JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. **2009,** *57,* 5413–5419 **5413**
DOI:10.1021/jf900786e

Lentils (*Lens culinaris* Medikus Subspecies *culinaris*): A Whole Food for Increased Iron and Zinc Intake

Dil Thavarajah,[†] Pushparajah Thavarajah,[†] Ashutosh Sarker,[‡] and Albert Vandenberg*,[†]

[†]Crop Development Centre, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8, Canada, and [‡]International Center for Agricultural Research in the Dry Areas (ICARDA), Post Office Box 5466, Aleppo, Syria

Micronutrient malnutrition, the hidden hunger, affects more than 40% of the world's population, and a majority of them are in South and South East Asia and Africa. This study was carried out to determine the potential for iron (Fe) and zinc (Zn) biofortification of lentils (*Lens culinaris* Medikus subsp. *culinaris*) to improve human nutrition. Lentils are a common and quick-cooking nutritious staple pulse in many developing countries. We analyzed the total Fe and Zn concentrations of 19 lentil genotypes grown at eight locations for 2 years in Saskatchewan, Canada. It was observed that some genetic variation exists for Fe and Zn concentrations among the lentil lines tested. The total Fe and Zn concentrations ranged from 73 to 90 mg of Fe kg^{-1} and from 44 to 54 mg of Zn kg^{-1}. The calculated percentages of the recommended daily allowance (RDA) for Fe and Zn were within the RDA ranges from a 100 g serving of dry lentils. Broad-sense heritability estimates for Fe and Zn concentrations in lentil seed were 64 and 68%, respectively. It was concluded that lentils have great potential as a whole food source of Fe and Zn for people affected by these nutrient deficiencies. This is the first report on the genetic basis for Fe and Zn micronutrient content in lentils. These results provide some understanding of the genetic basis of Fe and Zn concentrations and will allow for the development of potential strategies for genetic biofortification.

KEYWORDS: Iron; zinc; lentils; biofortification

1. INTRODUCTION

Micronutrient malnutrition affects more than half of the world's population, with most being women and preschool children in Asia and Africa. A one-third reduction in micronutrient iron (Fe) and zinc (Zn) deficiencies by the start of the third millennium was a major goal for the world's nutrition and public health communities (*1*). Traditional interventions such as dietary supplementation, food fortification, and dietary diversification are the major methods for reducing micronutrient malnutrition. Because of the lack of social and economic infrastructures, none of these efforts has been successful (*2*). For these reasons, there is an urgent need to develop long-term and sustainable solutions for reducing the micronutrient malnutrition in developing countries. A complementary solution to malnutrition termed "biofortification" has been proposed by world-leading nutritionists (*1*−*4*).

Iron, one of the most abundant metals on earth, is essential to human physiology. It is an integral part of many proteins and enzymes and is an essential component of proteins involved in oxygen transport, regulation of cell growth, and differentiation. Iron deficiency limits oxygen delivery to cells, leading to fatigue, poor work performance, decreased immunity, and death (*5*). A recommended dietary allowance (RDA) of 8 and 18 mg of Fe day^{-1} has been recommended for the average 19−50 year old males and females, respectively.

Zinc, another important trace element, exhibits antioxidant properties and is necessary for DNA replication, protein synthesis, oxidative stress reduction, and protection against brain tumors (*6*, *7*). Zinc supports normal growth and development during pregnancy for fetuses and during adolescence for children. It is also important for proper sense of taste in food and smell (*8*, *9*). A recommended daily intake of Zn is essential to maintain a constant concentration of Zn in humans because the human body has no specialized Zn storage system (*10*). For recommended Zn, RDAs of 11 mg of Zn day^{-1} are suggested for males and RDAs of 8 mg of Zn day^{-1} are suggested for females above 19 years of age.

The required amounts of Fe and Zn are generally met in the developed countries, yet large numbers of people in the developing world have daily intakes below the RDA standards. Approximately 40−45% of school-age children in developing countries are Fe- and Zn-deficient (*11*). The vast majority of the population in developing nations relies on a few staple starchy foods (rice, maize, wheat, and cassava) that are not good sources for meeting daily Fe and Zn requirements. For example, polished rice contains less than 2 mg kg^{-1} of Fe and 12 mg kg^{-1} of Zn. Although rice can provide more than 80% of daily

*To whom correspondence should be addressed. Telephone: +1 (306) 966-8786. Fax: +1 (306) 966-5015. E-mail: bert.vandenberg@usask.ca.

 Published on Web 05/22/2009

energy intake, it is an extremely deficient source of Fe and Zn. Improving the micronutrient content in pulse crops, such as lentil, pea, chickpea and common bean, may become a sustainable strategy to combat Fe and Zn deficiencies in the human population (*3*).

