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Genotype by Environment Interaction Effect on Grain Iron and Zinc Concentration of Indian and Mediterranean Lentil Genotypes

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Abstract: Lentil grains with high nutritional value qualify as a promising candidate for alleviation of micronutrient malnutrition in South Asia and North Africa. Genetic variation for micronutrient concentration in germplasm is prerequisite for biofortification of this crop. In the present study, ninety-six lentil genotypes consisting of Indian (released varieties, advanced breeding lines and germplasm lines) and Mediterranean (germplasm lines and landraces) line were evaluated for grain iron (Fe) and zinc (Zn) concentrations and the stability of these traits was studied across three different locations in India. The pooled analysis of variance revealed significant genotype, environment and genotype by environment interaction (GEI) mean squares for both the micronutrients. Stability analysis employing the AMMI model elucidated the first two interaction principal components as significant and cumulatively explained 100% of GEI variation. The first two components explained 55.9% and 44.1% of the GEI sum of squares for grain iron and 50.8% and 49.2% for grain zinc concentration, respectively. No correlation between grain iron and zinc concentration was observed. Among 96 lines, genotypes IG 49, P 16214, ILL 147 and P 2118 were found to be relatively stable, having higher mean iron and zinc concentrations with low modified AMMI stability value (MASV), modified AMMI stability index (MASI) and genotype selection index (GSI). The identified promising genotypes (high Fe: P16214, IG 115, P 2127 and IC 560812 and high Zn: P 8115, P3234, LL 461 and IC 560812) can be utilized for studying the genetics of grain Fe and Zn concentration by developing mapping populations and for biofortification of Indian lentil.

Keywords: lentil; grain Fe and Zn concentration; AMMI; stability parameters; biofortification

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1. Introduction

Lentil (*Lens culinaris* Medikus ssp. *culinaris)* grains are enriched with abundant protein, prebiotic carbohydrates, vitamins and macro- and micro-nutrients [1,2]. Lentil grains provide quality protein with significant concentration of endogenous amino acids viz. leucine, arginine, glutamic and aspartic acids [3]. Globally lentil is cultivated on over 4.8 Mha with a production of 5.73 M tons [4]. Lentil is the fifth most important grain legume grown in around 50 countries. Recommended daily allowance (RDA) for iron is 8.0 mg and 18.0 mg, while for zinc it is 11.0 mg and 8.0 mg for males and females, respectively, according to the National Institute of Health (NIH). Daily consumption of 100 g of lentil grains can provide a considerable amount of RDA for iron and zinc [5,6]. In

many of the developing countries lentil supplements cereals in daily diets of the resource-poor populations [7]. Being an ample source of iron (Fe) and zinc (Zn) and grown in micronutrient-deficient and resource-poor areas, lentil is a candidate crop for micronutrient biofortification [8].

Micronutrient deficiency persists as a serious global health concern affecting more than one fourth of the world's population [9]. Among micronutrient deficiencies, Fe and Zn deficiencies are the major manifestations of mineral malnutrition. Fe is integral to oxygen transport proteins, viz. hemoglobin and myoglobin [10]. Fe deficiency is the predominant cause of anemia, affecting 27% of global population [11]. Fe deficiency and anemia lead to impaired cognitive development, immune suppression, fatigue, low-birth weight of infants, increased mortality and morbidity [12,13]. About 17–29.6% of the global population is estimated to be at risk of low zinc intake with high prevalence in South Asia, South-East Asia, Sub-Saharan Africa and Central America [14]. High mortality rate has been reported among children resulting from infections associated with inadequate Zn intake. Zn performs major roles in biological systems as a catalyst, structural and regulatory ion [15]. Zn is involved in several metabolic pathways and hence is a key component in normal body growth and development. Zn deficiency causes delayed and reduced growth, hypogonadism, epidermal disorders and dysfunction of immune and central nervous systems [16]. Regular intake of Zn is required to avoid Zn deficiency as this micronutrient cannot be stored in the human body [15,16].

Agronomic fortification, post-harvest food fortification, diversified diet and oral supplementation are possible means to combat micronutrient deficiency but biofortification stands as a relatively feasible, effective, safe and sustainable micronutrient delivery approach. Biofortification, a conventional or molecular breeding-based approach, can enrich food crops nutritionally with enhanced bioavailability [17]. In order to breed micronutrient dense cultivars, substantial genetic variation in the gene pool for grain Fe and Zn concentration is a prerequisite.

