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Pushpa Jharia

Department of Genetics and
Plant Breeding, Jawaharlal
Nehru Krishi Vishwa
Vidyalaya, Jabalpur, Madhya
Pradesh, India

SK Singh

Department of Genetics and
Plant Breeding, Jawaharlal
Nehru Krishi Vishwa
Vidyalaya, Jabalpur, Madhya
Pradesh, India

Unravelling seed quality diversity through biochemical and nutritional profiling of field pea (*Pisum sativum* L.) lines

Pushpa Jharia¹, SK Singh²

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ABSTRACT

Field pea (*Pisum sativum* L.) is an important pulse crop in which improvement of seed nutritional quality is an important breeding objective. The present study was undertaken to assess variability for biochemical and nutritional quality traits among 70 field pea genotypes. The genotypes were evaluated for seed protein, carbohydrate, total sugar, reducing sugar, non-reducing sugar, zinc (Zn) and iron (Fe) concentrations. Considerable variation was observed for all traits. Protein content ranged from 16.35-23.54%, carbohydrate from 55.46-64.87%, total sugar from 5.10-7.91%, reducing sugar from 0.72-1.19%, non-reducing sugar from 4.21-7.05%, Zn from 23.16-36.87 mg kg⁻¹ and Fe from 40.71-50.98 mg kg⁻¹. The highest protein, carbohydrate, total sugar, reducing sugar and non-reducing sugar contents were recorded in HFP 94-12 (23.54%), HUV-12 (64.87%), KPMR-423 (7.91%), DDR-54 (1.19%) and NDVP-20 (7.05%), respectively. Among micronutrients, KPMR-504 (36.87 mg kg⁻¹) recorded the highest Zn, while B-22 (50.98 mg kg⁻¹) recorded the highest Fe concentration. The observed diversity identifies promising germplasm resources for trait-specific nutritional improvement. These genotypes may serve as potential parents in multi-trait breeding programmes aimed at combining enhanced seed nutritional quality with desirable agronomic performance. Further evaluation across environments is required to confirm the consistency and breeding value of these quality traits.

Keywords: *Pisum sativum* L., Nutritional quality, Protein, Carbohydrates, Seed sugars, Zinc, Iron, Plant breeding, Donor genotypes

1. Introduction

Field pea (*Pisum sativum* L. var. *arvense*) is a significant cool-season grain legume, valued for its protein-rich seeds, substantial carbohydrate content, and role in enhancing food and nutritional security. Dry seeds contain substantial amounts of protein, starch, soluble sugars, and essential minerals, making seed composition a critical determinant of grain quality and end-use value. Typically, field pea seeds contain about 25% protein and 70% carbohydrates; however, nutritional and mineral compositions vary considerably among diverse germplasm accessions (Gayacharan *et al.*, 2023; Anand *et al.*, 2024) [2, 1].

The biochemical composition of field pea seeds is a pivotal aspect of genetic diversity and a valuable selection criterion in quality-oriented breeding programmes. Substantial variation in protein, carbohydrate, sugar, and mineral nutrient contents has been documented within pea germplasm, thereby presenting opportunities to identify genotypes with superior nutritional profiles (Gayacharan *et al.*, 2023; Sharma *et al.*, 2015) [2, 3]. Recent investigations of field pea cultivars grown under Indian agro-climatic conditions have further revealed differences in protein, carbohydrate, and soluble carbohydrate fractions, underscoring the utility of biochemical characterisation for identifying valuable genetic resources (Tewari *et al.*, 2025) [4].

Protein is a principal determinant of the nutritional value of field pea and an important target for nutritional improvement through breeding. Genetic variation in seed protein enables the identification of high-protein genotypes and integration of nutritional traits into breeding programmes. Protein variation is also associated with other seed-quality traits, including starch and processing characteristics, highlighting the need to evaluate protein with other biochemical constituents (Sharma *et al.*, 2015; Windsor *et al.*, 2024) [3, 5].

Carbohydrates constitute a major fraction of field pea seeds and provide an important source of dietary energy, with starch as the main storage carbohydrate and soluble sugars contributing to seed quality. Genetic variation in carbohydrate composition facilitates the selection of genotypes with desirable biochemical profiles. Recent characterisation of Indian field pea cultivars has demonstrated variation in carbohydrate, protein and soluble carbohydrate fractions (Tewari *et al.*, 2025) [4].