Many international research groups have been investigating the genetic potential of increasing bioavailable Fe and Zn in staple food crops (*12*). Studies have demonstrated that there is significant genetic variation for Fe and Zn uptake in common bean (*2*) and lentil (*13*). Lentils are quick-cooking and require less processing than soybean and cereals. Lentils are rich in protein (20−36%) and are an excellent source of a large range of micronutrients (*14*, *15*). Annual world lentil production is approximately 4 megatons, of which an estimated 25% is grown in Saskatchewan, Canada (*16*), supplying lentils of diverse market classes to more than 100 countries. Concentrated regions of consumption include Europe, the Middle East, Africa, and most notably South Asia. On average, global pulse consumption is in decline, but lentil consumption is increasing faster than human population growth. Therefore, lentil is much more important than other crops as a vehicle for biofortification. One of the current goals of the Crop Development Centre (CDC) at the University of Saskatchewan, Canada, is to investigate the genetic potential for biofortification of lentils for key micronutrients (Se, Fe, Zn, and others) to improve global human health. The objectives of this study were (1) to determine Fe and Zn concentrations in seeds of elite lentil genotypes and cultivars developed at the University of Saskatchewan, (2) to determine the environmental variation in Fe and Zn concentrations, and (3) to access the genetic potential for using lentils in biofortification strategies to develop Fe- and Zn-dense lentils as a potential food source that could provide nutritional security to lentil consumers around the world.

2. MATERIALS AND METHODS

2.1. Materials. Standards and chemicals used for acid digestion to extract Fe and Zn were purchased from Alfa Aesar, A Johnson Matthey Company (Ward Hill, MA) and Sigma-Aldrich Co. (Canada). High-purity chemicals for atomic absorption spectroscopy (AAS) analysis were purchased from Alfa Aesar, A Johnson Matthey Company (Ward Hill, MA).

2.2. Soil Samples and Analysis. Locations of the field experiment sites in Saskatchewan and sample protocol were previously described (*17*). Locations of the study sites and soil zones in Saskatchewan included Saskatoon (moist dark brown), Kyle (brown), Swift Current (brown), Wilkie (dark brown), Melfort (black), Hodgeville (brown), Rosthern (thin black), and Rouleau (moist dark brown). These locations cover the major lentil growing areas in Saskatchewan. Four soil cores were collected from the 0−30 cm soil layer at each site. The samples were air-dried (≤40 °C), passed through a 2 mm sieve, homogenized into one composite sample, and stored in plastic vials at −20 °C until analysis. The soil samples were taken in October of 2005 and 2006, approximately 1 month after the lentil plots were harvested.

Approximately 1 g of soil underwent primary organic digestion in 3 mL of HNO_3 (70%) at 90 °C, followed by 1 mL of 30% H_2O_2 and further digestion in 3 mL of 70% HNO_3 and 9 mL of 35% HCl at 90 °C over 24 h. The resulting slurry was filtered and made up to 50 mL in deionized water. Measurements of total Fe and Zn content were acquired using this modified method (*17*) and were validated using National Institute of Standards and Technology (NIST) standard reference material 2586 (soils; [Fe] = 51 610 ± 20 mg kg^{-1} and [Zn] = 352 ± 2 mg kg^{-1}). The total Fe and Zn concentrations of different soils were presented as the mean of four replicates with standard error.

2.3. Lentil Seed Samples. Lentil seed samples were obtained from regional variety trials conducted in 2005 and 2006 by the Crop Development Centre (CDC), University of Saskatchewan, Canada. The selected lentil genotypes and their market classes were as follows: (1) extra small red, CDC Robin, CDC Rosetown, and CDC Imperial; (2) small red, CDC Blaze, CDC Impact, CDC Redberry, and CDC Rouleau; (3) large red, Red Chief; (4) large green, Laird, CDC Grandora, CDC Greenland, CDC Plato, CDC Sedley, and CDC Sovereign; (5) medium green, CDC Richlea and CDC Meteor; (6) small green, CDC Viceroy, CDC Milestone, and Eston. The selected lentil genotypes, market classes, protein contents, and countries of major consumption were described in our previous publication (*17*).

Subsamples of seeds for determination of Fe and Zn concentrations were taken randomly from the entire harvested lot of each replicated entry of the field plots at each location. Subsamples were 10−20 g of dry lentil seeds (14% moisture). Each replicated seed sample was prepared by a standard HNO_3 H_2O_2 digestion method (*18*). Measurements of total Fe and Zn concentrations using this modified method were validated using NIST standard reference material 1573a (tomato leaves; [Fe] = 51 610 ± 890 mg kg^{-1} and [Zn] = 352 ± 16 mg kg^{-1}). Total Fe and Zn concentrations were measured by AAS on a Varian SpectrAA150 (Varian Canada, Inc., Mississauga, Ontario, Canada).

