



Genetic variability for nutritional quality in Lentil (*Lens culinaris* Medikus Subsp. *culinaris*)

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ABSTRACT

Lentil (*Lens culinaris* Medikus Subsp. *culinaris*) is important dietary source of macro and micro-nutrients essential for human health. It provides essential amino acids to meet nutrient requirements. In order to develop macro/micro-nutrient rich lines, it is essential to know the level of genetic variability in the lentil accessions. A preliminary evaluation of seeds of 234 germplasm collected from various countries was carried out to understand the macro and micro-nutrient concentration level of phosphorus, potassium, calcium, magnesium, sulphur, iron, manganese, boron, copper and zinc which are essential nutrients for maintaining human health. Substantial variability of mineral concentration was estimated across the accessions. Highest seed concentrations (in mg kg⁻¹) were recorded in ILL8194 [P (5800); K (13600)], ILL8105 [Ca (1860)], ILL7200 [Mg (1300)], ILL6212 [S (3100)], ILL7547 [Fe (86)], ILL9886 [Mn (20)], ILL6459 [B (9.7)], ILL1005 [Cu (17)] and ILL5480 [Zn (60)]. Significant correlation was observed among the macro and micro-nutrients. Highest positive correlation was observed between Ca and Mg followed by K and S, P and K, and Ca and B; and K showed higher negative correlation with B and Ca. The high nutrient-content accessions identified in this study will be used as parents in developing nutritious lentils at ICARDA and by its cooperating national partner institutions.

Key words: Association, Correlation, Genetic variability, Lentil, Macro-nutrient, Micro-nutrient, Nutritional security.

INTRODUCTION

Lentil (*Lens culinaris* ssp. *culinaris*) is an ancient and early domesticated legume that continues to play an important role in human and animal diet, and modern agriculture. It is the fourth most important legume crop after bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum*) and chickpea (*Cicer arietinum*); with an area of 4.17 m ha and production of 4.41 m tons in 2011 (FAO, 2013). Lentil is an important source of protein, fibre, minerals, vitamins, and antioxidant compounds and macronutrients, micronutrients and trace elements. Dietary deficiencies in mineral elements can have significant negative impacts, such as learning disabilities in children, increased morbidity and mortality etc. It has been estimated that nearly 3.7 billion people worldwide are Fe deficient and 54% of these are severely deficient (Yang *et al.*, 2007). Nutrient deficiencies mainly result from their low concentrations in most of the plant foods and thus in the daily diet. Hence, there has been an interest in increasing the mineral concentrations of various seed crops. With these intentions, breeding strategies have been devised for increasing macro and micro-nutrient density in foods and are being assessed to develop sustainable and long-term solutions.

Producing micronutrient-enriched cultivars, particularly those with increased Zn and Fe either agronomically or genetically and improving the bio

availability of these minerals are considered a promising and cost effective method to manage micronutrient deficiencies. One approach that can be used to increase the level of mineral nutrients in food crops is to identify the sources of genetic diversity, screen for natural variants that have favourable traits and use these to develop new cultivars. Furthermore, the diversity in the lentil landraces has resulted due to differences in adaptability to traditional farming systems and taste preferences of the community in regions where lentil is cultivated.

In the present study, we examined the genetic variation in macronutrients (P, K, Ca, Mg and S) and micronutrients (Fe, Mn, B, Cu and Zn) to identify germplasm that could be used to improve the nutritional quality of lentil and provide information to international breeders. With a view the present study was undertaken to assess the variability in the germplasm for the various nutrients. The objectives of this study was supported by Harvest-Plus, CGIAR to a) assess the variability in commonly used nutrients, b) distribution of macro and micro-nutrients and potential of the germplasm resources, c) correlation between these elements, d) similarities in accessions for these elements and variation between and within the clusters, and e) association between the clusters with the origin of germplasm collected.