Lentil germplasm evaluation [18–20] has revealed wide variation for grain Fe and Zn concentrations. The inheritance of grain micronutrient concentration is complex with high environmental influence [6]. Genotypes expressing stable performance across environments for micronutrient concentrations can be utilized for breeding biofortified lentil varieties. Genotype by Environment Interactions (GEI) for complex traits can be dissected using statistical tools such as analysis of variance, whereas different statistical models, including univariate and multivariate, can facilitate identification of stable genotypes [21]. Additive main effects and multiplicative interaction (AMMI) model and the genotype main effects plus the G × E (GGE) model are the most conventionally followed multivariate statistical models for determining genotypic stability from multi-location trial data. While the AMMI model helps in understanding the structure of GEI in addition to estimating the total deviation of interaction and differentiating the main interactions from each another [22,23], the GGE model helps in determining wining genotypes suited to different environments and ranking them in tested environments based on their performances. The aim of the present investigation was to evaluate genetic diversity and decipher genotype by environment interaction effect among Indian and Mediterranean lentil genotypes for grain micronutrient concentrations for identification of Fe and Zn rich stable genotypes.

2. Materials and Methods

2.1. Plant Material and Field Experimentation

Grain iron and zinc concentrations were evaluated in a set of ninety-six lentil genotypes. The studied genotypes comprised of Indian (released varieties, advanced breeding lines, germplasm lines) as well as Mediterranean (landraces and ICARDA germplasm) genotypes (Table 1). The genotypes were raised at three locations: (i) Experimental Farm, Division of Genetics, Indian Agricultural Research Institute, New Delhi (28°38′23″ N, 77°09′27″ E, 228 m above mean sea level); (ii) Jawaharlal Nehru Krishi Vishwa Vidyalaya, Sagar (30.9° N, 75.85° E, 244 m amsl); and (iii) RAK, Sehore (23.06° N, 77.05° E, 498.77 m

amsl) during the winter season of 2016–17 following the standard package of practices. Entries were planted in randomized block designs in three rows of five-meter length with two replications and inter-row spacing of 25 cm, plant to plant spacing of 5 cm.

Table 1. Genotypes used and their source.

S.No.	Genotype	Type of Genotype	Source
1–13	IC 201704, IC 208326, IC 262839, IC 267663, IC 268248, IC 560135, IC 560169, IC 560181, IC 560206, IC 560212, IC 560333, IC 560372, IC 560812	Indian germplasm lines	NBPGR, New Delhi, India
14–38	IG 111996, IG 112078, IG 112128, IG 112131, IG 115, IG 129214, IG 129291, IG 129302, IG 129304, IG 129317, IG 130033, IG 195, IG 49, IG 5320, IG 569608, IG 70230, IG 73798, IG 73920, IG 73933, IG 9, ILL 10832, ILL 108331, ILL 147, ILL 2581, ILL 7663	Mediterranean landraces	ICARDA, Aleppo, Syria
39–46	L 11-243, L 11-273, L 11-279, L 11-282, L 11-289, L 11-291, L 11-294, L 11-297	Advanced breeding lines	AICRP MULLaRP, IIPR, Kanpur India
47	L 4076	Released variety	IARI, New Delhi, India
48–61	L 5253, L 7818, L 7903, L 7916, L 7920, LC 282-1444, LC 282-1485, LC 282896, LC 282907, LC 300-15, LC 300-16, LC 300-17, LC 300-19, LC 74151	Advanced breeding lines	IARI, New Delhi, India
62	LH 90-57	Advanced breeding lines	CCS, HAU, Hisar, India
63–66	LL 1122, LL 147, LL 461, LL 649	Advanced breeding lines	PAU, Ludhiana, India
67–85	P 13108, P 13129, P 13135, P 13138, P 13142, P 13143, P 15104, P 15121, P 15127, P 16214, P 2113, P 2116, P 2118, P 2125, P 2127, P 3233, P 3234, P 8112, P 8115	ICARDA Nursery selection	ICARDA, Aleppo, ICARDA, Aleppo, Syria
86–92	PL 02, PL 04, PL 05, PL 06, PL 07, PL 08, PL 406	Released varieties	GBPUAT, Pantnagar, India
93–96	PL 117, PL 24, PL 77-12, PL 97	Advanced breeding lines	GBPUAT, Pantnagar, India

2.2. Grain Iron and Zinc Analysis

Grains were harvested at physiological maturity and dried to less than 12% moisture content in dust-free conditions to avoid soil contamination. Grains were sorted manually to discard damaged and immature grains and stored in clean containers. For micronutrient analysis the grains were washed with Milli-Q water to remove the dust and oven dried at 35 °C for 5 days. Three grams of grains were grounded into fine powder using mortar and pestle manually. Powdered samples (0.5 g) were digested as per modified diacid protocol by Singh et al. [24] using a microwave digestion system (Multiwave ECO, Anton Paar, les Ulis, France). Iron and zinc concentrations (in ppm) were measured using atomic absorption spectrometry (Zeeman AAS, Z-Xpress 8000, Jena, Germany).

