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RESEARCH

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Genotype × environment interaction and stability analysis on iron, zinc, and selenium concentrations of lentil genotypes

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Abstract

Background: Pulses, particularly lentil (*Lens culinaris* L.), are an important component of the South Asian diet, and micronutrient biofortification provides a sustainable and cost-effective strategy to combat malnutrition in rural populations. Therefore, the present study aimed to identify stable lentil genotypes with enhanced zinc (Zn), iron (Fe), and selenium (Se) concentrations through genotype × environment interaction analyses using multiple stability models. *Methods*: Twenty-seven lentil genotypes including the check varieties BARI Masur-6, BARI Masur-7, and BINA Masur-7 selected from an initial screening of 152 genotypes were tested in three agroecological locations of Bangladesh, namely Gazipur, Ishwardi, and Jashore. Genotype × environment interactions and stability performance were assessed using the Eberhart and Russell, AMMI (additive main effects and multiplicative interaction), and BLUP (best linear unbiased prediction) models. *Results*: Analysis of variance revealed significant differences among genotypes, environments, and genotype × environment interactions for all studied traits. Based on the combined stability analyses, the genotypes LR 9-25 (G13), ILL-5151 (G23), and BARI Masur-7 (G11) exhibited superior stability with higher Se concentration. For Zn concentration, LRIL-22-39 (G25), LRIL-21-109 (G17), LRIL-22-39 (G27), and BARI Masur-7 (G11) demonstrated greater stability across environments. In the case of seed Fe concentration, LRIL-22-39 (G25), X96S/40-50134-12 (G12), ILL-5151 (G23), and LRIL-22-15 (G19) were identified as the most stable genotypes. Furthermore, ILL-5121 (G20), BARI Masur-6 (G3), and LRIL-21-109 (G17) showed higher and more stable seed yield performance across the tested locations. Among the environments, Ishwardi and Jashore were identified as the most suitable and stable locations for lentil cultivation in Bangladesh. *Conclusions*: The genotypes LRIL-22-39 (G25), LRIL-21-109 (G17), and ILL-5151 (G23) were identified as promising candidates for the development and release of biofortified lentil varieties combining enhanced micronutrient concentration with stable yield performance in Bangladesh.

Keywords: AMMI model, biofortification, Eberhart and Russell model, BLUP, lentil yield, stable genotypes

Introduction

Globally, more than two billion people are Fe and Zn deficient (Maggini *et al*., 2018; Wakeel *et al*., 2018; Kaur *et al*., 2023). These people are mostly poor rural people from developing countries. In these countries, the principal reason for rise in micronutrient deficiencies can be attributed to the cultivation of cereals (e.g. rice) as dominant crops, which are low in micronutrients. On the other hand, minimum attention is paid to the production of micronutrient-rich non-staples, such as pulses, vegetables, and fruits (Jahiruddin, 2020). The FAO has five objectives, of which objective 1 is to achieve a world free from poverty, hunger, and malnutrition in a sustainable manner, which is embedded in SDGs 1, 2, and 3.

Micronutrient malnutrition causes poor physical and mental development in children, vulnerability to or exacerbation of disease, mental retardation, blindness, and general losses in productivity and potential (Webb *et al*., 2018). In this scenario, dietary diversification,

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nutritional supplementation, and food fortification are effective in reducing micronutrient malnutrition.

Grain legumes have gained importance primarily because they are high in protein, low in saturated fat, and contain some key micronutrients (zinc, folate, selenium, calcium, and tocopherols). Biofortification is a new agro-based approach to help reduce micronutrient malnutrition, especially in rural areas at minimal cost. Research efforts have demonstrated that this agriculture-based method of addressing micronutrient deficiency through plant breeding works (Bouis, 2018). Recently, biofortification has mainly focused on iron (Fe), zinc (Zn), and vitamin A deficiencies. It can be achieved in three ways: (1) conventional biofortification, (2) agronomic biofortification, and (3) transgenic biofortification. Among these, agronomic biofortification offers a rapid solution to the problem and is required for optimizing and ensuring the success of genetic biofortification of the grains with micronutrients. Some of the biofortified crops include Fe-enriched common bean (*Phaseolus vulgaris L.*) variety in Congo and Rwanda (Harvest Plus, 2013); Fe-enriched rice (*Oryza sativa L.*) in Myanmar (Aung *et al*., 2013); Zn-rich maize variety in Colombia (Johnson, 2018); Fe- and Zn-rich wheat variety in India (Yashavanthakumar *et al*., 2023); Zn-enriched rice in Bangladesh (CGIAR, 2021); and vitamin A-enriched cassava (*Manihot esculenta Crantz*) in Congo and Nigeria (Harvest Plus, 2013). Bangladesh Agricultural Research Institute (BARI) released Fe- and Zn-rich legume crops lentil (*Lens culinaris* L.) in 2015 (CGIAR, 2015). Though it contains a high amount of se, but still there is no lentil variety released in Bangladesh that contains a combination of all those discussed micronutrients (Fe, Zn, and Se).

The performance of cultivars of a crop varies differently from one environment to another. The evaluation of genotypes is the key to utilization in breeding programs and is a continuous process. In the final stages of variety recommendation, multienvironmental trials should be done due to environmental variations (El-Kadi *et al*., 2011). A genotype may be stable if its environmental variance is small. To identify stable genotypes, researchers have traditionally relied on the Eberhart and Russell (1966) model; this foundational parametric tool uses linear regression to categorize genotypes based on their responsiveness to environmental improvements. However, its reliance on a strictly linear assumption often fails to capture the multidimensional complexity of real-world biological interactions inherent in lentil trials.

Bridging the gap between simplicity and complexity, the AMMI (additive main effects and multiplicative interaction) model employs a multivariate parametric approach that uses principal component analysis (PCA) to distinguish between main effects and the intricate patterns within the interaction (Gauch, 2013). In lentil breeding, AMMI is frequently utilized to visualize "mega-environments" and specific adaptations through biplots, identifying genotypes that perform consistently across varied agro-ecological zones (Tadesse *et al*., 2021). The usefulness of the method in different crops has been noted by many researchers (Annicchiarico *et al*., 2010; Pour-Aboughadareh *et al*., 2022; Akhtar *et al*., 2023; Madhusudhana *et al*., 2023; Mullualem *et al*., 2024; Rakotondramanana *et al*., 2024). Again, the best linear unbiased prediction (BLUP) method offers a robust, modern alternative by treating genotype effects as random. This approach "shrinks" environmental noise and provides superior predictive accuracy, which is especially critical for lentil researchers dealing with unbalanced datasets or missing observations in multilocation trials (Piepho *et al*., 2008; Pezeshkpour *et al*., 2024). Artiaga *et al.* (2025) utilized Eberhart and Russell's, Lin and Binns's modified by Carneiro's, AMMI, and weighted average of absolute scores (WAASB) methods for the evaluation of yield (kg ha^{-1}) values and stability of 15 chickpea genotypes under seven different environments.

A biofortified variety is useful when it is rich in several micronutrient elements, e.g., Fe, Zn, and Se, deficient concurrently. Several researchers have conducted research on single or two micronutrient efficiencies in lentil (Thavarajah *et al*., 2011a; Rasheed *et al*., 2020; Dhaliwal *et al*., 2021; Kumar *et al*., 2024). But research on multiple elements, namely Zn, Se, and Fe, in lentil grown under multiple environments in Bangladesh has not yet been reported. The major goal of the study was to investigate the potential of agronomic biofortification in lentil for Se, Fe, and Zn to improve human health. For this, the study was designed to achieve biofortification in lentil, which is started by a baseline survey at farmers' fields in Bangladesh. In view of the above discussion, the study was conducted to identify stable lentil genotypes for yield and their micronutrient concentration under three different agro-climatic conditions of Bangladesh using Eberhart and Russell, AMMI, and BLUP methods.

Methods

PLANT MATERIALS

The investigation was carried out with 27 lentil genotypes selected from the 152 genotypes of the previous year's experiments. Among the 27 genotypes, 24 genotypes were collected from the International Center for Agricultural Research in the Dry Areas (ICARDA), and 3 check varieties were collected from Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA). The list of the genotypes and their sources is presented in the Supplementary Table S1.

