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SCREENING OF PROMISING MUSTARD GENOTYPES FOR IRON AND ZINC ACQUISITION

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SUMMARY

Zinc (Zn) and iron (Fe) deficiency prevalence severely limit the mustard productivity, causing stunted growth, chlorosis, reduced yields and poor grain quality due to low availability of these micronutrients. This study aimed to identify high Zn and Fe acquisition efficient mustard genotypes under both (Zn and Fe) deficient condition separately. In order to distinguish tolerance 20 mustard genotypes, assess yield response, nutrient uptake and efficiency indices to identify superiority of nutrient-efficient genotype for breeding purposes and high-yielding varieties suitability under Fe and Zn deficient soils., two field experiments were conducted separately one on Fe deficient soil (pH 7.9, DTPA-Fe 4.34 mg/kg) and another on Zn deficient soil (pH 8.3, DTPA-Zn 0.30 mg/kg. In Fe deficient soil, iron sulphate @ 50 kg/ha was applies applied in half portion of the field and another is kept as untreated controls. Likewise, in Zn deficient soil, zinc sulphate heptahydrate @ 25 kg/ha was also applied in similar manner. Results of these studies, revealed that genotype RH 1529 was found the most Fe-tolerant genotype with 1.8% yield response, iron efficiency index (IEI) 98.22 and iron efficiency (IE) 94.11. the genotype RH 1499-7 exhibited peak Fe uptake (428.75 g/ha treated). For Zn stress, RH 119 showed highest tolerance with yield 2.4% response, zinc efficiency index (ZEI) 97.51 and zinc efficiency (ZE) 73.74. In Zn treated soil, genotype RH 725 achieved highest yield (25.4 q/ha with yield response of 3.4%). All genotypes classified as tolerant to semi-tolerant (yield responses <25%), with no susceptible was observed. These results imply that prioritizing genotypes like RH 1529, RH 1430 (Fe-efficient) and RH 119, RH 725 (Zn-efficient) in breeding programs, combined with targeted agronomic biofortification, can enhance mustard stability in deficient environments may narrow productivity gaps, boost farmer incomes and improve nutritional security in micronutrient-poor regions.

Key words: Genotypes, response, uptake, harvest index, content, zinc and iron

Mustard is a major oilseed crops and had a strong socio-economic importance in South Asia and it also cover significant acreage worldwide (Verma *et al.*, 2024). Nevertheless, globally rapeseed-mustard productivity remains below its potential and many parts of India exhibits a notable productivity gap when compared with leading rapeseed-mustard producer countries (Heidari *et al.*, 2025; Meena *et al.*, 2018). Mustard is facing significant threats from micronutrient imbalances, particularly zinc (Zn) and iron (Fe) deficiency/excesses that may prevalent in intensively cropped soils (Verma *et al.*, 2024). Both these nutrients required for optimal plant growth and had a special role human nutrition. In plants, zinc is essential for enzyme activation, protein synthesis and maintaining membrane integrity, while iron is crucial for chlorophyll formation, electron transport and energy transfer processes during photosynthesis (Dhaliwal *et al.*, 2021a; Devatwal *et al.*, 2023). Deficiencies of either micronutrient can lead to stunted plant growth, chlorosis, reduced grain yield and compromised grain quality. The deficiency of Zn and Fe were more prevalent in calcareous and alkaline soils, which are common in many wheat-growing regions worldwide (Cakmak, 2008). Mustard is also considered as staple food for a large portion of the population, especially in developing countries. However, the grain is often low in bioavailable zinc and iron, contributing to widespread micronutrient malnutrition, sometimes referred to as "hidden hunger". This is a serious health issue, as zinc and iron deficiency are linked to impaired immune function, anemia and developmental delays in children (Lafiandra et al., 2014; Kumar *et al.,* 2014). Recently genome-wide analysis and multi-environment trials revealed substantial genetic variation for key agronomic and quality traits of Indian mustard, indicating clear opportunities to breed genotypes that combine high yield potential with trait stability across environments (Barbero *et al.,* 2025).

