Indian J. Genet., 73(2): 169-176 (2013)
DOI : 10.5958/j.0975-6906.73.2.024

Characterization of elite lentil genotypes for seed iron and zinc concentration and genotype x environment interaction studies

Harish Kumar*, H. K. Dikshit, Anju M. Singh, D. Singh, Jyoti Kumari[1], Akanksha Singh and Dinesh Kumar[2]

Division of Genetics, IARI, New Delhi 110012; [1]Germplasm Evaluation Division, NBPGR, New Delhi 110012; [2]Division of Agronomy, IARI, New Delhi 110012

(Received: November 2012; Revised: January 2013; Accepted: January 2013)

Abstract

Forty-one lentil genotypes were evaluated to analyse genotype × environment interaction for iron and zinc concentration in the seeds. The analysis of variance for Fe and Zn concentration in grain at individual locations showed highly significant differences between genotypes. Pooled analysis of variance over locations displayed highly significant differences between genotypes, locations and genotype × location interaction. Among 41 genotypes, the maximum mean seed Fe concentration over the locations was observed in L4704 (136.91 mg kg^{-1}) while maximum Zn concentration in grain was observed in VL141 (81.54 mg kg^{-1}). The highest mean seed Fe and Zn was recorded at New Delhi (Fe -87.30 mg kg^{-1} Zn-68.60 mg kg^{-1}). Although both the micronutrients were influenced by environment, seed Fe was more sensitive to environmental fluctuations in comparison to seed Zn concentration. The G × E study revealed that the genotypes VL 141 was most stable and adapted to the diverse environmental conditions of North West Plain Zone for Zn concentration. The genotypes, VL 141 and LH84-8 had high mean value for Fe concentration with little deviation from regression. AMMI biplot revealed that for Fe concentration in all the three locations exhibited equal discriminating power, however, for Zn concentration Ludhiana was found to be most discriminative. The three locations exhibited poor correlation between them for both Fe and Zn concentration in seed. This is the first report on iron and zinc concentration in lentil from India.

Key words: *Lens culinaris*, Fe and Zn concentration, G x E interactions, AMMI analysis

Introduction

Lentil (*Lens culinaris* ssp. *culinaris*) is an annual diploid (2n = 2x = 14), self pollinated and cool season legume crop grown in dry areas of Indian subcontinent, Mediterranean region and North America. In India lentil was grown in about 1.48 m ha with production of 1.03 m ton during 2010-11. It is mainly grown as rainfed crop in Uttar Pradesh, Madhya Pradesh, Jharkhand, Bihar and West Bengal. The population density is high in these regions and so is the micronutrient deficiency. Micronutrient deficiency is caused due to non/poor availability of minerals in diet. It affects the human beings especially women and pre-school children [1]. Dietary diversification, supplements and fortified foods are the important means to reduce micronutrient malnutrition. Biofortification involves increasing the levels of specific, limiting micronutrients in edible tissues of crops by combining crop management, breeding, and genetic approaches. Biofortification is cost-effectiveness, highly sustainable and feasible [2-4]. Lentil is useful for human, animal and soil health. Lentil is important source of energy, protein, carbohydrate, fibre, mineral, vitamin and antioxidant compound. It is a good source of vitamin A, potassium, vitamin B complex and iron. Also lentil has an excellent macro and micronutrient profile and favourable levels of mineral bioavailability enhancing factors. Lentil is cheap source of quality protein for poor and vegetarians [5-7]. In India no report is available on estimation of Fe (iron) and Zn (zink) concentration and its stability across locations in lentil.

Genotype x environment (GE) interactions are extremely important in the development and evaluation of plant varieties because they reduce the genotypic-stability values under diverse environments [8]. The concept of stability has been defined in several ways and several biometrical methods including univariate and multivariate ones have been developed to assess

*Corresponding author's e-mail: harishmakhija10@gmail.com
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

it [9, 10]. The most widely used is the regression method, which is based on regressing the mean value of each genotype on the environmental index or marginal means of environments [11, 12]. AMMI analysis provides a graphical representation (biplot) to summarize information on main effects and interactions of both genotypes and environments simultaneously [13]. The present investigations were carried out with specific objective of identifying lentil elite lines with high Fe and Zn concentration and to study G x E interaction for these traits.

Materials and methods

Genetic material

The experimental material for the present study comprised of 41 lentil genotypes developed at different lentil breeding centres under All India Coordinated Research Project on mungbean, urdbean, lentil, lathyrus, rajmash and pea. These lines were tested in Initial Varietal Trial during 2010-11. These genotypes were analysed for seed Fe and Zn concentration (Table 1).