2.4. Statistical Analysis. The experiments were conducted following a randomized complete block design (RCBD) with three replicates of the same 19 genotypes at eight locations over 2 years. Data from both years and the eight locations were combined, and error variances were tested for homogeneity. Locations, replications, genotypes, and years were considered as random factors. Class variables included year, locations, replications, and genotypes. Mixed model analysis of variance was performed using the PROC GLM procedure of SAS version 8.2 (*19*). Means were separated by Fisher's protected least significance difference (LSD) at $p < 0.05$. Broad-sense heritability (H^2) is defined as the proportion of total phenotypic variation (V_p) attributable to genotypic variation (V_g). The total phenotypic variation (V_p) includes not only genetic variation (V_g) but also environmental variation (V_e) and the variation as a result of the interaction of genetics and the environment ($V_{g\times e}$). Broad-sense heritability (H^2) was calculated from the appropriate error mean squares using PROC GLM of SAS, version 8.2 (*20*).

3. RESULTS

3.1. Soil Fe and Zn Concentrations and Conditions. Plant available Fe and Zn concentrations are mainly governed by the soil organic fraction, soil pH, aeration, and the interaction between other metal cations, such as copper (Cu^{2+}) and manganese (Mn^{2+}). This study examined soils from eight different locations in Saskatchewan covering major soil zones where the majority of lentil crop is grown. The predominant soil texture was clay loam. The pH of the soil ranged from 5.9 to 7.9 (**Table 1**). The total Fe concentration in these soils ranged from 10 000 to 190 000 μg of Fe g^{-1}. Our soil analysis revealed that Rouleau had a significantly higher total Fe concentration than the other locations. The total soil Zn concentration ranged from 44 to 165 μg of Zn g^{-1} (**Table 1**). The soil at Rosthern had a significantly higher amount of total Zn than Wilkie. The Wilkie soil was slightly acidic and poorly aerated.

3.2. Total Fe and Zn Concentrations in Lentil Seeds. *3.2.1. Fe.* Analysis of variance showed that the following effects were highly significant for total Fe concentration in lentil seeds: year, location, genotype, replication, and year × location interaction (**Table 2**). As expected with most quantitative traits, the year × location interaction explained a large proportion of the variation in total Fe concentration in lentil seeds. This may be due to variable soil conditions (moisture, aeration, and soil pH), weather (precipitation and temperature), or other crop management practices. The broad-sense heritability estimate was 64% for the Fe concentration in lentil. This indicates that, for lentil genotypes grown in Saskatchewan, Canada, potential exists for increasing the Fe concentration in lentil seeds through genetic enhancement.

Significant genotypic differences in total Fe concentration were observed in both years at all locations, except Melfort and

Table 1. Chemical Properties of the Soils at 0−30 cm Depth from Various Locations Where Lentil Were Grown in Saskatchewan, Canada

location	soil texture	soil pH	total Fe[a] (μg g^{-1})	total Zn[a] (μg g^{-1})
Saskatoon	clay loam	6.3	10155 ± 100	71 ± 1
Melfort	clay loam	7.3	31000 ± 100	121 ± 2
Kyle	clay loam	6.3	31000 ± 100	115 ± 2
Rosthern	silt loam	6.5	50000 ± 300	165 ± 2
Hodgeville	clay loam	7.1	50000 ± 300	100 ± 2
Swift Current	clay loam	6.4	100000 ± 100	60 ± 1
Wilkie	clay loam	5.9	174000 ± 120	44 ± 1
Rouleau	heavy clay	7.9	191000 ± 120	67 ± 1

[a] Mean value ± standard error (n = 4).

Table 2. Pooled Analysis of Variance for Total Seed Fe and Zn Concentrations in 19 Lentil Genotypes Grown Across Locations over Years in Saskatchewan, Canada

		mean square[a]	
source	df[b]	Fe	Zn
year	1	302404*	268
location	7	12501*	2394*
genotype	18	946*	285*
replication (year, location)	32	865*	384*
year × location	7	9533*	3827*
genotype × year	18	318	63
genotype × location	126	275	90*
error	576	81	24
coefficient of variation (%)		11	10
broad-sense heritability[c] (%)		64	68

[a] Mean square was significantly different at $p < 0.05$. [b] Degrees of freedom based on three replicates. [c] Broad-sense heritability is the proportion of genotypic to phenotypic variance.

Rouleau in 2006 (**Table 3**). Between years, seed Fe concentration at a particular location varied up to 2-fold (**Table 3**). The year to year variation in Fe concentration at any specific location can be explained by both soil and environmental factors that influence Fe uptake during the growing season. Lentils grown at Kyle and Rosthern had higher mean total Fe concentration (66−120 mg kg^{-1}) compared to those from Hodgeville (67−70 mg kg^{-1}) and Swift Current (53−77 mg kg^{-1}). Furthermore, it was found that the Fe concentration in lentil seeds at any specific location was not directly influenced by the total soil Fe content. High seed Fe concentrations were not observed at locations with high soil Fe concentration.

