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# Genetic variability for mineral nutrients in lentil

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## ABSTRACT

**The present study was conducted to analyze the concentration of 8 important mineral nutrients namely calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), copper (Cu), manganese (Mn), selenium (Se), and cadmium (Cd) among the seeds of 96 accessions of lentil including breeding lines, varieties, parental lines and exotic lines. The mineral concentrations was ranged from 128 to 494 mg Ca, 4771 to 8927 for K, 140 to 829 mg for Mg, 3907 to 2762 mg for P, 5 to 10 mg for Cu, 10 to 24 mg for Mn, 240 to -630 µg for Se and 0 to 12 µg for Cd. Thus a range of significant variability was observed among the studied genotypes for these minerals. These results indicate that existing variability for these minerals can be utilized for developing high quality cultivars in lentil and can also be used in mapping and tagging of genes controlling Se and other minerals content.**

**Key words:** Diversity, minerals, seeds, lentil

Human body requires more than 21 mineral nutrients for its normal function (White and Broadley, 2005). These minerals are calcium (Ca), phosphorus (P), sodium (Na), chlorine (Cl), potassium (K), magnesium (Mg), iron (Fe), copper (Cu), cobalt (Co), iodine (I), zinc (Zn), manganese (Mn), molybdenum (Mo), fluoride (Fl), chromium (Cr), selenium (Se), sulphur (S), boron, silicon, arsenic, and nickel (Murray *et al*. 2000, Eruvbetine 2003). Based on the quantity required by our body, these minerals can be classified as macro- and micro-nutrients or ultra micro elements. Micro-nutrient deficiency affects more than two billion population worldwide (Welch and Graham 2003). Micronutrient malnutrition is commonly known as "hidden hunger" and causes many health burdens including low birth weight, anemia, learning disabilities, increased morbidity and mortality rates, low work productivity, and high healthcare costs especially in developing nations (Batra and Seth 2002, Welch and Graham 2003). In India, every year, 330,000 children die due to vitamin A deficiency and 22,000 people, mainly pregnant women, from severe anemia. Besides, 6.6 million children are borne mentally impaired every year due to I deficiency; intellectual capacity is reduced in 15% people due to I deficiency; and 200,000 babies are born every year with neural tube defects due to folic acid deficiency (Kotecha 2008). Therefore, it is essentially required to overcome these deficiencies by supplying the enriched diet with minerals and vitamins. Development of staple food cultivars with enriched micronutrients is one of the viable options for combating global micronutrient malnutrition (Thavarajah *et al*. 2011).

Lentil (*Lens culinaris* subsp. *culinaris*) is a cool-season food legume which is rich in protein (20-30%) and micronutrients including iron, zinc, selenium, folates, carotenoids, and vitamins (Thavarajah *et al*. 2011, Johnson *et al*. 2013, Sen Gupta *et al*. 2013). It is mostly cultivated in warm temperate, subtropical, and tropical regions of 52 countries on 3.6 million ha area with annual production of 3.6 million tons (FAOSTAT 2011). India is the major lentil producer in the world with 1.02 million tons of production harvested from 1.34 million ha area (AICRP on MULLaRP 2014-15). Past research in Canada and the United States has shown that lentil is an ideal crop for micronutrient biofortification and it can provide a solution to combat global micronutrient malnutrition (Thavarajah *et al*. 2011). Breeding techniques involving both conventional and modern biotechnology tools can be used to increase concentration and bioavailability of these essential elements in lentil (Welch 2002). However, identification of natural variants having favorable alleles for enhanced micronutrient content in the germplasm is required. Earlier studies showed significant variability for micronutrients in the germplasm from Turkey, Syria, Canada, and Pakistan (Thavarajah *et al*. 2009, 2011, Karakoy *et al*. 2012). These studies have identified lentil germplasm with favorable nutritional traits including folates, and macro- and micro-nutrients. In earlier studies, although the germplasm of south Asian origin had been analyzed for nutritional quality traits especially for micronutrients but these studies did not include genotypes having Indian origin. The objectives of this study were to determine (1) genetic variation for minerals in lentils grown under Indian condition, and (2) correlation of seed size with mineral concentration to identify genotypes for better nutritional quality.

## MATERIALS AND METHODS

### *Seed materials*

Ninety-six lentil genotypes including advanced breeding lines from Indian Institute of Pulses Research (IIPR), parental lines (varieties and breeding lines from

various agricultural research Institutes) of already developed breeding populations and mapping populations, and 16 elite lines from the International Center for Agricultural Research in the Dry Areas (ICARDA) were used in this study (Table 1). It is important to note that, breeding lines developed at IIPR are the derivatives from crosses involving parents adapted to short-season environments and represent diversity with regard to various morpho-physiological traits. On the other hand, the elite lines obtained from ICARDA are bred for Fe and Zn concentrations. The released cultivars developed at different research stations are recommended for cultivation in different agro-climatic zones of India.