Field trials

The test entries were evaluated at three locations in North Western Plain Zone (NWPZ) *viz*., New Delhi (S1) (28^{o}40'N, 77^{o}12'E, 218 metres above mean sea level (amsl), Ludhiana (S2) 30.9^{o}N, 75.85^{o}E 244 metres amsl, and Pantnagar (S3) 28^{o}58'N, 79^{o}25'E, 344 metres amsl during *rabi* 2010-11. The soils at these locations are deep well drained and light alluvium type. Soil Fe and Zn concentration at the experimental sites was estimated using standard procedures [14]. The entries were planted in Randomized Complete Block Design (RCBD) with three replications per entry (4 rows per replication) with plant to plant spacing of 5cm and row to row spacing of 30cm. Five random plants from each plot were handled following the procedure suggested by Harvest Plus [15].

Seed micronutrient analysis

Biochemical analysis for kernel Fe and Zn concentrations was carried out on triplicate ground samples of seeds from individual plant by digestion with 9:4 diacid mixture (HNO3: HClO4) followed by atomic absorption spectrometry (AAS) method using ECIL AAS (Perkin Elmer) as per the protocol described by Zarcinas *et al.* [16] and Singh *et al.* [14].

Statistical analysis

ANOVA of the seed micronutrient data from the three location trials was carried out using SAS 9.3. Stability analysis was performed following the Eberhart and Russell [17] model, using Windostat (Version 8.0, Indostat Services) software. In this analysis sum of square due to G x E were portioned into individual genotypes (X-i), regression of environmental means (bi) and deviation from regression (S 2d). The regression coefficients (bi) and mean square deviation from regression (S2d) were used to define genotype stability. The environmental mean was the mean of all genotypes in each environment. The pooled error was used to test the hypothesis that the mean square deviation did not differ significantly from 0 at 0.05 and 0.01% probability levels. The t-test employing the standard error of regression coefficient against the hypothesis that it did not differ from 1.0 was performed. It was assumed that genotype effects were fixed and year effects were random. Further, AMMI model, which combines ANOVA with principal component analysis (PCA) was used to study the agronomic nature of genotype x environment interactions using PROC IML procedure of SAS with version 9.3. Plots were prepared as described by Zobel *et al*. [18] using mean and first two PC scores.

Results and discussion

The ANOVA indicated that the genotypes under study differed significantly (P=0.01) for both seed Fe and Zn concentrations at all the three locations. The ranges for seed Fe concentration were 53.73-131.53 mg kg^{-1} (mean 87.30 ± 5.20 mg kg^{-1}) at Delhi, 40.13-160.66 mg kg^{-1} (mean 72.00 ± 2.97 mg kg^{-1}) at Ludhiana, and 40.63-141.42 mg kg^{-1} (mean 68.99 ± 3.68 mg kg^{-1}) at Pantnagar (Table 2), while the range across all the three locations was 50.85-136.91 mg kg^{-1} (mean 76.11 ± 10.48 mg kg^{-1}). The range for seed Zn concentration was 37.60-102.68 mg kg^{-1} (mean 68.602 ± 3.21 mg kg^{-1} at Delhi, while the ranges for Ludhiana and Pantnagar were 47.73-91.15 mg kg^{-1} (mean 64.630 ± 3.05 mg kg^{-1}) and 22.53-66.60 mg kg^{-1} (mean 45.004 ± 3.67 mg kg^{-1}), respectively. The range across the three environments was 40.20-80.57 mg kg^{-1} (mean 59.41 ± 5.96 mg kg^{-1}). The environmental index for Fe and Zn in seed varied from location to location. It was –7.1 and –14.4 (Zn) at Pantnagar, –4.1 (Fe) and 5.21 (Zn) at Ludhiana while at Delhi the index was 11.20 for Fe and 9.19 for Zn.

