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RESEARCH ARTICLE

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Genotype × Environment Interaction for Appraisal of Iron and Zinc Rich Stable Genotypes in Lentil (*Lens culinaris* Medik.)

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ABSTRACT

Background: Micronutrient malnutrition is a severe peril for wellbeing of humankind, which can be holistically addressed through genetic biofortification. Lentil, proclaim as poor man's meat can hold a great promise in global biofortification programme. The present study was designed to appraise genetic variability for Fe and Zn content and elucidate the role of Genotype × Environment interaction for delimitation of micronutrient enriched stable lentil genotypes integrating HA-GGE biplot with REML/BLUP.

Methods: Grain Fe and Zn content of 44 lentil genotypes grown at three different locations of West Bengal during two consecutive years were estimated for deciphering the G × E interaction combining HA-GGE and REML/BLUP.

Result: Results revealed substantial genetic variability for Fe (48.07 to 107.45 mg kg^{-1}) and Zn (38.72 to 60.07 mg kg^{-1}) in 44 lentil genotypes with significant influence of environment and GE interaction. The present study precisely detected ILL-10123 and VL-156 as the 'ideal' genotypes for Fe and Zn content, respectively in addition to non-redundant testing location. Identified genotypes and testing location aid in global biofortification programme for upscaling micronutrient concentration in lentil.

Key words: Biofortification, BLUP, GGE biplot, Lentil, Micronutrients.

INTRODUCTION

Nutritional security is cardinal for the salubrious sustenance of humanity. Presently, developing countries are extensively impacted by micronutrient malnutrition (MNM). Among the micronutrients, iron (Fe) and zinc (Zn) are vital in all life forms' sustenance and essential for physiological functions. However, 60% of the world population is Fe deficient, whereas, 33% is Zn deficit (Hotz and Brown, 2004), respectively. Genetic biofortification is a holistic approach, integrating conventional plant breeding and modern biotechnological tools for increasing the essential micronutrient concentration in their bioavailable forms in staple food crops without compromising agronomic superiority.

Lentil (*Lens culinaris* Medik.) is one of the healthiest pulses rich in starch, proteins, dietary fibres and notably essential micronutrients including Fe, Zn, selenium (Se), folate (vitamin B9), as well as several other dietary nutrients (Bhattacharya *et al*., 2022a). The recommended daily allowance (RDA) for Fe and Zn can be substantially met by incorporating 100 g of lentils into the daily human diet (Bhattacharya *et al*., 2022b). The biofortification of lentil paves the way forward as the grain Fe and Zn concentration reveals wide variation. However, the utilization of the wide variation among genotypes is arduous due to the complex inheritance pattern of grain micronutrient concentrations with low genetic gain (Gore *et al*., 2021). Over the years, efforts to comprehend the Genotype × Environment (GE) interaction for appraisal of stable Fe and Zn rich genotypes in lentil have utilized Eberhart and Russell model (Darai *et al*., 2020) and AMMI biplot (Gupta *et al*., 2021). Presently, the heritability-adjusted GGE (HA-GGE) biplot is the most precise method for delimitation of genotype and test environment (Yan and Holland, 2010). However, reports to decipher the GE interaction for appraisal of stable genotypes rich in micronutrients combining HA-GGE biplot and REML/BLUP analysis for sound statistical inference is sparse in lentil.

The present study was planned to unearth the stable Fe and Zn rich lentil genotypes using HA-GGE biplot combined with REML/BLUP analysis, which will facilitate global biofortification programme to mitigate hidden hunger.

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MATERIALS AND METHODS

Multilocational evaluation

The experiment was designed to evaluate 44 lentil genotypes consisting of 43 advance breeding lines and 1 released variety procured from Indian programme on lentil and International Centre for Agricultural Research on Dry

