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Genetic diversity among cultivated and wild lentils for iron, zinc, copper, calcium and magnesium concentrations

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Abstract

Information on the seed mineral concentration of cultivated and wild lentils is limited. The objective of this study was to determine the concentrations of iron (Fe), zinc (Zn), calcium (Ca), copper (Cu), and magnesium (Mg) in the seeds of 26 lentil genotypes representing 4 species and 3 subspecies of *Lens*. Plants were grown in a greenhouse using a completely randomized design with three replicates (n=78). Seed mineral concentrations were measured using acid digestion followed by inductively coupled plasma-optical emission spectroscopy. Concentrations of Fe, Zn, Ca, Cu, and Mg in seeds varied from 26-92, 17-51, 97-536, 3-12 and 272-892 mg kg^{-1}, respectively, among the *Lens culinaris* genotypes. Mineral concentrations for *L. lamottei* (Fe=64-80, Zn=26-40, Ca=311-434, Cu=2-6, Mg=754-839 mg kg^{-1}), *L. nigricans* (60-70, 33-39, 508-590, 3-4, 445-738 mg kg^{-1}) and *L. ervoides* (65, 37, 339, 6, 638 mg kg^{-1}) were within the range of *Lens culinaris* genotypes. No wild species was superior to cultivated ones for all micronutrients. A larger set of germplasm should be evaluated in future experiments to identify additional genetic variation in lentil for these micronutrients.

Keywords: *Lens* species, minerals, micronutrients, nutrition, germplasm characterization, phenotyping.

Introduction

Two billion people aroundthe world suffer from micronutrient malnutrition (IFAD/FAO/WFP, 2011). Micronutrient deficiency results from an inadequate intake of vitamins and minerals in people's diets. Different methods are available today to prevent micronutrient malnutrition, including food fortification, dietary supplementation, diet diversification, and biofortification. Biofortification, using traditional plant breeding practices combined with biotechnology, is a sustainable approach to the development of mineral-dense staple crops (Pfeiffer and McClafferty, 2007; Welch and Graham, 2004). Biofortification has been a success for several staple food crops and high protein maize (*Zea mays* L.) (QPM), β-carotene-rich sweet potato (*Ipomoea batatas*) and rice (*Oryza sativa*), and iron-rich commonbean (*Phaseolus vulgaris*) and pearlmillet (*Pennisetum glaucum*) cultivars aregrown in countries in Asia and Africa (Bouis et al., 2013). White and Broadley, (2009) reviewed different mineral biofortification research activities in various crops. They highlighted the potential of agronomic as well as the genetic biofortication to improve the availability of seven mineral nutrients in the human diet, namely, iron (Fe), zinc (Zn), copper (Cu), calcium (Ca), magnesium (Mg), iodine (I) and selenium (Se) (White and Broadley, 2009).The development of biofortified crop varieties, particularly nutrient rich food legumes, would have a positive impact in alleviating mineral malnutrition in Asian and African nations. Lentil (*Lens culinaris* Medik.) is a popular pulse crop, grown and consumed throughout the world. *Lens* is a small genus belonging to the Fabaceae family of the Viciae tribe. The genus contains one cultivated species (*L. culinaris* subsp. *culinaris*) with four subspecies (*L. culinaris* subsp. *culinaris*, *L.culinaris* subsp. *orientalis*, *L. culinaris* subsp. *odemensis* and *L. culinaris* subsp. *tomentosus)* and three wild species (*L. ervoides*, *L. nigricans*, and *L. lamottei)* (Ferguson et al., 2000). Lentil is a potential candidate for mineral biofortification as its nutritional profile is rich in Fe, Zn, and Se (Thavarajah et al., 2011; USDA National Nutrient Database, 2015). Identification of mineral dense lentil