There are several studies on agronomic bio-fortification and novel delivery forms (for e.g. chelates, nanoparticles) have been demonstrated to increase Zn and Fe content in tissues and to improve physiological status and antioxidant defences, yet responses are often genotype-dependent and interact with environment and management (Dhaliwal *et al.,* 2022). Because genotype × environment × management (G×E×M) interactions strongly influence both yield and nutrient accumulation, integrating genetic selection with targeted Zn/Fe management is a promising route to produce mustard cultivars that are both high-yielding and micronutrient-efficient under Zn/Fe stress (Patel *et al.,* 2024; Dhaliwal *et al.,* 2021b). This study therefore evaluates the performance and stability of selected mustard genotypes under Zn and Fe stress to identify genotypes with superior nutrient-use efficiency and stable seed yield and quality. Screening mustard genotypes under Zn and Fe stress is important to identify nutrient-efficient, high-yielding lines that maintain productivity and grain quality in deficient soils. It also provides suitable parents and selection criteria for breeding biofortified, stress-resilient mustard cultivars that help combat Zn and Fe malnutrition.

MATERIALS AND METHODS

To assess the response of mustard genotypes to Zn and Fe stress, two field experiments separately were conducted on Fe-deficient and Zn-deficient soils of villages Bhiwani Rohila and Kajla of Hisar district. Prior to conducting these experiments, the soils of said villages were tested for their respective element (i.e. Zn and Fe) status, thereby providing an ideal environment for evaluating mustard genotypes under stress condition and how the different mustard genotype cope with Fe and Zn deficiency.

Before sowing, initial soil physico-chemical properties were measured to establish the baseline characteristics of the experimental field. The soil of Bhiwani Rohila village exhibited a pH value of 7.9, with an electrical conductivity (EC) of 0.16 dS/m. The organic carbon (OC) content was 0.33% and the soil was found to be free of calcium carbonate (CaCOƒ). Texturally, the soil was classified as loamy sand, which is known for its moderate water retention and drainage properties. Most importantly, the soil was deficient in DTPA-extractable Fe, with a concentration of mere 4.34 mg/kg, confirming its suitability for Fe stress studies. Whereas, the initial soil properties of village Kajla revealed that pH of experimental field was 8.3, EC: 0.42 dS/m, OC: 0.53%, $CaCO_3$ present in traces and texturally the soil was loamy sand. The soil of the experimental field was deficient (0.30. mg/kg) in DTPA-extractable Zn.

Twenty-one promising mustard genotypes were selected for evaluation based on their potential for stress tolerance and agronomic performance. The experiment was laid out in a randomized block design, with each genotype subjected to two Fe treatments: control (without Fe considered here as Fe-untreated) and application of ferrous sulphate @ 50 kg/ha (considered here as Fe-treated). This approach allowed for a direct comparison of genotype performance under both Fe-deficient and Fe-supplemented conditions. Similarly, the performance of these genotypes was also evaluated for Zn under control (without Zn considered here as Zn-untreated) and application of zinc sulphate heptahydrate (21%) @ 25 kg/ha (considered here as Zn-treated).

Standard agronomic practices were followed throughout the growing season, including timely irrigation, weed management and pest control, to ensure that observed differences were primarily attributable to Fe and Zn availability. The crop was grown up to maturity and harvested. After harvesting, grain and straw yield for each genotype and nutrients were recorded separately in order to assess their impact on productivity under deficient/sufficient condition.

After harvesting, grain and straw samples were collected for laboratory analysis. The Fe and Zn content in the grain was recorded to determine the efficiency of Zn and Fe along with their uptake and translocation in each genotype. This provided insights into the ability of different mustard genotypes to utilize available Zn and Fe under treated and untreated conditions. The following parameters were calculated (Graham, 1984) using formula:

Iron/zinc uptake in grain =Grain yield ×Grain Fe/Zn content

Harvest index (HI) = Grain yield/ Biological yield

Iron/zinc efficiency index = (Grain yield at low Zn)/ (Grain yield at high Zn) ×100

Iron/zinc efficiency = (Grain Zn uptake at low Zn)/ (Grain Zn uptake at high Zn) ×100