The pooled ANOVA (Table 2) was carried out after ascertaining that the homogeneity of error variance using the Bartlett's test. G x E interactions were found to be significant (at P=0.01) for both the micronutrients. Although the accumulation of both the

Table 1. List of lentil genotypes studied and their location wise mean values of Fe and Zn across three locations

S.No	Entry	Seed source	Pedigree	New Delhi mean (Fe)	Ludhiana mean (Fe)	Pantnagar mean (Fe)	Seed Fe mean (mg kg^{-1} seed)	New Delhi mean (Zn)	Ludhiana mean (Zn)	Pantnagar mean (Zn)	Seed Zn (mg kg^{-1} seed)
1	2	3	4	5	6	7	8	9	10	11	12
1	L 4589	IARI, New Delhi	L 4603 x PKVL 1	77.60	63.00	66.53	69.04	78.86	48.36	66.60	64.61
2	DL 10-2	Dholi	ARUN x PL 406	83.46	80.40	56.16	73.34	82.93	61.80	50.33	65.02
3	VL 140	VPKAS, Almora	VL 501 x SEHORE 74-3	93.14	100.57	97.52	97.07	98.08	89.56	34.24	73.96
4	PL 406	GBPUAT, Pantnagar	Selection from P495	77.46	75.80	66.26	73.17	68.53	56.06	47.46	57.35
5	IPL 219	IIPR, Kanpur	ILL 7657 x DPL 61	106.40	52.66	81.80	80.28	66.60	71.80	42.53	60.31
6	RLG 109	Durgapura	RLG 14 x L 4076	91.73	75.40	81.30	82.81	72.93	66.86	38.20	59.33
7	PL 4	GBPUAT, Pantnagar	UPL 175 (PL 184 x P 288)	77.60	54.86	63.13	65.20	37.60	52.66	30.53	40.26
8	HPCL 649	Palampur	L 4148 x VIPASHA	85.93	105.27	62.86	84.68	54.66	54.56	34.86	48.03
9	PL 101	GBPUAT, Pantnagar	PL 5 x FLIP 9671	88.20	53.80	65.73	69.24	57.66	75.13	46.73	59.84
10	PL 063	GBPUAT, Pantnagar	DPL 59 x IPL 105	121.23	51.76	55.76	76.25	49.06	54.06	53.73	52.28
11	L 4588	IARI, New DelhI	L 4147 x PL 4	76.93	50.26	67.33	64.84	63.80	57.33	34.33	51.82
12	KSL 314	CSAU, Kanpur	DPL 62 x LG 6O	65.93	53.56	79.66	66.38	67.00	61.46	47.20	58.55
13	DL 10-1	Dholi	L 9-12 x PL 639	72.23	45.46	76.43	64.71	75.53	53.53	49.40	59.48
14	PL 104	GBPUAT, Pantnagar	PL 5 x DPL 58	71.67	52.66	53.60	59.31	55.60	65.33	43.40	54.77
15	L 4147	GBPUAT, Pantnagar	(L 3875 x P 4) PKVL 1	84.46	85.73	56.66	75.62	76.80	62.66	42.26	60.57
16	LL 1114	PAU, Ludhiana	LL 699 x LL 773	77.00	52.13	57.36	62.16	64.33	64.66	49.83	59.61
17	HPCL 617	Palampur	VIPASHA x PL 639	133.87	41.60	41.50	72.32	73.66	56.60	52.00	60.75
18	DL 10-3	Dholi	Not available	115.13	43.46	51.40	70.00	55.13	56.56	54.66	55.45
19	SL2-28	Shillongani	ILL 7617 x ILL 2573	115.27	64.86	64.00	81.37	69.33	55.46	38.93	54.57
20	VL 141	VPKAS, Almora	VL4 x VL 501	106.65	108.22	90.53	101.80	102.60	88.50	53.44	81.54
21	KSL 107	CSAU, Kanpur	KLS 224 x KLS 233	64.86	64.86	51.50	60.41	68.06	47.80	35.40	50.42
22	IPL 322	IIPR, Kanpur	(DPL44 x DPL62) x DPL 58	82.60	51.60	55.86	63.35	68.40	54.00	48.00	56.80
23	RVL 32	Sehore	SL 94-14 x JL 3	84.80	40.13	54.66	59.86	63.33	72.40	53.06	62.93
24	LH 07-28	CCS HAU Hisar	LH 84-8 x IPL 138 (L 9-104	76.53	53.20	51.66	60.46	65.60	73.06	43.93	60.86
25	DPL 62	IIPR, Kanpur	JL 1 x LG 171	100.37	125.30	113.08	112.92	79.68	81.65	54.94	72.09

