained superior across-location means despite clear site-to-site variation. QCHIIWBR-01 combined high Fe concentration with desirable kernel weight, suggesting good grain filling and acceptable market quality. QCHIIWBR-04, in contrast, coupled the highest protein content with the highest test weight, and also maintained Fe levels above both checks. Together, these patterns indicate complementary donor value: QCHIIWBR-01 appears especially useful where grain Fe enrichment and grain size are priorities, whereas QCHIIWBR-04 offers a stronger package for processing quality with substantial Fe enrichment.

A notable strength of the study is that the selected lines did not show an obvious quality penalty relative to the checks. This is important because one concern in biofortification breeding is that selection for micronutrients may be diluted by strong selection for yield or may compromise commercial grain quality. Contemporary breeding evidence suggests that simultaneous progress is possible when micronutrient targets are mainstreamed rather than treated as separate specialty goals (Govindan *et al.,* 2022). The present lines fit that concept well, because the best entries expressed either superior or at least competitive values for the core quality traits alongside improved Fe concentration.

The identified superior genotypes exhibited stable performance across multiple environments, indicating their potential adaptability and suitability for incorporation into wheat breeding programmes aimed at improving both end-use quality and nutritional value. These genotypes may therefore serve as valuable genetic resources for the development of wheat varieties with enhanced processing quality and micronutrient enrichment.

5. Conclusion

Considerable genetic variability was observed among the evaluated wheat genotypes for important grain quality traits including hectoliter weight, protein content, thousand kernel weight and grain iron content across environments. Genotypes QCHIIWBR-01 and QCHIIWBR-04 exhibited superior performance for multiple quality parameters compared to the check varieties, indicating their potential utility in wheat quality improvement and biofortification breeding programmes. Based on the available descriptive data, QCHIIWBR-01 combined higher mean grain Fe concentration with favourable TKW, whereas QCHIIWBR-04 combined higher mean protein content and hectoliter weight with Fe levels above the checks. These lines therefore appear promising as donor material for pre-breeding and crossing programmes aimed at integrating processing quality with Fe enrichment. However, the current evidence is limited to descriptive statistics and across-location means; formal inference on stability, genotype × environment interaction, and selection efficiency will require replicated multi-environment trials with complete raw datasets, including Zn observations. Future validation should therefore use replicated multi-environment trials with complete raw datasets, including Zn observations, so that the donor value of QCHIIWBR-01 and QCHIIWBR-04 can be tested more rigorously.

Author contributions

Sunil Kumar executed overall experiment including generation advancement and multilocation trials and manuscript writing. Pooja Verma and Vipin Kumar Malik were involved in field data recording. Narender Mohan, Vijeta Sagwal and Sonika Jakhar, executed lab studies. Vanita Pandey, Om Parkash Gupta, Anuj Kumar and Dinesh Kumar helped in draft preparation and data curation. Chandra Nath Mishra extended ED-XRF facility for Fe and Zn estimation and data curation. Vijay Singh helped in field preparation. Sewa Ram and Bhudeva Singh Tyagi and Ratan Tiwari extended the overall guidance.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Use of AI Tools

No.

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