Sugars, including reducing and non-reducing sugars, form an important part of the soluble carbohydrate pool and contribute to the biochemical and nutritional quality of pea seeds. Genetic variation in sugar-related traits supports their use in genotype characterisation and quality-oriented breeding. Therefore, simultaneous

Corresponding Author: Pushpa Jharia

Department of Genetics and
Plant Breeding,
Jawaharlal Nehru Krishi
Vishwa Vidyalaya,
Jabalpur, Madhya Pradesh,
India

evaluation of total, reducing and non-reducing sugars provides a broader assessment of biochemical diversity than total carbohydrate alone (Thakur *et al.*, 2024) [6]. Micronutrient concentration, particularly that of iron (Fe) and zinc (Zn), constitutes a crucial aspect of the nutritional quality of field pea. Natural variation in mineral accumulation within field pea germplasm offers prospects for the enhancement of micronutrient density through targeted genetic selection. Previous research has documented significant differences in Fe and Zn concentrations among field pea accessions, with certain accessions exhibiting relatively elevated levels of these minerals (Sharma *et al.*, 2015) [3]. Similarly, Indian germplasm resources have been identified with favourable mineral profiles, highlighting the potential of field pea for nutritional improvement and biofortification initiatives (Gayacharan *et al.*, 2023) [2].

The existence of substantial genetic diversity in field pea provides a basis for selecting genotypes with desirable combinations of agronomic and quality attributes (Pratap *et al.*, 2024) [7]. From a plant-breeding perspective, simultaneous evaluation of protein, carbohydrates, sugars and mineral nutrients provides a more comprehensive assessment of nutritional quality than consideration of any single biochemical constituent. Genotypes with favourable combinations of high protein and carbohydrate contents, desirable sugar composition, and enhanced Fe and Zn concentration can serve as valuable genetic resources and potential parents for improving nutritional quality. Such characterization complements conventional selection based on yield and morphological traits and aids the identification and utilization of elite germplasm.

Despite the recognised nutritional importance of field pea and the documented variability in individual seed-quality attributes, integrated evaluation of genotypes for major macro nutritional, sugar, and micronutrient traits remains important under Indian conditions. Assessing total protein, total carbohydrate, total sugar, reducing sugar, non-reducing sugar, zinc, and iron can provide an integrated measure of biochemical diversity and help identify genotypes with desirable nutritional profiles. Therefore, this investigation evaluated field pea genotypes for total protein, total carbohydrate, total sugar, reducing sugar, non-reducing sugar, zinc, and iron contents to assess biochemical variation and identify promising genotypes for future breeding programmes aimed at improving the nutritional quality of field pea.

2. Materials and Methods

A total of 70 field pea (*Pisum sativum* L.) genotypes, including three checks, were evaluated at the Breeder Seed Production Unit, Department of Genetics and Plant Breeding, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh, during the Rabi season 2020-21. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications and followed standard agronomic practices throughout the crop period.

Mature, well-dried seed samples of each genotype were collected and used to estimate total protein, total carbohydrate, total sugar, reducing sugar, non-reducing sugar, and zinc (Zn) and iron (Fe) contents. Seed samples were dried, powdered and subjected to biochemical analysis following the respective standard procedures. Total protein (%) was estimated by the Micro-Kjeldahl digestion and distillation method as described by AOAC (1984). Nitrogen content was determined by acid digestion followed by distillation and titration, and protein percentage was calculated using the appropriate conversion factor.

Total carbohydrate (%) was estimated by the anthrone method of Yemm *et al.* (1954) [8]. The absorbance of the developed colour was recorded at 620 nm using a UV-visible spectrophotometer, and carbohydrate content was expressed as a percentage on a dry-weight basis. Reducing and total sugars (%) were estimated by the Lane and Eynon volumetric method as described by Ranganna (1986) [9], using Fehling's solution and methylene blue as an indicator. Reducing sugar was determined directly from the clarified seed extract, whereas total sugar was estimated after inversion of sucrose. Non-reducing sugar

(%) was calculated as the difference between total sugar and reducing sugar.