### *Field experiments*

All these lentil genotypes were grown during the winter 2012-13 at the main research farm of IIPR, Kanpur, India (26?27_ N, 80? 14_ E and 152.4 m above mean sea level). The climate zone of the experimental location falls under the tropical sub-humid with the annual rainfall of 722 mm and maximum and minimum temperatures of 33 and 20?C. The soil of experimental site is classified as "*typic ustochrept"* with sandy loam texture having pH 8.1, bulk density 1.4 g $cm^{-3}$ and low organic carbon content (2.8 g $cm^{-3}$), at the initiation of the experiment (Ghosh *et al*. 2012). Ninety six genotypes were grown in single row plots and standard agronomical practices were followed to raise a successful crop.

### *Preparation of seeds for analysis*

A total number of 10-20 representative plants were selected from each test genotype, and pods were harvested and threshed separately. Seeds were air dried for 16 hrs. A random sample of 100 seeds (2-3g) from each genotype was finely ground by mortar and pestle.

### *Mineral analysis*

Total mineral concentration (Se, Ca, Mg, K, Cu, and Mn) in lentil seeds was determined using a modified $HNO_3$-$H_2O_2$ method (Thavarajah *et al*. 2009a, Jhonson *et al*. 2013). Ground seed samples (500 mg) were digested in nitric acid (70% $HNO_3$) at 90°C for 1 h. Samples were further digested with hydrogen peroxide (30%) before being diluted to 10 ml with nano-pure water. Mineral concentrations were measured using inductively coupled plasma emission spectroscopy (ICP-OES; ICP-6500 Duo, Thermo Fisher Scientific, Pittsburg, PA, USA). Measurements of all above minerals using this modified method were validated using National Institute of Standards and Technology (NIST) standard reference material 1576. A homogenized laboratory reference material (CDC Redberry) was also used periodically for quality control. All chemicals, and reagents used for mineral digestions in this study were purchased from Alfa Aesar, A Johnson Matthey Company, VWR International and Sigma-Aldrich Co. (St Louis, MO, USA). Water (distilled and deionized; dd$H_2O$) was purified by a Milli-Q Water System (Millipore, Milford, MA, USA) to a resistance of 18.2 MO.cm or greater (Nano-pure water).

### *Statistical analysis*

Mean, range, standard errors and correlations were calculated and histograms were prepared using statistical tools (MS-Excel 2010). A hierarchical cluster analysis was performed using Euclidean distances based on the traits studied which were used to compute the similarity between the genotypes and the average link criterion for cluster formation. A dendrogram was constructed using unweighted pair group method with arithmetic average (UPGMA) to graphically illustrate relationships among genotypes using NTSYS pc- 2.11x software (Rohlf 1998). Similarity/dissimilarity matrix formed from above analysis was further used for the principal component analysis (PCA) using the same software (Rohlf 1998).

## RESULTS

### *Variability for mineral concentration in lentil seeds*

A nutrient profile of 96 lentil genotypes representing elite breeding lines (39), varieties and parental lines (40) and exotic lines (17) was generated. Nutrient profiling was done for calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), copper (Cu), manganese (Mn), selenium (Se), and cadmium (Cd) for each genotype (Table 2). Mean, range and standard error of mean are presented in Table 2. Our results revealed significant differences in the nutrient concentrations of all the studied nutrients among the genotypes.

### *Macro-nutrients*

The average Ca concentration was estimated as 344 mg and varied as 128 - 495 mg among all breeding lines. In cultivars and parental lines, average Ca was assessed as 248 mg and it varied as 74 - 476 mg. The Ca concentration ranged from 43 to 373mg with an average of 202 mg. The highest amount of Ca concentration was observed in a variety, HUL 57 and in some breeding lines namely, IPL 220, IPL91158, IPL11737, and IPL11671 also possess higher amount of Ca. Among the exotic lines, FLIP95-34L had the lowest Ca content (43 mg).

The K content ranged from 4771.43 to 8927 mg with an average of 6062 mg in breeding lines. However, in varieties and parental lines, K content varied from 5488 to 9634 mg with a mean of 7151 mg. Exotic lines had an average K content 6232 mg and it varied from 5130 to 8672 mg. The highest K content was observed in parental line ILL6002 and variety PL02 and lowest in breeding line IPL10778.