2.3. Statistical Analysis

Combined analysis of variance (ANOVA) across environments was performed following homogeneity test of error variance based on Bartlett's test. Stability analysis was carried out using AMMI and genotype plus genotype × environment (GGE) models. AMMI1 biplot was graphed using means of the main effect vs. first interaction principal component (IPC1) score, as per Zobel et al. [25]. Modified AMMI stability index (MASI) and Modified AMMI stability value (MASV) were computed for grain iron and zinc concentrations as per Ajay et al. [26,27] as follows:

MASI was calculated as per Ajay et al. [26]:

$$\text{MASI} = \sqrt{\left(\sum_{n=1}^{N'} \text{PCn}\ 2\ \text{X}\ \theta n^2\right)} \quad (1)$$

MASV was calculated for studied nutritional traits according to Ajay et al. [27] as follows:

$$\text{MASV} = \sqrt{\sum_{n=1}^{N'-1} (\text{SSIPCn}/\text{SSIPCn} + 1\ \text{X}\ \text{PCn})^2 + (\text{PC}_{N'})^2} \quad (2)$$

where $SSIPC_1$, $SSIPC_2$, $SSIPC_n$ denote the sum of squares of 1st, 2nd and nth IPCs; PC_1, PC_2, ... PC_n denote the scores of 1st, 2nd and nth IPCs; θ_n is the percentage sum of squares explained by the nth principal component interaction effect and N' denotes the number of significant IPCs retained by the AMMI model. Lower MASI and MASV scores denote stability of genotypes across environments.

Simultaneous selection index for trait mean performance and stability was calculated using the genotype selection index approach suggested by Farshadfar et al. [28]:

$$\text{GSI} = \text{RMASI} + \text{R} \quad (3)$$

where RMASI is the ranking of the modified AMMI stability index and R is the ranking of the traits in all environments.

In addition, 'which-won-where'/GGE Biplots were plotted taking IPCA1 on *x*-axis and IPCA2 on *y*-axis as per Yan and Kang [29]. Data analysis was performed using software R (version 4.0.5; The R Foundation for Statistical Computing Platform, Vienna, Austria). The GGE biplot was graphed using GGEbiplotGUI package tool of the R software.

3. Results

3.1. Mean Performance of Genotypes

Mean performance and descriptive statistics of ninety-six genotypes evaluated across three locations are presented in Table 2. A wider array of variation was exhibited for studied traits in different environments. Genotypes constituting the panel followed normal distribution for grain Fe and Zn concentration (Figure 1). Analysis of variance indicated that the panel of lentil genotypes differed significantly ($p \leq 0.01$) among themselves for grain Fe and Zn concentrations across locations (Table 3). Mean grain Fe and Zn concentration over all locations and genotypes sampled was 70.46 mg/kg and 56.83 mg/kg, with genotypic mean Fe and Zn concentration ranging from 39.38 to 105.41 mg/kg and 27.4 to 87.3 mg/kg, respectively (Table 2). Genotypes P 16214 (105.41 mg/kg) followed by IG 115 (96.51 mg/kg), P 2127 (96.42 mg/kg) and IC 560812 (95.14 mg/kg) had the highest mean grain Fe concentrations while genotypes P 8115 (87.3 mg/kg), P 3234 (79.9 mg/kg), LL 461 (79.85 mg/kg) and IC 560812 (79.1 mg/kg) exhibited the highest mean grain Zn concentrations (Table 4). Mean performances for Fe and Zn concentrations were relatively higher at the Sehore location, while mean performances for both the traits were low at the Delhi location (Table 2) (Table S1). No correlation was observed between grain Fe and Zn concentration. Similar results have been previously observed in lentil [6,19].

Table 2. Descriptive statistics of ninety-six lentil genotypes for grain iron and zinc concentrations across three locations.

Variable	Locations	Mean	SD	SE	CV	Minimum	Maximum
Fe (mg/kg)	Delhi	68.97	13.82	1.41	20.04	37.7	116.75
	Sagar	69.85	14.18	1.45	20.30	38.95	106.73
	Sehore	72.55	15.78	1.61	21.75	34.4	107.26
	Combined locations	70.46	13.00	1.33	18.45	39.38	105.41
Zn (mg/kg)	Delhi	55.90	12.87	1.31	23.02	24.3	87
	Sagar	57.05	13.26	1.35	23.24	27.45	94.45
	Sehore	57.51	13.30	1.36	23.13	30.35	87.7
	Combined locations	56.83	11.92	1.22	20.97	27.4	87.3