1	2	3	4	5	6	7	8	9	10	11	12
26	KLB 102	CSAU, Kanpur	L 4076 x Precoz	77.90	62.93	66.00	68.94	75.53	60.86	47.86	61.42
27	LL 1161	PAU, Ludhiana	IPL 70 LL 811	64.86	88.40	63.36	72.21	53.13	80.00	55.53	62.88
28	LH 07-26	CCS HAU Hisar	LH 90-54 x L 4641	97.36	114.64	95.35	102.45	87.99	76.41	34.63	66.34
29	LH 84-8	CCS, HAU Hisar	L 9-12 x JLS 2	113.63	113.08	100.90	109.20	85.73	65.53	40.73	64.00
30	VL 520	VPKAS, Almora	DPL 15 x SEHORE 74-3	96.92	119.07	113.52	109.84	83.94	91.15	50.65	75.25
31	IPL 321	IIPR, Kanpur	K 75 x DPL 62	75.20	105.27	61.60	80.68	45.66	58.73	50.60	51.66
32	PL 024	GBPUAT, Pantnagar	L 4076 x DPL 15	64.86	105.43	53.90	74.73	67.66	72.13	37.73	59.17
33	KLB 104	CSAU, Kanpur	K 75 x KLB 137	53.73	65.26	40.63	53.21	61.53	47.73	38.06	49.11
34	L 4705	IARI, New Delhi	Precoz x LC 74-1-5-1	54.80	42.63	55.13	50.85	53.66	62.80	54.06	56.84
35	DPL 15	IIPR, Kanpur	PL 406 x L 4076	64.86	43.46	52.46	53.60	66.06	52.66	37.80	52.17
36	PL 100	GBPUAT, Pantnagar	PL 5 x DPL 15	88.20	53.03	64.26	68.50	60.60	63.93	37.00	53.84
37	LL 1190	PAU, Ludhiana	LL 699 x IPL 124	131.53	53.26	40.63	75.14	69.73	63.16	41.60	58.16
38	IPL 406	IIPR, Kanpur	DPL 35 x EC 157634 / 382	84.26	55.96	69.93	70.05	66.80	57.00	22.53	48.77
39	PL 099	GBPUAT, Pantnagar	PL 5 x L 4603	84.80	73.13	90.63	82.85	68.86	66.86	36.40	57.37
40	IPL 320	IIPR, Kanpur	ILL 6002 JL 1	87.60	49.4	56.80	64.60	73.33	70.46	56.93	66.91
41	L 4704	IARI, New Delhi	L 4149 x L 4076	108.66	160.66	141.42	136.91	79.64	78.26	57.16	71.69
Mean ± S.E.				87.30 ±5.20	72.00 ±2.97	68.99 ±3.68	76.11 ±10.48	68.60 ±3.21	64.63 ±3.05	45.00 ±3.67	59.41 ±5.96

micronutrients was influenced by environment, the accumulation of seed Fe is more sensitive to environmental fluctuations in comparison to that of seed Zn. Similar findings were also reported in maize [20].

The Eberhart and Russell model revealed that the variation due to G x E (linear) was non-significant for both seed Fe and Zn. However, the environment (linear) was significant for Zn (Table 3). Seed micronutrient concentration depends upon factors like soil type, soil fertility status, soil moisture, G x E, genotypic variation, soil profile, crop management practice and interaction among nutrients [3]. Soil Fe and Zn concentration at three locations were recorded as 5.01 mg kg^{-1} Fe and 1.68 mg kg^{-1} Zn at IARI New Delhi, 4.2 mg kg^{-1} Fe and 0.62 mg kg^{-1} Zn at Ludhiana and 4.5 mg kg^{-1} Fe and 0.985 mg kg^{-1} Zn at Pantnagar. The soil status exhibited variation for Fe and Zn concentration at the studied locations. In addition to this the factors like microclimatic effects and meteorological parameters play important role. Significant effects of genotype × location and genotype × year interaction for kernel Fe; and genotype × location × year interaction for kernel Zn were earlier reported in maize [19, 20].

Despite various factors affecting seed micronutrient status including differential behaviour of genotypes in locations, this study was successful in identifying promising lentil genotypes based

Table 2. Pooled ANOVA for seed Fe and Zn concentrations using general linear model

Source of variation	Degree of freedom	Mean sum of squares	
		Seed Fe	Seed Zn
Genotypes	40	2990.60**	579**
Environments	2	11856.93**	19631.04**
Genotype x environment	80	1017.08**	278.99**
Error	244	24.57	16.74

*Significant at P=0.05; **Significant at P=0.01

Table 3. ANOVA for stability of seed Fe and Zn concentrations using the Eberhart and Russell model

Source of variation	Degree of freedom	Mean sum of squares	
		Seed Fe	Seed Zn
Genotypes	40	996.87***	193.21***
Environment + (Genotype x environment)	82	427.27*	250.30***
Environment (Linear)	1	7905.06***	13089.60***
Gen. x Env. (Linear)	40	452.88*	113.05
Pooled deviation	41	219.90***	71.04***
Pooled error	240	8.25	5.52