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MATERIAL AND METHODS

Genetic material: Genetic material consisted of 234 lentil germplasm collected from various countries and breeding lines from ICARDA (Table 1). Breeding lines developed at International Center for Agricultural Research in the Dry Areas in an un-replicated completely randomized design, were derived from parents adapted to diverse traits. All these accessions were grown in an un-replicated single row plot of 4 m long and randomly allotted to a homogeneous field at the ICARDA Research station, Tel Hadya, Syria during 2006/07 cropping season.

Preparation for grain nutrient analysis: The seeds were harvested from each accession by selecting uniform 5 individual plants, bulk them and threshed. Harvested seed material were dried in sunlight and from each genotypes 6-8 g was sampled for grinding to make it powder. To avoid any contamination, gloves were used during grinding of the seeds. Biochemical analysis for grain macro and micro-nutrients was carried out in triplicate samples by digestion using 9:4 di-acid mixture (HNO_3 ; HClO_4) followed by Atomic Absorption Spectrometry method using AAS (make) at Waite Institute, University of Adelaide, Australia as per protocol described by Zarcinas *et al.* (1987) and Singh *et al.* (2005).

Statistical analysis: The simple statistics on means, minimum, maximum, standard deviation and coefficient of

variation (CV) were obtained. The distribution of the nutrients was also examined and tested for their normality using non-parametric tests such as Anderson-Darling, Cramer von Mises and Waston (Aitchison 1986). Association between various nutrients was assessed using simple correlation coefficient. The interrelationship between the accessions, for a given nutrient, was obtained using hierarchical clustering based on Euclidean distance between the accessions. The average link method, also known as UPGMA (Unweighted Pair Group Method with Arithmetic mean), was used to generate the clusters. Statistical significance of separation between the cluster means was assessed using within cluster variation in a one-way analysis of variance. Various computations were performed in Genstat software (Payne 2011) environment.

RESULTS AND DISCUSSION

Variability of macro and micro-nutrients: In current study, status of macro and micro-nutrient in seeds of 234 lentil germplasm lines including breeding lines from ICARDA and germplasm from different countries were studied (Table 1). Seeds were analysed for 10 important macro and micro-nutrients namely Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulphur (S), Iron (Fe), Manganese (Mn), Boron (B), Copper (Cu) and Zinc (Zn). Table 2 shows the mean, maximum and minimum of all the analyzed mineral

Table 1: Distribution of plant material by origin

Origin	No. of genotypes	ICARDA/International Lentil Line (ILL) numbers
Breeding Line, ICARDA, Syria	126	7657,7670,7686,7690,7701,7706,7949,7950,7960,7961,7972,8006,8074,8077,8090,8138,8140,8176,8189,8194,8195,8604,8605,8606,8618,8621,8623,8627,9830,9832,9837,9838,9839,9840,9841,9842,9843,9844,9845,9846,9847,9848,9849,9850,9854,9858,9859,9860,9861,9862,9864,9867,9870,9873,9877,9878,9885,9886,9887,9888,9897,9898,9899,9900,9901,9902,9903,9904,9905,9906,9907,9908,9909,9910,9911,9912,9913,9914,9915,9916,9917,9918,9919,9920,9921,9922,9923,9924,9925,9926,9927,9928,9929,9930,9931,9932,9933,9934,9935,9936,9937,9938,9939,9940,9942,9944,9945,9947,9948,9950,9951,7668,7678,7685,7940,7941,7947,8008,9833,9889,9890,9891,9892,9893,9894,9895,5684,5715,5722,5725,5755,5782,6004,6024,6031,6212,6238,6243,6246,6258,6467,6811,6821,6994,7127,7193,7199,7201,7204,7502,7504,7517,7521,7537,7979,7980,4400,5597,5748,5823,6002,6025,6027,6199,6207,6211,6459,6465,6972,6980,6982,7177,7200,7978
Syria	48	5588,5883,7982,7983,8095,5244,5582,7985,7991,8091,8093
Jordan (JOR)	11	2313,5540,465, 780, 1005,1851
Chile (CHL)	5	7207,7215,7219,7213,7217
Nepal (NPL)	5	4402,7616,7617,7618,7620, 9836
Pakistan (PAK)	5	590,8173,1878,8612,8634
Turkey (TUR)	5	96,7711,7553,7558
Morocco (MAR)	4	4605,8108,8109
Argentina (ARG)	4	784,818,813
Egypt (EGY)	3	7713, 7717
Bangladesh (BGD)	2	8105,8106
Canada (CAN)	2	857, 7547
Algeria (DZA)	2	8174, 9896
USA	2	6408 (Bulgaria/BGR), 5480 (Czechoslovakia/CSK), 7981 (Ecuador/ECU), 2439 (Ethiopia/ETH), 7543 (India/IND), 759 (Iran/IRN), 358 (Mexico/MEX), 4606 (Palestine/PAL), 6829 (Poland/POL), 323 (Serbia/SRB)
Ten countries	1 (each)	
Total	234	