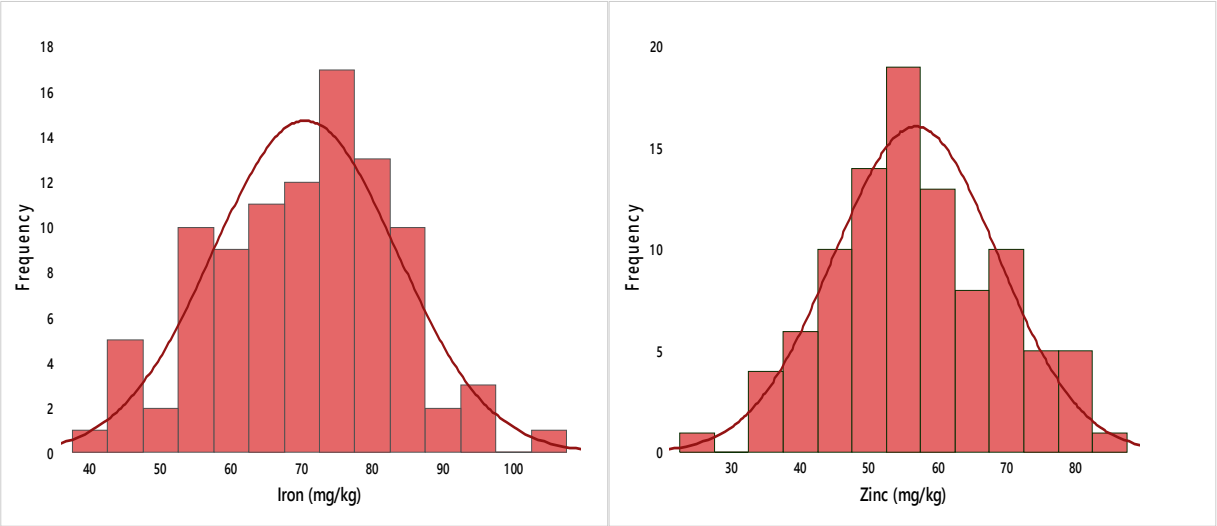

Figure 1. Frequency distribution of ninety-six lentil genotypes for grain iron and zinc concentration pooled across three locations.

Table 3. Mean sum of squares for grain iron and zinc concentrations obtained by pooled ANOVA across three locations for ninety-six lentil genotypes.

Source	DF	Mean Sum of Squares	
		Iron	Zinc
Genotype (G)	95	1014.02 **	851.84 **
Environment (E)	2	668.06 *	131.74 *
Genotype × Environment (G × E)	190	134.2 **	92.46 **
Replication within Environment	3	3.784	9.29
IPCA1	96	156.23 **	111.05 **
IPCA2	94	123.70 **	107.81 **
Total	575	1832.29	1091.27

Significance * and ** significant at, $p < 0.05$ and $p < 0.01$.

Table 4. Mean grain iron and zinc concentrations, genotype ranking, modified AMMI stability values (MASV), modified AMMI stability indices (MASI), rank orders (rank MASV/MASI) and genotypic selection index (GSI) of 41 high iron and zinc genotypes tested across three locations.