EXPERIMENTAL SET-UP

A series of experiments were conducted over the years on Rabi season (November–March) at the experimental fields of Bangladesh Agricultural Research Institute (BARI), Gazipur, Regional Agricultural Research Station (RARS) Ishwardi and RARS Jashore. These are the major lentil production regions of Bangladesh. The soil properties and weather data of these locations are presented in Table 1 and Fig. 1A and B, respectively. According to Table 1, the soil pH ranged from acidic in Gazipur (5.46) to near-neutral in Ishwardi (6.91) and Jashore (6.89). Organic matter concentration was relatively low across all sites, with values increasing slightly from Gazipur (1.07%) to Jashore (1.28%). Micronutrient availability also varied among sites. Iron concentration was highest in Gazipur soil (29.1 ppm) and lower in Ishwardi (15.6 ppm) and Jashore (14.0 ppm). Selenium concentration ranged from 165 μg g^{-1} in Gazipur to 204 μg g^{-1} in Jashore. Available zinc was lowest in Gazipur (0.245 ppm), highest in Ishwardi (0.379 ppm), and intermediate in Jashore (0.275 ppm). The study was laid out in a Randomized Complete Block Design (RCBD) with three (3) replications. The plant-to-

Table 1. Soil properties of three experimental locations Gazipur, Ishwardi, and Jashore (soil analysis according to Soil Science Laboratory, Bangladesh Agricultural Research Institute, Gazipur).

	Soil test value		
Soil characteristics	**Gazipur**	**Ishwardi**	**Jessore**
pH	5.46	6.91	6.89
Organic matter (%)	1.07	1.20	1.28
Exchangeable K (meq/100 g)	0.130	0.11	0.15
Total nitrogen (%)	0.059	0.056	0.061
Available phosphorus (ppm)	6.69	30.00	14.80
Available sulfur (ppm)	14.55	12.00	20.00
Iron (ppm)	29.1	15.6	14.00
Selenium (μg/kg)	165	193	204
Zinc (ppm)	0.245	0.379	0.275

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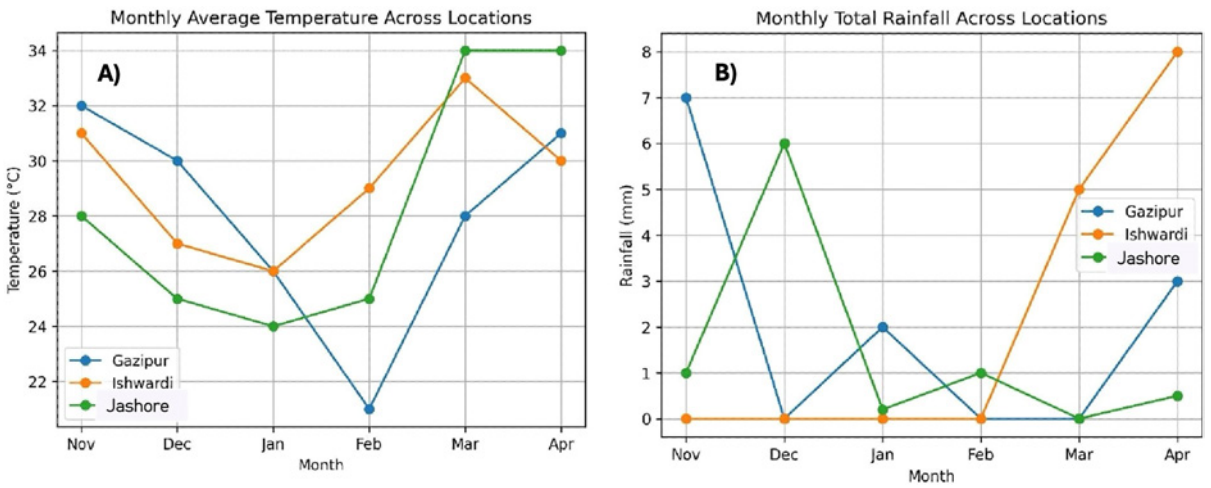


Fig. 1. Graphical representation of weather data of three locations, namely, Gazipur, Ishwardi, and Jashore during experimental periods.

plant distance was 6–8 cm and line-to-line distance was 30 cm. The unit plot size was 4.0 m × 0.6 m. The experimental plots were prepared by plowing with tractor, followed by harrowing and laddering. Weeds and stubbles were removed. Fertilizers were applied as per the national recommendation (FRG-2012) before the final land preparation. Seeds of 27 lentil genotypes were sown in the field in mid-November 2019. Thinning was done 25 days after sowing, and weeding was done during thinning and after 2 months after sowing. Different genotypes matured at different times, so the crops were harvested after 100–120 days.

PLANT SAMPLE COLLECTION AND PREPARATION

To determine the Se, Fe, and Zn concentrations of lentil seeds, three samples from each replication were collected randomly from each field of three experimental locations. The collected lentil plants were manually harvested and stored separately in cotton bags at room temperature. The lentil plants were dried at ≤40°C inside the cotton bags, threshed, hand-cleaned, and seeds were separated. Immature seeds and foreign materials were removed to make a homogeneous seed sample. To increase the storage period and keep the seeds viable and healthy, the cleaned seeds were oven-dried at 40°C to reduce the moisture concentration to 14% (Bhattarai *et al*., 2023) and then weighed. About 20 g of dry seeds for each sample were stored at –20°C. Then, dry lentil seed samples were shipped from Bangladesh to the Department of Plant Sciences, University of Saskachewan, Canada, for analysis.

LENTIL SEED SELENIUM, IRON, AND ZINC CONCENTRATION

The concentrations of Se, Zn, and Fe in lentil seeds were determined by the modified HNO_3–H_2O_2 method as outlined by Thavarajah *et al.* (2010). Approximately 500 mg of finely ground lentil seeds was weighed into digestion tubes. Digestion was conducted at 90°C using the following acids for specific durations: (a) 6 mL of concentrated (70%) nitric acid (HNO_3) for 1 h, (b) 3 mL of hydrogen peroxide (H_2O_2) for 15 min, and (c) 3 mL of 6 M hydrochloric acid (HCl) for another 5 min. Upon complete digestion, the tubes were removed from the digestion block and cooled to room temperature. The total volume was adjusted to 10 mL, and then the solution was filtered (Whatman No. 1 filter paper) using a vacuum system (Gardner Denver Thomas Inc., Welch Vacuum Technologies, LA, USA). The digestion was repeated twice, and the average data was used for analysis. The total Se concentration was determined using ICP-Mass Spectrometer (iCAP 6500 series, Thermo Jarrell Ash Corp., Franklin, MA, USA) with a detection limit of 75 µg l^{-1}. The Se concentration was quantified using a calibration curve that ranged from 1 to 50 mg l^{-1}. The digested samples were subsequently analyzed for Fe and Zn concentrations using F-AAS, one of the most widely used instruments for micronutrient analysis because of its lower cost, higher accessibility, reliability and analytical performance (Ma and Gonzalez, 1979). The quantification of Fe and Zn concentrations using F-AAS was done following the protocol described by Analytik Jena, novAA®300 (F-AAS). It was done using absorption analysis.

STATISTICAL ANALYSIS

All the data was subjected to statistical analysis. Here, the multivariate stability analysis was performed to explain the genotype (G) × environment (E) interaction. In stability analysis, relevant biometrical methods cited in standard text were followed (Singh and Chaudhary, 1985; Dabholkar, 1992). Eberhart and Russell's model (1966) was used to study the stability of a genotype under several environments.

$$Y_{ij} = \mu + b_i I_j + \delta_{ij} \; (i = 1, 2, \ldots, n \text{ and } j = 1, 2, \ldots, l)$$

Where, Y_{ij} = mean performance of $_i$th variety in the $_j$th environment; µ = mean of all genotypes across the environment; b_i = regression coefficient for the $_i$th genotype; I_j = environmental index for the $_j$th environment; δ_{ij} = captures the deviation from expected performance.

Phenotypic index (P_i) is considered as one of the stability parameters and it is the deviation of the genotype mean over environment from grand mean (Ram *et al*., 1970). The formula is:

$$Pi = \bar{Y}_i - \bar{Y}_{..}$$

Where, $\bar{Y}_i$ = mean of ith genotype over the environments and $\bar{Y}_{..}$ = grand mean.

Environmental index (Ij) is the deviation of mean of all genotypes at a given environment from grand mean and was calculated as:

$$I_j = \bar{Y}_{.j} - \bar{Y}_{..}$$

Where, $\bar{Y}_{.j}$ = mean at jth environment, $\bar{Y}_{..}$ = grand mean.