nutrients in 234 germplasm lines. The studied germplasm showed considerable variation in mineral levels (Table 2). The top five lines for each macro and micro-nutrients are listed in Table 3. Correlations among the macro and micro-nutrients present in the germplasm are presented in Table 4. Bar Chart representing nutrient wise frequency distribution in all the germplasm studied (Figure 1). The variation between the cluster means was found statistically significant ($P < 0.001$) (Table 5). The distributions of lines in various clusters are given in Table 6 along with basic statistics.

Macronutrients: Mean P content of germplasm was 4239 mg kg⁻¹ and ranged from 3000 to 5800 mg kg⁻¹ (Table 2). The genotypes viz. ILL 8194, ILL 7978 and ILL 784 had the highest while ILL 8612, ILL9908, ILL 9900 and ILL 9899 had the lowest P levels. The mean K and Ca concentrations of the germplasm are 10795 mg kg⁻¹ (range: 8600 to 13600 mg kg⁻¹) and 410.9 mg kg⁻¹ (range: 189.9 to 1860 mg kg⁻¹), respectively. The genotypes viz. ILL 8194, ILL, 590 and ILL 6002 had the highest while ILL 9899 and ILL 9900 had the lowest K levels. The genotype ILL 8105 had the highest while the genotypes ILL 7686 and ILL 5480 had lowest levels of Ca. The mean level of Mg was 969.7 mg kg⁻¹ for all the germplasm lines. Its level ranges from 700 to 1300 mg kg⁻¹. The genotypes ILL7200 and ILL 7553 had highest while the genotypes viz. ILL 7207, ILL7686 and ILL 5755 had lowest level of Mg. The mean S concentration of 234 lines was 2278 mg kg⁻¹. It ranged from 1790 to 3100 mg kg⁻¹. The line ILL

6212 had the highest and the lines ILL 780, ILL 8612 had shown the lowest S level.

Micronutrients: In the studied germplasm lines, the amount of Fe varied from 40.54 to 81.39 mg kg⁻¹, and the mean was 66.40 mg kg⁻¹. The lines ILL 7547, ILL 1878 and ILL 7668 were shown the highest Fe levels and the lines ILL 9848 and ILL 9951 recorded the lowest Fe levels (Table 3). Mean Mn concentration of all germplasm lines was 13.7 mg kg⁻¹ and its concentration level was ranged from 9.25 to 20.29 mg kg⁻¹. The lines ILL 9886, ILL 8195 and ILL 590 have the highest Mn levels and ILL 4605 and ILL 5715 had the lowest Mn levels. The concentration of B varied from 3.62 to 9.67 mg kg⁻¹. The lines ILL 6459, ILL 4402 and ILL 8105 have found with the highest levels of B; and the lines ILL 9841, ILL 9936, ILL 5588 showed the lowest B levels. The mean of Cu concentration level recorded was 12.27 mg kg⁻¹ for all germplasm. It ranged from 7.46 to 17.33 mg kg⁻¹. The lines ILL 1005, ILL 7678 and ILL 7127 recorded the highest levels of Cu and the lines ILL 7177 and ILL 358 showed the lowest levels of Cu. The average Zn concentration of the all 234 germplasm lines studied was 44.98 mg kg⁻¹ and the range was 22.36 to 60.40 mg kg⁻¹. The germplasm lines ILL 5480, ILL 9888, ILL 6211 and ILL 5725 are found with the highest Zn levels; and the lines ILL 9945, ILL 5722 and ILL 5540 with lowest Zn levels.