S. No.	Genotype	Mean Fe (mg/kg)	$Rank_{Fe}$	$MASV_{Fe}$	$MASI_{Fe}$	Rank $MASV_{Fe}$/ $MASI_{Fe}$	GSI_{Fe}	Mean Zn (mg/kg)	$Rank_{Zn}$	$MASV_{Zn}$	$MASI_{Zn}$	Rank $MASV_{Zn}$/ $MASI_{Zn}$	GSI_{Zn}
1	L 4076	76.84	14	2.84	1.25	40	54	70.41	12	1.40	0.69	21	33
2	ILL 7663	61.56	36	1.63	0.72	20	56	67.80	16	1.93	0.95	38	54
3	P 3234	77.26	12	1.60	0.70	19	31	79.91	2	1.38	0.68	17	19
4	P 2116	75.29	15	1.89	0.83	26	41	64.71	18	1.35	0.66	15	33
5	L 11-289	60.77	41	2.28	1.00	37	78	60.80	26	2.30	1.13	41	67
6	IC 560333	78.52	10	1.47	0.65	12	22	56.50	35	1.57	0.77	26	61
7	PL 77-12	65.98	32	2.06	0.91	30	62	65.41	17	1.64	0.80	29	46
8	L 7916	69.48	25	1.59	0.70	17	42	61.01	25	1.14	0.56	4	29
9	PL 04	77.11	13	1.50	0.66	14	27	68.27	15	1.19	0.58	5	20
10	LL 1122	80.61	9	2.03	0.89	28	37	73.98	8	1.91	0.94	37	45
11	LC 282-907	67.21	29	1.56	0.69	15	44	62.01	22	1.97	0.97	39	61
12	IC 208326	88.05	5	2.05	0.90	29	34	62.58	21	1.39	0.68	18	39
13	P 15121	61.27	38	1.05	0.47	1	39	55.68	39	1.40	0.68	20	59
14	P 16214	105.41	1	3.24	1.42	41	42	73.35	9	1.21	0.59	7	16
15	PL 02	60.82	40	2.30	1.01	38	78	63.35	20	1.78	0.87	33	53
16	IG 115	96.51	2	1.91	0.84	27	29	59.63	29	1.80	0.88	34	63
17	L 7903	63.72	35	1.36	0.60	9	44	53.63	41	1.90	0.93	36	77
18	LH 90-57	81.76	7	1.76	0.77	23	30	60.00	28	1.52	0.75	25	53
19	LL 461	66.79	31	2.24	0.98	35	66	79.85	3	1.68	0.82	31	34
20	PL 05	81.14	8	2.26	0.99	36	44	56.30	37	1.28	0.63	12	49
21	P 2118	65.34	33	1.09	0.48	2	35	73.25	10	1.22	0.60	9	19
22	IC 560812	95.14	3	2.19	0.96	34	37	79.06	4	1.76	0.86	32	36
23	IC 560206	68.79	27	1.36	0.60	8	35	64.31	19	1.50	0.73	24	43
24	P 2113	60.91	39	1.24	0.54	4	43	69.71	13	1.67	0.82	30	43
25	L 11-291	71.19	23	2.18	0.96	33	56	56.06	38	1.31	0.64	13	51
26	L 11-294	72.59	20	1.56	0.69	16	36	54.15	40	2.19	1.07	40	80
27	IC 262839	61.48	37	2.53	1.11	39	76	77.50	6	1.61	0.79	27	33
28	IG 112128	73.46	19	1.49	0.65	13	32	61.68	23	1.33	0.65	14	37
29	ILL 147	88.36	4	1.74	0.76	21	25	61.03	24	1.05	0.51	3	27
30	LC 74151	67.78	28	2.14	0.94	32	60	58.98	30	1.42	0.70	22	52
31	LC 282896	74.05	17	1.31	0.57	6	23	77.63	5	1.46	0.71	23	28
32	IG 9	82.06	6	1.81	0.80	25	31	60.15	27	0.99	0.48	1	28
33	L 11-282	69.37	26	1.34	0.59	7	33	57.66	33	1.37	0.67	16	49
34	LC 300-17	73.71	18	1.79	0.79	24	42	69.26	14	1.26	0.62	11	25
35	PL 07	70.78	24	1.31	0.57	5	29	58.61	31	1.03	0.50	2	33
36	IG 49	77.69	11	1.37	0.60	10	21	58.50	32	1.22	0.60	8	40
37	IC 560135	74.15	16	2.13	0.94	31	47	75.05	7	1.40	0.68	19	26
38	LC 282-1485	71.44	22	1.10	0.48	3	25	70.90	11	1.83	0.90	35	46
39	P 8115	64.34	34	1.43	0.63	11	45	87.26	1	1.63	0.80	28	29
40	P 13142	71.49	21	1.59	0.70	18	39	56.98	34	1.20	0.59	6	40
41	P 13143	66.96	30	1.75	0.77	22	52	56.36	36	1.24	0.60	10	46

3.2. Pooled Analysis of Variance

Bartlett's test-based homogeneity of variance test indicated homogeneous error variance for both the micronutrients, permitting for pooled ANOVA across environments. Pooled ANOVA of ninety-six lentil genotypes was performed considering locations as random effect and genotypes as fixed effects. Pooled ANOVA revealed highly significant

GEI ($p < 0.001$). Genotype effects were also highly significant ($p < 0.001$) and environmental effects were significant ($p < 0.05$) (Table 3). The relative magnitudes of genotype, environment and GEI variances to the total sum of square accounted for 76.05%, 1.05% and 20.13% for grain Fe concentration and 80.54%, 0.26% and 17.48% for grain Zn concentration, respectively. Percentage share of GEI variances for grain Fe and Zn concentrations indicates the traits are highly influenced by environmental factors including soil pH, micronutrient status, their availability to plants, etc. Several studies have reported significant effect of GEI for grain Fe and Zn concentrations in various crops including wheat [30–32], maize [33,34] and pearl millet [35]. Though both the micronutrients exhibited considerable environmental interactions, the magnitude of GEI variances to the total sum of the square indicated greater sensitivity of grain iron concentration to environmental factors than grain zinc concentration. Substantial GEI observed in the study implies difference in response of genotypes across environments. The observed magnitude of genotypic variation suggests it should be possible to improve nutritional qualities of lentils through breeding approaches despite significant GEI.

3.3. AMMI Aanalysis

The three sums of squares (SS) from ANOVA, viz. genotype, GEI signal and GEI noise, indicate appropriateness of AMMI analysis for a given dataset. AMMI analysis is relevant for datasets with considerable GEI signal. If SS for GEI noise and GEI are nearly equal, then GEI is buried in noise and AMMI analysis is not relevant. To find the relevance of the AMMI analysis, sum of squares GE noise and GE signal were calculated as per Gauch [36]. Sum of squares for genotype and GEI were obtained directly through ANOVA, whereas sum of square for GE noise was estimated by multiplying error mean square by number of degrees of freedom for GEI. GE signal was then obtained by subtracting GE noise from GEI. Sum of squares obtained for GE noise for both Fe (2325.6) and Zn (1126.7) were substantially less than SS for GEI (25,449 for Fe and 17,568 for Zn), indicating outcomes from AMMI analysis are worthwhile.