*Significant at P=0.05; **Significant at P=0.01

on mean value of genotypes, regression values and deviations from regression. Six lentil genotypes *viz*., L4589, VL140, PL104, LH07-28, KLB 102, LL1190 were identified as stable genotypes for seed Fe concentration, while VL140, RLG109, HPCL649, PL063, L4588, KSL314, LL114, DL10-3, VL141, LH07-26, LL1190, IPL406, PL099, IPL320, L4704 were identified as stable genotypes for seed Zn concentration (Table 4). The present study indicated higher sensitivity of seed Fe to environmental fluctuations. The study further revealed no correlation between seed Fe and Zn concentrations at all the three locations in the analysed genotypes.This indicated the possibility of genetically improving the two target traits independent of each other using this specific set, including the most promising genotypes.

AMMI analysis

Pooled ANOVA was carried out after analyzing the homogeneity of error variance using the Bartlett's test and the total iron and zinc concentration using GLM method is presented in Table 5. There were significant differences among the genotypes, environments and G × E interactions. Significant G × E interactions explained 36.21% and 26.33% of total sum of squares for Fe and Zn concentration respectively, which shows that although both the micronutrients are influenced by environments, seed Fe is relatively more sensitive to environmental fluctuations than seed Zn.Genotypic contribution towards total sum of squares were 53.23% for Fe and 27.33% for Zn concentration. Significant genotypic differences suggested that genes necessary for micronutrient enrichment traits are available within the *Lens* genome that could allow for substantial increases in seed Fe and Zn concentrations by recombination and directional selection. However, the ranges and means of seed Fe and Zn concentration varied widely at different locations due to the differences attributable to genotypes, environments as well as G × E interactions.

In the AMMI analysis employing Gollob's test, first two PC explained 100% of the G × E variation. PC 1 and PC 2 explained 77.96% and 22.03% of total G × E interactions for Fe and Zn concentration; the first two PC explained 61.91% and 38.08%, respectively. The graphical method was employed by using two PC [21] to investigate environmental variation and interpret the G × E interaction for Fe (Fig. 1a) and Zn (Fig. 2a) concentration. Also, the AMMI biplot analysis between the mean and the first PCA of G x E interactions (Figs. 1b&2b) indicated the distinct behaviour of the environment.

The lentil entries closer to the origin of biplot were stable across the three test locations. For Fe concentration, the genotypes DL 10-2, RLG 109, L 4147, PL 099 were high and widely adapted for three locations. The genotype VL 141 and LH84-8 were high in Fe concentration but slightly deviated from the origin of biplot. Whereas genotype L 4704 showed highest Fe concentration but displayed more deviation from the origin of biplot (Fig. 1b). Similarly, the genotypes close to the X-axis (zero value of the first PCA of G x E interaction) were the most stable genotypes across environments for that particular trait. Genotypes PL 063, HPCL 617, RVL 32, LH 07-28, DPL 62, KLB 102, VL 520, IPL 320, L 4704 are high in Zn concentration and are widely adapted for three locations. From Fig. 2b, it is clear that the genotype VL 141 is significantly superior to others in mean value and simultaneously had less G x E interaction due to being closer to X axis. The lines connecting the bipolt origin and the margins for the environment are called environment vectors. The angle between two environment vectors is related to the correlation coefficient between them [22]. Based on the angles of the environment vectors the three locations have poor correlation between them for both the traits (Figs. 1a and 2a). Another observation related to length of the environment vector which depicts standard deviation within each environment. The critical perusal of biplot revealed that all the three locations have equal discriminating power for Fe concentration but for Zn concentration Ludhiana location was found to be most discriminative.