Frequency distribution of 234 lentil germplasm for grain concentration of all ten nutrients viz., P, K, Ca, Mg, S,

Table 2: Mean, standard deviation, minimum and maximum, and CV of macro and micro-nutrients in the lentil collection (N=234)

Variable ^(mg kg⁻¹)	Mean	Standard Deviation	Minimum	Maximum	CV (%)
P	4239	594.2	3000	5800	14.0
K	10795	884.9	8600	13600	8.2
Ca	410.9	219.4	189.9	1860	53.4
Mg	969.7	118.8	700	1300	12.3
S	2278	196.3	1790	3100	8.6
Fe	66.40	5.834	40.54	85.61	8.8
Mn	13.70	1.835	9.251	20.29	13.4
B	5.361	1.284	3.623	9.665	24.0
Cu	12.27	1.351	7.458	17.33	11.0
Zn	44.98	6.642	22.36	60.40	14.8

^(a)Based on common tests for normality, Anderson-Darling, Cramer von Mises and Waston, Cu was normally distributed while other micro-nutrients departed significantly ($P < 0.05$) from the normal distribution.

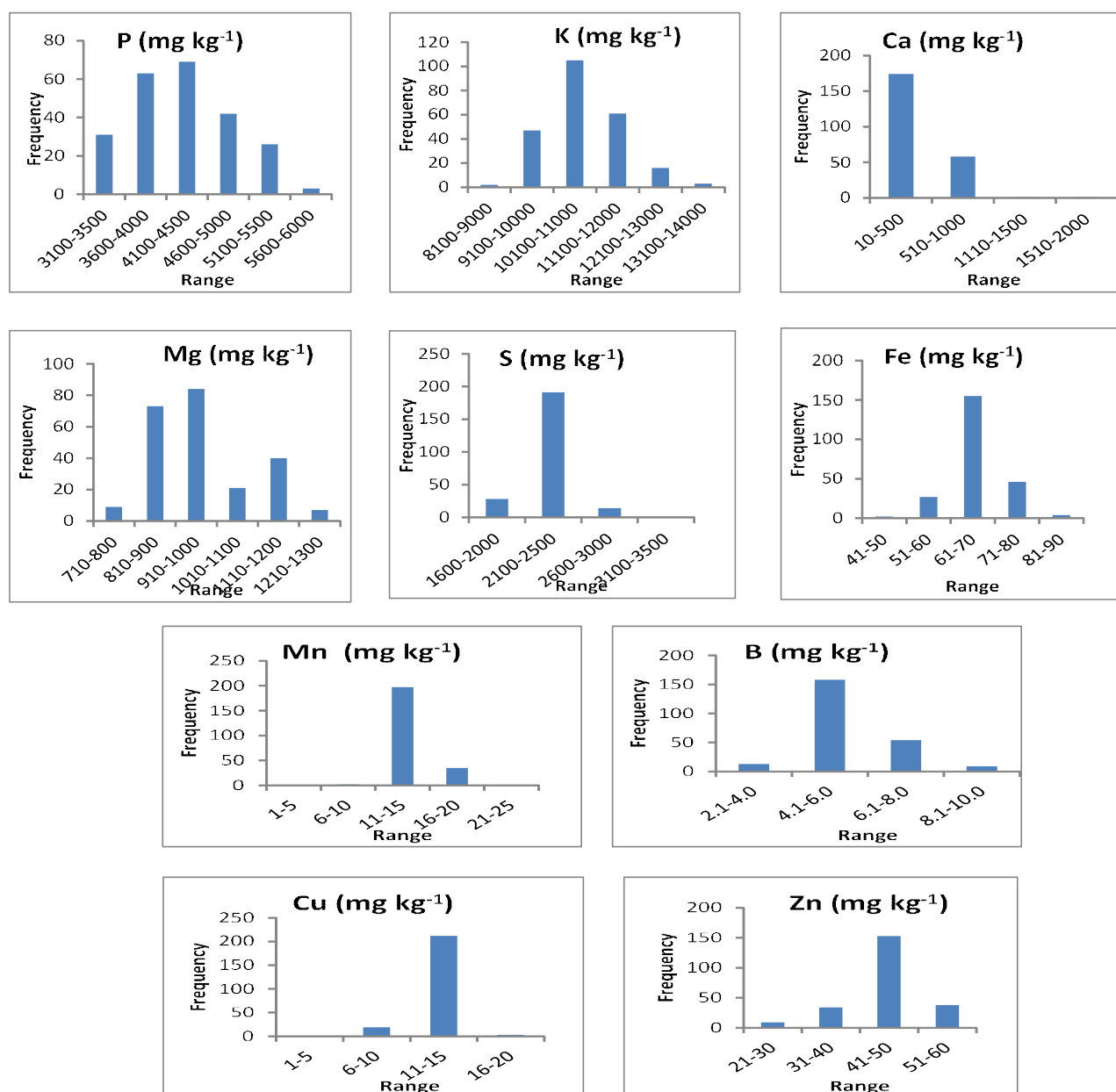
Table 3: Top five accessions containing high values for macro and micro-nutrients from 234 lines