genotypes is a priority for biofortification research. Karaköy et al., (2012) evaluated mineral concentration of a set of Turkish landraces and cultivated genotypes of lentil and reported considerable variability for Fe, Zn, Cu, calcium (Ca), and Mg concentrations. Alghamdi et al., (2014) evaluated 35 advanced ICARDA breeding lines in Saudi Arabia at one field location over two seasons and reported significant variation for Fe, Zn, Cu, Ca, Mg, phosphorus (P), potassium (K), and manganese (Mn) concentrations. However, there is limited information regarding the variation in mineral concentrations among the subspecies of *L. culinaris* and the wild relatives. If high mineral concentrations exist in the subspecies or wild relatives, interspecific hybridization could be used to introgress alleles associated with improved nutritional quality into cultivated lentil (Ladizinsky, 1985). The *Lens* subspecies and wild relatives are routinely utilized in breeding programs as sources of novel traits, such as disease resistance, not found in cultivated lentil (Fialaet al., 2009). In order to identify potential candidate donors, lentil and its wild relatives need to be evaluated to determine the variability for mineral concentrations. The objective of this study was to determine the seed mineral concentrations of 26 *Lens* genotypes grown under greenhouse conditions.

Results

Iron concentration in cultivated and wild lentils

Mean Fe concentration was 61 mg kg^{-1} across all 26 lentil genotypes tested (Table 2). Among the 20 *L. culinaris* genotypes, Fe concentration ranged from 26 (IG72830) to 92 mg kg^{-1} (CDC Red Rider) with a mean of 58 mg kg^{-1}. CDC Redberry and CDC Red Rider had significantly higher concentration of Fe compared to other tested genotypes. Fe concentration was significantly lower in the genotypes belonging to different *L.culinaris* subspecies (*L.culinaris* subsp. *culinaris*, *L.culinaris* subsp. *orientalis*, and *L. culinaris* subsp. *tomentosus*) than in improved cultivars or breeding lines (*L.culinaris*subsp.*culinaris*). *L.lamottei* genotype IG110810 had a significantly higher concentration (80 mg kg^{-1}) of Fe compared to other non-*culinaris* wild types. All the non-*culinaris* wild type genotypes differed significantly ($P < 0.05$) in terms of Fe concentration except IG110812 (*L. lamottei*) and IG72815 (*L. lervoides*). Percent recommended dietary allowance (RDA) of Fe for the genotypes evaluated ranged from 14-51% per serving.

Zinc concentration in cultivated and wild lentils

For the 26 lentil genotypes evaluated, the mean Zn concentration was 33 mg kg^{-1} (Table 2). Zn concentration ranged from 17 (IG72830) to 51 mg kg^{-1} (CDC Rosetown) among the 20 *L. culinaris* genotypes with a mean of 32 mg kg^{-1} (Table 2). Within *L. culinaris* subsp. *culinaris* genotypes CDC Rosetown (51 mg kg^{-1}) had significantly higher concentration of Zn compared to other genotypes. Among the other subspecies (*L. culinaris* subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, and *L. culinaris* subsp. *tomentosus*) IG72614 had significantly higher Zn concentration (43 mg kg^{-1}) compared to other genotypes. All the non-*culinaris* wild type genotypes differed significantly in terms of Zn concentration. Among the non-*culinaris* wild types IG110812 had significantly higher concentration of Zn (40 mg kg^{-1}). Each serving of lentil accounts for 21-64% of RDA of Zn (8 mg) (Otten et al., 2006).

Copper concentration in cultivated and wild lentils

The mean Cu concentration across all lentil genotypes was 6 mg kg^{-1} (Table 3). Cu concentration among the 20 *L. culinaris* genotypes ranged from 2.6 (IG72688) to 12.0 mg kg^{-1} (CDC Rosetown) with a mean of 6 mg kg^{-1}. Within *L. culinaris* subsp. *culinaris* genotypes CDC Rosetown (12 mg kg^{-1}) had significantly higher concentration of Cu compared to other genotypes. Among the other subspecies (*L. culinaris* subsp. *culinaris*, *L.culinaris* subsp. *orientalis*, and *L. culinaris* subsp. *tomentosus*) genotypes IG72614and IG72616, belonging to the *tomentosus* subspecies, had equal and significantly higher concentrations of Cu (12 mg kg^{-1}) than other genotypes. Among the non-*culinaris* wild type genotypes IG110812 and IG72815 both had seed Cu concentrations of 6 mg kg^{-1}, which was significantly greater than the other wild, genotypes. A serving of the lentil genotypes tested can provide 22-133% of the Cu RDA (0.9 mg) (Otten et al., 2006).