Table 4. Stability parameters of 41 lentil studied genotype across 3 locations

Entry	Seed Fe			Seed Zn		
	Mean (mg kg^{-1})	bi	S^2Di	Mean (mg kg^{-1})	bi	S^2Di
L 4589	69.04	0.71	8.16	64.61	0.05	464.51***
DL 10-2	73.34	1.07	217.74 ***	65.02	1.12	136.75 ***
VL 140	97.07	-0.32	-0.19	73.96	2.74	-2.68
PL 406	73.17	0.44	25.91 *	57.35	0.74	40.69 **
IPL 219	80.28	2.04	629.03 ***	60.31	1.17	44.18 **
RLG 109	82.81	0.73	25.15 *	59.33	1.46	-5.54
PL 4	65.20	1.01	57.17 **	40.26	0.57	148.70 ***
HPCL 649	84.68	0.43	855.69 ***	48.03	0.89	0.50
PL 101	69.24	1.55	132.92 ***	59.84	0.77	211.14 ***
PL 063	76.25	3.89	119.71 ***	52.28	-0.12	4.75
L 4588	64.84	0.92	192.94 ***	51.82	1.22	-4.25
KSL 314	66.38	-0.24	321.12 ***	58.55	0.80	-2.75
DL 10-1	64.71	0.41	522.79 ***	59.48	0.82	174.5 ***
PL 104	59.31	1.07	0.38	54.77	0.71	75.19 ***
L 4147	75.62	0.99	339.91 ***	60.57	1.32	34.68 **
LL 1114	62.16	1.25	32.99 *	59.61	0.66	-1.09
HPCL 617	72.32	5.36	122.80 ***	60.75	0.69	99.12 ***
DL 10-3	70.00	3.87	187.46 ***	55.45	0.04	-4.24
SL2-28	81.37	2.96	24.53 *	54.57	1.14	38.93 **
VL 141	101.56	0.52	124.58 ***	80.57	1.90	1.48
KSL 107	60.41	0.49	63.94 **	50.42	1.14	121.22 ***
IPL 322	63.35	1.64	34.95 *	56.80	0.68	64.34 ***
RVL 32	59.86	2.06	211.36 ***	62.93	0.61	62.17 ***
LH 07-28	60.46	1.41	-4.65	60.86	1.10	66.23 ***
DPL 62	112.91	-0.99	110.13 ***	72.09	1.14	16.32 *
KLB 102	68.94	0.75	6.18	61.33	1.00	50.12 **
LL 1161	72.21	-0.44	347.60 ***	62.88	0.33	401.55 ***
LH 07-26	102.45	-0.29	199.79 ***	66.34	2.21	-1.65
LH 84-8	109.20	0.48	50.64 **	64.00	1.70	87.15 ***
VL 520	109.83	-1.08	31.26 *	75.25	1.62	89.84 ***
IPL 321	80.68	-0.13	986.47 ***	51.66	-0.00	81.47 ***
PL 024	74.73	-0.45	1424.87 ***	59.17	1.42	46.95 **
KLB 104	53.21	0.23	284.53 ***	49.11	0.83	50.83 **
L 4705	50.85	0.24	81.33 **	56.84	0.13	42.21 **
DPL 15	53.60	0.91	62.13 **	52.17	1.05	37.85 **
PL 100	68.50	1.63	124.73 ***	53.84	1.12	25.45 *
LL 1190	75.14	5.01	-5.14	58.16	1.16	-3.62
IPL 406	70.05	1.13	145.86***	48.77	1.83	-2.36
PL 099	82.85	0.03	150.17 ***	57.37	1.43	1.40
IPL 320	64.60	1.94	81.42 **	66.91	0.69	-5.57
L 4704	136.91	-2.31	342.54 ***	71.69	0.99	-2.21
Mean±SE	76.11 ±10.48			59.41 ±5.96		

Table 5. Pooled analysis of variance for total Fe and Zn concentration in 41 lentil genotypes grown across three locations

Source	df	SS		MSS		% of total SS	
		Fe	Zn	Fe	Zn	Fe	Zn
Replications	2	212.08	134.03	106.04	67.01		
Genotypes	40	119624.25	23160.07	2990.60**	579**	53.23	27.33
Environments	2	23713.85	39262.08	11856.93**	19631.04**	10.55	46.33
Genotype × Environment	80	81366.66	22319.37	1017.08**	278.99**	36.21	26.33
PC 1	41	63436.07	13818.66	1547.22**	337.04**	77.96	61.91
PC 2	39	17930.60	8500.72	459.76**	217.96**	22.03	38.08
Pooled error	244	5996.80	3984.47	24.57	16.32		
Total	368	230913.67	88860.04	-	-		
CV(%)		6.51	6.8				
R2		0.974	0.955				

The critical analysis of pedigree *vis-a-vis* micronutrient concentration did not reveal any correlation. The pedigree of genotypes showed high Fe (> 75 mg kg^{-1}) and Zn (> 60 mg kg^{-1}) concentration but did not indicate specific parent contributing to these traits. The present study clearly indicated the presence of significant variation for seed Fe and Zn concentration among the studied lentil genotypes and presence of genotype x environment interaction, besides