Nutrients Concentration	Name of Accessions (Rank wise)				
	1	2	3	4	5
P(mg kg ⁻¹)	ILL8194(5800)	ILL7978(5600)	ILL784(5600)	ILL9877(5500)	ILL7200(5500)
K(mg kg ⁻¹)	ILL8194(13600)	ILL590(13300)	ILL6002(13100)	ILL6212(12900)	ILL9930(12800)
Ca(mg kg ⁻¹)	ILL8105(1860)	ILL6980(1060)	ILL7200(970)	ILL813(960)	ILL7213(940)
Mg(mg kg ⁻¹)	ILL7200(1300)	ILL7553(1270)	ILL6211(1240)	ILL6980(1230)	ILL7685(1220)
S(mg kg ⁻¹)	ILL6212(3100)	ILL6238(2800)	ILL9940(2700)	ILL6027(2700)	ILL5540(2700)
Fe(mg kg ⁻¹)	ILL7547(85.61)	ILL1878(83.44)	ILL7668(81.78)	ILL9892(80.83)	ILL7558(79.76)
Mn(mg kg ⁻¹)	ILL9886(20.29)	ILL8195(19.03)	ILL590(18.97)	ILL6024(18.61)	ILL7204(18.53)
B(mg kg ⁻¹)	ILL6459(9.67)	ILL4402(9.66)	ILL8105(9.31)	ILL4605(8.99)	ILL8093(8.64)
Cu(mg kg ⁻¹)	ILL1005(17.33)	ILL7678(15.79)	ILL7127(15.67)	ILL9892(15.19)	ILL9910(15.09)
Zn(mg kg ⁻¹)	ILL5480(60.40)	ILL9888(60.35)	ILL6211(59.00)	ILL5725(58.95)	ILL96(57.51)

Table 4: Correlation among the macro and micro-nutrient based on the N= 234 lentil accessions