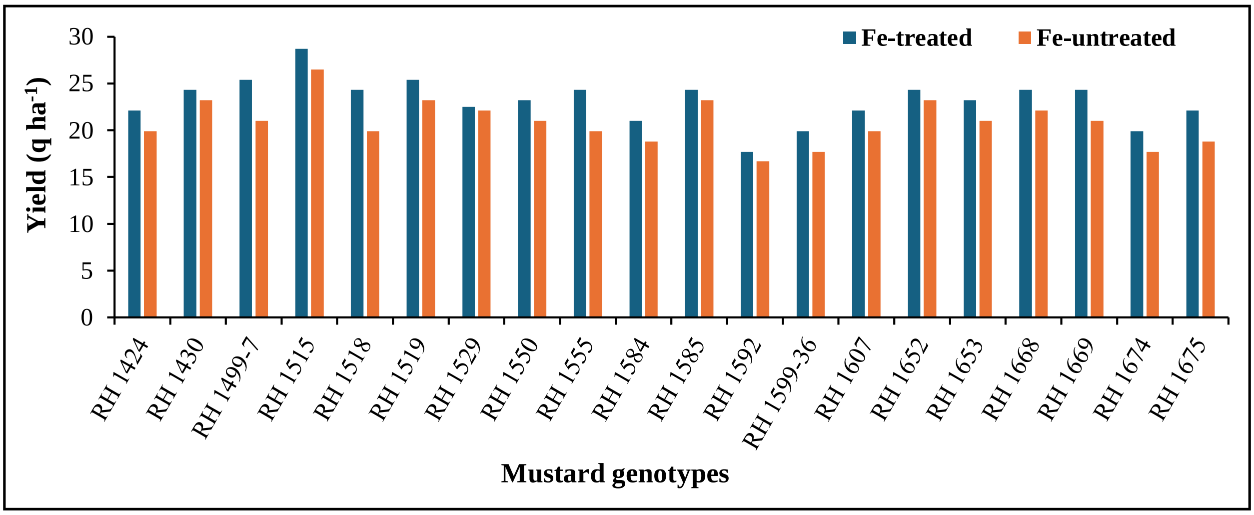

Fig. 1. Effect of Fe application on grain yield of 20 mustard genotypes.

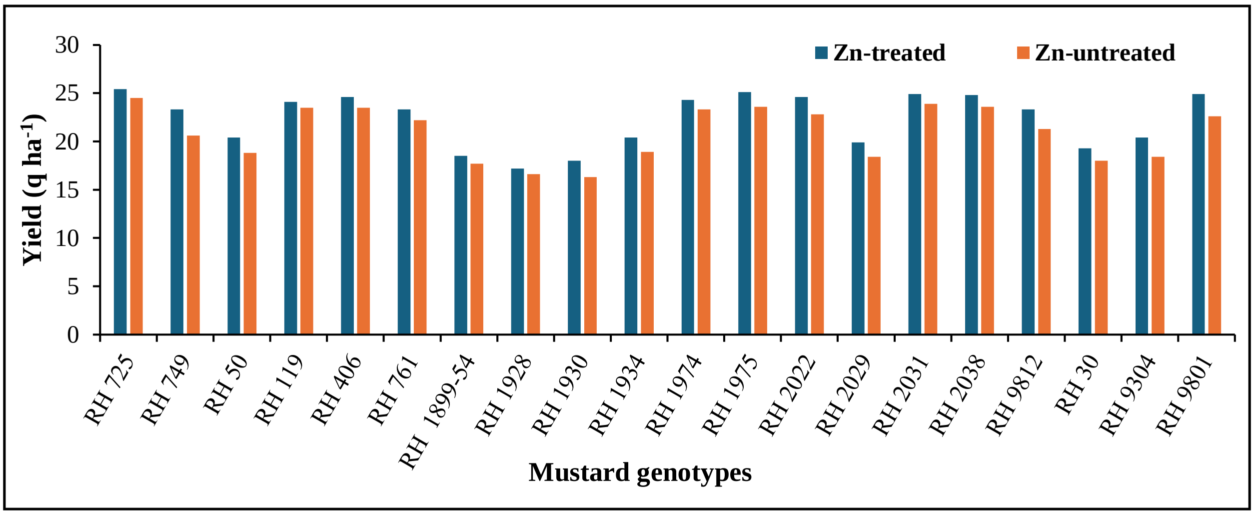

Fig. 2. Effect of Zn application on grain yield of 20 mustard genotypes.

RESULTS AND DISCUSSION

The data pertaining to seed yield of mustard genotypes under Fe and Zn- treated condition are depicted in fig.1 whereas, content of nutrient (Fe) and percent response given in Tables 1. Inflated yield of genotype RH 1515 was observed in Fe-treated, with the extant of 8.30% as compared to Fe-untreated (26.5 q/ha). Whereas, genotype RH 1592 yielded lowest of 17.7 and 16.7 q/ha among Fe-treated and Fe-untreated condition, respectively. The Fe content was highest in genotype RH 1499-7 in Fe-treated condition which consist about 84.89% higher than Fe-untreated condition. Yield response of genotype RH 1529 was 1.80% that reflects highest tolerance for Fe among the all genotypes followed by RH 1430 (4.7%) and RH 1585 and RH 1652 (4.8%, both) whereas, genotype shown lowest tolerance for Fe was RH 1518 (22.2%). The genotype RH1499-7 reflects highest (123.62%) Fe uptake under Fe-treated condition over Fe-untreated. The harvest index varied from 48-49 wheras, iron efficiency index (IEI) and iron efficiency (IE) of value (98.22 and 94.11, respectively) was found highest for RH 1529 (Table 3).