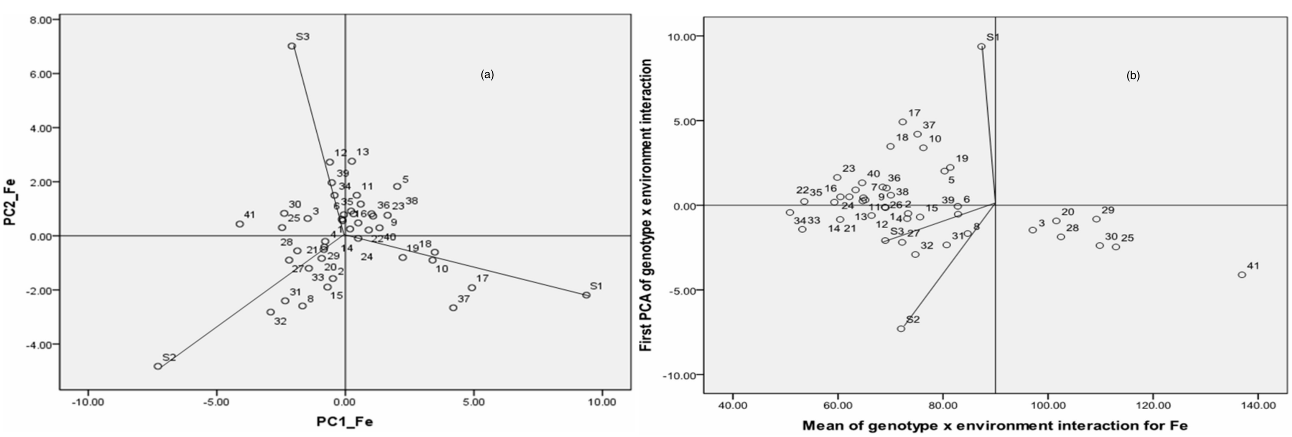

Fig. 1. AMMI (additive main effects and multiplicative interactions model) plots for Fe (a) between two PC and (b) between mean and first PC

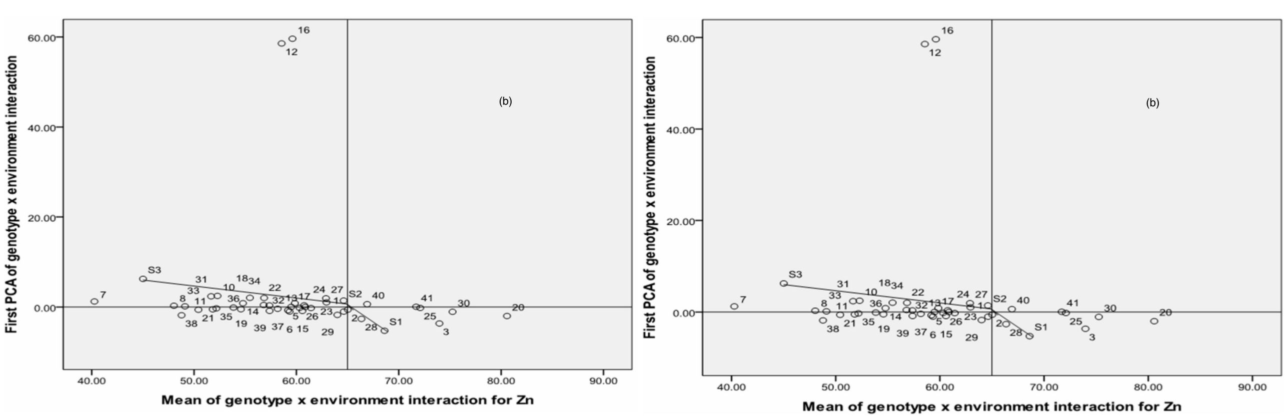

Fig. 2. AMMI (additive main effects and multiplicative interactions model) plots for Zn (a) between two PC and (b) between mean and first PC

identification of a set of promising and stable genotypes for seed micronutrient traits. The study further revealed no correlation between seed Fe and Zn concentrations at all the three locations in the analysed genotypes. Among all genotypes VL 520 was most stable along with relatively better Zn concentration although VL 141 had highest mean value with some G x E interaction. These genotypes are recommended for commercial cultivation and can also be effectively utilized in the recombination breeding programs to enhance genetic variability in lentil and also for generation of mapping population(s) for undertaking QTL analysis of seed micronutrient traits.This is the first report of the iron and zinc study in lentil from India.

Acknowledgements

Authors are thankful to Professor and Head, Division of Genetics, IARI for providing the necessary facilities for the research work. The first author is thankful to the Indian Council of Agricultural Research, New Delhi, India for the grant of Junior Research Fellowship for the Master's Degree programme. The study was partially supported by Harvest Plus: Lentil Biofortification project funded through ICARDA.