Calcium concentration in cultivated and wild lentils

Among all evaluated lentil genotypes, the mean Ca concentration was 339 mg kg^{-1} (Table 3). Mean Ca concentration among the 20 *L. culinaris* genotypes was 323 mg kg^{-1}, with the lowest concentration in Eston (97 mg kg^{-1}) and the highest in Pennell (536 mg kg^{-1}). Within *L. culinaris* subsp. *culinaris* genotypes Pennell (536 mg kg^{-1}) had significantly higher concentration of Ca compared to other genotypes. Among the other subspecies (*tomentosus, orientalis, odemensis*) IG71594 (*orientalis* subspecies) had significantly higher concentration of Ca (534 mg kg^{-1}) than other genotypes. Among the non-*culinaris* wild type genotypes belonging to *L. nigricans*, IG72548 and IG72549, had significantly higher concentration of Ca (508 mg $kg^{-1}$and 590 mg kg^{-1}, respectively). Percent RDA (1000 mg) of Ca (Otten et al., 2006) ranged from 1-6% per serving.

Magnesium concentration in cultivated and wild lentils

The mean Mg concentration among all tested lentil genotypes was 638 mg kg^{-1} (Table 3). Magnesium concentration ranged from 272 (CDC Redberry) to 892 mg kg^{-1} (Pennell) among the 20 *L. culinaris* genotypes, with a mean of 616 mg kg^{-1}. Within *L. culinaris* subsp. *culinaris* genotypes, the concentration of Mg in Penell seeds (892 mg kg^{-1}) was significantly greater than other genotypes. Among the other subspecies (*tomentosus, orientalis, odemensis*) genotypes belonging to *tomentosus* subspecies IG72614 and IG72616 had significantly higher concentration of Mg (807 mg kg^{-1} and 865 mg kg^{-1}, respectively) than other genotypes. Among the non-*culinaris* wild types, *L. lamottei*, IG110813 had significantly higher concentration of Mg (839 mg kg^{-1}) compared to the other species. Percent RDA (310 mg) of Mg (Otten et al., 2006) ranged from 9-29% per serving.

Discussion

Lentil is a cool season food legume with a narrow genetic base, therefore genetic variability for individual traits is generally low (Eujayl et al., 1998). This low genetic variability is further exacerbated in breeding programs by the extensive use of superior genotypes with common ancestors (Kumar et al., 2004). Interspecific hybridization, either directly between cross compatible species or indirectly between cross-incompatible species using a bridge species, can be used in the genetic improvement of lentils (Kumar et al., 2011). This technique is utilized when a desirable

Table 1. Brief description of plant materials used.

Species	Genotype/accession	Remark	Origin
L. culinaris subsp. *culinaris*	CDC Redberry	Small red cultivated type	University of Saskatchewan, Canada
L. culinaris subsp.*culinaris*	CDC Rosetown	extra small red cultivated type	University of Saskatchewan, Canada
L. culinaris subsp. *culinaris*	CDC Rouleau	small red cultivated type	University of Saskatchewan, Canada
L. culinaris subsp. *culinaris*	CDC LeMay	small french green cultivated type	University of Saskatchewan, Canada
L. culinaris subsp. *culinaris*	CDC Red Rider	medium red cultivated type	University of Saskatchewan, Canada
L. culinaris subsp. *culinaris*	CDC Greenland	large green cultivated type	University of Saskatchewan, Canada
L. culinaris subsp. *culinaris*	Barimasur-2	small red cultivated type	BARI, Bangladesh
L. culinaris subsp. *culinaris*	Barimasur-3	small red cultivated type	BARI, Bangladesh
L. culinaris subsp. *culinaris*	Barimasur-4	small red cultivated type	BARI, Bangladesh
L. culinaris subsp. *culinaris*	Riveland	large green cultivated type	WSU, USA
L. culinaris subsp.*culinaris*	Eston	small green cultivated type	University of Saskatchewan, Canada
L. culinaris subsp. *culinaris*	Pennell	large green cultivated type	WSU, USA
L. culinaris subsp. *orientalis*	IG72594	small seeded wild type	WSU, USA
L. culinaris subsp. *orientalis*	IG72603	small seeded wild type	WSU, USA
L. culinaris subsp. *orientalis*	IG72618	small seeded wild type	WSU, USA
L. culinaris subsp. orientalis	IG72896	small seeded wild type	WSU, USA
L. culinaris subsp. *tomentosus*	IG72830	small seeded wild type	WSU, USA
L. culinaris subsp. *tomentosus*	IG72614	small seeded wild type	WSU, USA
L. culinaris subsp. *tomentosus*	IG72616	small seeded wild type	WSU, USA
L. culinaris subsp. *odemensiss*	IG72688	small seeded wild type	WSU, USA
L. ervoides	IG72815	small seeded wild type	WSU, USA
L. lamottei	IG110810	small seeded wild type	WSU, USA
L. lamottei	IG110812	small seeded wild type	WSU, USA
L. lamottei	IG110813	small seeded wild type	WSU, USA
L. nigricans	IG72548	small seeded wild type	WSU, USA
L. nigricans	IG72549	small seeded wild type	WSU, USA