Stability analysis employing AMMI model elucidated that only the first two interaction principal components, IPC1 and IPC2, were significant based on Gollob's F-test [37] and together explained 100% of GEI variation with no residual left. IPC1 and IPC2 explained 55.9% and 44.1% of the total GEI sum of squares for grain Fe concentration, whereas they explained 50.8% and 49.2% of the total GEI sum of squares for grain Zn concentration, respectively. Of the two AMMI biplots, the AMMI1 biplot was plotted between the main effects of trait mean (genotypic and environmental mean) and IPCA1 scores for both genotype and environment, whereas the AMMI2 biplot has been plotted taking scores of IPC1 vs. IPC2. The biplots were constructed comprising genotypes having high grain mean Fe (>60 mg/kg) and Zn (>53 mg/kg) concentrations for better interpretation and visualization. Hence, forty-one genotypes exhibiting higher mean for both micronutrients were included for plotting AMMI1 and AMMI2 biplots (Table 4). Fifteen genotypes showed above-average performance for grain Fe concentration in the AMMI1 biplot between mean grain Fe concentration vs. IPCA1 of GEI (Figure 2a). Out of fifteen genotypes, only two genotypes IC 560812 (G22) and IG 49 (G36) were found having higher mean Fe concentration while lying closer to the origin lower IPCA1 score (Figure 2a). These two genotypes had adaptability across locations based on their IPCA1 scores. Similarly, sixteen genotypes showed above-average performance for grain Zn concentration in AMMI1 biplot. Among these sixteen genotypes, three genotypes, IC 262839 (G27), P 16214 (G14) and P2113 (G24), were the most adapted across locations, having higher mean Zn concentration (Figure 2c).

