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Research Article

Genetic Variability for Grain Iron and Zinc Concentration in Lentil

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Abstract

Micronutrient deficiency is reported in more than 2 billion people and is more serious problem in developing countries. Food fortification, agronomic fortification and biofortification are the possible solutions. Biofortification is most feasible and sustainable for resource poor. It requires multilocation germplasm evaluation, study of genotype and environment interaction (GEI) and use of conventional and molecular tools for development of Fe and Zn rich varieties. In the present study, 96 lentil genotypes were evaluated at three locations to determine the extent of variability and to study GEI for grain Fe and Zn concentration.Large variation was recorded for grain Fe and Zn concentration. Using Eberhart and Russel model for grain Fe concentration, genotypes P 2124, P 2126 and P 2127 having unit regression coefficient (bi=1) and non-significant deviation from regression ($S^2di = 0$) were found to be stable. Similarly for grain Zn concentration genotypes NDL 11-1 and L 4648 were found to be stable. The stable genotypes identified in the present study can be to develop bio fortified lentils.

Keywords: Lentil, grain Fe and Zn concentration, variability, stability, heritability

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Introduction

Lentil (*Lens culinaris* ssp. *culinaris*) is a self-pollinating diploid (2n = 2x = 14) cool season legume with genome size of genome size of 4 Gbp [1]. This crop is mainly cultivated in the Indian subcontinent, Western Canada, Australia, Middle East, Southern Europe and North America. Lentil grains are rich in protein, vitamins, minerals and prebiotic carbohydrates [2]. They are also rich in micronutrients such as grain Fe and Zn concentration and have potential to provide adequate amount of these nutrients [3]. Lentil is suited for micronutrient biofortification as it is grown in areas inhabited with micronutrient deficient and resource poor. This crop is grown in about 52 countries. During 2013 lentil was cultivated on 4.34 million hectares with production of 4.95 million tons [4]. South Asia contributed 2.32 million hectare area and 1.53 million ton production. In India lentil was grown in 1.34 million hectares with production of 1.13 million tons. In India the major lentil area includes Uttar Pradesh, Madhya Pradesh, Jharkhand, Bihar and West Bengal. In these regions lentil is grown by resource poor farmers and these regions have high population density along with high malnutrition and micronutrient deficiency. Billions of people worldwide and particularly in developing countries suffer from micronutrient malnutrition resulting in reduced likelihood that mothers survive during childbirth, damaged cognitive development and lower disease resistance in children. This problem is more prevalent in developing countries where most of the population depends on cereal based diets which lacks micronutrients.

In developing countries with poor income, staple food is priority the key source for energy. As the income increases non staple plant foods and animal products are procured. Poverty and micronutrient malnutrition are linked. Both Fe and Zn have specific metabolic roles. Fe is necessary for transport of oxygen by Red Blood Corpuscle within hemoglobin molecules, for normal neurotransmitter chemistry and morphology of neuronal networks [5]. Fe deficiency can lead to maternal mortality [6], reproductive performance [7], mental damage of children [5] and reduced capacity for physical work in children and adults. Zinc plays important role in several metabolic pathways [8] including protein synthesis, cellular growth and cellular differentiation [9]. Inadequate dietary intake of Zn can cause mortality and stunting in children [10]. Zinc deficiency may result in anorexia, depression, altered reproductive biology, gastro-intestinal problems and impaired immunity. Caulfield et al. [11] have reported about 800,000 deaths annually from diarrhea, pneumonia, and malaria in children under five due to zinc deficiency. The means to reduce micronutrient deficiency include biofortification, dietary diversification, supplements and fortified foods. Among these biofortification is most sustainable. Biofortification is the development of micronutrient dense crops. It target resource poor having a regular dietary intake of food in sufficient quantity. It provides a means of providing naturally fortified food to rural population. Genetic variability is essential to improve lentil cultivars for high grain concentration of Fe and Zn.

Means of traits across environments are sufficient to indicate genotypic performance in the absence of the genotype × environment interaction (GEI). GEI complicates breeding, testing, and selection of superior genotypes for quantitative traits. Several methods have been utilized by different workers for study of G x E interaction (Joint regression by Eberhart and Russel [12], multivariate clustering technique by Crossa [13] and multiplicative formulation by Gauch [14]. G x E interactions for grain Fe and Zn concentration have been reported in different crops by Mallikarujun et al. [15] (maize), Chakraborti et al. [16] (maize), Velu et al. [17] (wheat), Karimizadeh et al. [18] (lentil) and Kumar et al. [19] (lentil). The objectives of present study were to study, genetic variability and genotype x environment interactions for grain Fe and Zn concentration.