Combined statistical analysis showed that the effect of lentil genotypes was significant at most locations for both elements. The most highly significant genotypic differences in total Fe concentration for both years were observed at Saskatoon and Hodgeville. **Figure 1** presents the significant differences in total Fe concentration of selected lentil genotypes grown in Saskatchewan. For Saskatoon, the extra small red lentil genotype CDC Rosetown and small red lentil genotype CDC Blaze had significantly higher total Fe concentration (93−99 mg kg^{-1}) than those of green lentil genotypes CDC Grandora and Eston (74−77 mg kg^{-1}) (**Figure 1**). In addition, at the Saskatoon location, the green seed coat genotype Laird (96 mg kg^{-1}) had significantly greater total Fe concentration (66−77 mg kg^{-1}) compared to CDC Grandora, Eston, and CDC Greenland (data not shown). We observed similar genotypic differences at Hodgeville (**Figure 1**), except for large green lentil genotype Laird that had significantly lower amounts of total Fe concentration compared to CDC Rosetown. Saskatchewan-grown lentils contained 73−90 mg of Fe kg^{-1} depending upon location, soil zone, and growing conditions (**Table 4**).

Table 3. Mean Total Fe Concentration in Seeds of 19 Lentil Genotypes Grown at Different Locations in Saskatchewan, Canada, in 2005 and 2006

		total Fe concentration in lentil seeds (mg kg^{-1})			
year	location	minimum	maximum	mean (SE)[a]	genotype effect[b]
2005	Saskatoon	53	87	72 (2)	*
	Kyle	55	88	70 (4)	*
	Hodgeville	53	80	67 (2)	*
	Rosthern	49	80	66 (3)	*
	Melfort	53	87	62 (2)	*
	Rouleau	39	53	45 (1)	*
	Swift Current	37	75	53 (5)	*
	Wilkie	49	87	62 (3)	*
2006	Kyle	98	129	113 (4)	*
	Saskatoon	76	116	93 (4)	*
	Rouleau	82	115	99 (4)	NS
	Melfort	98	124	109 (6)	NS
	Hodgeville	56	100	70 (10)	*
	Wilkie	67	143	107 (5)	*
	Swift Current	57	94	77 (3)	*
	Rosthern	100	142	120 (8)	*

[a] SE, pooled standard error of the mean calculated from the mean square of ANOVA for each location (n = 57). [b] The genotype effect was significantly different at $p < 0.05$. NS = not significant at $p < 0.05$.

The amount of Fe found in a 100 g serving of dry lentils potentially provides 91−113% of the minimum RDA for males and 41−50% for females (**Table 4**). Our field data were derived from small plot field trials. The average total Fe concentration available in commercial lentil shipments would reflect a blended average across environments and years in Saskatchewan.

3.2.2. Zn. Our results demonstrated that significant genetic variability exists for the total Zn concentration in lentil grown in Canada. Combined statistical analysis (mixed model) showed that the following effects were significant for total Zn concentration in lentil seeds: location, genotype, replication, and year × location and genotype × location interactions (**Table 2**). Significant location and genotype × location effects demonstrated the pronounced influence of genetic and environmental factors on total Zn content in lentil seeds. Broad-sense heritability estimates for Zn were fairly high (68%), indicating that potential exists for genetic improvement of the Zn content in lentil genotypes (**Table 2**). The range in seed Zn concentrations in lentil genotypes across the locations was narrower than seed Fe concentrations, ranging from 32 to 56 mg of Zn kg^{-1} (**Table 5**). Significant genotypic variations in total Zn concentration were observed in both years at all locations, except at Saskatoon in 2005 (**Table 5**). Furthermore, our results indicated that the Zn concentration in lentils is fairly stable across the environments. High-Zn genotypes accumulated more Zn compared to low-Zn genotypes grown at locations with high and low soil Zn (**Figure 2**). For both locations, Rosthern (high soil Zn) and Swift Current (low soil Zn), the red lentil genotypes CDC Blaze, CDC Impact, and Red Chief had significantly higher total Zn concentration (50−60 mg kg^{-1}) compared to CDC Meteor, Eston, and CDC Rouleau (42−50 mg kg^{-1}) (**Figure 2**). We calculated that, on average, the lentils contained 44−54 mg of Zn kg^{-1} depending upon location, soil zone, and growing conditions (**Table 6**). This concentration can potentially provide 40−49% of the RDA for males and 55−68% RDA for females from 100 g of dry lentils (**Table 6**). In this study, we found no significant correlation between Fe and Zn concentrations among lentil genotypes (data not shown). Thus, genetic factors conferring Fe uptake are likely different than Zn uptake, and