Areas (ICARDA). Genotypes were evaluated at three different locations over two consecutive years as L1 and L2 (District Seed Farm, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Kalyani; latitude of 22°99′N; longitude of 88°42′E; altitude of 11 m above mean sea level) during winter season of 2020-21 and 2021-2022, respectively; L3 and L4 (Regional Research Sub-Station (RRSS), Chakdah; latitude of 23°05′N; longitude of 88°54′E and altitude of 14 m) during 2020-2021 and 2021-22, respectively; L5 and L6 (Indian Institute of Technology (IIT), Kharagpur; latitude of 22°31′ N; longitude of 87°31′E with altitude of 61 m) during 2020-2021 and 2021-2022, respectively. The soil of Kalyani and Chakdah is well-drained, clay loam in texture (35% clay in Kalyani and 38.5% in Chakdah) having neutral pH (7.1: Kalyani; 6.9: Chakdah). The soil at IIT, Kharagpur is red and lateritic in nature (13% clay) and acidic in reaction (pH: 5.2). The DTPA extraction procedure (Lindsay and Norvell, 1978) was followed to analyse the soil Fe and Zn contents in mg kg^{-1} for all the test locations. The field experiments laid in randomised complete block design (RCBD) with three replications in each location. The cropping practices were in accordance with standard agronomic practices of lentil with optimal plant geometry of 3 m row length and 25 × 5 cm row to row and plant to plant spacings, respectively. Recommended dosage of fertilizers was applied (20 kg N, 40 kg P_2O_5 and 20 kg K ha^{-1}) in all the locations.

Estimation of grain Fe and Zn content

Five plants were randomly selected during harvest for grain Fe and Zn estimation in each location. The seeds collected from the selected plants were dried under sun until grain moisture content was under 12%. This was followed by washing of seed samples from each genotype in distilled water and drying at 35°C for 5 days in a contamination and corrosion free oven to remove all moisture from the seed samples. Subsequently, grain Fe and Zn were estimated under atomic absorption spectrophotometer (AAS) (Perkin Elmer Aanalyst 300) using standard protocol by Zarcinas *et al.* (1987).

Statistical analysis

The effects of environment, genotype and GE related to each location across the years were elucidated through analysis of variance (ANOVA). The mean significant difference between G and E was calculated using the LSD test at P=0.05 probability level. The restricted maximum likelihood (REML) method was utilised to find the genetic value of each genotype for appraisal of variance component employing best linear unbiased prediction (BLUP). Summation rank index (SRI) was calculated by combining grain Fe and Zn content of each genotype and finally heat plot was generated.

The HA-GGE biplot model was created following environment-focused singular value partitioning and concomitantly broad sense heritability (H) was calculated (Yan and Holland, 2010). Finally, the first principal component (PC1) was put against the corresponding second principal component (PC2) for the genotypes and environment originating from singular value decomposition (SVD) of environment-centred data for generation of basic GGE biplot model utilising the formula proposed by Yan and Kang (2003):

$$Y_{ij} = \mu + e_j + \sum_{n=1}^{N} \lambda_n \gamma_{in} \delta_{jn} + \varepsilon_{ij}$$

Where,

Y_{ij}= Mean response of i^{th} genotype (i=1,…,I) in the j^{th} environment (j=1,..,J).

μ = Grand mean.

e_j= Environment aberrations from the grand mean.

λ_n= The eigen value of the PC analysis axis.

γ_{in} and δ_{jn}= The principal component scores of the genotype and environment at axis n.

N = Number of principal components in the model.

ε_{ij}= Residual effect ~ N (0, σ^2).

Data was computed statistically using R software (R Development Core Team, 2012).

RESULTS AND DISCUSSION

Significance of G × E interaction

Grain Fe and Zn content showed highly significant (P<0.01) effect of genotype, environment and GE interaction across the locations over the years, as depicted by the pooled ANOVA and genetic parameter (Table 1). The relative contribution of the components portrayed that the environmental factors shared 60.68% and 73.96% of the total variation for grain Fe and Zn content, respectively. This propounds the importance of MET for the selection of stable lentil genotypes rich in Fe and Zn content. The dissection of the variation in the present study revealed higher GE interaction in case of Fe (σ^2gl: 20.63) rather than in the case of Zn (σ^2gl: 12.51) which was in accordance with the earlier finding (Gore *et al.*, 2021).