Materials and Methods

Experimental material and field trials

The experimental material included 96 lentil genotypes representing released varieties, advanced lines developed at different lentil breeding centres of India, and exotic germplasm lines from the International Centre for Agricultural Research in the Dry Areas (ICARDA). The material included both large-seeded (macrosperma) and small-seeded (microsperma) lentil types. The genotypes were evaluated at (i) Delhi (North Western Plain Zone) [28°40′N 77°12′E 218 masl (meters above mean sea level)], (ii) Indore (Central Zone) (30.9°N 75.85°E 244 masl), and (iii) Dharwad (Peninsular Zone) (28°58′N 79°25′E 344 masl) during the winter season of 2015–16. The Fe concentration in the soil was estimated using a procedure suggested by Singh et al. [20] The entries were grown in randomized complete block design with three replications per entry (3 rows per replication) with a plant distance of 5 × 30 cm and row length of 4 m. Standard packages of practices were followed for crop cultivation.

Determination of grain Fe and Zn concentrations

At physiological maturity, a single plant was harvested and dried under shade. The plant was threshed and grains were placed in a clean plastic tray manually (using contaminant-free gloves) on a clean surface. The grains were dried at 35 °C for five days in a contamination and corrosion free oven. From each sample, 5 gm of grains were grounded manually into a fine powder using a mortar and pestle. Dust and metal contamination was avoided. The grain powder sample (0.5 g) was digested following the modified diacid protocol [20] using a microwave digestion system (Multiwave ECO, Anton Paar, les Ulis, France). Fe and Zn concentrations (in ppm) were estimated through inductively coupled plasma-mass spectrometry (ICP-MS) (Perkin Elmer, model: NexION 300 ICP-MS, USA) with an automatic sampling protocol at Division of Soil Science and Agricultural Chemistry, IARI, New Delhi.

Statistical analysis

The data was subjected to analysis of variance (ANOVA) for testing the significance of variation due to variety, years and their interaction for four seed quality traits following Gomez and Gomez [21].

The data were analyzed for stability parameters following Eberhart and Russell [12] model.

$$Yij = \mu + bi\ Ij + \delta ij + \varepsilon ij$$

where Y_{ij} is the mean for the genotypes i at location j; m is the general mean for genotype i; b_i is the regression coefficient for the ith genotype at a given location index, which measures the response of a given genotype to varying location; I_j is the environmental index, which is defined as the mean deviation for all genotypes at a given location from the overall mean; δ_{ij} is the deviation from regression for the ith genotype at the jth location and εij is the mean for experimental error. A genotype having non-significant deviation from regression (s^2 di = 0) and unit regression coefficient (bi=1) was considered as stable.

Results and Discussion

In this study, 96 lentil entries were evaluated at three different locations for grain Fe and Zn concentrations. Analysis of variance (ANOVA) indicated that varieties differed significantly for grain Fe and Zn concentrations. The mean performance (X), the regression coefficient (bi) and the deviation mean square (S^2di) are the parameters to study GEI. Finlay and Wilkinson [22] considered the linear regression (bi) as a measure of stability. However, Eberhart and

Russell[12] emphasized the need of both bi and S^2di in judging the stability of a genotype. Paroda and Hayes [23] advocated that the bi could simply be regarded as a measure of responsiveness and S^2di as a measure of stability. Analysis of variance for grain Fe and Zn concentration in three environments revealed that both the traits were significantly ($P<0.01$) affected by genotype, environment and genotype and environment interactions (GEI) (**Tables 1** and **2**). Grain Fe exhibited higher influence of GEI in comparison to grain Zn concentration. Significant effect of genotype and environment interactions for both grain Fe and Zn concentrations revealed that genotypes performed differently across the tested environments.