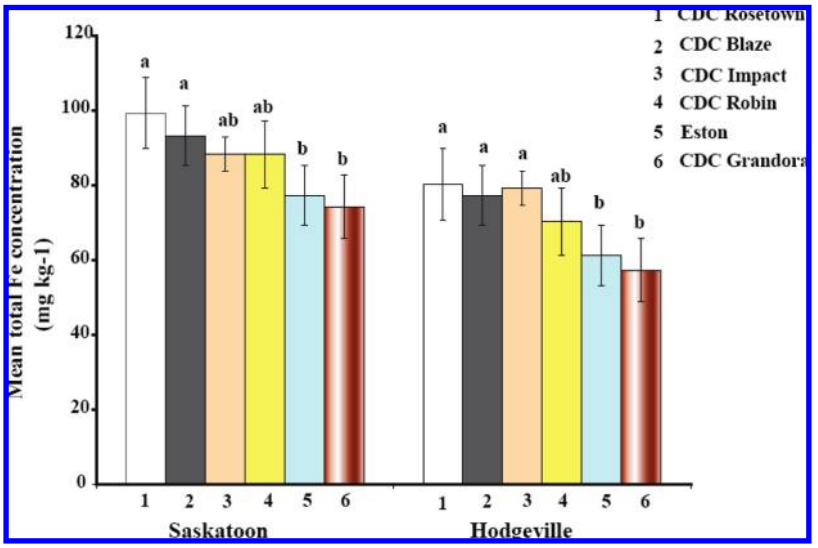

Figure 1. Total Fe concentration in selected lentil genotypes grown at Saskatoon and Hodgeville (2005 and 2006). Comparisons were made for each location separately. Within a location, different letters above bars indicate significant differences at $p < 0.05$ among genotypes ($n = 114$). Pooled standard error of the mean calculated from the mean square of ANOVA for Saskatoon (SE ±1) and Hodgeville (SE ±3).

Table 4. Comparison of the Total Fe Concentration in 19 Lentil Genotypes Grown in Saskatchewan, Canada, in 2005 and 2006

		% RDA for adults 18−50 age group[a] (100 g of lentil)	
genotype	mean total Fe for eight locations[b] (mg kg^{-1})	males (8 mg day^{-1})	females (18 mg day^{-1})
CDC Rosetown	90 a	113	50
CDC Sedley	88 ab	110	49
CDC Impact	85 bc	106	47
CDC Redberry	84 cd	105	47
CDC Blaze	83 cd	104	46
CDC Viceroy	83 cd	104	46
Laird	83 cd	104	46
Red Chief	83 cd	104	46
CDC Robin	82 cde	103	46
CDC Imperial	81 def	101	45
CDC Sovereign	80 defg	100	44
CDC Plato	79 efgh	99	44
CDC Rouleau	78 fghi	98	43
CDC Grandora	77 ghi	96	43
CDC Richlea	76 hij	95	42
Eston	76 hij	95	42
CDC Greenland	75 ij	94	42
CDC Milestone	75 ij	94	42
CDC Meteor	73 j	91	41
SE[c]	3		

[a] % RDA was calculated on the basis of the mean total Fe concentration across eight locations ($n = 912$) in Saskatchewan. [b] Means within a column followed by different letters are significantly different at $p < 0.05$. [c] SE, pooled standard error of the mean calculated from the mean square of ANOVA for each location ($n = 114$) and the mean of eight locations ($n = 912$).

Table 5. Mean Total Zn Concentration in Seeds of 19 Lentil Genotypes Grown at Different Locations in Saskatchewan, Canada, in 2005 and 2006

		total Zn concentration in lentils (mg kg^{-1})			
year	location	minimum	maximum	mean (SE)[a]	genotype effect[a]
2005	Saskatoon	31	57	45 (5)	NS
	Kyle	43	64	51 (2)	*
	Hodgeville	27	41	32 (1)	*
	Rosthern	41	60	49 (2)	*
	Melfort	45	73	56 (2)	*
	Rouleau	42	59	53 (1)	*
	Swift Current	46	69	54 (2)	*
	Wilkie	38	79	51 (6)	*
2006	Kyle	33	40	37 (1)	*
	Saskatoon	37	62	49 (3)	*
	Rouleau	30	43	36 (1)	*
	Melfort	44	58	52 (1)	*
	Hodgeville	43	60	53 (3)	*
	Wilkie	38	60	50 (2)	*
	Swift Current	37	60	53 (1)	*
	Rosthern	47	59	53 (1)	*

[a] SE, pooled standard error of the mean calculated from the mean square of ANOVA for each location ($n = 57$). [b] Genotype effect was significantly different at $p < 0.05$. NS = not significant at $p < 0.05$.

there is sufficient genetic variability to increase the Fe and Zn concentrations in lentil seeds.