The range of Mg content was 140 - 829 mg with an average of 344 mg in breeding lines, 342- 699 mg with an average of 538mg in varieties and 315 - 618 mg with an

**Table 1. Concentration of eight mineral nutrients in diverse lentil genotypes**

| Genotypes | Ca mg/kg | K mg/kg | Mg mg/kg | P mg/kg | Cu mg/kg | Mn mg/kg | Se µg/kg | Cd µg/kg |
|---|---|---|---|---|---|---|---|---|
| **Breeding lines** | | | | | | | | |
| IPL-10800 | 274 | 4790 | 250 | 5403 | 7 | 16 | 330 | 10 |
| IPL-10778 | 363 | 4771 | 146 | 6243 | 9 | 20 | 290 | 20 |
| IPL-11735 | 469 | 5086 | 234 | 7026 | 9 | 20 | 260 | 30 |
| IPL-10666 | 300 | 4960 | 140 | 6190 | 9 | 17 | 290 | 30 |
| IPL-11737 | 484 | 5057 | 204 | 6153 | 10 | 17 | 260 | 20 |
| IPL-11700 | 300 | 4972 | 207 | 6503 | 9 | 17 | 350 | 20 |
| IPL-91114 | 346 | 5603 | 208 | 6779 | 11 | 23 | 400 | 30 |
| IPL-11671 | 495 | 5484 | 321 | 5778 | 8 | 16 | 310 | 20 |
| IPL-10799 | 333 | 5213 | 283 | 5838 | 9 | 15 | 340 | 0 |
| IPL-8618 | 322 | 5657 | 312 | 5918 | 10 | 22 | 280 | 0 |
| IPL-10762 | 308 | 5501 | 265 | 6210 | 10 | 17 | 380 | 0 |
| IPL-91159 | 234 | 5764 | 279 | 5805 | 11 | 16 | 340 | 10 |
| IPL-10819 | 467 | 5913 | 228 | 5846 | 11 | 21 | 360 | 20 |
| IPL-91116 | 395 | 6044 | 381 | 6217 | 10 | 19 | 400 | 0 |
| IPL-11702 | 467 | 5264 | 240 | 7262 | 10 | 20 | 390 | 30 |
| IPL-8639 | 268 | 6509 | 191 | 5012 | 12 | 19 | 380 | 10 |
| IPL-91267 | 356 | 6033 | 225 | 5799 | 11 | 22 | 450 | 20 |
| IPL-7199 | 278 | 5624 | 328 | 4265 | 10 | 15 | 240 | 10 |
| IPL-10816 | 364 | 5965 | 235 | 5615 | 12 | 19 | 390 | 30 |
| IPL-91158 | 483 | 5269 | 348 | 5840 | 10 | 19 | 360 | 10 |
| IPL-91155 | 346 | 6144 | 176 | 5385 | 12 | 19 | 370 | 50 |
| IPL-8728 | 378 | 6294 | 268 | 5284 | 12 | 20 | 430 | 20 |
| IPL-10829 | 429 | 6419 | 275 | 5762 | 12 | 23 | 380 | 120 |
| IPL-8692 | 317 | 6824 | 252 | 5699 | 14 | 23 | 450 | 10 |
| IPL-8529 | 271 | 6635 | 281 | 5315 | 11 | 19 | 390 | 0 |
| IPL-6196 | 336 | 6128 | 374 | 6016 | 10 | 17 | 360 | 70 |
| IPL-215 | 265 | 6500 | 555 | 5372 | 8 | 13 | 470 | 0 |
| IPL-220 | 482 | 8088 | 551 | 6871 | 11 | 20 | 630 | 0 |
| IPL-221 | 469 | 6787 | 534 | 5872 | 9 | 16 | 450 | 0 |
| IPL-222 | 180 | 6723 | 545 | 6102 | 9 | 14 | 470 | 0 |
| IPL-223 | 197 | 5598 | 459 | 3907 | 6 | 10 | 280 | 0 |
| IPL-224 | 386 | 6301 | 562 | 5329 | 9 | 13 | 430 | 0 |
| IPL-325 | 471 | 6607 | 542 | 5012 | 8 | 16 | 430 | 0 |
| IPL-326 | 128 | 6404 | 396 | 4533 | 5 | 11 | 400 | 0 |
| IPL-327 | 357 | 6667 | 520 | 5055 | 7 | 24 | 400 | 0 |
| IPL-328 | 376 | 8927 | 830 | 6773 | 10 | 19 | 460 | 0 |
| IPL-529 | 209 | 6772 | 459 | 5257 | 7 | 15 | 470 | 0 |
| IPL532 | 185 | 6698 | 384 | 4973 | 8 | 14 | 370 | 0 |
| IPL-534 | 314 | 6480 | 427 | 4969 | 8 | 15 | 410 | 0 |
| **Cultivar/parental lines** | | | | | | | | |
| WBL-77 | 203 | 6667 | 525 | 4952 | 8 | 13 | 330 | 0 |
| PL-4 | 221 | 6047 | 490 | 4813 | 9 | 15 | 330 | 0 |
| Shalimar M1 | 155 | 7297 | 387 | 4723 | 7 | 9 | 370 | 0 |
| L-4147 | 330 | 7264 | 671 | 5830 | 9 | 14 | 400 | 0 |
| PL-5 | 189 | 6833 | 509 | 4934 | 6 | 11 | 270 | 0 |
| LL-147 | 420 | 7292 | 643 | 5603 | 9 | 13 | 370 | 0 |
| VL-103 | 180 | 7212 | 437 | 5296 | 7 | 11 | 350 | 0 |
| L-4076 | 303 | 7121 | 534 | 5866 | 9 | 15 | 420 | 0 |
| VL-4 | 276 | 7483 | 588 | 5764 | 9 | 17 | 410 | 0 |
| DP-15 | 213 | 6746 | 627 | 5835 | 7 | 12 | 310 | 0 |
| B-77 | 298 | 8188 | 600 | 6237 | 9 | 17 | 420 | 0 |
| Ranjan | 327 | 8096 | 606 | 5975 | 10 | 15 | 440 | 0 |
| HUL-57 | 476 | 6803 | 563 | 5572 | 9 | 13 | 440 | 0 |
| JL-1 | 141 | 6738 | 342 | 4576 | 7 | 12 | 400 | 0 |
| NDL 1 | 318 | 7269 | 543 | 5259 | 9 | 13 | 330 | 0 |
| PL-234 | 291 | 6878 | 681 | 5644 | 9 | 13 | 390 | 0 |
| LH-84-8 | 317 | 6700 | 430 | 4584 | 7 | 13 | 430 | 0 |
| IPL-406 | 310 | 7399 | 566 | 5754 | 5 | 13 | 460 | 0 |
| T-36 | 213 | 6363 | 435 | 5056 | 8 | 15 | 320 | 0 |
| KLS-218 | 299 | 6703 | 596 | 5405 | 8 | 14 | 350 | 0 |
| DPL-62 | 200 | 7125 | 548 | 5351 | 7 | 12 | 360 | 0 |