Linear regression coefficient (b_i) for the seed Se, Zn, and Fe and grain yield of 27 genotypes on environmental indices was done to determine the adaptability and stability of individual genotype. The formula is:

$$b_i = SPXY / SSX$$

Where, SSX = sum of means of environment; SPXY = sum of the product of Y (genotype) and X (environment)

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Mean square deviation (S^2_{di}) from linear regression was calculated using the formula:

$$S^2_{di} = \text{Reminder MS} - \text{Error MS}$$

Where,

$$\text{Reminder MS} = \text{Reminder SS}/n - 2(n = \text{number of environments})$$

$$\text{Remainder SS} = \text{SS}(Y) - \text{Regression SS},$$

$$\text{Regression SS} = \text{SP}(XY)^2 / \text{SS}(X)$$

The GGE biplots and AMMI are graphical images to exemplify G × E interaction and genotype ranking based on mean and stability.

To get a straightforward interpretation of the G × E interaction, the multivariate stability analysis was performed graphically based on AMMI model (Kempton, 1984; Gauch *et al*., 1988). The AMMI model was used to quantify the effect of different factors (genotype, location) of the experiment. The AMMI statistical model is most appropriately termed as a hybrid model. The hybrid model is:

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum_{n=1}^{N} \lambda n \gamma_{gn} \delta_{en} + \rho_{ge}$$

Where, Y_{ge} = yield of the genotype (G) in the environment (e); μ = grand mean; α_g = genotype mean deviation; β_e = environment mean deviation; N = number. of IPCAs (interaction principal component axis) retained in the model; λn = singular value for IPCA axis n; γ_{gn} = genotype eigenvector values for IPCA axis n; δ_{en} = environment eigenvector values for IPCA axis n; ρ_{ge} = the residuals.

MIXED-EFFECTS MODEL ANALYSIS FOR MULTIPLE ENVIRONMENTS

The study utilized a mixed-effects methodology through the application of BLUP analysis (Moehring *et al*., 2015). BLUPs can mitigate environmental variance and assess the genuine individual breeding value, and they are increasingly utilized by plant breeders to precisely quantify genotypic values. BLUPs were computed utilizing the subsequent model with the "lme4" package in R software version 4.5.2 (R Core Team, 2025):

$$y = X\beta + Zu + e$$

In this context, y represents the vector of the examined trait, β denotes the vector of fixed effects, u denotes the vector of random effects, e signifies the vector of residuals, X indicates the interception and Z is the matrix of random effects.

Results and discussion

The combined analysis of variance of 27 lentil genotypes presented in Supplementary Table S2. It revealed highly significant effects of genotype, environment, and genotype × environment interactions (GEI) for Fe, Se, and Zn concentrations, and seed yield in lentil genotypes evaluated across three locations in Bangladesh. These results indicate substantial genetic variation among the tested genotypes and strong environmental influence on both micronutrient accumulation and yield expression. Genotype-environment interaction affects micronutrient concentrations by influencing their incorporation by roots, translocation through shoots, and assimilation in grains (Singh *et al*., 2017). Significant GEI for all traits suggests differential genotypic responses across environments, indicating that performance rankings of genotypes change with location. Such environment-dependent performance has also been reported in lentil by Bhattacharya *et al.* (2025) and Gupta *et al.* (2021).

STABILITY ANALYSIS FOR YIELD AND NUTRIENT CONCENTRATION OF 27 LENTIL GENOTYPES BY EBERHART AND RUSSELL MODEL

The results of the response of different genotypes under three different environments following Eberhart and Russell Model are presented in Tables 2–5. The stability parameters under test included superiority index (Pi), regression coefficient (bi) and deviation from regression (S^2di) for grain yield, and Se, Zn, and Fe concentrations of lentil seeds. Variance of deviation from regression is more important than genotype × environment interaction in stability.

SEED SE CONCENTRATION

The mean value of Se concentration of lentil seeds of different genotypes (Table 2) averaged over environments indicates that genotypes LR 9-25 (G13) and LRIL-22-39 (G27) have the highest (388 μg g^{-1}) and the lowest (155 μg g^{-1}) Se concentration, respectively. The environment's mean values ranged from 214 μg g^{-1} to 251 μg g^{-1}. Considering the stability index, the genotypes LR 9-25 (G13) followed by BARI Masur-6 (G3) and LRIL-22-205 (G15) showed lower superiority index (Pi) for Se concentration in lentil seeds. The Ishwardi and Jashore environments showed the positive environmental index (Ij) and the Gazipur environment showed the negative environmental index (Ij) for Se biofortification. The higher selenium availability in the soils of Ishwardi and Jashore (Table 1) was consistent with their positive environmental index (Ij) values in the Eberhart and Russell stability model, indicating that greater soil Se availability favored higher and more stable selenium accumulation in lentil seeds, as previously reported in lentil biofortification studies by Rahman *et al.* (2013). The regression coefficient (bi) values of those genotypes ranged from −0.11 to 2.50, indicating that the bi values of genotypes LR 9-25 (G13), ILL-5090 (G4), LRIL-22-15 (G19), and LRIL-22-215 (G21) had low values, which were very close to 1, while the S^2di value of LR 9-25 (G13) tended near to zero. Based on the results of the regression analysis, the genotypes LR 9-25 (G13), ILL-5090 (G4), LRIL-22-15 (G19), and LRIL-22-215 (G21) were identified as highly stable over environments because their regression coefficient was close to unity (b = 1) (Table 2). Rahman *et al.* (2013) observed that Indian and local cultivars had the highest seed Se but lower seed yield at four locations in Bangladesh.

SEED ZN CONCENTRATION

The highest seed Zn concentration was recorded in genotype LRIL-21-220 (G25) (45.8 μg g^{-1}), while the lowest was observed in LRIL-22-15 (G19) (30.2 μg g^{-1}). Mean Zn concentration across locations ranged from 35.4 μg g^{-1} to 38.7 μg g^{-1} (Table 3). Genotypes LRIL-21-220 (G25), LRIL-22-39 (G27), LIRL-21-224 (G18), LRIL-21-109 (G17), LRIL-22-15 (G19), ILL-5086 (G10), ILL-5075 (G9), LRIL-22-215 (G21), LR 9-25 (G13), ILL-5096 (G14), and BARI Masur-7 (G11) exhibited lower superiority index (Pi), indicating better performance for Zn concentration. Ishwardi and Jashore exhibited positive environmental index (Ij) values in the Eberhart and Russell stability model, which may be associated with their comparatively higher soil Zn availability than in Gazipur, thereby supporting greater Zn accumulation and stable performance in lentil seeds, as previously reported by Akter *et al.* (2023). Regression coefficients (bi) close to unity and deviations from regression (S^2di) near zero for genotypes LRIL-21-109 (G17), LIRL-21-224 (G18), LRIL-22-15 (G19), BARI Masur-7 (G11), LRIL-22-39 (G27), and LRIL-21-220 (G25) indicated low environmental sensitivity and high stability. These genotypes combined higher Zn concentrations with wide adaptability across environments. Darai *et al.* (2020) identified RL-11 and Shital as stable genotypes for seed Zn concentration based on regression coefficient (bi), while accessions ILL-7723, WBL-77 were identified as stable genotypes for Zn concentration founded on the value of mean square deviation (S^2di).

SEED FE CONCENTRATION

The mean seed Fe concentration among 27 lentil genotypes ranged from 62.1 μg g^{-1} to 82.9 μg g^{-1}, with LRIL-21-220 (G25) showing the highest and LRIL-22-215 (G21) the lowest values (Table 4). Mean Fe concentration across environments varied from 71.0 μg g^{-1} to 74.0 μg g^{-1}. Genotypes BARI Masur-6 (G3), X96S/40-50134-12 (G12), LR 9-25 (G13), ILL-5121 (G20), LRIL-21-220 (G25), and ILL-5151 (G23) exhibited lower superiority index (Pi), indicating better performance for seed

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Table 2. Stability analysis for seed Se concentration of different lentil genotypes at three locations.