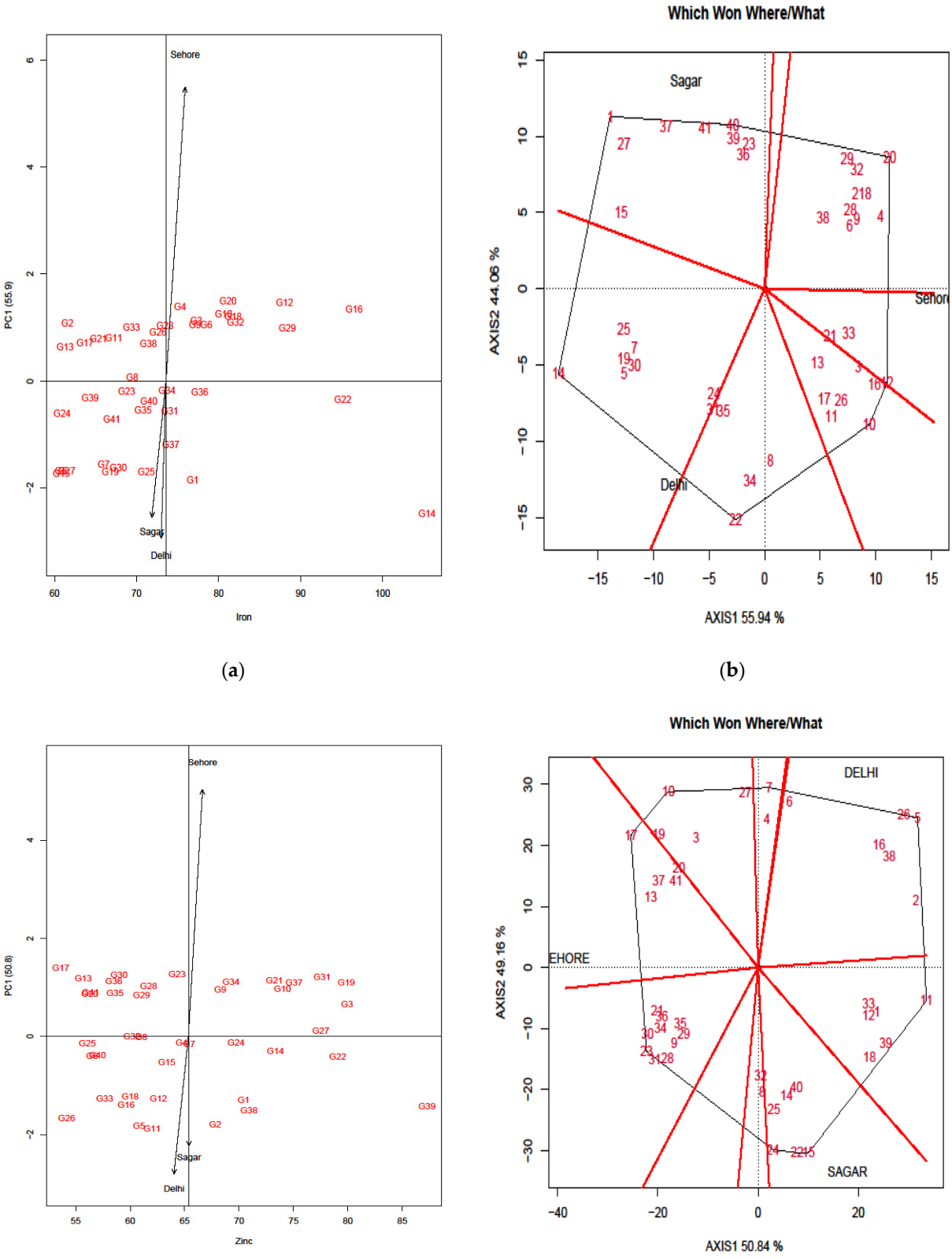

Figure 2. AMMI1 biplot between mean iron and zinc values over three locations vs. IPC1 and AMMI2/GGE biplot between IPC1 vs. IPC2 (**a**) AMMI1 biplot for 41 high iron lines, (**b**) AMMI2/GGE biplot for 41 high iron lines, (**c**) AMMI1 biplot for 41 high zinc lines, (**d**) AMMI2/GGE biplot for 41 high zinc lines.

'Which-won-where' biplot enables identification of stable genotypes, representative environment and best performing genotypes to mega-environments. The AMMI2 biplot plotted between IPCA1 and IPCA2 revealed genotypes specifically adapted to different locations investigated in the study. Genotype P 16214 (G14) performed well in Delhi and L 4076 (G1) in Sagar, whereas IC 208326 (G12) performed well in Sehore for grain Fe concentration (Figure 2b). 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 (Figure 2d).

3.4. Stability Analysis Using MASI and GSI

The AMMI model, as a measure of stability, does not furnish stability quantitatively. To rectify the problem, Purchase [38] and Purchase et al. [39] proposed the AMMI stability value (ASV) to quantify and rank genotypes, which is required for facilitating genotypic selection. Later, Zali et al. [40] developed modified ASV (MASV), incorporating the relative weight of all significant IPCAs to compute stability measure. Retaining all significant IPCA axes improves reliability of stability measure providing more comprehensive and precise information. Recently, Ajay et al. [27] rectified the existing MASV by Zali et al. [40] and proposed a new corrected formula for MASV as MASV2, which significantly improved ranking of genotypes. Ranking of genotypes based on both MASV and MASI values indicated P 15121 (G13), P 2118 (G 21) and LC 282-1485 (G38) had the lowest MASV and MASI values and hence were the most stable lines for grain Fe concentration. Similarly, genotypes IG 9 (G32) followed by PL 07 (G35) and ILL 147 (G29) were identified as stable lines for grain Zn concentration. PL 07 (G35), P2118 (G21) and IG 49 (G36) had relatively low MASV and MASI values for both the micronutrients. Selection of genotypes cannot be made considering stability per se as the most stable lines may not be the best performer and vice versa. Hence, both mean and stability of performance should be simultaneously considered for selection of genotypes to be effective and precise. GSI, a simultaneous selection index approach for trait mean performance and stability, was employed where the lowest GSI implies stability of line along with high mean performance. In the study, genotypes IG 49 (G36), IC 560333 (G6), LC 282896 (G31), ILL 147 (G29) and IG 115 (G16) scored lowest in GSI for grain Fe, while genotypes P 16,214 (G14), P 2118 (G21), PL 04 (G9), LC 300-17 (G34) and IC 560135 (G37) had the lowest GSI for grain Zn concentration. Genotypes IG 49 (G36), P 16214 (G14), ILL 147 (G29) and P 2118 (G21) were identified as promising stable lines considering the mean Fe and Zn performances, AMMI1 biplot, MASV/MASI and GSI.

4. Discussion

Breeding for grain Fe and Zn is challenging, owing to polygenic inheritance and environmental influence on the target traits. Identification of stable genotypes through multi-location evaluation is integral for varietal development and release. The present study was conducted to evaluate the genetic variability for grain Fe and Zn concentrations among diverse lentil genotypes while simultaneously deciphering environmental influence on the studied traits. Mean performance for grain micronutrients at different environments indicated a wide array of variation among genotypes which can be harnessed by including them in breeding programs aiming towards micronutrient enrichment. High genetic variability has been reported in the lentil gene pool for grain micronutrient concentration [6,18,20,41,42]. Greater magnitude of genetic variability for grain iron and zinc is pivotal for biofortification breeding and to achieve significant genetic gain through conventional/molecular breeding, provided the trait has high heritability per se [43]. As per this investigation, daily consumption of 100 g of lentil can potentially meet RDA of iron and zinc depending on the bioavailability of minerals post-consumption.