Table 1 Pooled analysis of variance for grain Fe concentration

Source	df	SS	MS	F value
Genotypes	95	161423.3	1699.192	76.22**
Environments	3	3413.551	1137.85	51.04**
Replications in environments	4	17380.79	4345.199	194.93**
Genotypes x environments	285	29292.73	102.7815	4.61**
Error	380	8470.582	22.291	
Total	767	219980.9		

Table 2 Pooled analysis of variance for grain Zn concentration

Source	df	SS	MS	F value
Genotypes	95	78452.11	825.8117	38.20**
Environments	3	1769.215	589.7385	27.28**
Replications in environments	4	21662.48	5415.62	250.53**
Genotypes x environments	285	7921.688	27.7954	1.28*
Error	380	8214.327	21.6166	
Total	767	118019.8		

Mean performance and stability statistic

Mean performance and stability statistic for grain Fe and Zn concentration are shown in (**Table 3**). Genotype L 4591 followed by L 4590 exhibited high mean performances for grain Fe concentration. While genotype like 10-3-Y-26 followed by P 14103 exhibited low mean performances for grain Fe concentration. In case of grain Zn entries RL 1 followed by LC 300-1 showed high mean performances. While genotypes like P 13129 followed by LC 74-1-5-1 showed low mean performances for grain Zn concentration. Distribution of studied lentil genotypes for grain Fe and Zn concentration in different groups is depicted in **Figure 1**. The presence of GE interaction (Tables 1 and 2) indicated that decision based solely on genotypes means were not reliable. Genotypes responded differently to changes in environments; therefore, measure of stability parameters is important to decide the stable genotypes.

Table 3 Stability analysis for grain Fe and Zn concentration using Eberhart and Russel model

S.no.	Genotype	Grain Fe concentration (mg/kg)			Grain Zn concentration (mg/kg)		
		Mean	Regression coefficient (bi)	Stability parameter (S^2di)	Mean	Regression coefficient (bi)	Stability parameter (S^2di)
1	L 404	75.963	1.4285	326.920**	53.115	0.427	10.133
2	L 830	71.963	-0.4112	326.920**	48.615	0.427	10.133
3	L4596	76.250	-0.2165	393.319**	51.413	-1.079	32.763*
4	L4602	75.950	0.417	320.610**	49.000	0.343	-3.766
5	L4603	61.939	1.427	188.488*	54.655	0.864	3.094
6	L4618	58.688	0.3219	163.425*	50.155	0.864	3.094
7	L4620	60.288	-0.6911	132.892*	51.403	1.529	-10.581
8	L4648	62.188	0.6223	148.971*	54.280	1.214	-0.255
9	L4649	76.038	-0.3832	205.982**	55.355	0.444	-3.900
10	L4650	72.038	0.4131	205.982**	50.855	0.444	-3.900
11	L4698	74.525	0.0833	100.356*	50.998	0.292	-9.188
12	L5120	70.325	-0.3313	235.770**	51.588	0.292	16.169*
13	L5126	76.413	1.9832	74.410*	47.100	3.231	525.757**
14	L5253	72.413	0.2665	74.410*	44.100	2.852	379.881**