		Selenium (Se)						
		Location (μg g^{-1})						
ID	Genotype	Gazipur	Iswardi	Jashore	Overall mean (μg g^{-1})	Superiority index (Pi)	Regression coefficient (bi)	Deviation from regression (S^2di)
G1	ILL-8406-122	250	297	300	282	5652	1.37	234.9
G2	BINA Masur-7	170	201	217	196	18,470	1.30	−29.1
G3	BARI Masur-6	326	335	341	334	1471	0.40	−65.2
G4	ILL-5090	147	161	186	164	25,010	1.06	−44.9
G5	ILX-87015	215	282	231	243	10,839	0.46	2239.4
G6	LRIL-21-220	185	210	202	199	17,828	0.48	107.4
G7	FAIP-92-54L	277	304	358	313	3152	2.20	63.7
G8	ILL-5080	187	173	184	181	21,403	−0.11	31.9
G9	ILL-5075	172	250	255	226	13,552	2.27	767.2
G10	ILL-5086	208	200	220	209	15,977	0.34	61.2
G11	BARI Masur-7	246	254	284	261	8042	0.57	18.7
G12	X96S/40-50134-12	191	198	219	202	17,244	0.76	−30.2
G13	LR 9-25	375	393	396	388	0.00	1.02	19.8
G14	ILL-5096	167	183	183	178	22,075	0.45	−27.1
G15	LRIL-22-205	298	324	390	337	1778	2.50	234.9
G16	ILL-4684	186	184	196	189	19,835	0.27	−29.0
G17	LRIL-21-109	276	354	362	331	2065	2.36	696.4
G18	LRIL-21-224	149	145	209	167	24,594	1.61	752.1
G19	LRIL-22-15	164	185	200	183	21,035	0.98	−62.7
G20	ILL-5121	186	251	269	235	11,979	2.30	281.8
G21	LRIL-22-215	163	173	197	178	22,112	0.92	−27.9
G22	ILL-6336	297	317	305	306	3357	0.21	95.8

Continued

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Table 2. Continued.

		Selenium (Se)						
		Location (µg g^{-1})						
ID	Genotype	Gazipur	Iswardi	Jashore	Overall mean (µg g^{-1})	Superiority index (Pi)	Regression coefficient (bi)	Deviation from regression (S^2di)
G23	ILL-5151	217	207	266	230	12,743	1.31	746.2
G24	ILL-5139	201	205	245	217	14,703	1.20	166.8
G25	LRIL-22-39	163	153	165	160	25,987	0.03	−29.3
G26	ILL-6325	215	210	233	219	14,297	0.48	71.7
G27	LRIL-22-70	156	146	165	155	27,056	0.26	82.9
	Mean	214	233	251				
	E.Index (Ij)	−19.9	18.9	1.03				

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Table 3. Stability analysis for seed Zn concentration of different lentil genotypes at three different locations.

		Zinc (Zn)						
		Location (μg g^{-1})						
Entry ID	Genotype	Gazipur	Iswardi	Jashore	Overall mean (μg g^{-1})	Superiority index (Pi)	Regression coefficient (bi)	Deviation from regression (S^2di)
G1	ILL-8406-122	31.5	39.5	35.77	35.6	58.6	1.66	14.26
G2	BINA Masur-7	35.2	36.5	39.83	37.2	39.0	1.26	−1.52
G3	BARI Masur-6	36.1	33.4	38.77	36.1	50.2	0.45	11.50
G4	ILL-5090	31.6	30.0	31.10	30.9	114.7	−0.25	−0.55
G5	ILX-87015	31.2	38.9	33	34.5	72.14	1.08	23.69
G6	LRIL-21-220	33.5	32.2	34.43	33.4	78.0	0.15	−1.46
G7	FAIP-92-54L	34.8	35.8	31.13	33.9	77.9	-0.83	6.33
G8	ILL-5080	35.4	37.2	40.60	37.7	34.3	1.42	1.44
G9	ILL-5075	38.7	39.1	42.90	40.2	16.8	1.58	2.51
G10	ILL-5086	37.6	40.3	43.17	40.4	16.3	1.56	−0.59
G11	BARI Masur-7	41.0	42.9	46.80	43.5	15.3	1.07	0.71
G12	X96S/40-50134-12	32.1	37.0	38.13	35.8	53.5	1.86	−1.23
G13	LR 9-25	39.5	39.1	45.33	41.3	124.4	1.42	11.26
G14	ILL-5096	36.4	43.4	41.83	40.5	17.5	1.85	4.78
G15	LRIL-22-205	30.6	31.1	31.83	31.2	109.7	0.33	−1.47
G16	ILL-4684	30.7	35.3	33.17	33.1	84.9	0.66	3.65
G17	LRIL-21-109	41.7	45.5	47.67	45.0	1.3	0.95	0.22
G18	LRIL-21-224	38.8	40.7	41.87	40.5	3.6	0.90	0.49
G19	LRIL-22-15	28.5	29.6	32.53	30.2	12.6	1.09	0.22
G20	ILL-5121	30.9	36.6	33.03	33.5	80.5	0.73	9.44
G21	LRIL-22-215	40.0	41.1	43.00	41.4	10.6	0.81	−0.87
G22	ILL-6336	27.6	33.6	30.53	30.6	121.3	1.19	8.56
G23	ILL-5151	37.1	40.8	39.67	39.2	23.9	0.90	−1.31
G24	ILL-5139	38.0	37.6	42.00	39.2	23.3	1.75	4.81

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Table 3. Continued.

		Zinc (Zn)						
		Location (μg g^{-1})						
Entry ID	Genotype	Gazipur	Iswardi	Jashore	Overall mean (μg g^{-1})	Superiority index (Pi)	Regression coefficient (bi)	Deviation from regression (S^2di)
G25	LRIL-22-39	44.5	45.7	47.13	45.8	0.05	0.94	0.65
G26	ILL-6325	28.1	30.3	33.63	30.7	117.4	1.52	−1.31
G27	LRIL-22-70	43.3	44.4	45.70	44.5	1.20	0.94	0.75
	Mean	35.4	37.7	38.70				
	E.Index (Ij)	-2.02	1.51	0.50				

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Table 4. Stability analysis for seed Fe concentration of different lentil genotypes at three locations.

		Iron (Fe)						
		Location (μg g^{-1})						
ID	Genotype	Gazipur	Iswardi	Jashore	Overall mean (μg g^{-1})	Superiority index (Pi)	Regression coefficient (bi)	Deviation from regression (S^2di)
G1	ILL-8406-122	71.68	75.5	70.5	72.5	58.3	−1.04	5.12
G2	BINA Masur-7	62.0	64.2	66.5	64.2	178.7	−0.97	2.30
G3	BARI Masur-6	80.8	76.0	77.3	78.1	9.0	1.24	−0.96
G4	ILL-5090	68.9	77.5	71.8	72.7	62.7	−2.67	1.41
G5	ILX-87015	83.0	79.8	78.8	80.5	12.1	1.63	−3.70
G6	LRIL-21-220	78.8	75.7	70.7	75.1	34.6	1.48	19.63
G7	FAIP-92-54L	64.3	61.9	68.5	64.9	166.4	0.39	18.25
G8	ILL-5080	71.0	62.2	67.2	66.8	132.7	2.79	−3.41
G9	ILL-5075	69.0	61.1	62.1	64.0	180.3	2.73	−2.13
G10	ILL-5086	67.3	70.9	73.3	70.4	83.7	−1.46	4.18
G11	BARI Masur-7	78.3	74.1	77.8	76.7	19.9	1.25	−3.73
G12	X96S/40-50134-12	81.1	78.4	78.0	79.2	7.1	1.02	−2.73
G13	LR 9-25	82.6	74.8	81.2	79.5	8.8	2.34	5.29
G14	ILL-5096	70.5	70.4	67.4	69.5	91.4	0.26	2.20
G15	LRIL-22-205	70.1	65.0	64.3	66.4	136.5	1.88	−3.70
G16	ILL-4684	82.2	76.1	74.4	77.5	17.1	2.32	5.04
G17	LRIL-21-109	80.5	78.8	78.7	79.3	44.2	0.61	−0.95
G18	LRIL-21-224	75.5	70.1	75.1	73.6	45.3	1.59	2.79
G19	LRIL-22-15	83.8	75.0	78.3	79.0	10.5	2.92	−3.63
G20	ILL-5121	74.9	72.5	73.2	73.5	6.5	0.82	−0.45
G21	LRIL-22-215	64.9	61.1	60.4	62.1	216.4	1.41	−1.37
G22	ILL-6336	64.1	60.0	66.0	63.4	192.9	1.04	10.06
G23	ILL-5151	84.4	76.0	80.3	80.2	6.3	2.74	−2.27

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Table 4. Continued.

		Iron (Fe)						
		Location (µg g^{-1})						
ID	Genotype	Gazipur	Iswardi	Jashore	Overall mean (µg g^{-1})	Superiority index (Pi)	Regression coefficient (bi)	Deviation from regression (S^2di)
G24	ILL-5139	61.3	65.8	65.5	64.2	180.1	−1.60	−2.88
G25	LRIL-22-39	84.6	81.7	82.4	82.9	0.00	0.96	−0.18
G26	ILL-6325	78.4	72.6	71.2	74.1	41.2	2.19	3.12
G27	LRIL-22-70	64.6	60.8	63.8	63.1	196.4	1.15	−1.81
	Mean	74.0	71.0	72.0				
	E.Index (Ij)	1.87	−0.37	−1.50				

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Table 5. Stability analysis for seed yield of different lentil genotypes at three locations.