Table 2. Mean iron (Fe) and zinc (Zn) concentration and Percent Recommended Daily Allowance (%RDA) of 26 lentil genotypes.

Genotype (species)	Species	Fe Concentration ($mg \cdot kg^{-1}$)	%RDA	Zn concentration ($mg \cdot kg^{-1}$)	%RDA
CDC Redberry	*L. culinaris* subsp. *culinaris*	91 a	51	37 c,d	46
CDC Rosetown	*L. culinaris* subsp. *culinaris*	82 a,b,c	46	51 a	64
CDC Rouleau	*L. culinaris* subsp. *culinaris*	71 a,b,c,d,e	39	46 a,b	58
CDC LeMay	*L. culinaris* subsp. *culinaris*	68 b,c,d,e,f	38	31 d,e,f,g,h	39
CDC Red Rider	*L. culinaris* subsp. *culinaris*	92 a	51	45 a,b	56
CDC Greenland	*L. culinaris* subsp. *culinaris*	64 c,d,e, f,g,	36	43 b,c	54
Barimasur-2	*L. culinaris* subsp. *culinaris*	52 e,f,g,h,i,j	29	33 d,e,f,g	41
Barimasur-3	*L. culinaris* subsp. *culinaris*	46 f,g,i,j,k,h	26	31 d,e,f,g,h	39
Barimasur-4	*L. culinaris* subsp. *culinaris*	36 i,j,k	20	25 h,i,j	31
Riveland	*L. culinaris* subsp. *culinaris*	62 c,d,e,f,g,h	34	30 e,f,g,h,i	38
Eston	*L. culinaris* subsp. *culinaris*	39 h,i,j,k	22	17 k	21
Pennell	*L. culinaris* subsp. *culinaris*	87 a,b	48	36 c,d,e	45
IG72594	*L. culinaris* subsp. *orientalis*	54 e,f,g,h,i	30	33 d,e,f	41
IG72603	*L. culinaris* subsp. *orientalis*	34 j,k	19	18 k	23
IG72830	*L. culinaris* subsp. *tomentosus*	26 k	14	17 k	21
IG72688	*L. culinaris* subsp. *odemensiss*	36 i,j,k	20	22 j,k	28
IG72614	*L. culinaris* subsp. *tomentosus*	58 d,e,f,g,h	32	43 b,c	54
IG72616	*L. culinaris* subsp. *tomentosus*	61 c,d,e,f,g,h	34	36 c,d,e	45
IG72618	*L. culinaris* subsp. *orientalis*	43 g,h,i,j,k	24	24 i.j.k	30
IG72896	*L. culinaris* subsp. *orientalis*	67 b,c,d,e,f	37	26 g,h,i,j	33
IG110810	*L. lamottei*	80 a,b,c,d	44	26 f,g,h,i,j	33
IG110812	*L. lamottei*	64 b,c,d,e,f,g	36	40 b,c	50
IG110813	*L. lamottei*	70 a,b,c,d,e	39	31 d,e,f,g,h	39
IG72548	*L. nigricans*	60 c,d,e,f,g	33	28 f,g,h,i,j	35
IG72549	*L. nigricans*	71 a,b,c,d,e	39	33 d,e,f	41
IG72815	*L. ervoides*	65 b,c,d,e,f,g	36	37 c,d,e	46
Mean		61	39	33	41
SE		2.4		1.1	
SD		21.0		10.1	
Range		26-92	14-51	17-51	21-64

Means within a column followed by different letters are significantly different at $p < 0.05$ (n = 78), Percent RDA values were calculated with daily requirement of 18 mg of Fe and 8 mg of Zn (females, age 19+ years) (Otten et al., 2006). Percent RDAs were calculated based on the serving size of 100 g of cooked lentil.