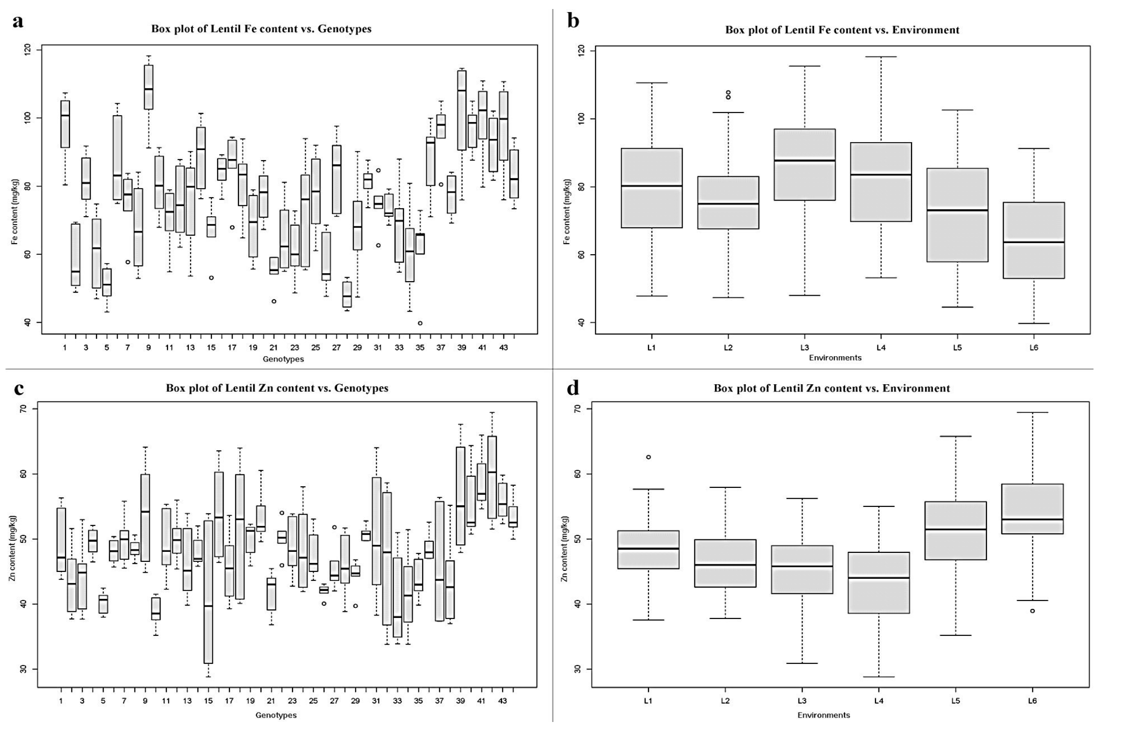
Variability appraisal for grain Fe and Zn content

The variation in the average grain Fe content between the genotypes ranged from 107.45 mg kg^{-1} (ILL-10123) to 48.07 mg kg^{-1} (RKL-14-112) (Table 2). On the other hand, the variation in Zn content within the genotypes ranged from 60.07 mg kg^{-1} (VL-157) to 38.72 mg kg^{-1} (L-4603) (Table 1). The distribution of lentil genotypes and environments for Fe and Zn content over the years was depicted through the boxplot analysis (Fig 1). It was observed that, IPL-342 (G7), PLE-1801 (G11), LL-1427 (G16), PLE-1802 (G25) and RKL-14-276 (G29) were the consistent genotypes for grain Fe content (Fig 1a). Considering the test locations, the average grain Fe content was recorded the highest at L3 (86.32 mg kg^{-1}) while the lowest was at L6 (63.73 mg kg^{-1}) with L2 and L6 having the congruous response (Fig 1b). The study also depicted genotypes PLR-1802 (G4), PL-254 (G6), PL-269 (G22), PLL-1801 (G26) and RVL-17-1 (G34) as the consistently performing genotypes for Zn content (Fig 1c). Between the locations, it was the highest at L6 (54.25 mg kg^{-1}) and the lowest at L4 (43.64 mg kg^{-1}) with locations L1 and

L2 having consistent median values (Fig 1d). The present study corroborated with the earlier findings as significant variation in the grain Fe and Zn content was detected among the tested lentil genotypes over the locations in consecutive years though the variability was less for Zn content than Fe (Shrestha *et al*., 2018). Regarding the position of the genotypes across the locations, there was presence of both consistent and variable responses by lentil genotypes indicating the existence of cross-over (COI) and non-cross-over (non-COI) interaction over the

Table 1: Pooled ANOVA and descriptive statics for grain Fe and Zn content in the tested locations over the years among lentil genotypes.