Variables	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn
P	-									
K	0.61	-								
Ca	0.27	-0.13**	-							
Mg	0.57	0.19**	0.77	-						
S	0.55	0.70	-0.03	0.22	-					
Fe	0.09*	0.10*	0.35	0.36	0.13**	-				
Mn	0.31	0.40	0.06*	0.09*	0.28	0.04	-			
B	0.23	-0.15**	0.59	0.51	-0.11*	0.14*	-0.07*	-		
Cu	0.30	0.39	0.01	0.23	0.40	0.34	0.13**	-0.07*	-	
Zn	0.57	0.52	0.17**	0.39	0.54	0.34	0.27	0.01	0.54	-

Significance: *, ** and the values in bold indicate statistical significance at 5%, 1% and 0.1% respectively. All other estimates were non-significant ($P > 0.05$)

**Fig-1:** Histograms of the macro and micro-nutrients as observed in 234 lines of lentil

Fe, Mn, B, Cu and Zn showed bell shaped curves except in Ca which is positively skewed (Right skewed). (Figure 1). The distributions of each element, except Cu, were statistically different ($P < 0.05$) from normal as indicated by each of the three tests *viz.*, Anderson-Darling, Cramer- von Misses and Watson (Aitchison, 1986).

Correlation among macro and micro-nutrients:

Significant correlation was observed among the macro and micronutrients at $P < 0.05$ or higher probability (Table 4). It was positively significant and highest in Ca and Mg (0.77) followed by K and S (0.70), P and K (0.61), and Ca and B (0.59). The K showed significant and higher negative correlation with B (-0.15) and Ca (-0.13). The significant and positive correlation of P was also observed with Mg (0.57), Zn (0.57) and S (0.55). The Zn also had positively significant correlation with K (0.52), S (0.54) and Cu (0.54). Similarly, Mg had positive and significant correlation with B (0.51).

Similarity of lines for the macro and micro-nutrients:

Based on the hierarchical cluster analysis, similarity groups of lines were identified for each macro/micro-nutrient at 90% similarity (Table 5). For each element, except Mg, the 234 lines were found grouped into three clusters, while for Mg

Table 5: Extent of variability between the clusters mean relative to the variability within the clusters in terms of variance ratio

Variables	Clusters formed at 90% similarity level	
	No. of clusters	variance ratio
P	3	553.92
K	3	374.56
Ca	3	666.21
Mg	2	521
S	3	101.2
Fe	3	154.65
Mn	3	351.56
B	3	607.94
Cu	3	143.24
Zn	3	340.48

P- value observed for the between the clusters variance and within the clusters variance ratio was < 0.001 for each of the nutrients.

into two. It gives between the group variance relative to the within the group as variance ratio (VR), which varied from 101 to 666. The highest VR was observed for Ca followed by B, P, Mg and K.

For each cluster, distributions of the elements are given in Table 6. For most of the elements, within the cluster CV is reasonably low, e.g., $< 10\%$. However, there were a few clusters with a relatively larger variability, *i.e.*, less

Table 6: Distribution of lines in various clusters, and the means, standard deviation, minimum and maximum of the clusters.

Variables (mg kg ⁻¹)	90% Similarity						
	Clusters	No. of lines	Mean	SD	CV (%)	Minimum	Maximum
P	1	42	5167	218.3	4.2	4900	5800
	2	127	4289	272.6	6.4	3900	4800
	3	65	3542	212.1	6.0	3000	3800
K	1	127	11065	417	3.8	10500	11900
	2	24	12437	448	3.6	12000	13600
	3	83	9907	448.2	4.5	8600	10400
Ca	1	175	301.8	68.75	22.8	189.9	510
	2	58	715.2	120.96	16.9	560	1060
	3	1	1860	a	a	1860	1860
Mg	1	142	890.4	49.98	5.6	700	960
	2	92	1092.3	85.25	7.8	970	1300
S	1	194	2220	153.9	6.9	1790	2400
	2	39	2549	75.6	3.0	2500	2800
	3	1	3100	a	a	3100	3100
Fe	1	199	65.91	3.808	5.8	57.02	72.71
	2	24	76.64	3.438	4.5	73.21	85.61
	3	11	52.99	4.984	9.4	40.54	56.48
Mn	1	158	12.69	0.9179	7.2	9.25	14.07
	2	10	18.62	0.7055	3.8	17.86	20.29
	3	66	15.38	0.9392	6.1	14.11	17.48
B	1	164	4.639	0.5089	11.0	3.623	5.743
	2	49	6.582	0.4362	6.6	5.82	7.364
	3	21	8.156	0.7062	8.7	7.438	9.665
Cu	1	196	12.28	0.9174	7.5	10.53	13.81
	2	19	14.73	0.8395	5.7	13.96	17.33
	3	19	9.75	0.852	8.7	7.46	10.43
Zn	1	80	51.36	3.053	5.9	47.66	60.4
	2	139	43.04	3.547	8.2	33.3	47.58
	3	15	28.87	3.065	10.6	22.36	32.47