For Zn and Fe estimation, seed samples were subjected to nitric-perchloric acid digestion followed by measurement using Atomic Absorption Spectroscopy (AAS), following Boline *et al.* (1977) [10]. Zinc and iron concentrations were recorded at 213.9 and 248.3 nm, respectively, and expressed as mg kg⁻¹ of seed sample.

3. Results and Discussion

Significant phenotypic diversity was observed among the 70 field pea genotypes for all biochemical and nutritional quality traits evaluated, indicating the presence of useful variability within the germplasm for quality-oriented selection. The magnitude of variation differed among traits, with substantial differences observed in seed protein, carbohydrate, sugar fractions, zinc and iron concentrations. Such diversity provides an opportunity to identify trait-specific donor genotypes and to incorporate nutritional quality attributes into field pea improvement programmes.

3.1 Protein content

Seed protein content ranged from 16.35 to 23.54%, with a mean of 19.37%. The maximum protein content was recorded in HFP 94-12 (23.54%), followed by KFP-151 (22.91%), DDR-23 (22.12%), B-22 (22.08%) and VL-1 (21.97%). The observed range was comparable with earlier reports in field pea germplasm. Srivastava *et al.* (2009) [11] reported soluble protein contents of 16.3–22.9% among 12 field pea genotypes, with JP 885, KFP 103 and Rachna showing relatively high protein concentrations. Similarly, Mishra *et al.* reported a protein range of 18.20–24.67% among 30 field pea genotypes, confirming substantial variation for this nutritional trait.

The identification of HFP 94-12 with a protein concentration of 23.54% places this genotype within the upper range reported for field pea germplasm and indicates its potential value in protein-oriented breeding. The occurrence of KFP-151, DDR-23, B-22 and VL-1 among the higher-protein genotypes further demonstrates the availability of multiple sources that could be exploited for recombination. Franck *et al.* (2024) [12], in a multi-location evaluation of seven pea cultivars, reported significant cultivar effects on grain protein concentration but no significant cultivar × location interaction for protein, indicating relatively consistent genotypic differences under their test environments. In contrast, Soncin Alfaro *et al.* (2025) [13], while evaluating eight yellow pea genotypes across four growing locations, observed significant effects of both genotype and growing location on seed protein concentration, with a significant genotype × location interaction, demonstrating that environmental conditions can modify the expression of protein content in field pea.

Thus, HFP 94-12, KFP-151, DDR-23 and B-22 may be considered promising parental sources for improving seed protein concentration. However, their effectiveness as stable donor parents should be confirmed through multi-environment evaluation and by assessing their performance in combination with yield and other agronomic traits.

3.2 Carbohydrate content (%)

Total carbohydrate content varied from 55.46 to 64.87%, with a mean of 60.08%. HUPV-12 (64.87%) recorded the highest value, followed by KPMR-503 (62.96%), KPMR-502 (62.10%), KPMR-144 (62.08%) and Kalamatar (61.98%). Mishra *et al.* reported a comparable range of 57.95–63.53% carbohydrates among 30 field pea genotypes, with significant genotypic differences for the nutritional traits studied.

Srivastava *et al.* (2009) [11] also observed considerable variation in seed starch (41.3–51.2%) and identified HUP 2, KPMR 144, KFP 103 and IPF 14 as relatively high-starch genotypes. Although starch concentration and total carbohydrate estimated by the anthrone method are not equivalent analytical parameters, the consistency of genotypic differences across studies supports the existence of exploitable variation in carbohydrate-rich seed composition.

The relatively high carbohydrate concentration of HUV-12 therefore identifies it as a potential source for improving this trait. The occurrence of KPMR-144 among the higher-carbohydrate genotypes in the present study is also of interest because this genotype has previously been reported for relatively high starch and soluble sugar content.

3.3 Total sugar content (%)

Total sugar content ranged from 5.10 to 7.91%, with a mean of 6.69%. KPMR-423 (7.91%) recorded the highest concentration, followed by KPMR-144, Kalamatar, KPMR-504 and Kashi Samridhi, each recording 7.62%. [Srivastava et al. \(2009\)](#) [11] reported soluble sugar concentrations ranging from 6.1 to 7.8% among 12 field pea genotypes, with KPMR 144, IPF 14 and IPFD 98-9 showing relatively higher sugar concentrations.