**Table 1. Contd....**

| Genotypes | Ca mg/kg | K mg/kg | Mg mg/kg | P mg/kg | Cu mg/kg | Mn mg/kg | Se µg/kg | Cd µg/kg |
|---|---|---|---|---|---|---|---|---|
| VL-1 | 298 | 7424 | 699 | 6068 | 9 | 15 | 430 | 5 |
| PL-639 | 280 | 7415 | 636 | 5976 | 7 | 14 | 430 | 0 |
| L-9-12 | 285 | 7591 | 582 | 5783 | 7 | 16 | 480 | 0 |
| WBL-58 | 266 | 7051 | 589 | 5396 | 9 | 14 | 430 | 0 |
| Br-25-1 | 236 | 7133 | 673 | 6117 | 10 | 16 | 490 | 0 |
| JL-3 | 225 | 7516 | 479 | 5029 | 8 | 13 | 300 | 0 |
| PL-406 | 281 | 6929 | 665 | 5542 | 9 | 15 | 410 | 0 |
| K-75 | 240 | 7325 | 584 | 5697 | 7 | 14 | 420 | 0 |
| IPL-81 | 215 | 8392 | 603 | 6829 | 11 | 14 | 480 | 3 |
| IPL 98/193 | 74 | 6000 | 361 | 4123 | 5 | 9 | 440 | 0 |
| L-602 | 137 | 5807 | 368 | 3993 | 4 | 9 | 340 | 0 |
| L-4603 | 275 | 5922 | 500 | 4444 | 6 | 10 | 290 | 0 |
| IPL-316 | 272 | 6494 | 502 | 4187 | 6 | 12 | 370 | 0 |
| LL-57 | 238 | 5488 | 467 | 3513 | 5 | 8 | 330 | 0 |
| Precoz | 133 | 7597 | 343 | 3725 | 8 | 9 | 400 | 0 |
| PL-02 | 205 | 9355 | 453 | 5717 | 9 | 11 | 610 | 0 |
| ILL-7663 | 273 | 7868 | 607 | 4708 | 7 | 11 | 460 | 2 |
| EC-208362 | 150 | 6868 | 468 | 4391 | 7 | 10 | 500 | 0 |
| ILL-6002 | 165 | 9634 | 610 | 5723 | 11 | 11 | 580 | 0 |
| **ICARDA lines** | | | | | | | | |
| Flip-2000-19 | 252 | 6371 | 550 | 5129 | 6 | 12 | 370 | 0 |
| Flip-98-15L A | 243 | 7161 | 578 | 5133 | 6 | 11 | 510 | 0 |
| Flip-98-15LB | 197 | 6534 | 563 | 4873 | 6 | 11 | 430 | 0 |
| Flip-90-257 | 149 | 5857 | 447 | 4521 | 6 | 10 | 500 | 0 |
| Flip-95-1L | 123 | 5452 | 396 | 4452 | 6 | 9 | 380 | 0 |
| Flip-2002-7L | 96 | 6282 | 355 | 4447 | 6 | 10 | 400 | 0 |
| Flip-2000-13L | 223 | 5130 | 412 | 3924 | 5 | 10 | 410 | 0 |
| Flip-95-34L | 43 | 5893 | 315 | 4320 | 7 | 8 | 360 | 0 |
| Flip-2003-25L | 229 | 7987 | 443 | 5059 | 8 | 10 | 490 | 0 |
| Flip-96-51LA | 202 | 5403 | 365 | 4358 | 6 | 9 | 430 | 0 |
| Flip-2003-9L | 180 | 5791 | 476 | 4257 | 5 | 9 | 430 | 0 |
| Flip-98-3 LA | 168 | 5560 | 375 | 4238 | 5 | 9 | 430 | 0 |
| Flip-98-3 LB | 237 | 5610 | 501 | 4428 | 6 | 10 | 460 | 0 |
| Flip-96-51LB | 213 | 5423 | 445 | 4083 | 5 | 9 | 350 | 0 |
| Flip-2000-7L | 132 | 6189 | 386 | 4538 | 6 | 11 | 360 | 0 |
| Flip-2000-25L | 370 | 8672 | 535 | 5617 | 10 | 13 | 560 | 0 |
| Flip-2002-56L | 373 | 6622 | 618 | 5118 | 8 | 12 | 600 | 0 |