SD= Standard deviation. a = only one observation

similar lines, for Ca (CV%=16.9 to 22.8%). The cluster size can be used to identify the lines with desired level of the nutrients.

This study is one of the early studies on genetic variability of a range of macro and micro-nutrients involving a diverse collection of lentil germplasm including promising breeding lines developed at ICARDA. On average, global pulse consumption is in decline, but lentil consumption is increasing faster than human population growth, making this species ideal for bio-fortification. Thavarajah *et al.*, (2011) presented that lentil has great potential as a fortifiable crop. Seeds of this crop can be utilised as whole food for providing the most of essential nutrients, foliate and anti-nutritional factors (Gupta *et al.*, 2013).

Seed mineral concentrations were highly variable across the accessions reflecting the presumed diversity of these germplasm. This suggests that genotypic variation for macro and micro-nutrient levels in lentil germplasm provides good opportunities for improvement of cultivated lentil. The genotypes *viz.*, ILL 8194 (5800 mg kg⁻¹), ILL 8194 (13600 mg kg⁻¹), ILL 8105 (1860 mg kg⁻¹), ILL 7200 (1300 mg kg⁻¹), ILL 6212 (3100 mg kg⁻¹), ILL 7547 (86 mg kg⁻¹), ILL 9886 (20 mg kg⁻¹), ILL 6459 (9.7 mg kg⁻¹), ILL 1005 (17 mg kg⁻¹) and ILL 5480 (60 mg kg⁻¹) were found with highest level of concentrations for P, K, Ca, Mg, S, Fe, Mn, B, Cu and Zn, respectively. A few genotypes showed high concentrations of two or more than two nutrients (ILL8194- P and K; ILL7200- Mg, Ca and P; ILL8105- Ca and B; ILL590- K and Mn; ILL 6980- Ca and Mg; ILL6211-Mg and Zn; ILL6212- S and K and ILL9892- Fe and Cu). Hence

these genotypes showing high concentrations for two or more than two nutrients may be utilised in breeding programs for combined enhancement of nutrient status. The relationship among macro and micro-nutrients provide opportunity to improve two or more nutrients together at a time. The positive correlation of several minerals with each other might be possibly due to common uptake pathways (Table 4). Zn had significant and positive correlation with all the macro and micro-nutrients. Similar results were also reported in lentil by Karaköy *et al.*, 2012. It has been reported that positive relationships between Zn and P, and Zn with Mg may be due to the well-known effect of phytate on binding of Zn and Mg in seeds (Marschner, 1995). Positive association of Zn with other minerals demonstrate that selection of high Zn concentration accessions may indirectly select for higher levels of other macro and micro-nutrients. Similarly, Mn, Fe and Cu had significant and positive correlation with all other macro and micro-nutrients except for Fe-B and Cu-B which had negative correlations. In addition, genotypes with high macro and micro-nutrient levels might be suitable for studying the mechanisms of mineral element accumulation and transport. In summary, we identified considerable variation in the macro- and micronutrient contents of lentil germplasm. Our results provide a useful foundation for the development of new cultivars of lentil that have high mineral content.

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