Sources of		Fe concentration			Zn concentration		
variation	DF	MS	% Contribution	P value	MS	% Contribution	P value
			Pooled ANOVA				
ENV	5	7934.51	60.68	0.00	2015.21	73.96	0.00
GEN	43	4312.57	32.98	0.00	542.50	19.91	0.00
ENV*GEN	215	771.70	5.90	0.00	156.68	5.75	0.00
			Pooled genetic parameters				
Min			39.77			28.81	
Max			118.3			69.44	
LSD			11.00			4.81	
CV			9.79			6.50	
Heritability			0.81			0.79	
$\sigma^2 g$			250.72			38.19	
$\sigma^2 p$			309.83			48.23	
$\sigma^2 gl$			20.63			12.51	



(1a) Distribution of Fe content of 44 lentil genotypes.
(1b) Distribution of Fe content across 6 testing locations.
(1c) Distribution of Zn content of 44 lentil genotypes.
(1d) Distribution of Zn content across 6 testing locations.
Numbers correspond to genotypes and locations as listed in Tables 2 and 3 respectively.

Fig 1: Boxplot view depicting the distribution of Fe and Zn of 44 genotypes tested over 6 testing locations.

locations. This finding is in accordance with earlier literatures (Das *et al*., 2019; Das *et al*., 2020), which represented COI and non-COI within the same dataset. Breeders often search for COI when breeding for specific adaptation, which is prevalent for some lentil genotypes in the present study.

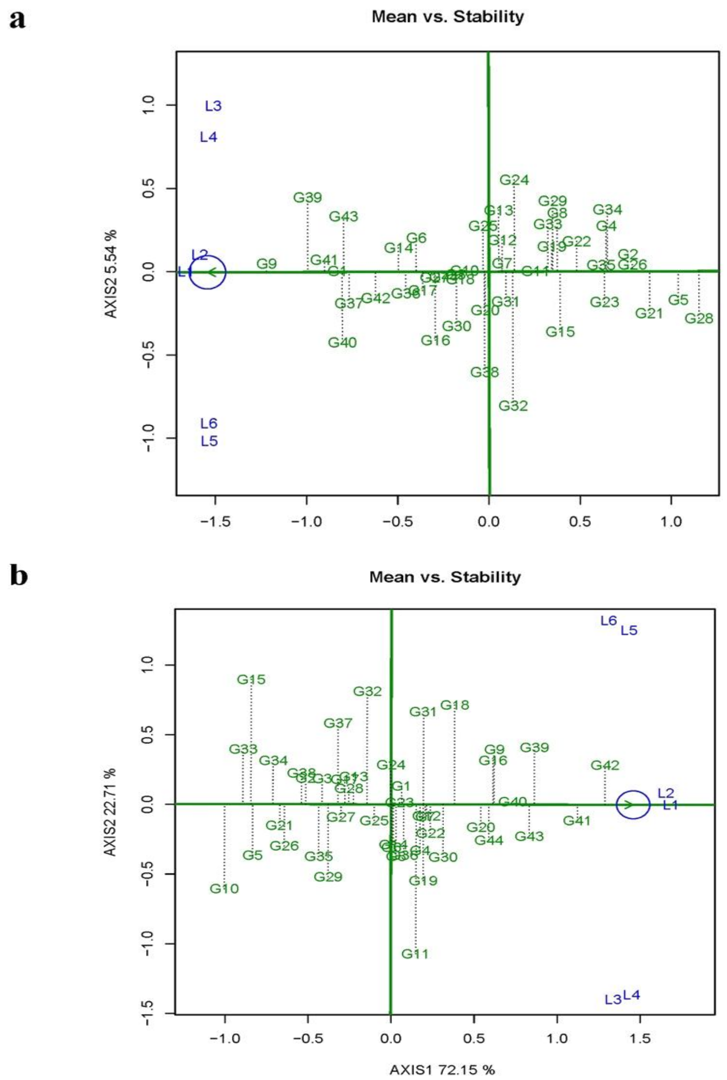
Table 2: Mean performance of lentil genotypes utilized in the study.