average of 457 mg in exotic lines. The results showed wide variability for Mg among the breeding lines when compared to varieties, parental lines and exotic lines. Breeding line IPL328 had highest Mg content and IPL10666 the lowest.

The average P content was 5723 mg and ranged from 3907 to 7262 mg in breeding lines. In varieties and parental lines, average P was estimated 5250 mg with a range of 3513 to 6829 mg. It ranged from 3924 to 5617 mg with an average of 4617 mg in exotic lines. The highest P content was observed in breeding line IPL11702 and the lowest in parental line LL57.

### *Micro-nutrients*

The average Cu content was 10 mg with a range of 5 - 14 mg in breeding lines, 8 mg with a range of 4 - 11 in varieties and 632 mg with a range of 5- 10 mg in exotic lines. The Cu content was highest in breeding line IPL 8692 and lowest in parental line L 602.

The Mn content in the seeds of breeding lines varied from 10 to 24 mg with an average of 18 mg. Average content of Mn in varieties and parental lines was 13 mg with a range of 8 -17 mg. Among exotic lines, the range of Mn was from 9 to 13 mg with an average of 10 mg. Maximum amount of Mn was observed in breeding line IPL327 and minimum in parental line L57.

### *Ultra micro-nutrients*

A wide range of variability was observed for Se among the lentil genotypes. It ranged from 240 to 630 µg with an average of 380 µg in breeding lines. The Se content among varieties and parental lines ranged from 270 to 610 µg with an average 400 µg. In exotic lines, mean Se content was 440 µg and it ranged from 350 to 600 µg. The highest and lowest amounts of Se were observed in breeding lines IPL220 and IPL7199, respectively. The content of Cd among the germplasm studied ranged from 0 to 120 µg. The genotypes with high Cd content are IPL10829 and IPL-91155 and with low content are FLIP 2003-9L and IPL 326.

Based on mean performance, few genotypes (IPL-220, IPL-328, IPL-81, VL-1, IPL-10829, IPL-8692, ILL-6002,

PL-02) showed richness for more than two elements. Genotype IPL220 had maximum concentrations of Ca, Se, Zn, Mg, and Cd, making it a valuable germplasm for the nutritional point of view. The standard error of mean was high for Ca (15.5), Mg (24.0), Mn (0.5) and P (116.7) in breeding lines. It was high for Cu (0.3), K (232.0) and Zn (1.9) in exotic lines. However, cultivars and parental lines showed lowest standard error of mean for most of the micronutrients (Table 2).