The present results are therefore within the broad range reported previously. The repeated occurrence of KPMR-144 among higher-sugar material is noteworthy, although direct numerical comparisons should be interpreted cautiously because sugar fractions and analytical procedures may differ among studies. The present variation indicates that selection for seed sugar composition can be incorporated into quality-oriented breeding programmes.

KPMR-423 was the most promising genotype for total sugar in the present material and may serve as a potential donor for this trait. KPMR-144 and Kalamatar may also be useful in crossing programmes where high sugar concentration is targeted together with other desirable quality or agronomic attributes.

3.4 Reducing sugar content (%)

Reducing sugar concentration varied from 0.72 to 1.19%, with a mean of 0.90%. The highest value was observed in DDR-54 (1.19%), followed by KPMR-402 (1.12%), Kalamatar (1.08%), Jayanti (1.07%) and KPMR-503 (1.00%). The observed variation indicates differences among genotypes in the accumulation and partitioning of reducing sugar fractions.

Compared with protein, carbohydrate and mineral concentration, relatively limited breeding literature is available specifically on reducing sugar variation in field pea germplasm. Consequently, the present results provide additional information on biochemical diversity within the evaluated material. The identification of DDR-54 as the highest-reducing-sugar genotype indicates its potential utility where this specific biochemical attribute is included as a breeding target.

However, reducing sugar should preferably be considered as one component of a multi-trait selection strategy, rather than as an independent criterion, because improvement in a single biochemical constituent may not necessarily coincide with improvement in yield, seed size, protein or micronutrient concentration.

3.5 Non-reducing sugar content (%)

Non-reducing sugar content ranged from 4.21 to 7.05%, with a mean of 5.78%. NDVP-20 (7.05%) recorded the highest concentration, followed by Jayanti (6.78%), KPMR-504 (6.71%), Kashi Samridhi (6.71%) and KPMR-144 (6.71%). The variation observed among genotypes indicates differential accumulation of non-reducing sugar fractions.

The contrasting performance of KPMR-423 for total sugar and NDVP-20 for non-reducing sugar illustrates that the component sugar fractions do not necessarily follow the same genotype ranking. Such variation may be useful in breeding programmes requiring targeted modification of seed biochemical composition. NDVP-20 can therefore be considered a potential source for higher non-reducing sugar concentration, while KPMR-504, Kashi Samridhi and KPMR-144 may provide additional sources for this trait.

3.6 Zinc content

zinc concentration exhibited considerable variation among the evaluated genotypes, with a mean of 30.35 mg kg⁻¹ and a range of 23.16–36.87 mg kg⁻¹. The highest zinc concentration was recorded in KPMR-504 (36.87 mg kg⁻¹), followed by HFP94-13 (36.80 mg kg⁻¹), KPMR-485 (36.80 mg kg⁻¹), Dhanauli Batri (36.64 mg kg⁻¹) and DDR-54 (36.55 mg kg⁻¹). The observed variation is consistent with previous reports demonstrating substantial genetic diversity for mineral accumulation in pea.

Dissanayaka *et al.* evaluated diverse pea accessions across multiple location-years and reported substantial variation in seed Zn concentration, with a mean of 29.42 µg g⁻¹ and a range of 12.83–51.47 µg g⁻¹. Their results also demonstrated significant accession, year and location effects, indicating that both genetic and environmental factors contribute to variation in seed mineral concentration. [Franck et al. \(2024\)](#) [12] similarly reported significant cultivar and location effects on pea grain minerals, with location generally exerting a stronger influence than cultivar for several mineral traits.

3.7 Iron content

Iron concentration ranged from 40.71 to 50.98 mg kg⁻¹, with a mean of 47.53 mg kg⁻¹. B-22 (50.98 mg kg⁻¹) recorded the highest Fe concentration, followed by NDVP-4 (50.70 mg kg⁻¹), Dhanauli Batri (50.64 mg kg⁻¹), Somnath Pili Batri (50.43 mg kg⁻¹) and RP-3 (50.27 mg kg⁻¹).