Sl. no.	Genotype	Yield plant^{-1} (g)	Seed index (g)	Fe content in grain		Zn content in grain	
				Mean (mg kg^{-1})	BLUP	Mean (mg kg^{-1})	BLUP
1	BCL-1242	4.8	3.1	97.61	23.45	49.02	0.79
2	BCL-1296	5.1	2.9	57.97	-19.80	43.55	-4.61
3	PLS-1802	9.3	2.5	81.54	4.80	44.28	-3.85
4	PLR-1802	6.5	2.0	60.97	-16.72	49.56	1.30
5	RLG-250	7.4	2.8	51.02	-27.09	40.27	-7.90
6	PL-254	8.5	2.0	87.05	10.61	48.11	-0.14
7	IPL-342	4.6	3.6	75.23	-1.85	49.89	1.68
8	PLR-1801	7.6	2.3	67.70	-9.68	48.41	0.15
9	ILL-10123	8.2	2.1	107.45	31.85	53.97	5.75
10	L-4603	6.8	1.9	80.31	3.50	38.72	-9.45
11	PLE-1801	6.1	2.6	70.58	-6.70	49.10	0.81
12	IPL-341	5.4	3.0	75.19	-1.84	50.07	1.85
13	IPL-601	5.6	2.9	75.74	-1.35	46.27	-1.91
14	KLB-1442	4.3	3.4	89.33	12.92	48.01	-0.25
15	KLS-1451	4.9	2.3	67.18	-10.25	40.95	-7.16
16	LL-1427	5.1	3.4	84.26	7.67	54.01	5.77
17	LL-1522	8.4	2.2	86.08	9.51	45.68	-2.50
18	LL-1525	6.5	2.9	81.06	4.29	51.80	3.62
19	LL-1576	5.3	3.4	68.33	-9.04	50.06	1.77
20	PL-247	5.2	3.2	77.53	0.64	53.35	5.09
21	PL-252	5.4	2.8	54.89	-23.05	41.90	-6.26
22	PL-269	6.3	3.0	64.98	-12.51	50.15	1.89
23	PL-276	9.2	1.9	61.10	-16.62	48.71	0.49
24	PL-279	8.8	2.2	73.57	-3.59	48.42	0.22
25	PLE-1802	9.1	2.8	77.84	0.90	47.45	-0.78
26	PLL-1801	6.2	3.0	57.25	-20.54	41.96	-6.22
27	PLL-1802	5.8	2.9	84.17	7.47	45.46	-2.72
28	RKL-14-112	7.1	2.8	48.07	-30.16	45.86	-2.32
29	RKL-14-276	6.2	3.1	68.44	-8.94	44.34	-3.84
30	RKL-16-304	4.3	3.4	81.37	4.65	50.83	2.55
31	RKL-58F-3715	4.8	3.1	74.57	-2.49	50.44	2.27
32	RKL-61F-215	5.1	3.4	73.47	-3.55	47.03	-1.11
33	RL-10	4.1	3.1	68.91	-8.42	40.47	-7.65
34	RVL-17-1	4.9	2.9	60.90	-16.81	41.80	-6.32
35	RVL-17-11	5.0	2.6	61.70	-16.03	43.74	-4.42
36	Sehore-74-3	7.1	3.1	88.53	12.10	48.69	0.47
37	SJL 6-3	5.6	3.0	96.10	19.96	45.72	-2.47
38	TCADL-18-2	9.7	2.5	77.47	0.60	43.63	-4.52
39	VL-126	7.8	2.3	101.87	21.54	56.45	7.01
40	VL-152	8.5	2.4	97.01	20.97	55.29	8.19
41	VL-156	8.7	2.4	99.48	25.93	58.65	11.77
42	VL-157	7.9	2.5	92.55	16.35	60.07	10.34
43	VL-531	5.4	3.3	96.93	20.81	55.82	7.51
44	VL-532	6.1	3.5	83.15	6.51	53.34	5.09

Appraisal of stable lentil genotypes

In the current dataset, the ranking of the genotypes regarding grain Fe and Zn content was illustrated graphically through the "Average Environment Coordination (AEC)" view (Fig 2). Observed values for PC1 (Grain Fe/Zn) presented 88.17% and 72.15% of the total variation for Fe and Zn, respectively whereas PC2 (stability of the genotype) portrayed 5.54% and 22.71% of the total variation observed for Fe and Zn content, respectively. Earlier literature recommended that, the cumulative contribution of PC1 and PC2 should be more than 80% for judging the fitness of the methodology (Tamang *et al.*, 2022).