4. DISCUSSION

More than 20 minerals and nutrients are essential to maintain human health. Human diets however often lack or are deficient in one or more of these essential nutrients. Poor-quality diets, including high intake of staple carbohydrate foods (rice, wheat, corn, and cassava) and low intake of animal proteins, pulses, fruits, and vegetables, are the cause of micronutrient malnutrition in most populations. It has been estimated that more than one-half of the world's population, mainly women and preschool children in developing nations, faces serious threats from micronutrient malnutrition (*11*).

Despite ongoing attempts to minimize micronutrient malnutrition through supplementation and food fortification, it is our view that the approach known as "biofortification by genetic means" can be adopted to increase human micronutrient intake through diet. On the basis of the findings from our present study, we consider genetic biofortification to have great potential. We observed that Saskatchewan-grown lentils contain 73−90 mg of Fe kg^{-1}, with an average of 81 mg of Fe kg^{-1}. For instance, for males, this level could potentially provide 91−113% of the RDA of Fe just by eating 100 g of dry lentils. Moreover, Zn concentrations in these same lentil genotypes ranged from 44 to 54 mg of Zn kg^{-1}, with an average of 49 mg Zn kg^{-1}. Breeding for the development of micronutrient-dense cultivars in staple crops has been reported and discussed in many reports (*2*, *4*, *21*, *22*). However, success in crop improvement through plant breeding

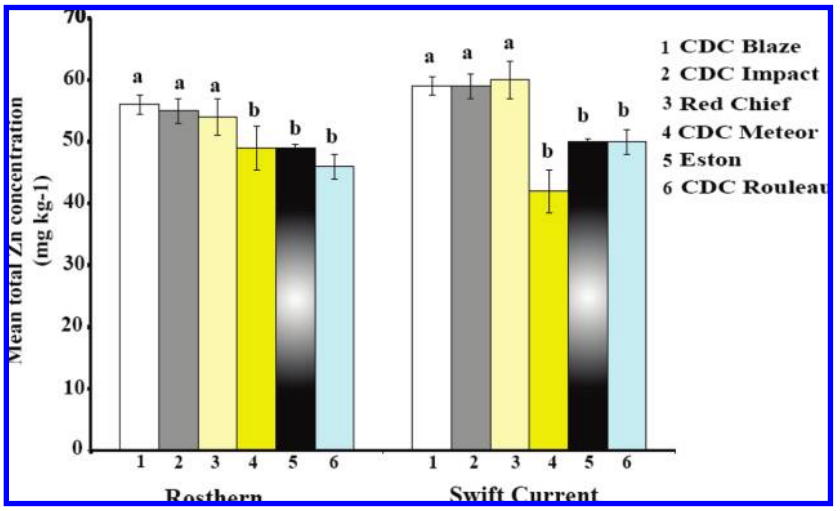

Figure 2. Total Zn concentration in selected lentil genotypes grown at Rosthern and Swift Current (2005 and 2006). Comparisons were made for each location separately. Within a location, different letters above bars indicate significant differences at $p < 0.05$ among genotypes ($n = 114$). Pooled standard error of the mean calculated from the mean square of ANOVA for Rosthern (SE ±1) and Swift Current (SE ±1).

Table 6. Comparison of the Total Zn Concentration in 19 Lentil Genotypes Grown in Saskatchewan, Canada

		% RDA for adults 19+ age group[a] (100 g of lentil)	
genotype	mean total Zn for eight locations[b] (mg kg^{-1})	males (11 mg day^{-1})	females (8 mg day^{-1})
CDC Blaze	54 a	49	68
CDC Sedley	52 ab	47	65
CDC Impact	52 ab	47	65
Red Chief	51 bc	46	64
CDC Robin	51 bcd	46	64
CDC Redberry	50 bcde	45	63
Laird	49 cdef	45	61
CDC Viceroy	49 cdef	45	61
CDC Rosetown	49 cdef	45	61
CDC Imperial	49 cdef	45	61
CDC Greenland	49 cdef	45	61
CDC Grandora	49 cdef	45	61
CDC Sovereign	48 fghi	44	60
CDC Richlea	47 ghij	43	59
CDC Plato	47 ghij	43	59
Eston	46 ijk	42	58
CDC Meteor	46 ijk	42	58
CDC Rouleau	45 jk	41	56
CDC Milestone	44 k	40	55
SE[c]	2		

[a] % RDA was calculated on the basis of the mean total Fe concentration across eight locations ($n = 912$) in Saskatchewan. [b] Means within a column followed by different letters are significantly different at $p < 0.05$. [c] SE, pooled standard error of the mean calculated from the mean square of ANOVA for each location ($n = 114$) and the mean of eight locations ($n = 912$).

depends upon the existence of genetic variability in the gene pool for the target traits. To our knowledge, this is the first scientific report that provides heritability estimates for Fe and Zn concentrations in lentil seeds.