References

1. **UNSCN.** 2004. 5th Report on the world nutrition situation. Nutrition for improved development outcomes. United Nations System Standing Committee on Nutrition, Geneva.
2. **Bouis H. E., Hotz Christine, McClafferty Bonnie, Meenakshi J. V., Pfeiffer and Wolfgang H.** 2011. Biofortification: A new tool to reduce micronutrient malnutrition, Food Nutri. Bull.,**32** (Supplement 1): 31S-40S.
3. **Pfeiffer W. H. and McClafferty B.** 2007. Harvest Plus: breeding crops for better nutrition. Crop Sci., **47**: 88-105.
4. **Welch R. M.** 2002. Breeding strategies for biofortified staple plant foods to reduce micronutrient malnutrition globally. J. Pl. Nutri., (Suppliment). **132**: 495S-499S.
5. **Thavarajah D., Thavarajah P., Sarker A. and Vandenberg A.** 2009. Lentils (*Lens culinaris* Medikus subsp. *culinaris*): a whole food for increased iron and zinc intake. J. Agri. Food Chem., **57**: 5413-5419.
6. **Thavarajah D., Thavarajah P., Wejesuriya A., Rutzke M., Glahn R. P., Geral F. and Vandenberg A.** 2011. The potential of lentil (*Lens culinaris* L.) as a whole food for increased selenium, iron, and zinc intake: preliminary results from a 3 year study. Euphytica, **180**: 123-128.
7. **Thavarajah P., Thavarajah D. and Vandenberg A.** 2009. Low phytic acid lentils (*Lens culinaris* L.): a potential solution for increased micronutrient bioavailability. J. Agri. Food Chem., **57**: 9044-9049.
8. **Hebert Y., Plomion C. and Harzic N.** 1995. Genotypic x environment interaction for root traits in maize as analysed with factorial regression models. Euphytica, **81**: 85-92.
9. **Lin C. S., Binns M. R. and Lefkovitch L. P.** 1986. Stability analysis: where do we stand? Crop Sci., **26**: 894-900.
10. **Backer H. C. and Leon J.** 1988. Stability analysis in plant breeding. Pl. Breeding, **101**: 1-23.
11. **Ramagosa I. and Fox P. N.** 1993. Genotype x Environment interaction and Adaptation, *In*: Plant Breeding: Principals and Prospects (eds.). Chapman and Hall, London, p. 373-390.
12. **Tesemma T., Tsegaye S., Belay G., Bechere E. and Mitiku D.** 1998. Stability of performance of tetraploid wheat landraces in Ethiopian highland. Euphtytica, **102**: 301-308.
13. **Crossa J.** 1990. Statistical analysis of multilocation trials. Adv. Agro., **44**: 55-85.
14. **Singh D., Chonkar P. K. and Dwivedi B. S.** 2005. Manual on soil, plant and water analysis. Westville Publishers, New Delhi.
15. http://www.harvestplus.org/concentration/crop-sampling-protocals-micronutrient-analysis.
16. **Zarcinas B. A., Cartwright B. and Spouncer L. R.** 1987. Nitric acid digestion and multi element analysis of plant material by inductively coupled plasma spectrometry. Comm. Soil Sci. Pl. Analysis, **18**: 131-146.
17. **Eberhart S. A. and Russell W. A.** 1966 Stability parameters for comparing varieties. Crop Sci., **6**: 36-40.
18. **Zobel R. W., Wright M. J. and Gauch H. G.** 1988. Statistical analysis of a yield trial. Agro. J., **80**: 388-39.
19. **Chakraborti Mridul, Prasanna B. M., Hossain Firoz, Mazumdar Sonali, Anju M. Singh, Guleria Satish and Gupta H. S.** 2011. Identification of kernel iron- and zinc-rich maize inbredsand analysis of genetic diversity using microsatellite markers, J. Pl. Bioc. Biot., **20**: 224-233.
20. **Oikeh S. O., Menkir A., Maziya-Dixon B., Welch R. M., Glahn R. P. and Gauch G. Jr.** 2004. Environmental stability of iron and zinc concentrations in grain of elite early-maturing tropical maize genotypes grown under field conditions. J. Agri. Sci., **142**: 543-551.
21. **Ebdon J. S. and Gauch H. G.** 2002. Additive main effect and multiplicative interaction analysis of national turfgrass performance trials II: Cultivar recommendations. Crop Sci., **42**: 497-506.
22. **Yan W.** 2002. Singular-value partitioning in biplot analysis of multi-environment trial data. Agro. J., **94**: 990-996.