		Yield						
		Location (kg/ha)			Overall mean (kg/ha)	Superiority index (Pi)	Regression coefficient (bi)	Deviation from regression (S^2di)
ID	Genotype	Gazipur	Iswardi	Jashore				
G1	ILL-8406-122	535	657	716	636	594,733	3.53	−306.3
G2	BINA Masur-7	1066	1584	1652	1434	37,249	3.00	1184.3
G3	BARI Masur-6	1253	1804	1968	1675	407	0.86	−32.9
G4	ILL-5090	428	551	559	512	737,610	0.68	−352.78
G5	ILX-87015	570	629	651	617	626,201	0.40	−556.50
G6	LRIL-21-220	518	537	572	542	713,549	0.23	−245.0
G7	FAIP-92-54L	597	608	663	623	623,312	0.26	425.9
G8	ILL-5080	478	560	578	539	711,245	0.50	−569.2
G9	ILL-5075	639	659	679	659	586,948	0.18	−500.1
G10	ILL-5086	571	566	621	586	665,681	0.17	595.5
G11	BARI Masur-7	1175	1836	2006	1672	1005	1.05	5.4
G12	X96S/40-50134-12	616	668	733	672	564,875	0.51	441.3
G13	LR 9-25	1049	1770	1816	1545	13,629	0.88	7126.4
G14	ILL-5096	391	459	502	451	816,378	0.52	−330.3
G15	LRIL-22-205	545	582	631	586	660,813	0.37	−568.6
G16	ILL-4684	584	690	680	652	585,976	0.53	−429.9
G17	LRIL-21-109	771	844	827	814	434,086	0.33	−88.5
G18	LRIL-21-224	402	447	455	435	842,629	0.27	−567.5
G19	LRIL-22-15	406	505	479	463	803,280	0.43	426.2
G20	ILL-5121	562	694	741	666	563,287	0.87	−508.7
G21	LRIL-22-215	332	451	468	417	854,994	0.70	−496.9
G22	ILL-6336	617	737	750	702	531,185	0.69	−439.7

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Table 5. Continued.

ID	Genotype	Yield						
		Location (kg/ha)			Overall mean (kg/ha)	Superiority index (Pi)	Regression coefficient (bi)	Deviation from regression (S^2di)
		Gazipur	Iswardi	Jashore				
G23	ILL-5151	537	686	646	623	613,648	0.65	1789.9
G24	ILL-5139	465	566	709	580	652,159	4.13	4551.0
G25	LRIL-22-39	434	531	551	505	748,017	0.59	−560.4
G26	ILL-6325	475	618	652	581	653,687	4.01	−568.9
G27	LRIL-22-70	591	667	730	663	571,954	0.63	142.8
	Mean	615	774	816				
	E. Index (Ij)	-120.1	80.99	39.12				

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Fe concentration. Gazipur exhibited a positive environmental index (Ij) in the Eberhart and Russell stability model for Fe concentration, which may be associated with its comparatively higher soil Fe availability (29.1 ppm, Table 1) than in Ishwardi and Jashore, thereby favoring greater Fe accumulation in lentil seeds, consistent with previous reports that environmental and soil factors significantly influence Fe accumulation and biofortification efficiency in lentil (Thavarajah *et al*., 2011b; Dhaliwal *et al*., 2021). Regression coefficients (bi) close to unity and deviation from regression (S^2di) near zero for genotypes LRIL-21-220 (G25), ILL-5121 (G20), BARI Masur-6 (G3), and LRIL-21-109 (G17) indicated low environmental sensitivity and high stability. These genotypes combined higher seed Fe concentration with wide adaptability across environments. Darai *et al*. (2020) identified five lentil accessions, namely, Sagun, WBL-77, LG12, RL-6 as stable genotypes for seed Fe concentration based on regression coefficient (bi), while accessions Black Masuro, ILL7723, RL-4 were classified as stable genotypes based on S^2di.

SEED YIELD

Genotype BARI Masur-6 (G3) produced the highest seed yield (1675 kg ha^{-1}), whereas LRIL-22-215 (G21) showed the lowest yield (417 kg ha^{-1}) across three locations (Table 5). Similar yield variation among lentil genotypes was reported by Khatun *et al*. (2022). Genotypes BARI Masur-6 (G3) and BARI Masur-7 (G11) exhibited lower superiority index (Pi), indicating better yield performance. Eberhart and Russell proposed that an ideal population of genotypes has the highest yield over a broad range of environments, a regression coefficient (b) value of 1.0 and deviation mean square of zero. Ishwardi and Jashore had positive environmental indices, while Gazipur was unfavorable. Regression coefficients (bi) close to unity and deviation from regression (S^2di) near zero for BARI Masur-6 (G3) and BARI Masur-7 (G11) indicated low environmental sensitivity and high stability. These genotypes combined higher yield with wide adaptability across environments. In a study of G × E interactions, Hossain *et al.* (2023) reported that genotypes G6, G7, G9, and G10 showed positive Pi, non-significant bi, and S^2di, indicating that they were stable over the locations. They postulated that Jashore and Ishwardi were favorable locations and Mymensingh and Rangpur were unfavorable locations in the context of seed yield performances. Tadesse *et al.* (2021) categorized four lentil accessions, namely, G4, G15 G8, G6, G10 were identified as stable genotypes for seed yield concentration based on the value of mean square deviation (S^2di).

STABILITY ANALYSIS FOR YIELD AND NUTRIENT CHARACTERS BY AMMI MODEL

Stability analysis by AMMI 1 Biplot

The AMMI 1 biplot statistical model has been used to diagnose the G (genotype) × E (environment) interaction pattern in respect of four studied traits. For the selenium (Se), the highest portion (59.8%) of variation is explained by genotype effects, while the least variation is explained by environment effects (25.0%). The interaction effect explained 15.2% of the total Se concentration variation. The interaction term was partitioned into three terms of AMMI extracted from genotype (G) × environment (E) interaction by this method (Fig. 2A). Here, the figure showed that genotypes that remain at the right side of perpendicular line, i.e., BARI Masur-6 (G3), ILL-6336 (G22), LR 9-25 (G13), and BARI Masur-7 (G11), are less affected by genotype (G) × environment (E). Genotypes ILX-87015 (G5), BARI Masur-7 (G11), ILL-5139 (G24), ILL-5151 (G23), X96S/40-50134-12 (G12), and LRIL-22-70 (G6) indicated IPCA1 score close to center point, meaning they are stable genotypes across the environments. Darai *et al.* (2017) exhibited the same thing, where biofortified lentil genotypes, i.e., HUL-57, PL-4, RL-11, and LG-12, showed the best stability across the environments.

Genotype effects explained the largest proportion of variation in seed Zn concentration (53.0%), followed by environmental effects (27.4%) and genotype × environment interaction (19.3%) (Fig. 2B). Genotypes ILL-5080 (G8), ILL-5086 (G10), LIRL-21-224 (G18), LRIL-22-215 (G21), ILL-5151 (G23), ILL-5075 (G9), and ILL-5139 (G24) whereas environment Jashore showed superior Zn concentration, and the recommended genotypes are less affected by genotype (G) × environment (E) interaction. These genotypes and the environment Jashore were found in the same quadrants, so they interact positively (Fig. 2B). Gazipur and Jashore, having IPCA1 scores close to zero, were identified as favorable environments for these genotypes. The genotypes BINA Masur-7 (G2), ILL-5080 (G8), X96S/40-50134-12 (G12), and ILL-5151 (G23) seem the most stable across the environments as they tend toward zero or center point. Gupta *et al.* (2021) showed 16 genotypes with above-average performance for grain Zn concentration in AMMI 1 biplot. Among the 16 genotypes, IC262839 (G27), P16214 (G14), and P2113 (G24) were the most adapted genotypes across the locations, having a higher mean Zn concentration. Darai *et al.* (2020) identified three lentil genotypes WBL-77, RL-11, and Sagun, which contained a high Zn concentration and were widely adapted to three locations based on the AMMI 1 biplot display.