15	ILL7663	77.000	0.661	114.135*	46.253	3.102	339.480**
16	L7818	75.288	1.4392	61.582*	41.163	2.550	155.307**
17	L7903	76.525	0.1468	674.149**	54.498	-0.598	72.345*
18	DPL15	72.525	1.8565	674.149**	49.998	-0.598	72.345*
19	DPL 21	78.575	0.4008	661.512**	52.333	-1.094	43.061*
20	DPL 58	76.738	-0.064	590.473**	50.805	0.020	112.096*
21	PL 02	74.000	1.0227	212.545**	40.838	-1.172	68.911*
22	P 13129	70.000	-1.4292	212.545*	36.338	-1.172	68.911*
23	PL 101	77.638	-0.5943	257.023*	43.425	-2.182	258.480**
24	PL 639	64.013	0.5718	616.742*	46.175	-2.793	266.579**
25	RL 1	70.900	0.138	346.857*	57.978	0.488	124.944*
26	ILL 2581	66.900	0.2669	346.857**	53.478	0.488	124.944*
27	SKL 259	68.213	0.6965	641.641**	54.263	-0.205	157.091**
28	EC 1	62.800	0.6965	437.330**	56.375	-0.261	152.539**
29	10-2-B-2	54.788	0.6965	0.366	51.600	-1.671	10.181
30	10-3-Y-26	50.788	0.6965	0.366	47.100	-1.671	10.181
31	Globe mutant	59.663	0.6965	62.538*	48.075	-1.424	133.169*
32	Fasciated mutant	68.500	0.6965	518.547**	48.563	-3.090	3.164
33	HM 1	67.188	0.6965	49.902*	53.678	2.116	-7.143
34	MC 6	63.188	0.6965	49.902*	47.678	2.495	-9.315
35	K 75	66.938	0.6965	45.648*	53.238	1.564	34.952*
36	VL 103	62.438	5.1828	145.995*	54.438	2.295	40.693*
37	FLIP 96-57	70.578	0.862	352.169**	44.050	1.572	83.344*
38	LL 147	66.578	3.2194	352.169**	39.550	1.572	83.344*
39	LL 931	67.400	0.2023	292.250**	40.925	-0.314	88.447*
40	LC 74-1-5-1	69.113	-0.1414	488.381**	36.288	0.228	72.981*
41	LC 300-1	78.838	-0.1057	225.179**	57.825	0.196	39.357*
42	PL 4	74.838	0.8858	225.179**	53.325	0.196	39.357*
43	LL 1231	76.850	0.6701	218.402**	53.988	0.180	38.565*
44	IPL 221	77.538	-0.5202	239.766**	52.538	1.647	176.155**
45	VL 143	62.788	0.6965	402.813**	53.325	0.037	28.249*
46	PL 406	58.788	0.6965	402.813**	48.825	0.037	28.249*
47	PL 117	79.975	0.6965	48.259*	45.900	0.850	81.525*
48	IPL 220	62.213	0.6965	96.735*	47.888	2.039	102.832*
49	L 4590	86.425	0.6965	145.460*	45.400	1.502	201.884**
50	PL 4	82.425	0.6965	145.460*	40.900	1.502	201.884**
51	L 4591	88.900	0.6965	253.254**	45.550	1.483	202.815**
52	LL 1203	85.338	0.6965	175.108**	43.225	1.742	335.787**
53	RLG 147	65.425	0.6965	25.357*	50.113	-0.530	84.657*
54	DL 11-4	61.425	3.4457	25.357*	45.613	-0.530	84.657*
55	KLS 113	71.913	0.3532	76.953*	47.600	-0.126	43.355*
56	NDL 11-1	56.975	2.1326	22.794*	46.250	1.078	11.535
57	PL 122	76.338	1.7058	15.266	52.225	3.038	323.741**
58	SKUAL 9	72.338	-1.8497	15.266	47.725	3.038	323.741**
59	L 4706	77.388	0.7718	-3.858	48.488	3.212	424.510**
60	DPL 15	80.263	1.6838	138.607*	49.288	3.510	473.784**
61	LL 1210	64.200	1.0672	195.834*	57.225	0.383	226.848**
62	KLB 345	60.200	1.6851	195.834*	52.725	0.383	226.848**
63	PL 024	66.445	1.443	129.929*	55.450	0.218	334.985**
64	PL 129	69.588	1.7056	431.080**	55.275	-0.215	204.303**
65	IPL 324	68.075	1.5114	400.966**	48.400	0.823	0.131
66	IPL 406	64.075	0.6965	400.966**	43.900	0.823	0.131
67	L 4707	72.163	0.6965	780.894**	48.700	3.913	2.414
68	LL 1204	57.388	0.6965	579.903**	50.830	3.319	84.027*
69	LH 84-8	55.950	0.6965	79.270*	50.075	0.991	83.288*

70	RVL 48	51.950	0.6965	79.270*	45.575	0.991	83.288*
71	KLB 314	63.200	0.6965	219.200**	45.163	0.807	179.302*
72	IPL 325	59.000	1.7413	173.128*	45.963	0.706	133.957*
73	DPL 62	70.250	0.6965	0.051	44.713	4.702	481.208**
74	P 2102	66.250	0.6965	0.051	40.213	4.702	481.208**
75	P 2124	65.638	1.3459	78.343*	43.000	4.012	544.903**
76	P 2125	64.700	1.4538	188.791*	42.133	3.227	428.237**
77	P 2126	72.663	1.1686	36.466	51.150	2.053	2.605
78	P 2127	68.663	1.6192	36.466	46.650	2.053	2.605
79	P 2130	72.788	1.7288	9.243	49.175	1.045	31.080*
80	P 2205	64.225	0.7119	818.164**	51.950	0.832	28.086*
81	P 2215	67.100	1.1506	-6.447	54.660	0.579	22.015*
82	P 2230	63.100	1.7901	-6.447	50.160	0.579	22.015*
83	P 2233	68.425	0.1913	-5.662	52.013	-1.638	63.137*
84	P 2239	67.788	6.598	-7.002	52.038	0.526	50.080*
85	P 3113	63.575	0.4764	302.147**	50.025	5.007	65.405*
86	P 3204	59.575	2.4448	302.147**	50.525	3.746	168.484*
87	P 3208	60.950	0.6673	106.834*	50.825	4.288	128.471*
88	P 3220	72.050	0.5201	70.979*	50.913	4.172	78.448*
89	P 13104	66.188	4.8786	132.694**	54.588	0.066	55.388*
90	P 13113	62.188	3.3423	132.694**	50.088	0.066	55.388*
91	P 13122	66.875	-1.1179	296.565**	53.775	0.222	26.188*
92	P 13135	55.375	3.6956	432.974**	55.188	0.070	115.528*
93	P 13143	53.325	1.0858	101.914*	47.200	2.187	373.829**
94	P 14103	47.825	0.3004	84.789*	42.700	2.187	373.829**
95	P 14201	58.838	3.6124	14.099*	44.188	0.439	131.359*
96	P 15104	61.250	3.2295	207.924*	45.713	1.247	297.163**
	Mean	68.04		Mean	49.068		