### *Genetic relationship*

In this study, genetic relationship was studied for the analysed elments among the 96 genotypes. A dendogram constructed on the basis of dissimilarity matrix using UPGMA method of cluster analysis was presented in Figure 1. These genotypes were grouped in two main clusters (I and II). Cluster I had only 8 genotypes comprising a mixture of breeding lines, varieties, exotic lines and parental lines. This group of genotypes had highest genetic dissimilarity with rest of genotypes belonging to cluster II. However, cluster I had no further sub-clusters, while cluster II was comprised of two sub clusters (IIa and IIb). The subcluster IIa showed clearly two groups having exotic lines in one group and all varieties and parental lines along with 16 breeding lines and few exotic lines in another group. The subcluster IIb had only breeding lines and genotypes belonging to this sub-cluster showed higher genetic dissimilarity with the genotypes of sub cluster IIa. PCA analysis based on 10 micronutrients in the seeds of 96 genotypes revealed that first three most informative components individually accounted 63.44%, 35.43% and 0.73% of total variation, respectively, and collectively these three components explained 99.6% of total variability.

### *Correlation among minerals and with seed weight*

Significant correlation was observed among the nutrients analysed in this study (Table 3). It was significantly positive between Cu and Mn (0.76) followed by Ca and Mn (0.70), and K and Mg (0.66). The Mg showed significantly negative correlation with Cd (-0.40) and Mn (-0.36). A significant positive correlation of Cu was also observed with Ca (0.54), Cd (0.62) and P (0.63). Ca also had positive correlation with Cd (0.51) and P (0.62). Se had positive significant correlation with K (0.60), and Mg (0.43).

In order to study the relationship between seed size and level of micronutrients in the seeds, we studied the correlation of 100-seed weight with each micronutrient (Table 3). The results showed no correlation of seed weight with the concentration of all micronutrients except Mg and Mn. Correlation of 100-seed weight was significantly positive with Mn (0.20) and negative with Mg (-0.29). However, correlations between 100-seed weight and other minerals were weak (Table 3).

## DISCUSSION

Minerals malnutrition is a serious health problem that affect more than one-half of world population especially women and preschool children (Thavarajah *et al*. 2009, Zeng *et al*. 2010, Thavarajah *et al*. 2011). Seeds of leguminous crops especially pulses are generally rich in proteins and micronutrients. Therefore, their use as supplement with

**Table 2. Mean and range for eight minerals of lentil genotypes**

| Type of elements | Breeding lines | | | Cultivars / Parental lines | | | Exotic lines | | |
|---|---|---|---|---|---|---|---|---|---|
| | Range | Average | S.E. | Range | Average | S.E. | Range | Average | S.E. |
| Macro elements (mg/kg) | | | | | | | | | |
| Ca | 128- 495 | 344 | 15.5 | 74- 476 | 248 | 12.4 | 43 -373 | 202 | 20.7 |
| K | 4771- 8927 | 6062 | 137.1 | 5488 - 9634 | 7151 | 131.6 | 5130 - 8673 | 6232 | 232.0 |
| P | 3907 - 7262 | 5723 | 116.7 | 3513 - 6829 | 5250 | 117.6 | 3924 - 5617 | 4617 | 110.9 |
| Mg | 140- 830 | 344 | 24.0 | 342 - 699 | 538 | 15.7 | 315 - 618 | 457 | 21.5 |
| Micro elements (mg/kg)) | | | | | | | | | |
| Cu | 5.0- 14.1 | 10 | 0.3 | 5 -11 | 8 | 0.2 | 5 - 10 | 6 | 0.33 |
| Mn | 10- 24 | 18 | 0.5 | 8 -17 | 13 | 0.4 | 8 - 13 | 10 | 0.3 |
| Trace elements (µg/kg) | | | | | | | | | |
| Se | 240- 630 | 380 | 0.0 | 270-.610 | 400 | 1 | 350 - 600 | 440 | 0.0 |
| Cd | 0.0- 12.0 | 15.1 | 0.0 | 0.0- 5.0 | 0.25 | 0.0 | 0.0 | 0.0 | 0.0 |

**Table 3. Relationship of seed weight with different minerals in lentil genotype.**

| Minerals | Ca | P | K | Mg | Cu | Mn | Se | Cd |
|---|---|---|---|---|---|---|---|---|
| P | 0.62** | - | | | | | | |
| K | -0.13 | 0.13 | - | | | | | |
| Mg | -0.11 | -0.01 | 0.66** | - | | | | |
| Cu | 0.54** | 0.63** | 0.16 | -0.25** | - | | | |
| Mn | 0.70** | 0.70** | -0.14 | -0.36** | 0.76 | - | | |
| Se | -0.06 | 0.09 | 0.60** | 0.43** | 0.06 | -0.14 | - | |
| Cd | 0.51** | 0.50** | -0.26** | -0.49** | 0.62** | 0.66** | -0.28** | - |
| 100-SW | -0.01 | 0.04 | 0.01 | -0.29** | 0.11 | 0.20** | -0.08 | 0.16 |