The mean Fe concentration in the present study was close to the 50.70 µg g⁻¹ mean reported by Dissanayaka *et al.* across diverse pea accessions evaluated over multiple location-years. Their reported Fe range (29.22–90.53 µg g⁻¹) was considerably wider than that observed in the present material, demonstrating the potential breadth of genetic variation available within broader pea germplasm collections. The same study also established significant accession and environmental effects on seed mineral concentration.

Table 1. Biochemical and micronutrient analysis of field pea genotypes

S.N.	Genotype	Total protein (%)	Carbohydrate (%)	Total Sugar (%)	Reducing Sugar	Non-Reducing sugar	Zinc Content (mg/kg)	Iron Content (mg/kg)
1	DDR-39	18.10	61.65	6.10	0.79	5.31	35.20	49.03
2	HFP94-13	21.70	58.87	6.49	0.90	5.59	36.80	46.93
3	VL-1	21.97	60.14	6.58	0.81	5.77	31.66	48.17
4	P ⁻³	18.14	56.12	6.14	1.00	5.14	33.94	46.63
5	Late Sown	18.20	57.84	6.89	0.90	5.99	25.92	48.11
6	PP-14	19.12	60.12	6.41	0.88	5.53	34.83	47.07
7	JP-80	19.78	60.04	6.87	0.89	5.98	30.61	45.11
8	Triple branching	18.63	59.36	6.35	0.91	5.44	28.06	49.38
9	DDR-23	22.12	61.75	6.87	0.89	5.98	24.89	49.67
10	VL-3	19.25	60.16	7.51	0.97	6.54	35.34	47.22
11	PP-86	20.61	60.91	6.94	0.80	6.14	30.22	50.09

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S.N.	Genotype	Total protein (%)	Carbohydrate (%)	Total Sugar (%)	Reducing Sugar	Non-Reducing sugar	Zinc Content (mg/kg)	Iron Content (mg/kg)
12	RP-3	16.60	58.45	7.12	0.91	6.21	24.72	50.27
13	Aman	17.52	58.36	6.10	0.89	5.21	31.53	46.85
14	B-22	22.08	60.84	7.08	0.88	6.20	34.74	50.98
15	DDR-52	21.69	61.12	6.65	0.96	5.69	31.48	48.15
16	IPFD 12-2	19.81	56.27	7.16	0.88	6.28	25.73	46.99
17	DDR-54	21.62	61.69	6.41	1.19	5.22	36.55	48.54
18	KPMR-302	19.85	61.21	6.78	0.95	5.83	30.82	46.92
19	KPMR-504	19.21	61.65	7.62	0.91	6.71	36.87	48.34
20	KPMR-402	20.95	60.78	7.10	1.12	5.69	28.81	48.60
21	KPMR-420	21.84	61.82	7.52	0.97	6.28	33.23	47.10
22	KPMR-423	18.78	60.56	7.91	0.86	5.22	36.24	49.80
23	KPMR-485	21.96	55.46	6.70	0.81	5.83	36.80	46.64
24	KPMR-144	18.89	62.08	7.62	0.84	6.71	32.33	47.23
25	KPMR-327	20.48	61.02	6.56	0.89	5.98	23.93	48.06
26	JP-885 (Purple)	19.83	60.07	6.10	0.89	6.55	25.62	49.39
27	NDVP-20	20.62	61.78	6.41	0.82	7.05	30.03	44.12
28	Double branching	17.10	58.10	6.10	0.81	5.89	25.21	49.04
29	Jayanti	16.91	60.47	6.17	1.07	6.78	32.06	48.14
30	JFP-27	19.97	60.65	7.46	0.90	5.67	36.33	45.21
31	JFP 99-25	20.86	61.74	6.59	0.87	5.72	24.24	46.07
32	IPF 99-25	19.34	59.69	7.49	0.94	6.55	30.23	44.53
33	PP-96	19.37	59.32	6.32	0.89	5.43	27.27	40.71
34	HUP-2	20.74	60.21	7.48	0.92	6.56	34.55	46.65
35	Kashi samridi	19.67	60.74	7.62	0.91	6.71	33.78	48.73
36	KPMR-30	19.10	60.82	6.87	0.89	5.98	35.46	46.70
37	DDR-44	16.89	60.34	7.19	0.92	6.27	30.31	45.95
38	Kalamatar	20.95	61.98	7.62	1.08	6.54	30.10	49.06
39	DDR-43	20.73	59.19	6.81	0.72	6.09	34.99	49.22
40	DDR-27	20.41	60.27	6.10	0.90	5.20	31.13	45.91
41	JM 91-01	18.15	59.98	6.75	0.91	5.84	29.67	44.07
42	JM-6	20.78	61.65	6.65	0.89	5.76	27.54	48.61
43	KPMR-486	18.50	59.36	7.20	0.90	6.30	25.92	47.56
44	IPFD 10-12	19.85	60.89	6.54	0.90	5.64	24.47	45.48
45	KPMR-502	17.87	62.10	6.78	0.91	5.87	35.58	49.51
46	KPMR-400	20.94	61.41	6.14	1.00	5.14	32.40	47.83
47	HUVP-12	17.25	64.87	6.19	0.84	5.35	23.51	49.96
48	IPFD 11-5	18.62	60.28	6.32	0.89	5.43	34.46	48.97
49	HFP 94-13	20.12	61.83	7.05	0.88	6.17	25.80	45.25
50	Batra Patiram	17.64	58.95	6.84	0.87	5.97	33.27	49.84
51	Kali Batri	17.85	59.10	5.49	0.88	4.61	32.20	45.16
52	KFP-151	22.91	61.85	6.87	0.89	4.78	34.72	41.50
53	HFP 94-12	23.54	59.18	6.69	0.90	5.98	23.28	48.73
54	Batra Chhoti Badwah	16.35	58.96	5.19	0.89	5.79	36.34	46.33
55	Shikha	18.69	58.10	6.48	0.90	4.30	34.05	45.85
56	KPMR-503	21.94	62.96	7.36	1.00	5.58	27.15	48.71
57	Chhoti safed anju	17.65	57.63	5.10	0.89	6.36	27.54	47.70
58	PP-155	20.96	60.12	6.98	0.91	4.21	30.11	49.77
59	Somnath Pili Batri	17.35	58.10	6.85	0.89	5.10	33.21	50.43
60	DDR 94-14	19.73	61.13	7.47	0.87	6.07	33.55	47.94
61	Atru Matar	16.56	58.74	6.12	0.88	5.24	23.57	47.39
62	<i>Dhanauli batri</i>	17.85	58.89	6.32	0.88	5.44	36.64	50.64
63	Matar Rangpur	17.32	59.08	5.12	0.90	4.22	35.20	46.10