Highest mustard grain yield was recorded for RH 725 under Zn-treated condition and it was about 3.67% higher over Zn-untreated condition (Fig. 2). The seed Zn content was highest (37.3 mg/kg) in treated plot for RH 1975 whereas, lowest (18.9 mg/kg) was observed in Zn-untreated condition for genotype RH 749 (Table 2). However, highly tolerant genotype against Zn stress in terms of response was in genotype RH 119 (2.4%) followed by RH 725 (3.4%), RH 1928 (3.5%) and RH2031 (4.20%). In Zn-untreated condition, highest Zn uptake (84.8 g/ha)

TABLE 1
Effect of iron (Fe) application to different genotypes of mustard on Fe content, uptake and yield response

Genotypes	Content (mg/kg)		Uptake (g/ha)		Yield response (%)
	Fe-treated	Fe-untreated	Fe-treated	Fe-untreated	
RH 1424	117.8	96.6	260.34	192.23	11.1
RH 1430	100.3	94.3	243.73	218.78	4.7
RH 1499-7	168.8	91.3	428.75	191.73	21.1
RH 1515	101.5	77.3	291.31	204.85	8.3
RH 1518	105.2	78.1	255.64	155.42	22.2
RH 1519	95.5	72.1	242.57	167.27	9.5
RH 1529	98.0	93.9	220.50	207.52	1.8
RH 1550	129.1	84.9	299.51	178.29	10.5
RH 1555	94.3	93.5	229.15	186.07	22.1
RH 1584	92.7	69.2	194.67	130.10	11.7
RH 1585	85.8	81.6	208.49	189.31	4.8
RH 1592	103.6	93.7	183.37	156.48	5.9
RH 1599-36	131.8	122.1	262.28	216.12	12.4
RH 1607	108.6	87.9	240.01	174.92	11.1
RH 1652	125.9	111.3	305.94	258.22	4.8
RH 1653	121.7	66.7	282.34	140.07	10.5
RH 1668	131.3	74.7	319.06	165.09	10.0
RH 1669	95.9	72.7	233.04	152.67	15.7
RH 1674	109.0	82.0	216.91	145.14	12.4
RH 1675	98.9	96.5	218.57	181.42	17.6

TABLE 2
Effect of Zinc (Zn) application to different genotypes of mustard on Zn content, uptake and yield response

Genotypes	Zn content (mg/kg)		Uptake (g/ha)		Yield response (%)
	Zn-treated	Zn-untreated	Zn-treated	Zn-untreated	
RH 725	33.2	22.8	84.33	55.86	3.4
RH 749	25.4	18.9	59.18	38.93	13.0
RH 50	33.5	23.6	68.34	44.37	8.7
RH 119	28.3	21.4	68.20	50.29	2.4
RH 406	34.2	24.0	84.13	56.40	4.6
RH 761	33.5	25.6	78.06	56.83	5.0
RH 1899-54	29.7	21.4	54.95	37.88	4.8
RH 1928	26.5	19.5	45.58	32.37	3.5
RH 1930	29.8	23.5	53.64	38.31	10.5
RH 1934	33.6	21.6	68.54	40.82	7.8
RH 1974	34.8	24.2	84.56	56.39	4.3
RH 1975	37.3	28.4	93.62	67.02	6.4
RH 2022	29.8	21.5	73.31	49.02	7.9
RH 2029	34.8	25.8	69.25	47.47	7.9
RH 2031	33.9	24.5	84.41	58.56	4.2
RH 2038	30.5	22.0	75.64	51.92	5.1
RH 9812	36.4	26.4	84.81	56.23	9.3
RH 30	33.2	24.8	64.08	44.64	7.6
RH 9304	25.6	19.4	52.22	35.70	10.7
RH 9801	28.6	21.3	71.21	48.14	10.4

was recorded by genotype RH 9812 which was 50.83% higher than Zn-untreated condition. Harvest index (HI) for genotypes under Zn-untreated condition were found almost similar to tested for Fe-untreated condition, whereas zinc efficiency index (ZEI) and zinc efficiency (ZE) of RH 119 quantified as 97.51 and 73.74, respectively which found inflated over other genotypes (Table 4).