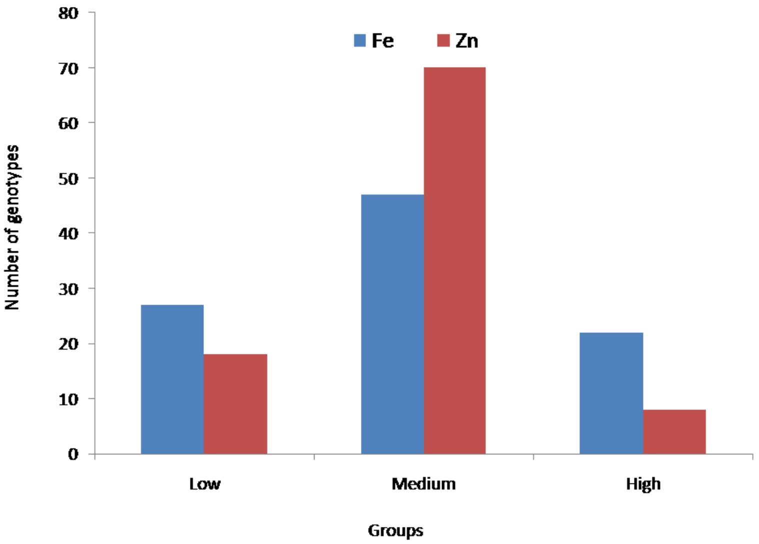

Figure 1 Distribution of studied lentil genotypes for grain Fe and Zn concentration in different groups

Genotypes like P 2124, P 2126 and P 2127 having unit regression coefficient (bi=1) and non-significant deviation from regression (S^2di = 0) was considered as stable. However, genotypes L 4706, DPL 62, P2233 and SKUL 9 possessed high mean with less than unity regression, the genotype should be suitable for poor environmental conditions. High value of regression (bi>1) indicates that the variety is more responsive for input rich environment like L 404, L 5126 DPL 15, PL 129 and PL 02. While, low value of regression (bi<1), is an indication that the variety may be adopted in poor environment like PL 4, FLIP 95-57, PL 117, L 4590, LL 1203, L 4591, L 470 L 4650, DPL 21, KLS 113, L 5253, ILL 7663, P 3220, L 4602, L 7903, RL 1, L830, LC 3001, LC 74-1-5, L 4596, L 5120 PL 101, IPL 221 L 4649 and L 4698.For grain Zn concentration genotypes NDL 11-1 and L 4648 having unit regression coefficient (bi=1) and non-significant deviation from regression ($S^2di = 0$) was considered as stable. However, genotypes L 4603, L 4618, IPL 324, IPL 406, L 4649, L 4650, L 830, L 4602, L 4698, 10-2-B-2, 10-3-Y-26 Fasciated mutant and L 404 possessed high mean with less than unity regression, the genotype should be suitable for poor environmental conditions. High value of regression (bi>1) indicates that the variety is more responsive for input rich environment, genotypes like L 4707, MC 6, HM 1, P 2126, P 2127, L 4648 and NDL 11-1. While, low value of regression (bi<1), is an indication that the variety may be adopted in poor environment like P2233, DPL 21, L 4596, RLG 147, EC 1, PL 129, SKL, 259, DPL 58, VL 143, P 13104, P 13113, PL 024, LL 1210 and KLB 345. Stable genotypes identified from the present study can be utilized in biofortification of lentil. Bio fortified lentil varieties will reduce micronutrient deficiency in human beings by regularly consuming grains of these varieties. The bioavailability of grain Fe and Zn concentration and concentration of phenolic acids and flavonoids which contribute to antioxidant value [24] needs comprehensive study.

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