The largest percentage of variance in seed Fe concentration was explained by genotype effects (56.6%), followed by environmental effects (23.2%) and genotype × environment interaction (20.3%) (Fig. 2C). The best-performing genotypes that show superior seed Fe concentration are LIRL-21-224 (G18), LRIL-22-70 (G6), ILL-6325 (26), BARI Masur-7 (G11), and BARI Masur-6 (G3). These genotypes and the environment Gazipur remain in the same quadrant, so there is presence of positive interactions and genotypes show similar adaptations. The genotypes LIRL-21-224 (G18), ILL-5121 (G20), LRIL-22-70 (G6), ILL-6325 (26), BARI Masur-7 (G11), and ILL-5096 (G14) are suited for widespread adaptation and exhibit little G × E interaction. Gupta *et al.* (2021) identified 15 genotypes with above-average grain Fe concentration in an AMMI1 biplot, but only two – IC 560812 (G22) and IG 49 (G36) – combined high Fe concentration with stability (low IPCA1 scores). Similarly, Darai *et al.* (2020) reported four genotypes (Black Masuro, Sagun, RL-4, and LG-12) with high seed Fe concentration and wide adaptation across three environments. Chourkri *et al*. (2025) reported that the genotype contributed less to the total variance for iron and zinc concentrations.

Grain yield variation was mostly explained by genetic effects (58.3%), followed by environmental effects (25.0%) and genotype × environment interaction (16.8%) (Fig. 2D). Genotypes BINA Masur-7 (G2), BARI Masur-6 (G3), LR 9-25 (G13), and BARI Masur-7 (G11), whereas the environments Ishwardi and Jashore showed highest mean grain yield above the grand mean with positive IPCA1 scores, The AMMI1 biplot effectively characterized G×E interaction patterns for grain yield in lentil. The genotypes ILL-5121 (G20), ILL-6336 (G22), ILL-8406-122 (G1), ILL-5151 (G23), LRIL-22-70 (G27), ILL-4684 (G16), and X96S/40-50134-12 (G12) showed the highest stability and less G × E interaction because they remained very close to the center point. Hossain *et al.* (2023) reported G10 (BARI Masur-8) as a high-yielding and stable genotype based on an AMMI1 biplot. Similarly, Tadesse *et al.* (2021) identified 11 lentil genotypes with low G × E interaction using AMMI1 analysis. Darai *et al.* (2020) also found Khajura-2 and RL-12 to be the most stable genotypes due to their low PCA1 scores.

Stability analysis by AMMI 2 Biplot

The first two AMMI interaction PCs explained 99.9 % of the total interaction effect for seeds Se concentration (PC1 = 58.1%, PC2 = 41.9%) (Fig. 3A). In AMMI 2 biplot (Fig. 3A), the environmental scores are joined to the origin by side lines. Since IPCA 2 scores also play a significant role in explaining the GEI, the IPCA 1 score was plotted against the IPCA 2 score to further explore adaptation. An example of this is shown in Fig. 3A, where the

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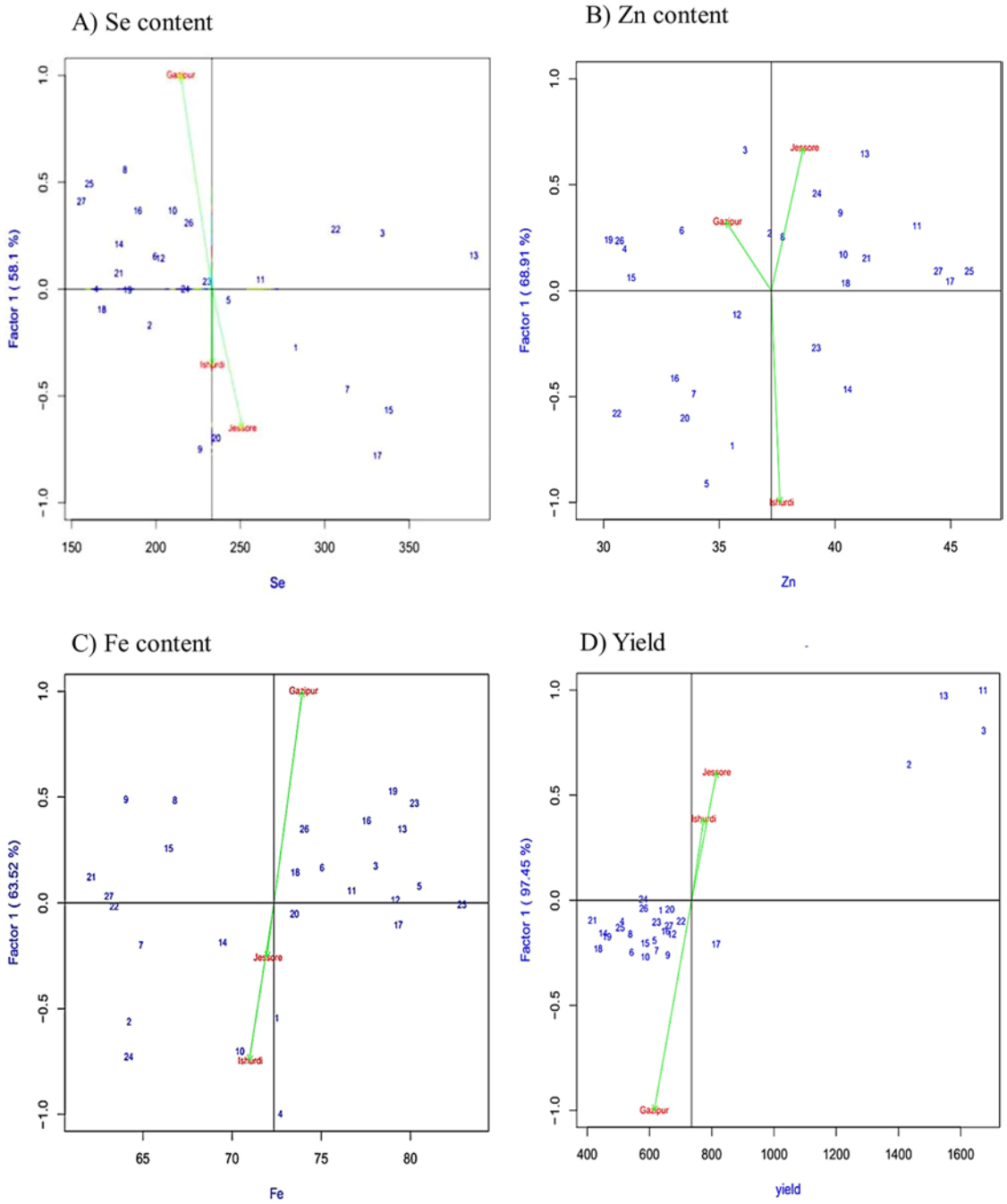


Fig. 2. AMMII biplot for 27 lentil genotypes (A) IPCA 1 score plotted Y axis against mean Se concentration in X-axis (B) IPCA 1 score plotted Y axis against mean Zn concentration in X-axis (C) IPCA 1 score plotted Y axis against mean Fe concentration in X-axis (D) IPCA 1 score plotted Y axis against mean yield in X-axis. Here, 1 = ILL-8406-122, 2 = BINA Masur-7, 3 = BARI Masur-6, 4 = ILL-5090, 5 = ILX-87015, 6 = LRIL-21-220, 7 = FAIP-92-54L, 8 = ILL-5080, 9 = ILL-5075, 10 = ILL-5086, 11 = BARI Masur-7, 12 = X96S/40-50134-12, 13 = LR 9-25, 14 = ILL-5096, 15 = LRIL-22-205, 16 = ILL-4684, 17 = LRIL-21-109, 18 = LRIL-21-224, 19 = LRIL-22-15, 20 = ILL-5121, 21 = LRIL-22-215, 22 = ILL-6336, 23 = ILL-5151, 24 = ILL-5139, 25 = LRIL-22-39, 26 = ILL-6325, 27 = LRIL-22-70.

points representing the environments Gazipur, Ishwardi, and Jashore are connected to the origin. Among all the environments, Gazipur had strong interaction because of long arrows found in that case. Environments, i.e., Ishwardi and Jashore, which had a shorter distance from the origin, showed stability and less deviation to most of the genotypes as well as higher mean Se concentration. In the present study, ILL-5090 (G4), LRIL-22-15 (G19), LRIL-22-215 (G21), X96S/40-50134-12 (G12), and BARI Masur-7 (G11) were close to the origin and hence they were non-sensitive to environmental interactive forces and showed stable performance. The farthest genotypes, such as ILX-87015 (G5), LRIL-22-205 (G15), LIRL-21-224 (G18), ILL-5121 (G20), LRIL-21-109 (G17), ILL-5075 (G9), and ILL-5151 (G23), depicted more specific adaptation to environments.