TABLE 3
Impact of Iron (Fe) application to different genotypes on harvest index, iron efficiency index and iron efficiency

Genotypes	Harvest Index (HI)		Iron efficiency Index (IEI)	Iron efficiency (IE)
	Fe-treated	Fe-untreated		
RH 1424	0.49	0.49	90.05	73.84
RH 1430	0.48	0.48	95.47	89.76
RH 1499-7	0.48	0.49	82.68	44.72
RH 1515	0.48	0.48	92.33	70.32
RH 1518	0.48	0.49	81.89	60.80
RH 1519	0.48	0.48	91.34	68.96
RH 1529	0.49	0.49	98.22	94.11
RH 1550	0.49	0.49	90.52	59.53
RH 1555	0.48	0.49	81.89	81.20
RH 1584	0.49	0.49	89.52	66.83
RH 1585	0.48	0.49	95.47	90.80
RH 1592	0.49	0.49	94.35	85.33
RH 1599-36	0.49	0.49	88.94	82.40
RH 1607	0.49	0.49	90.05	72.88
RH 1652	0.48	0.48	95.47	84.40
RH 1653	0.49	0.49	90.52	49.61
RH 1668	0.48	0.49	90.95	51.74
RH 1669	0.48	0.49	86.42	65.51
RH 1674	0.49	0.49	88.94	66.91
RH 1675	0.49	0.49	85.07	83.00

TABLE 4
Impact of Zinc (Zn) application to different genotypes on harvest index, iron efficiency index and iron efficiency

Genotypes	Harvest Index		Zinc efficiency Index (ZEI)	Zinc efficiency (ZE)
	Zn-treated	Zn-untreated		
RH 725	0.48	0.48	96.46	66.24
RH 749	0.48	0.49	88.41	65.79
RH 50	0.49	0.49	92.16	64.92
RH 119	0.48	0.48	97.51	73.74
RH 406	0.48	0.48	95.53	67.04
RH 761	0.48	0.48	95.28	72.81
RH 1899-54	0.49	0.49	95.68	68.94
RH 1928	0.49	0.49	96.51	71.02
RH 1930	0.49	0.49	90.56	71.41
RH 1934	0.49	0.49	92.65	59.56
RH 1974	0.48	0.48	95.88	66.68
RH 1975	0.48	0.48	94.02	71.59
RH 2022	0.48	0.48	92.68	66.87
RH 2029	0.49	0.49	92.46	68.55
RH 2031	0.48	0.48	95.98	69.37
RH 2038	0.48	0.48	95.16	68.64
RH 9812	0.48	0.49	91.42	66.30
RH 30	0.49	0.49	93.26	69.67
RH 9304	0.49	0.49	90.20	68.35
RH 9801	0.48	0.48	90.76	67.60

Relative susceptibility of mustard genotype against Fe and Zn

Based on yield response to Fe and Zn, all the 20 genotypes were categorized as tolerant (response <10%), semi-tolerant (10-25%) and susceptible (>25%). In case of Fe, based on percent yield response, 7 genotypes viz: RH 1430, RH 1515, RH 1519, RH 1529, RH 1585, RH 1592 and RH 1652 were found tolerant (<10%) to Fe stress and for Zn, 16 genotypes viz: RH 725, RH 50, RH 119, RH 406, RH 761, RH 1899-54, RH 1928, RH 1934, RH 1974, RH 1975, RH 2022, RH 2029, RH 2031, RH 2038, RH 9812 and RH 30 were found tolerant (<10%) to Zn stress. Response of mustard genotypes for semi tolerant (10-25%) genotypes to Fe stress includes viz: RH 1424, RH 1499-7, RH 1518, RH 1550, RH 1555, RH 1584, RH 1599-36, RH 1607, RH 1653, RH 1668, RH 1669, RH 1674 and RH 1675 whereas, semi tolerant genotypes for Zn stress includes RH 749, RH 1930, RH 9304 and RH 9801. However, none of the mustard genotype were categorized under susceptible to Fe and Zn stress.

CONCLUSION

This study underscores the potential of genetic variability among mustard genotypes to address micronutrient deficiencies, enabling the development of stress-tolerant varieties that maintain productivity without heavy reliance on external inputs. Superior genotypes demonstrate inherent efficiency in nutrient utilization, highlighting opportunities for targeted breeding programs that prioritize stability across deficient environments and integrate with agronomic practices for enhanced crop performance. Such selections promise to narrow productivity gaps, support sustainable farming systems and contribute to nutritional security by improving micronutrient supply through staple crops in vulnerable regions. Future efforts should focus on validating these traits through multi-environment testing and molecular approaches to accelerate the release of resilient cultivars for farmers.

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