Table 3. Mean copper (Cu), calcium (Ca) and magnesium (Mg) concentration and Percent Recommended Dietary Allowance (%RDA) of 26 lentil genotypes.

Genotype	Species	Cu ($mg \cdot kg^{-1}$)	%RDA	Ca ($mg \cdot kg^{-1}$)	%AI	Mg ($mg \cdot kg^{-1}$)	%RDA
CDC Redberry	*L. culinaris* subsp. *culinaris*	10 b	111	323 b,c,d,e	3	272 l	9
CDC Rosetown	*L. culinaris* subsp. *culinaris*	12.0 a	133	257 e,f	3	423 j,k,l	14
CDC Rouleau	*L. culinaris* subsp. *culinaris*	9.0 b,c,d	100	318 b,c,d,e	3	556 h,i,j	18
CDC LeMay	*L. culinaris* subsp. *culinaris*	7 d,e,f	78	409 b	4	842 a,b,c	27
CDC Red Rider	*L. culinaris* subsp. *culinaris*	10 b	111	361 b,c	4	656 d,e,f,g,h,i	21
CDC Greenland	*L. culinaris* subsp. *culinaris*	9.0 b,c,d	100	205 f,g	2	610 f,g,h,i	20
Barimasur-2	*L. culinaris* subsp. *culinaris*	4.0 g,h,i,j	44	337 b,c,d,e	3	697 c,d,e,f,g,h,i	22
Barimasur-3	*L. culinaris* subsp. *culinaris*	4.0i,j,k	44	314 b,c,d,e	3	707 b,c,d,e,f,g,h	23
Barimasur-4	*L. culinaris* subsp. *culinaris*	6.0 f,g,h,i	67	344 b,c,d,e	3	662 d,e,f,g,h,i	21
Riveland	*L. culinaris* subsp. *culinaris*	8.0 b,c,d	89	355 b,c,d	4	537 1i,j	17
Eston	*L. culinaris* subsp. *culinaris*	4.0 g,h,i,j	44	97 h	1	331 1k,l	11
Pennell	*L. culinaris* subsp. *culinaris*	7.0 d,e,f	78	536 a	5	892 a	29
IG72594	*L. culinaris* subsp. *orientalis*	3.0 j,k	33	534 a	5	584 g,h,i,j	19
IG72603	*L. culinaris* subsp. *orientalis*	3.0 j,k	33	313 b,c,d,e	3	581 g,h,i,j	19
IG72830	*L. culinaris* subsp. *tomentosus*	4.0 j,k	44	112 g,h	1	375 1k,l	12
IG72688	*L. culinaris* subsp. *odemensiss*	3.0 j,k	33	352 b,c,d,e	4	643 e,f,g,h,i	21
IG72614	*L. culinaris* subsp. *tomentosus*	6.0 e,f	67	304 c,d,e	3	807 a,b,c,d	26
IG72616	*L. culinaris* subsp. *tomentosus*	6.0 e,f,g	67	341 b,c,d,e	3	865 a,b	28
IG72618	*L. culinaris* subsp. *orientalis*	4.0 h,i,j	44	264 d,e,f	3	540 i,j	17
IG72896	*L. culinaris* subsp. *orientalis*	3.0 j,k	33	368 b,c	4	732 a,b,c,d,e,f,g	24
IG110810	*L. lamottei*	2.0 k	22	357 b,c,d	4	754 a,b,c,d,e,f	24
IG110812	*L. lamottei*	6.0e,f,g,h	67	311 c,d,e	3	789 a,b,c,d,e	25
IG110813	*L. lamottei*	5.0 e,f	56	434 c,d,e	4	839 a,b,c,d	27
IG72548	*L. nigricans*	4.0 i,j,k	44	508 a	5	738 a,b,c,d,e,f,g	24
IG72549	*L. nigricans*	3.0 j,k	33	590 a	6	445 j,k	14
IG72815	*L. ervoides*	6.0 e,f	67	292 c,d,e,f	3	756 a,b,c,d,e,f	24
Mean		6	67	339	3	638	21
SE		0.3		13.9		21	
SD		2.7		122		185.45	
Range		2-12	22-133	97-590	1-6	272-892	9-29