Significant at P = 0.05

cereal grains in daily diets can play a significant role in reducing the prevalence of nutrient deficiency and malnutrition (Yadav *et al*. 2008, Ozer *et al*. 2010). Lentil has already been identified as one of the most important pulse crops that have great potential for biofortification (Thavarajah *et al*. 2011). The nutrient profile of lentils has been already analyzed earlier in a number of studies, which showed lentil grains as whole food to meet the body requirement of essential micronutrients and vitamins (Thavarajah *et al*. 2011, Gupta *et al*. 2013). However, earlier studies mainly exploited the variability for micronutrients in the breeding materials generated from ICARDA and no source of variability was identified in Indian germplasm (Thavarajah *et al*. 2009, 2011, Karakoy *et al*. 2012, Gupta *et al*. 2013). Hence, in the present study, diversity for micronutrients was analysed among the breeding lines, varieties developed in Indian conditions along with the parental varieties involved in the development of breeding lines, and exotic lines received from ICRADA. Our results showed a wide range of variation among the materials under investigation (Table 2). Among the macronutrients, the highest amount of Ca (up to 494 mg) was observed among breeding lines, followed by varieties and parental lines and exotic lines. However, the amount of Ca recorded in the present study was much lower than the earlier reported Ca content (850 mg) in Turkish germplasm (Karakoy *et al*. 2012). Similarly, Mg content (139.98 - 829.46 mg) was also lower in the present study compared to the Turkish land races and varieties (890 - 1260 mg with an average of 1060 mg). Therefore, the present study indicated that germplasm of Indian origin has lower concentration of Ca and Mg when compared to Turkish germplasm. The reasons for these differences for Ca and Mg between germplasm materials of two countries could be due to the level of soil fertility, soil type, seed characteristics, seed composition and climatic factors. Although, Mg level in Indian germplasm was low compared to entries of other countries (Karakoy *et al*. 2012). Genotypes with the highest level of Mg can fulfill about 58% of RDA. Further improvement can increase the availability of P (3907.0 – 7261.6 mg) and K (4771.43-9633.90 mg) compared to Turkish land races (2860-5330 mg for P and 6380-9500 mg for K). Malnutrition of micronutrients especially for Se is a significant problem among the women and preschool children in Asia and Africa. In the present study, we observed wide range of variation for Se among the Indian varieties and breeding lines when compared to exotic lines.

Cadmium (Cd), an ultra-micro nutrient, is toxic element to human health because its excess amount can cause adverse health effect. Particularly, kidney is the critical target organ where it can be accumulated for a relatively