Present study validated the preciseness of HA-GGE biplot as for both the micronutrients the cumulative contribution of first two PCs were more than 80%. The AEC view illustrations bear a single arrowhead line passing through the origin of the biplot known as "AEC abscissa" representing the direction of higher Fe and Zn content of the tested lentil genotypes along with a double-arrowed line perpendicular to the AEC abscissa, denoted as "AEC ordinate," which displays the stability of genotypes. The lower projection length would determine the more stable genotypes and *vice versa*. Genotypes *viz.*, ILL-10123 (G9), VL-126 (G39), VL-156 (G41), BCL-1242 (G1), VL-531 (G43), SJL 6-3 (G37), VL-152 (G40), VL-157 (G42), KLB-1442 (G14),



(2a) Mean vs. Stability view of the GGE biplot for grain Fe content across 6 testing locations.

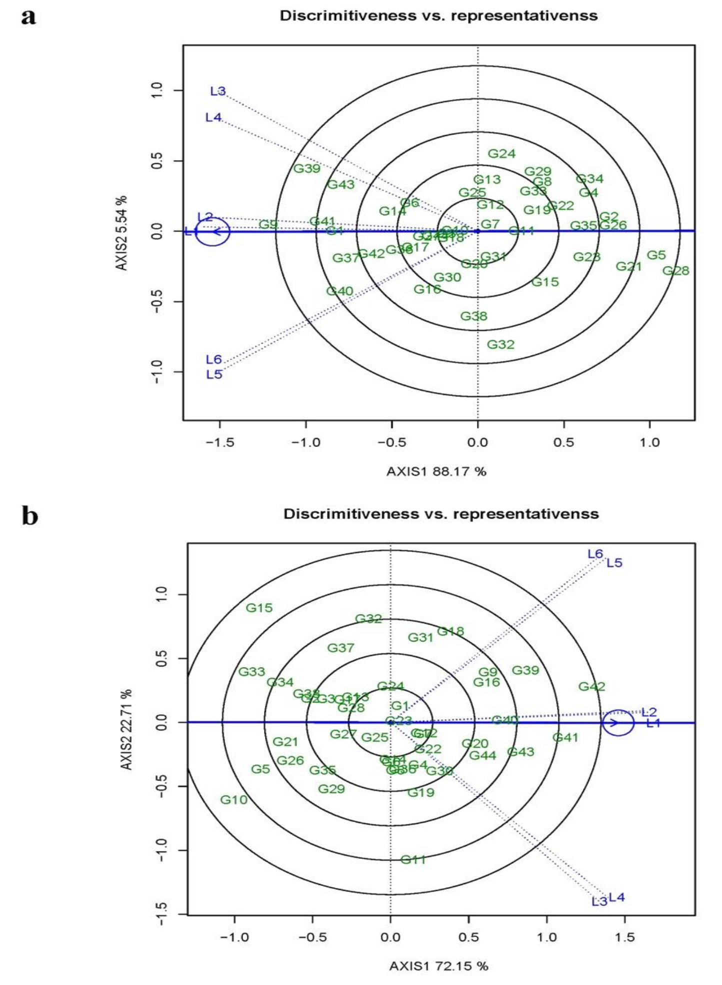
(2b) Mean vs. Stability view of the GGE biplot for grain Zn content across 6 testing locations.

Numbers correspond to genotypes as listed in Table 2.

Fig 2: Mean vs. Stability view of the GGE biplot of 44 lentil genotypes over 6 testing locations.

PL-254 (G6), Sehore-74-3 (G36), LL-1522 (G17), LL-1427 (G16), LL-1525 (G18), RKL-16-304 (G30), VL-532 (G44), L-4603 (G10) and PLS-1802 (G3) have more than average Fe content (Fig 2a) as they were allocated in the positive direction of "AEC abscissa". Among the genotypes, ILL-10123 (G9) was the 'ideal' genotype as it exhibited highest grain Fe content along with very high stability, followed by BCL-1242 (G1). The superiority was also expressed by the highest REML/BLUP value (Table 2). The two genotypes *viz*., BCL-1242 (G1) and VL-156 (G41) were detected as the 'desirable' genotypes as they exhibited proximity with "ideal" genotype with good stability and high mean value for Fe content which was in accordance with their REML/BLUP values.