Means within a column followed by different letters are significantly different at $p < 0.05$ (n = 78), Percent RDA were calculated with daily requirement of 900 µg for Cu , 1000 mg for Ca, and 310 mg for Mg (females age 19+) (Otten et al., 2006). Percent RDAs were calculated based on the serving size of 100 g of cooked lentil. For Ca, Adequate Intake (AI) values are available, not the RDA (Otten et al., 2006).

characteristic is present in another related or crossable species (Tullu et al., 2011). Biofortification for mineral traits is a priority research area in food legumes (including lentil) (Grusak, 2009; Thavarajah et al., 2009, 2011; Johnson et al., 2013; Iqbal et al., 2006; Hunt 2003). Identifying and utilizing donors with alleles that result in high seed concentrations of mineral nutrients is required for the successful development of biofortified cultivars. This study has documented that the *Lens* wild species and subspecies that were evaluated are usually an inferior source for seed mineral concentration compared to cultivated types. However, generation of transgressive segregants, due to the accumulation of additive genes, from crosses between or within different species or subspecies is not ruled out..The present study may provide suitable genotypes to make crosses to develop breeding populations. The selection of genotypes based on concentration of micronutrients (Fe, Zn, Cu, Ca, Mg) (Table 2 and Table 3) could be utilized to develop intraspecific or interspecific recombinant inbred line populations to identify and map QTL associated with seed mineral concentrations.

While making interspecific crosses the cross-compatibility has to be taken into consideration. The primary gene pool members are easily cross-compatible (*Lens culinaris* subsp. *culinaris*, *Lens culinaris* subsp. *odemensis*, *Lens culinaris* subsp. *orientalis*, *Lens culinaris* subsp. t*omentosus*) (Ferguson et al., 2000). Crossing between primary and secondary/tertiary gene pools members (*L. ervoides*, *L. lamottei*, *L. nigricans*) may require the use of tissue culture based techniques, such as embryo rescue, or the use of bridge species (Ferguson et al., 2000).

In the present study, significant variation in mineral (Fe, Zn, Cu, Ca, Mg) concentration was observed. Similarly, Karaköy et al., (2012) studied the mineral status of Turkish lentil landraces and cultivars in lentil. They reported Fe concentration from 49.40 to 81.39 mg kg^{-1}. The concentrations reported for Zn, Cu, Ca, and Mg were 46.90-73.10 mg kg^{-1}, 9.10-16.92 mg kg^{-1}, 480-1280mg kg^{-1} and 850-1260mg kg^{-1}, respectively. Similarly, Solanki et al., (1999) evaluated improved lentil cultivars in India. They reported Fe concentration from 80 to 92 mg kg^{-1} and Ca concentration from 1150 to 1650 mg kg^{-1}. The Ca concentrations Solanki et al., (1999) reported were higher than those from the present study, possibly due to thegenotypic differences in Indian lentil cultivars and or different field soilconditions. Thavarajah et al., (2009) reported Fe and Zn concentrations of 73-90 and 44-54 mg kg^{-1}, respectively, in a set of lentil cultivars by analyzing seeds harvested from regional varietal trials of 19 genotypes from 9 locations in Saskatchewan, Canada over 2 years. The present study demonstrated more variation for these two micronutrients, which is attributed to the inclusion of related species in addition to *L. culinaris*. Zia-Ul-Haq et al., (2011) evaluated four improved lentil cultivars from Pakistan for different micronutrients and reported that Fe, Zn, Cu, and Ca concentrationranged from 27-32, 39-44, 89-99, and 1180-1210 mg kg^{-1}, respectively. In a study comparing micronutrient concentrations in different legumes, Iqbal et al., (2006) found that Fe, Zn, Cu, Ca and Mg concentration was 31, 44, 99, 1200, and 45 mg kg^{-1}, respectively, in lentil. In these studies, the reported Fe concentration was very low and the Ca and Cu concentrations were very high compared to the data presented here. The differences may be due to the fact that seeds were not from the single uniform trials, as they did not report the methods of growing the plants. In addition, differences might be due to their use of the less sensitive or accurate flame/graphite atomic absorption spectrophotometer (AAS), compared to the more sensitive ICP-OES, to determine micronutrients. AAS is more vulnerable to physical and chemical interferences than is ICP-OES. Alghamdi et al., (2014) studied 35 advanced breeding lines of cultivated lentilin Saudi Arabia from a field trial over two years and reported concentrations for Mg (1261-1573 mg kg^{-1}), Ca (64.9-84 mg kg^{-1}), Fe (65.7- 85.7 mg kg^{-1}), Zn (26.3 - 45.1 mg kg^{-1}), and Cu (8.6 -13.7 mg kg^{-1}). This corresponds closely to the concentrations of Fe, Zn, and Cu but not for Mg and Ca concentration reported in the present study.