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S.N.	Genotype	Total protein (%)	Carbohydrate (%)	Total Sugar (%)	Reducing Sugar	Non-Reducing sugar	Zinc Content (mg/kg)	Iron Content (mg/kg)
64	Batana mulchand	17.78	59.16	6.49	0.89	5.60	23.67	45.58
65	Gol batra tenduna	17.96	58.85	6.32	0.86	5.46	23.16	49.68
66	Rachna	18.48	60.14	7.48	0.85	6.63	25.40	47.60
67	Dhan batri	17.32	59.78	6.87	0.81	5.29	24.22	48.10
68	Gol batri Amar	18.02	59.14	6.18	0.88	6.06	23.62	44.87
69	NDVP-4	19.21	60.47	6.85	0.82	5.30	24.70	50.70
70	Safed batri gudda	17.39	58.94	5.94	0.84	5.10	27.22	46.24

4. Conclusion

The present investigation revealed substantial phenotypic variability for seed nutritional quality among the 70 field pea genotypes, providing useful diversity for quality-oriented breeding and parent selection. Protein content ranged from 16.35–23.54%, carbohydrate from 55.46–64.87%, total sugar from 5.10–7.91%, reducing sugar from 0.72–1.19%, non-reducing sugar from 4.21–7.05%, Zn from 23.16–36.87 mg kg⁻¹ and Fe from 40.71–50.98 mg kg⁻¹. HFP 94-12, HUVF-12, KPMR-423, DDR-54, NDVP-20, KPMR-504 and B-22 were identified as potential sources for high protein, carbohydrate, total sugar, reducing sugar, non-reducing sugar, Zn and Fe, respectively. These genotypes can serve as potential parents in trait-specific and multi-trait breeding programmes. Since biochemical traits were evaluated at a single time point, their stability and association with yield and other agronomic traits require further validation before their wider breeding utilization.

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