The first two AMMI interaction PCs explained 99.9% of the total interaction effect for biofortified Zn concentration (PC1 = 68.9%, PC2 = 31.1%) (Fig. 3B). In AMMI 2 biplot (Fig. 3B), the environmental scores are joined to the origin by side lines. Sites with short spokes or arrows do not exert strong interactive forces. Those with long spokes exert strong interaction. An example of this is shown in Fig. 3B, where the points representing the environments Gazipur, Ishwardi, and Jashore are connected to the origin. The environment of Gazipur had short spokes and

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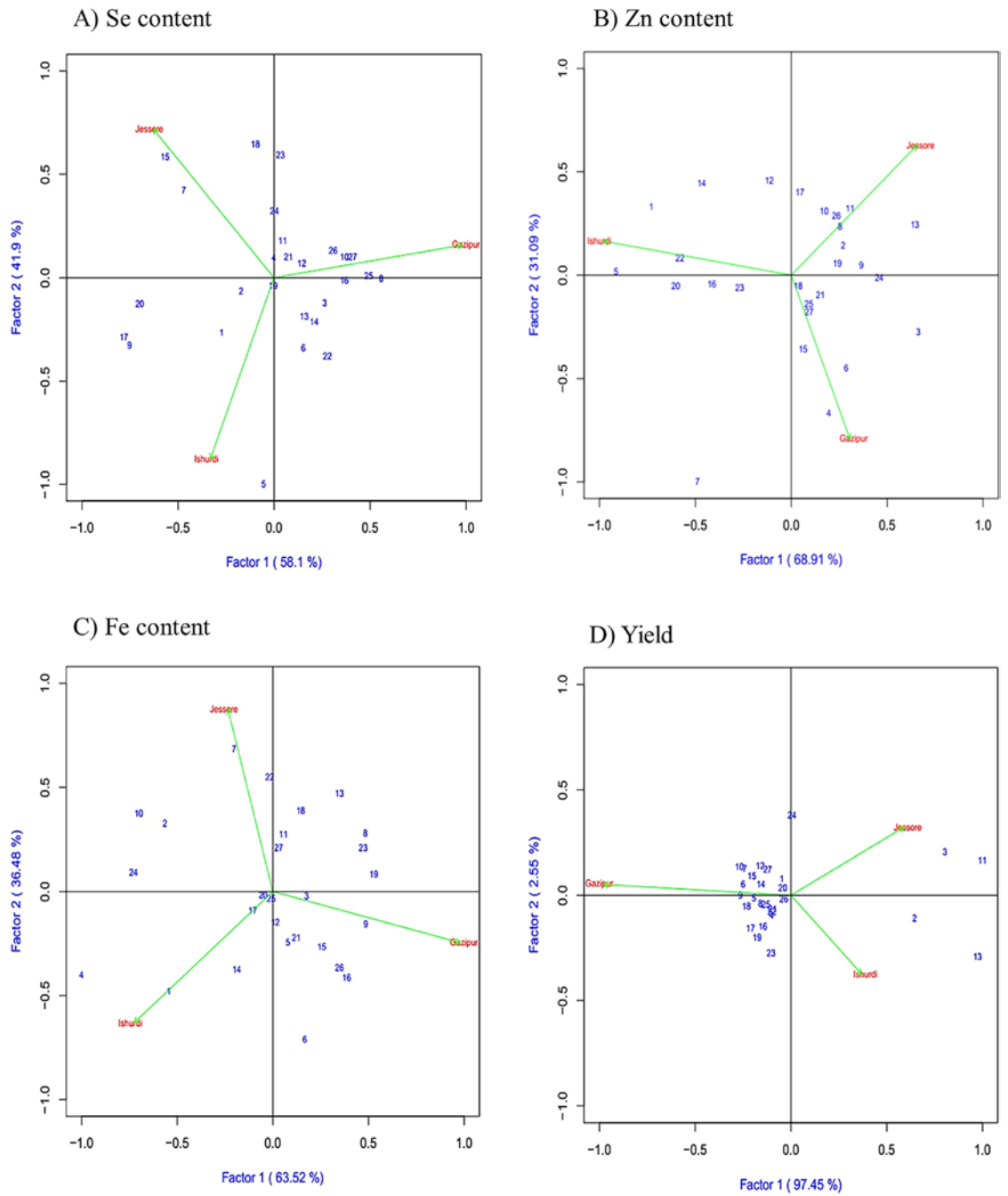


Fig. 3. AMMI2 biplot for 27 lentil genotypes in three locations (A) IPCA 2 score plotted Y axis against AMMI 1 interaction IPCA1 score in X-axis for mean Se concentration (B) IPCA 2 score plotted Y axis against AMMI 1 interaction IPCA1 score in X-axis for mean Zn concentration (C) IPCA 2 score plotted Y axis against AMMI 1 interaction IPCA1 score in X-axis for mean Fe concentration (D) IPCA 2 score plotted Y axis against AMMI 1 interaction IPCA1 score in X-axis for mean yield. Here, 1 = ILL-8406-122, 2 = BINA Masur-7, 3 = BARI Masur-6, 4 = ILL-5090, 5 = ILX-87015, 6 = LRIL-21-220, 7 = FAIP-92-54L, 8 = ILL-5080, 9 = ILL-5075, 10 = ILL-5086, 11 = BARI Masur-7, 12 = X96S/40-50134-12, 13 = LR 9-25, 14 = ILL-5096, 15 = LRIL-22-205, 16 = ILL-4684, 17 = LRIL-21-109, 18 = LRIL-21-224, 19 = LRIL-22-15, 20 = ILL-5121, 21 = LRIL-22-215, 22 = ILL-6336, 23 = ILL-5151, 24 = ILL-5139, 25 = LRIL-22-39, 26 = ILL-6325, 27 = LRIL-22-70.

they did not exert strong interactive forces, whereas Ishwardi exerted strong interactive forces. In the present study, FAIP-92-54L (G7), ILX-87015 (G5), ILL-5090 (G4), BARI Masur-6 (G3), X96S/40-50134-12 (G12), ILL-5096 (G14), ILL-8406-122 (G1), and ILL-5090 (G4) were more responsive since they were away from the origin, whereas genotypes LIRL-21-224 (G18), LRIL-22-215 (G21), LRIL-21-220 (G25), LRIL-22-39 (G27), and LRIL-22-15 (G19) were close to the origin and hence they were non-sensitive to environmental interactive forces. Gupta *et al.* (2021) found for grain Zn concentration, L 11-289 (G5) followed by L 11-294 (G26) were specifically adapted to Delhi, PL 02 (G15) followed by IC 560812 (G22) to Sagar, and L 7903 (G17) had specific adaptation to Sehore based on AMMI 2 biplot.

For seed Fe concentration, the first two AMMI interaction PCs explained 99.9% of the total interaction effect (PC1 = 63.5%, PC2 = 36.5%) (Fig. 3C). In this case, the environment of Gazipur had long spokes and they exerted strong interactive forces. In the present study, ILL-5090 (G4), ILL-8406-122 (G1), LRIL-22-70 (G6), ILL-5086 (G10), FAIP-92-54L (G7), ILL-4684 (G16), and LR 9-25 (G13) were found more responsive since they were away from the origin whereas genotypes LRIL-21-220 (G25), ILL-5121 (G20),

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BARI Masur-6 (G3), X96S/40-50134-12 (G12), and LRIL-21-109 (G17) were close to the origin and hence they are regarded as non-sensitive to environmental interactive forces (Fig. 3C). The AMMI 2 biplot plotted between IPCA1 and IPCA2 revealed genotypes specifically adapted to different locations that were investigated in the study. Gupta *et al.* (2021) identified genotype P 16214 (G14) that performed well in Delhi and L 4076 (G1) in Sagar, whereas IC 208326 (G12) performed well in Sehore for grain Fe concentration using AMMI 2 biplot display.

For yield of lentil, the first two AMMI interaction PCs explained 100% of the total interaction effect (PC1 = 97.5%, PC2 = 2.6%) (Fig. 3D). According to Fig. 3D, the environments of Gazipur had long spokes and it exerted strong interactive forces, whereas Ishwardi and Jashore had shorter distance from the origin which showed little deviation to most of the genotypes and more stability. ILL-5151 (G23) was unstable followed by ILL-5139 (G24). Genotypes ILL-8406-122 (G1), ILL-5121 (G20), and ILL-6325 (G26) showed the most stability for high-yielding lentil genotypes over the locations when IPCA 1 and IPCA 2 scores were plotted (Fig. 3D). Since IPCA 2 scores also play a significant role in explaining the GEI, the IPCA 1 score was plotted against the IPCA 2 score to further explore adaptation. In the present study, BARI Masur-7 (G11), LR 9-25 (G13), ILL-5139 (G24), and ILL-5151 (G23) were more responsive since they were away from the origin, whereas genotypes ILL-5121 (G20), ILL-6325 (G26), and ILL-8406-122 (G1) were close to the origin and hence they were non-sensitive to environmental interactive forces. Tadesse *et al.* (2021) conducted a similar study where the genotypes DZ -2012-LN-00118 (G4), DZ -2012-LN-0051 (G15), and DZ -2012-LN-0058 (G8) were the most stable genotypes and showed wider adaptation over the studied environments.