The Food and Nutrition Board of the Institute of Medicine, The National Academies, USA established percent recommended dietary allowance (RDA) for the minerals (Otten et al., 2006). The RDA is the average recommended daily level of intake of a particular nutrient that is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy people (Otten et al., 2006). The values vary by age and gender and in this study, the RDA used was for females, 19 to 50 years old.This category was chosen as the daily intake requirements for most minerals were higher for adult females than adult males and children. Percent RDA values were calculated based on a 100g serving size of cooked lentils for each of the minerals (Otten et al., 2006). A considerable proportion (for Fe 14-51%, for Zn 21-64%, for Cu 22-133%, Mg 9-29%) of RDA for minerals would be obtained from consuming 100 g of cooked lentils (Table 2 and 3). This is similar to data reported in previous studies (Thavarajah et al., 2009, 2011). Percent RDA of Ca was only 1-6% for the genotypes we evaluated, indicating that lentil as a not good source Ca. Developing lentil varieties with high concentrations of Fe and Zn would be especially beneficial for Asian and African countries where 40-45% of school-age children are Fe- and Zn-deficient (de Benoist et al., 2008).

Materials and Methods

Chemicals

Chemical reagents and standards used for mineral digestion and analytical determinations were purchased from Alfa Aesar, VWR International and Sigma–Aldrich Co. (St. Louis, MO, USA) and used without further purification. Water (distilled and deionized; ddH_2O) was purified by a Milli-Q Water System (Millipore, Milford, MA, USA) to a resistance of 18.2 MΩ cm or greater.

Plant materials

The experimental genotypes included 12 *L. culinaris* subsp. *culinaris*, 4 *L. culinaris* subsp. *orientalis*, 3 *L. culinaris* subsp. *tomentosus*, 1 *L. culinaris* subsp. *odemensis*, 1 *L. ervoides*, 3 *L. lamottei* and 2 *L. nigricans* genotypes (Table 1). This set of genotypes was selected as it represents different market classes of cultivated lentil as well as the subspecies of *L. culinaris* and the wild relatives. The seeds were obtained from the USDA-ARS Grain Legume Genetics and Physiology Research Unit, WSU, Pullman, Washington, USA and maintained as single plant selections in the former Pulse Quality Laboratory, NDSU, Fargo, ND, USA.

Greenhouse experiment

Ten surface sterilized seeds from each lentil genotype were placed in sterile petri dishes with absorbent filter paper saturated with Millipore filtered water. The petri dishes were placed in the dark at room temperature (22°C). Every second day, the absorbent paper was saturated with 2-3 mL of Millipore water. Plastic pots (15.25cm) were filled with approximately 300 g of a peat-perlite-vermiculite mixture (Sunshine Grow Mix Number 1, Sun Gro Horticulture Canada Inc., ON, Canada) and saturated with deionized water. The pots were allowed to drain overnight, and then the weight of each pot recorded. At seeding, three germinated seeds of each lentil genotype were sown in pots at 70% field capacity. A total of 78 pots were seeded: three replicates of the 26 genotypes with randomization among the pots following a completely randomized design. Greenhouse conditions were as follows: day/night temperatures of 22 °C/ 16 °C; photosynthetically active radiation levels of 300 µmol $m^{-2}{\cdot}s^{-1}$ using a 16 h photoperiod beginning at 0600 local time, and 50-60% relative humidity. Pots were watered to approximately 70% of free draining moisture concentration every day. Every two weeks, 250 mL of nutrient solution was added to each pot. Nutrient concentrations of the all-purpose Plants-Prod 20-20-20 Classic fertilizer solution (Plant Products Co. Ltd., Brampton, ON, Canada) were 20% total N, 20% total P, 20% soluble K, 0.02% B, 0.05% chelated Cu, 0.1% chelated Fe, 0.05% Mo, 0.05% Zn, and