Our results also showed that Fe uptake in lentil is affected by soil and environmental conditions. Soil redox potential has a foremost influence on Fe solubility. Reduced ferrous ion (Fe^{2+}) is distinctly more soluble than oxidized ferric ion (Fe^{3+}). The Fe^{3+} concentration in soil solution is very low compared to other cations in the soils. In well-drained, oxidized soils, Fe^{3+} is the most pronounced form in the soil solution. In acid soils, Fe deficiencies are less frequent than in high-pH and calcareous soils (*23*). Chelated ferric ion is the key form of Fe available for absorption by the plant. Some plants reduce ferric chelates in the rhizosphere through ferric reductase at the outer surface of the root cell plasma membrane and absorb the ferrous irons. Iron is then transported to the shoot through the transpiration stream and imported into leaf cytoplasm (*24*). Soils at Wilkie, Swift Current, and Rouleau had significantly higher total Fe concentrations compared to the other locations. Although Swift Current and Rouleau had high concentrations of total soil Fe, most of this Fe may not be available to the lentil plants. This may be due to high pH, soil texture, poor soil aeration, and moisture of these soils.

Plant available Zn concentration is mainly governed by pH, clay mineral composition, and organic fraction of soils. In general, plants absorb Zn^{2+} as a component of synthetic and natural organic complexes. The availability of Zn decreases with increased soil pH, as observed with Fe. Most pH-induced Zn deficiencies occur in neutral and calcareous soils. Not all of these soils show Zn deficiency however, as a result of increased availability from chelation of Zn^{2+} (*23*). Zinc in soils from this study ranged from 44 to 165 μg g^{-1} and had an average of 93 μg g^{-1}. Soils derived from shale contain more Zn (95 μg g^{-1}) compared to the soils derived from igneous (70 μg g^{-1}) or limestone (16 μg g^{-1}) soil parent material (*23*). The variation in soil Zn content at any specific location can be explained by both soil and weather factors that influence the uptake of Zn. Most Saskatchewan soils are derived from shale-based glacial till, which is rich in Zn. In addition, the predominant soil texture is clay loam, and the soil pH at most of these locations ranged from 5 to 7. These factors do affect an increase in Zn^{2+} availability to the lentil plants.

Our results demonstrated that Fe and Zn concentrations in lentil seeds are influenced by location, genotype, replication, year × location, and genotype × location. For both of these elements, the genotype effect was significant. In contrast, the genotype × location interaction was significant only for Zn, demonstrating that the environment does influence the Zn concentration in lentil seeds. Additionally, we found substantial genotypic differences in Fe and Zn accumulation in lentils across different environments. We observed that high Fe genotypes, such as CDC Rosetown, CDC Blaze, and CDC Impact, will likely accumulate more Fe compared to low Fe genotypes, such as Eston, CDC Grandora, and CDC Meteor, across different locations. Also, a similar pattern was observed for high

Zn genotypes, CDC Blaze, CDC Impact, and Red Chief, which accumulated more Zn compared to low Zn genotypes, CDC Meteor, Eston, and CDC Rouleau, grown at different locations.

Plant breeders frequently use heritability estimates to distinguish the proportion of total phenotypic variation as a result of genotype and environmental influences. This estimate is then used to design appropriate breeding methodologies. In this study, the estimate of genetic variance was 12.6 for Fe and 4.1 for Zn. The estimate of phenotypic variance was 19.7 for Fe and 5.9 for Zn (data not shown). The broad-sense heritability estimate was 64 and 68% for Fe and Zn concentrations, respectively, which indicates that Fe and Zn accumulation in lentil seeds has medium high heritability. Our results imply that, it may be possible to breed lentil cultivars with enhanced ability to accumulate Fe and Zn in seeds despite environmental influence.

We conducted a preliminary analysis of the Fe and Zn content of lentil seeds grown in other regions of the world (USA Pacific Northwest, >50 samples; Australia, >50 samples; India, 10 samples; Bangladesh, 12 samples; and Syria, 7 samples). We also analyzed the Fe concentration in lentil seeds from five different high Fe lentil parents screened from the lentil germplasm collection maintained by the International Center for Agricultural Research in the Dry Areas (ICARDA). All of the samples from other regions of the world and the high Fe lentil parents had moderate to low Fe (<65 mg of Fe kg^{-1}) and Zn (20−40 mg of Zn kg^{-1}), on average 10−30% less than Fe and Zn content in lentils grown in Saskatchewan (data not shown).