Genotypes *viz*., VL-157 (G42), VL-156 (G41), VL-126 (G39), VL-531 (G43), VL-152 (G40), ILL-10123 (G9), LL-1427 (G16), PL-247 (G20), VL-532 (G44), LL-1525 (G18), RKL-58F-3715 (G31), RKL-16-304 (G30), LL-1576 (G19), PL-269 (G22) and IPL-341(G12) exhibited moderate to higher than average values for Zn content (Fig 2b) and placed in the positive direction of the "AEC abscissa". Overall, VL-156 (G41) has both high mean performance and good stability in the AEC view as well as highest value in the REML/BLUP analysis, thus, considered as 'ideal' genotype. Moreover, VL-152 (G40) and VL-157 (G42) were detected as the 'desirable' genotypes considering the REML/BLUP values (Table 1) and AEC view of GGE biplot. Summation rank index considering both Fe and Zn content



(3a) "Discriminativeness vs. Representativeness" view of test locations regarding grain Fe content.

(3b) "Discriminativeness vs. Representativeness" view of test locations regarding grain Zn content.

Numbers correspond to genotypes and locations as listed in Tables 2 and 3 respectively.

Fig 3: "Discriminativeness vs. Representativeness" view of test locations based on GGE biplot of 44 lentil genotypes across 6 testing locations.

was culminated to detect VL-156 (G41) as the 'ideal' genotype having high Fe and Zn content combined with good stability across the locations (Fig 3).

Several factors are at play during nutrient homeostasis and this complex network involves multiple genes and transcription factors that vary from genotype to genotype leading to genotypic variation. Moreover, further research suggested the existence of quantitative traits modulating the pathway revealing the importance of understanding the GE interaction, which impacted the results of genetic analysis for these complex traits (Bhattacharya *et al*., 2022b).

Delimitation of ideal test location

HA-GGE biplot can provide a win-win opportunity to identify suitable testing environment and evaluate their superiority along with appraisal of genotypes. Square root of heritability ($\sqrt{H}$) provides the differentiating factor for testing the superiority of the test environment, which is presented as the vector length of the test environment in the graphical representation and denoted as "discriminating ability" on to the target environment. Along with that, the angle between the environmental vectors and "AEC abscissa" denotes the 'representativeness' of the test location wherein a more acute angle indicates more 'representativeness' and *vice versa*. Keeping in mind both the factors, the environments L1 and L2 were indicated as the superior environments having highest discriminating ability (longest vector length) combined with representativeness (acute angle with AEC abscissa) and can facilitate in delineating the genotypes regarding grain Fe and Zn content with specific adaptation (Fig 4). The combination of "representativeness" and "discriminating ability" provides the "desirability index" which