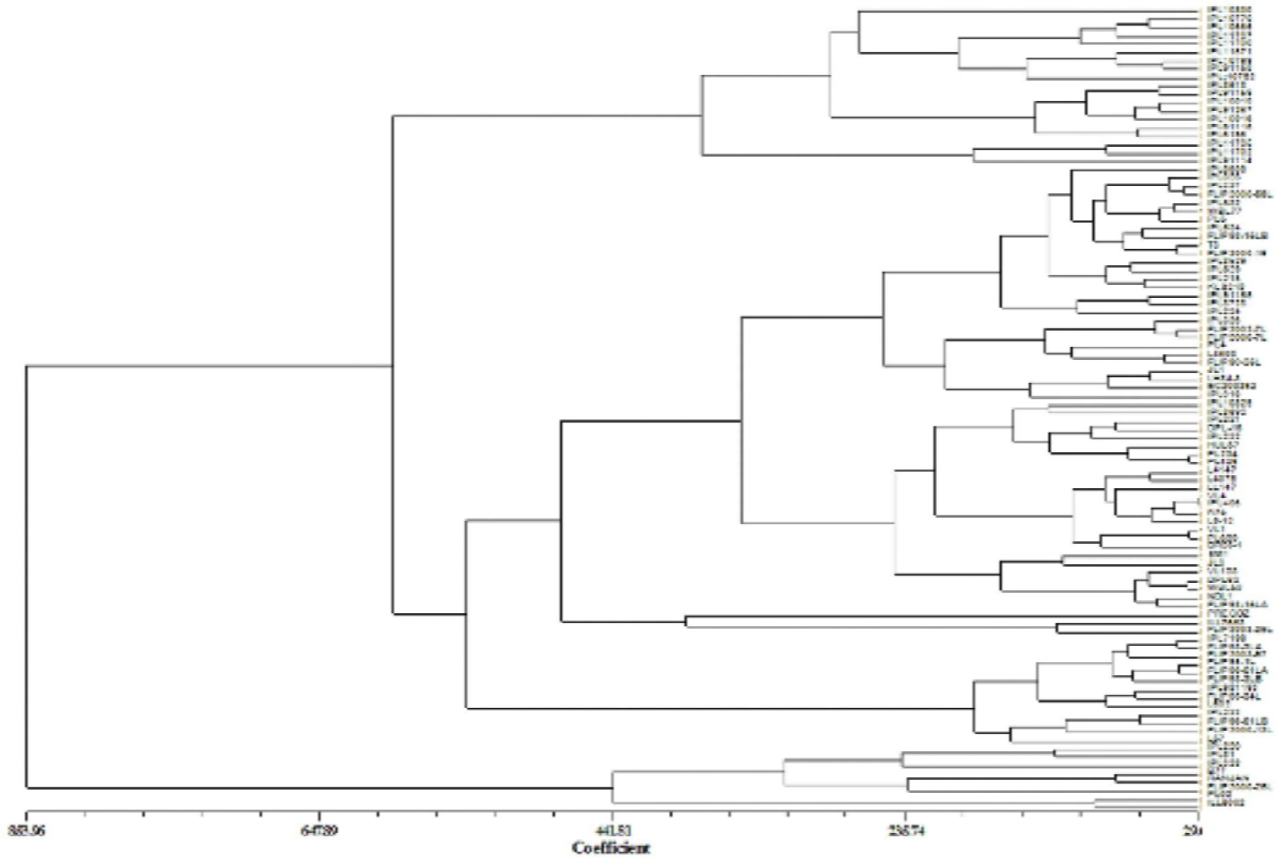


Figure 1. Dendogram showing relationships among the 96 genotypes on the basis of mineral content in lentil

long time from 20 to 30 years and causes subsequent damages and renal failure (Jarup *et al*. 2000). Its higher dose affects the respiratory system and has always been associated with bone disease. It is estimated that 98% of the ingested cadmium comes from terrestrial foods. It transfers to edible food from soil and also deposited out of the atmosphere on edible plant parts which establishes the vast majority of human cadmium intake (van Assche 1998). Therefore, low amount of this element in our foods is desirable. In the present study, impressive variation was observed among the present genotypes. Most of the genotypes are free from Cd in the seeds, except in breeding line, IPL10829. However, it is still very low than the toxic level; in fact Satarug *et al*. (2000) recommended permissible dietary intake of Cd is 30µg/day and the amount of Cd present in IPL10829 is 2.5 times lower than the permissible limit. Therefore, use of lentil is generally considered very safe to human health and variability observed in the present study for low Cd content.

These results suggest that the genotypic variation for the micro- and macro-nutrient levels in the present germplasm provides good opportunities for biofortification of lentil cultivars as well as provides opportunity for further studies related to the mechanisms of uptake and transport of mineral nutrients in lentils. Since, mineral characteristics of the crop plants are largely influenced by the genetic and environmental factors such as soil fertility, soil type, seed characteristics, seed composition and climatic factors, hence further studies are required to conduct experiments under different environmental conditions to validate the results.

The relationships among the micro- and macro-nutrients provide opportunity to combine two or more nutrients together in the same genotype. The positive correlation of several minerals with each other might be possibly due to common uptake pathways or transporters. Mn had the highly positive and significant correlation with Ca, Cd, Cu, and P. Seed weight is the most important trait associated with crop yield. In other crops, inverse relationship of micronutrient concentration and seed size has been reported (Garvin *et al*. 2006; Zhao *et al*. 2009; Gomez-Galera *et al*. 2010). Our results showed no significant relationship between mineral nutrient contents and hundred-seed weight. Similar results were also reported among the Turkish land races of lentil by Karakoy *et al*. (2012). Correlation between desirable and undesirable traits was observed due to linkage or pleiotropic effect (Hotz *et al*. 2004).

The considerable variation identified for macro- and micro-nutrient contents among lentil genotypes suggest scope for bio-fortifying cultivars for macro- and micro-nutrients. Identification of breeding line, IPL220 in the present study is currently under multi-location evaluation and can be recommended as a biofortified lentil which can be a boon in reducing deficiencies of micronutrients in developing countries like India. These materials could also pave the way for developing mapping populations for their use in identification of quantitative trait loci (QTL) associated with mineral uptake and transport in the near future.

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