ANOVA revealed significant genotypic effect for both the traits studied in each sampled environment. ANOVA indicated significant GEI along with environmental effects on grain micronutrient concentrations, which indicated genotypic performance is influenced

in different environments. Genotype by environment interaction influences micronutrient concentrations by affecting their uptake by roots, translocation through shoots and assimilation in grains [41]. Many researchers have documented significant effect of GEI for grain Fe and Zn concentrations in various crops including wheat [30–32], maize [34], sorghum [43], pearl millet [35,44] and lentil [45]. The high magnitude of genotypic effect implies hereditary factors do govern these traits but the presence of GEI and the environmental effect led differential responses of genotypes across environments. Kumar et al. [6] reported significant genotype × year interactions for Fe and Zn concentration in lentil, indicating the influence of rainfall, temperature and soil parameters. The percentage share of GEI for grain iron and zinc concentrations indicated both the micronutrients exhibited considerable environmental interactions, though Fe concentration had greater sensitivity towards environmental factors. A similar study in maize by Chakraborti et al. [33] and Agrawal et al. [34] reported kernel Fe to be more greatly influenced by environmental conditions than Zn concentration, whereas Kumar [46] observed both the micronutrients in lentil to be equally sensitive. The outcomes clearly illustrate those genetics as well as environmental factors determine the micronutrient profile in lentil grains. Hence, both should be given due consideration in selection of superior genotypes and varietal development.

GGE biplot help in identifying genotypes suitable to different environments, interpreting representativeness of test environment and ranking genotypes in tested environments based on their performance. GGE biplot for grain Fe and Zn concentrations formed a polygon with best performing genotypes at the vertices (Figure 2b,d). Different environments had different winning genotypes, indicating crossover genotype by environment interaction. This agrees with previous reports on grain micronutrient concentration assessment in rice [21], pearl millet [35] and sorghum [43].

Of the several stability measures, AMMI stability value (ASV) proposed by Purchase et al. [39,47] is the most popular. ASV considers only the first two IPCs for computation, whereas the MASV considers all the significant IPCs for computation [27]. Unlike ASI, MASI calculates stability value considering all significant IPCs in the AMMI model [26]. In the study, two stability measures, viz. MASV and MASI, were employed. As only IPCA1 and IPCA2 together explained 100% variability, MASV and MASI measures of the AMMI model became equivalent to drawing a conclusion based on ASV and ASI. Results obtained by MASI and MASV were consistent, indicating the accuracy and usefulness of different methods in deciphering GEI for complex traits and identifying stable genotypes. In the present study, P 15121, P 2118 and LC 282-1485 were identified as the most stable genotypes for grain Fe, while IG 9, PL 07 and ILL 147 were stable for grain Zn concentration. The results are in accordance with studies on stability analysis applying ASV [35,48] and MASV parameters of AMMI model [49,50].

The GSI approach was used for selection of desirable genotypes employing trait mean performance and stability across tested environments simultaneously. Low GSI value indicates high trait mean and stable performance. In the present study, genotypes IG 49, P 16214, ILL 147 and P 2118 were promising stable lines, having higher mean Fe and Zn concentrations based on AMMI1 biplot, MASV/MASI and GSI analysis. These lines could be further tested for their ability to combine for yield and grain micronutrient concentrations for developing stable superior performance and mineral rich lentil varieties.

5. Conclusions

The indigenous and exotic lentil lines investigated in this study exhibited wide variability for grain micronutrient concentrations which can be tapped by including them in breeding programs for micronutrient alleviation. Genetic variation for investigated micronutrients can be utilized for mapping genes/quantitative trait loci (QTL) governing iron and zinc uptake and accumulation in grains. Stability analysis revealed genotypes IG 49, P 16214, ILL 147 and P 2118 were high performing, promising and stable exotic lines for grain micronutrient concentration which can be used as donors in lentil biofortification breeding for developing nutrient-dense cultivars. These exotic lines can be hybridized

with indigenous lines to broaden their genetic base while breeding for high micronutrient concentration simultaneously. The genotypes with high Fe (P16214, IG 115, P 2127 and IC 560812) and Zn (P 8115, P3234, LL 461 and IC 560812) can be used for developing the mapping populations for deciphering the mode of inheritance of these traits. As no correlation was observed between grain iron and zinc concentrations, high iron and zinc containing lentil lines may be crossed to get recombinants for these micronutrients.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11091761/s1, Table S1: Mean performance (grain Fe and Zn concentration) of studied genotypes over three location.

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