STABILITY ANALYSIS FOR YIELD AND NUTRIENT CHARACTERS BY BLUP ANALYSIS

The precise assessment of genotype, means performance, is one of the most significant benefits of BLUP method (Nardino, 2016). The results from using the BLUP method can be more accurate and dependable. BLUP performed better in genotype-by-environment data and according to Smith *et al.* (2005), BLUP also enables the best prediction of random effects. This approach is a highly effective way to estimate the average performance of genotypes in mixed models. The overall mean values over three locations for four biofortified and yield traits are presented in Table 6. Among 27 genotypes, the highest combined BLUP values were exhibited by the genotypes LR 9-25 (G13), LRIL-22-205 (G15), BARI Masur-6 (G3), LRIL-21-109 (G17), and FAIP-92-54L (G7) for Se concentration; LRIL-21-220 (G25), ILL-5151 (G23), LRIL-22-15 (G19), LR 9-25 (G13), and X96S/40-50134-12 (G12) for Fe concentration; LRIL-21-109 (G17), BARI Masur-7 (G11), LRIL-21-220 (G25), LRIL-22-39 (G27), and LRIL-22-215 (G21) for Zn concentration and LRIL-21-109 (G17), X96S/40-50134-12 (G12), ILL-5121 (G20), LRIL-22-39 (G27) and ILL-5075 (G9) for yield. Hossain *et al.* (2023) found high-yielding stable lentil genotypes, namely, G7, G10, and G4 according to BLUP-based simultaneous selection stability characteristics.

In the current study, we employed three methods, namely, Eberhart and Russell, AMMI, and BLUP to find out stable genotypes. Eberhart and Russell remains a popular introductory tool for its simplicity and ability to categorize varieties via linear regression; it is fundamentally limited by its assumption of linearity and sensitivity to unbalanced data. AMMI improves upon this by using PCA to reveal complex, non-linear patterns of adaptation, providing high-quality visual biplots that identify specific “mega-environments.” However, for modern, large-scale breeding programs with “noisy” or incomplete datasets, BLUP is the superior choice; it uses mixed-model equations to provide the most statistically accurate predictions of genetic value by minimizing environmental bias. Here, the findings of stability methods such as AMMI, Eberhart and Russell and BLUP were very similar. Considering all the stability models, LR 9-25 (G13), and BARI Masur-6 (G3) were the best for Se concentration; LRIL-22-39 (G25), X96S/40-50134-12 (G12), ILL-5151 (G23), and LRIL-22-15 (G19) were superior and common genotypes for Fe concentration; LRIL-22-39 (G25), LRIL-21-109 (G17), LRIL-22-39 (G27), and BARI Masur-7 (G11) were the best for Zn concentration. Bhattacharya *et al.* (2025) observed ILL-10123 and VL-156 as the “ideal” genotypes for Fe and Zn concentrations, respectively, in three testing locations using GGE biplot combined with BLUP analysis. Finally for yield performances, the genotypes ILL-5121 (G20), BARI Masur-6 (G3) and LRIL-21-109 (G17) showed more yield and stability. Considering such all-stability models, Hossain *et al.* (2023) observed well-adapted genotypes, namely, G2, G9, and G7 with moderate grain yield in all tested environments. Amiri *et al.* (2025) observed that the genotypes 10, 18, 23, 74, and 93 in chickpea were the more stable genotypes using both BLUP and AMMI models. Karimizadeh *et al.* (2026) integrated AMMI and BLUP methods and identified G2, G9, G12, G16, and G8 as superior-performing and widely stable in lentil. The superior and stable biofortified lentil genotypes identified in this study may reflect genotype-specific efficiency in micronutrient uptake, internal translocation, and seed accumulation, resulting in enhanced Se, Fe, and Zn concentrations in edible grains, particularly under micronutrient-favorable environments where soil-available nutrients positively support grain nutrient accumulation. Such genotype- and environment-dependent responses are important for lentil biofortification, as stable enrichment of Se, Fe, and Zn in grains can contribute to improving dietary micronutrient intake and human nutrition, supporting lentil as a promising whole-food source for increased micronutrient intake (Thavarajah *et al*., 2011b).

CONCLUSION

This study demonstrated considerable genotypic variation in grain selenium, iron, and zinc concentrations among lentil genotypes grown across different environments. The combined use of the Eberhart and Russell model, AMMI, and BLUP approaches enabled the identification of superior and stable genotypes for micronutrient biofortification. Genotypes such as LR 9-25 (G13), ILL-5151 (G23), and BARI Masur-7 (G11) were superior for selenium concentration; LRIL-22-39 (G25), X96S/40-50134-12 (G12), ILL-5151 (G23), and LRIL-22-15 (G19) performed better for iron concentration; while LRIL-22-39 (G25), LRIL-21-109 (G17), LRIL-22-39 (G27), and BARI Masur-7 (G11) were identified as promising genotypes for zinc concentration. In addition, ILL-5121 (G20), BARI Masur-6 (G3), and LRIL-21-109 (G17) showed notable stability and superior yield performance across the analyses.

The findings further suggest that micronutrient accumulation in lentil is influenced by both genotype and environment, with Ishwardi and Jashore appearing more favorable for biofortified lentil production. The positive association between soil-available micronutrients and grain nutrient concentration in specific environments indicates that suitable growing conditions may enhance nutrient uptake, translocation, and seed deposition. From a breeding perspective, the identified superior and stable genotypes can be used as potential donor parents in crossing programs to develop high-yielding, nutrient-dense lentil varieties. Genotypes with high selenium, iron, or zinc concentration may be intercrossed to combine multiple micronutrient traits, while stable genotypes with good yield performance can be advanced directly or used in multi-location trials for variety development. Overall, these findings provide valuable genetic resources and practical breeding guidance for developing biofortified lentil cultivars with improved nutritional quality and potential benefits for human health.

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Table 6. Overall mean values of BLUP (best linear unbiased prediction) method over three locations.

ID	Genotype	Se	Zn	Fe	Yield
G1	ILL-8406-122	281	35.7	72	640
G2	BINA Masur-7	210	40.1	70.5	592
G3	BARI Masur-6	261	43.1	76.4	1.64
G4	ILL-5090	204	35.8	78.7	675
G5	ILX-87015	384	41.0	79.0	1.51
G6	LRIL-21-220	180	40.3	69.6	462
G7	FAIP-92-54L	335	31.6	66.8	591
G8	ILL-5080	190	33.3	77.1	655
G9	ILL-5075	328	44.5	63.9	811
G10	ILL-5086	170	40.2	73.4	446
G11	BARI Masur-7	185	30.7	78.5	474
G12	X96S/40-50134-12	197	37.2	64.7	1.41
G13	LR 9-25	236	33.7	73.4	668
G14	ILL-5096	180	41.1	62.8	429
G15	LRIL-22-205	304	31.0	63.9	703
G16	ILL-4684	230	39.0	79.7	627
G17	LRIL-21-109	218	39.1	64.7	586
G18	LRIL-21-224	163	42.4	82.1	514
G19	LRIL-22-15	220	31.1	73.9	587
G20	ILL-5121	158	41.2	63.7	665
G21	LRIL-22-215	331	36.2	77.6	1.64
G22	ILL-6336	167	31.3	72.6	521
G23	ILL-5151	243	34.6	76.2	621
G24	ILL-5139	200	33.6	74.8	550
G25	LRIL-22-39	311	34.1	65.3	627
G26	ILL-6325	183	37.7	67.1	546
G27	LRIL-22-70	226	40.0	64.6	662

Se = selenium concentration, Fe = iron concentration, Zn = zinc concentration.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest exists.

ETHICS STATEMENT

The authors confirm that the research meets any required ethical guidelines, including adherence to the legal requirements of the study country.

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AUTHOR CONTRIBUTIONS

AS and AKH contributed in conceptualization and methodology; AKMMR contributed in investigation, writing – draft, and funding acquisition; MA contributed in data analysis; MAA and MSA, and MAH contributed in review; MJ, MAH, and AKH contributed in editing.

DATA AVAILABILITY

The availability of data and materials in this research is subject to the policy set by the corresponding author upon reasonable request.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the online version of this article.

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