Over the past few years, researchers have been accumulating data on the potential for increasing concentrations of Fe and Zn in rice, wheat, maize, sweet potato, common bean, and cassava. The International Center for Tropical Agriculture (CIAT) reported genetic variability for Fe and Zn concentrations in common bean (*25*). Their study evaluated a core collection of over 1000 accessions of common bean and found that Fe concentrations ranged from 34 to 89 mg of Fe kg^{-1}, with the average of 55 mg of Fe kg^{-1}. Zn concentrations in seeds of the same accessions ranged from 21 to 54 mg of Zn kg^{-1}, with the average of 35 mg of Zn kg^{-1} (*26*). At ICARDA, evaluation of >1600 lentil genotypes comprising landraces, breeding lines of red and green lentils, for Fe and Zn contents showed wide variability, with a range of 43−132 mg of Fe kg^{-1} and 22−78 mg of Zn kg^{-1} (*13*). Similar to common bean, lentil has a great potential to deliver significant amounts of Fe and Zn in food systems to mitigate micronutrient deficiencies in the developing world.

The chemical form of Fe is an important factor that affects the Fe bioavailability in humans. About 40% of Fe present in meat products is in the heme form, which is more bioavailable to humans than the nonheme form of Fe (*27*). Heme Fe is better absorbed (15−40%) than nonheme Fe (1−15%) (*28*, *29*). Plant food products, especially seeds of pulses and cereals, contain many antinutritional factors (phytic acid and tannins) that can reduce the bioavailability of dietary nonheme Fe and Zn to human (*30*). Studies have shown that tannins in common bean are mostly localized in the seed coat and that phytic acid is the major antinutrient present in the cotyledon (*31*). Phytic acid can be significantly reduced by 50−60% in lentils during cooking (*32*). Thus, to breed lentils for increased bioavailability, low contents of phytic acid and tannins should be taken into consideration when selecting lentil genotypes that accumulate higher levels of Fe and Zn.

Antinutrients are the compounds that reduce the Fe, Zn, and other mineral bioavailability. Although fiber, tannins, oxalic acid, and heavy metals are antinutrients, phytic acid is one of the most important ones in whole legumes and cereal grains. High phytic acid in foods can severely limit the availability of essential micronutrients, especially for human populations that are mainly dependent upon cereals and legumes. We have completed a preliminary analysis of phytic acid in lentils. High-performance liquid chromatography separation with a conductivity detector was used for phytic acid analysis of whole lentil seed (*33*). We selected lentil genotypes with high Fe (CDC Rosetown), medium Fe (CDC Robin), and low Fe (Eston) from the Saskatoon and Hodgeville locations for 2005 and 2006. The mean phytic acid concentration for these lentil genotypes from both locations ranged from 2.2 to 3.6 mg g^{-1}. Eston had 2.2 mg g^{-1} of phytic acid (or total 0.58 mg g^{-1} phytic acid P) compared to CDC Rosetown (3.6 mg g^{-1} or total 0.96 mg g^{-1} phytic acid P) and CDC Robin (3.5 mg g^{-1} or 0.93 mg g^{-1} total phytic acid P). Our preliminary data show that Saskatchewan-grown commercial lentils are naturally low in phytic acid compared to the reported low phytic acid mutant wheat (1.24−2.51 mg g^{-1} of total phytic acid P) and common bean (0.52−1.38 mg g^{-1} of total phytic acid P) (*34*, *35*). Our next paper will present more in depth analysis of phytic acid levels and micronutrient bioavailability in Saskatchewan lentils.

Breeding for Fe- and Zn-dense genotypes of crops, their cultivation, and consumption may be an effective strategy to mitigate global micronutrient malnutrition. Recent studies have strongly suggested such possibilities of genetic improvement of Fe and Zn content in common bean, rice (*25*), and wheat (*25*, *26*). Future research will be carried out to include a more diverse set of lentil genotypes comprising wild relatives, commercial cultivars, elite landraces, and breeding lines to help develop an effective strategy to further improve Fe and Zn levels in lentil genotypes that are more suited to different geographical regions in Asia, Europe, Australia, and North America.

In summary, our present study demonstrated that lentils have great potential as a Fe- and Zn-rich natural food product. Furthermore, these results indicate that it should be possible to improve Fe and Zn levels in lentil seed through genetic improvement. Success in genetic biofortification efforts could substantially enhance human micronutrient intake, especially for micronutrient-deficient populations around the globe.

ACKNOWLEDGMENT

We thank B. Barlow and staff for providing the lentil seeds and soil samples from Crop Development Centre, University of Saskatchewan, Canada. We also thank Barry Goetz, Jamie Ruszkowski, and Desiree Lalonde for providing the technical assistance for AAS analysis and Dr. Kofi Agblor for reviewing the manuscript.

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Received March 9, 2009. Revised manuscript received April 23, 2009. Support for this research was provided by the Saskatchewan Pulse Growers (PRO0806) Saskatoon, Saskatchewan.