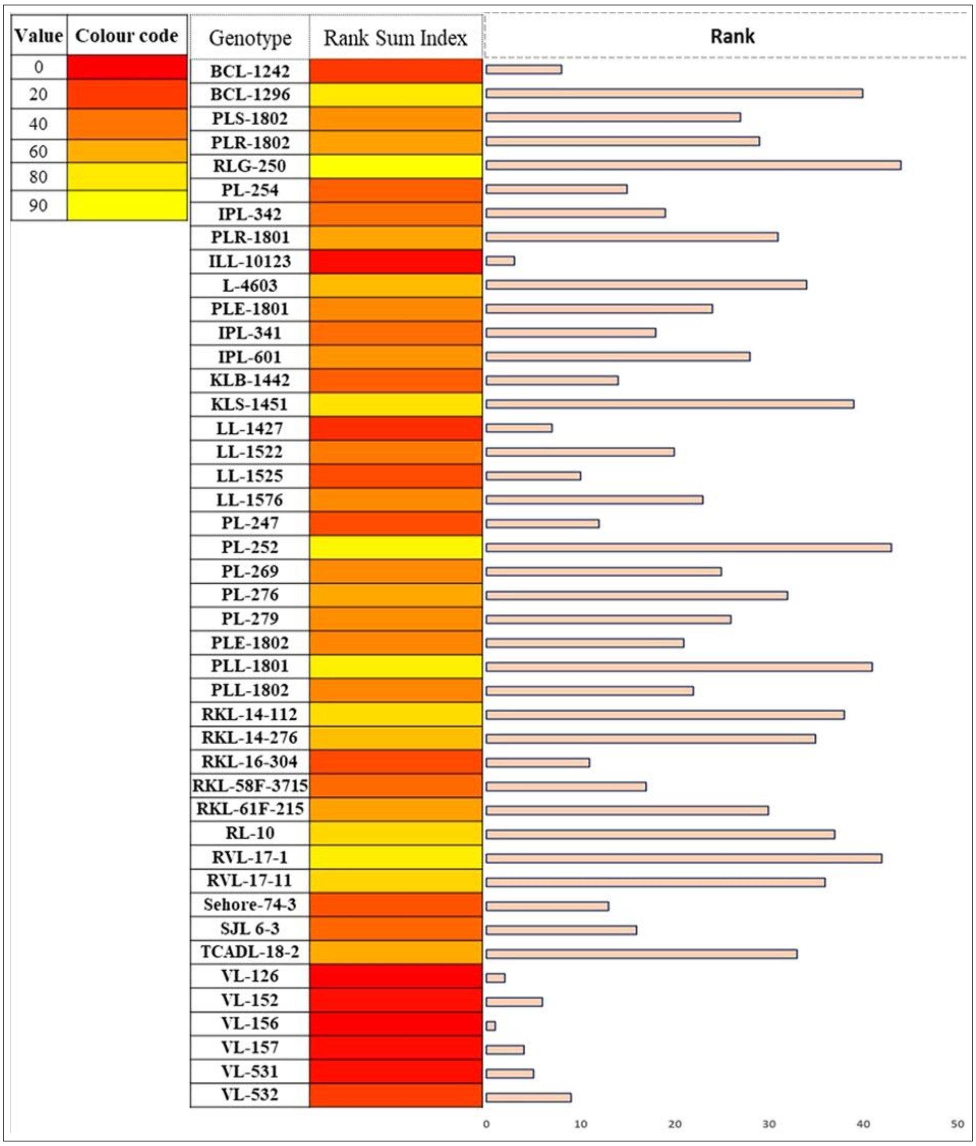


Fig 4: Heatmap for Summation Rank Index of 44 lentil genotypes combining grain Fe and Zn content over the locations and years.

Table 3: Parameters for judging the suitability of test locations.

Locations	Code	Fe concentration			Zn concentration		
		Discriminating power	Representativeness	Desirability index	Discriminating power	Representativeness	Desirability index
BCKV, Kalyani-2020-21	L1	6.26	0.97	6.06	6.15	0.95	5.87
BCKV, Kalyani-2021-22	L2	6.32	0.95	5.99	6.12	0.97	5.92
RRSS, Chakdah-2020-21	L3	6.22	0.92	5.72	6.19	0.78	4.81
RRSS, Chakdah-2021-22	L4	6.19	0.94	5.82	6.32	0.80	5.03
IIT, Kharagpur-2020-21	L5	6.22	0.93	5.80	6.26	0.80	4.98
IIT, Kharagpur-2021-22	L6	6.12	0.92	5.64	6.08	0.79	4.78

is one of the crucial factors for ascertaining the ideal test location. L1 (6.06) and L2 (5.92) were identified as 'ideal' or type-I locations for appraisal of precious genotypes in case of grain Fe and Zn content, respectively considering the highest "desirability index" (Table 3).

Over the years, Bhattacharya *et al*. (2022b) have utilised the HA-GGE biplot for appraisal of genotypes and environment. In addition to this, the adequacy and accountability of the methodology combined with the overall environmental influence, which is portrayed by the vector projection on the "AEC abscissa" is an indirect selection criterion for self-pollinated crops like lentil as the additive component of variation is predominant (Yan and Holland, 2010). Breeders can utilize the identified ideal locations for appraisal of lentil genotypes in future experiments.

CONCLUSION

It is evident that the tested lentil genotypes possess substantial variability for grain Fe and Zn content and there is also significant influence of environment during the expression of these characters. The mixed approach combining the REML/BLUP and GGE biplot extended in the present study could precisely detected "ideal" genotypes as well as decisive test locations for the appraisal of the lentil genotypes. Lentil is the major component of Asian and African food platter where eradication of hidden hunger is the most seminal social priority. Adoption of micronutrient rich lentil genotypes may provide new frontier for better nutritional subsistence.

Conflict of interest

The authors declare that there is no conflict of interest.

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