1% EDTA. Plants were thinned to two per pot after one week. Plants were harvested at physiological maturity and threshed individually. Seeds were ground using a stainless steel coffee grinder to obtain fine quality flour.

Mineral concentration

Mineral (Fe, Zn, Cu, Ca, Mg) concentrations in lentil seeds were determined using a previously described modified HNO_3-H_2O_2 method (Alcok et al., 1987; Thavarajah et al., 2009). Finely ground seed samples (500 mg) were placed in individual digestion tubes. Six mL of concentrated (70%) nitric acid (HNO_3) was added to each digestion tube. The digestion tubes were placed in a 90 ℃ digestion block for one hour, and they were shaken at 15 and 45 minutes. Three mL of 30% w/w hydrogen peroxide (H_2O_2) was then added to each tube. The tubes were kept for 15 m at 90 °C. Finally, 3 mL of 6 M hydrochloric acid (HCl) was added to each digestion tube, and the tubes were kept in the digestion block for 5 minutes. Upon complete digestion (the time required for complete digestion was determined in earlier laboratory experiments, the complete digestion is indicated by the discontinuation of brown smoke coming from the digestion tubes), the tubes were removed from the digestion block, the volume was adjusted to 10 mL, and then filtered (Whatman No. 1 filter papers) using a vacuum system (Gardener Denver Thomas Inc., Welch Vacuum Technologies, LA, USA). Mineral concentrations of the filtrates were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES); ICP-6500 Duo, Thermo Fisher Scientific, Pittsburg, PA, USA). Measurements of total minerals were validated using National Institute of Standards and Technology (NIST) standard reference material (SRM) 1576a (wheat flour; [Fe]=14.11±0.13 mg kg^{-1}, [Zn]=11.61±0.26 mg kg^{-1}, [Ca]=191.4±3.3 mg kg^{-1}, [Mg]=398±12 mg kg^{-1}, [Cu]=2.03±0.14 mg kg^{-1}). Calibration curves for Fe, Zn, and Cu concentration were made using serial dilutions from 0.5 to 50.0 mg L^{-1}. The detection limit was 5 µg L^{-1}. Calibration curves for Ca and Mg concentration were made using serial dilutions from 10 to 500 mg L^{-1}.

Statistical analysis

The experimental design was a complete randomized design (CRD) with three replicates of 26 *Lens* genotypes (n=78). Analysis of variance was performed using the General Linear Model (PROC GLM) of SAS version 9.3 (SAS Institute, 2009). Means were separated using Fisher's protected least significant difference (LSD) at $P < 0.05$.

Conclusion

Lentils are an integral part of peoples' diets in many countries in Asia, including Bangladesh, Nepal, India, and Pakistan. People living in these areas are affected with mineral deficiencies, particularly iron deficiency anemia. Biofortification of minerals in lentil will have a positive impact on maternal and child health in these mineral deficiency affected areas. This study reports on the mineral status of different *Lens* species for Fe, Zn, Cu, Ca and Mg. This information could be for breeding programs to help direct choices of parents for intra- or interspecific hybridization. While this study is not exhaustive, it may serve as a caution for potential linkage drag on seed mineral nutrient concentration when introgressing a desired trait, e.g. disease resistance, from a *Lens* subspecies or wild relative into current cultivars. Utilization of different genotypes with very high and very low mineral concentrations may be used to develop mapping populations used to identify QTL associated with these micronutrients in lentil. Genomic approaches (Kaur et al., 2011; Verma et al., 2013; Sharpe et al., 2013; Wong et al., 2015) could be used to map or tag genes involved in seed mineral concentration in lentil and for precise introgression of novel traits from the *Lens* species and wild relatives.

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