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High Potential for Selenium Biofortification of Lentils (*Lens culinaris* L.)

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Beneficial forms of selenium (Se) and their impact on human health are a global topic of interest in public health. We are studying the genetic potential for Se biofortification of pulse crops to improve human nutrition. Lentils (*Lens culinaris* L.) are an important protein and carbohydrate food and are a valuable source of essential dietary components and trace elements. We analyzed the total Se concentration of 19 lentil genotypes grown at eight locations for two years in Saskatchewan, Canada. We observed significant genotypic and environmental variation in total Se concentration in lentils and that total Se concentration in lentils ranged between 425 and 673 μg kg^{-1}, providing 77−122% of the recommended daily intake in 100 g of dry lentils. Over 70% of the Se was present as selenomethionine (SeMet) with a smaller fraction (<20%) as inorganic Se and very small amounts as selenocysteine (SeCys). We found that soils from the locations where the lentils were grown were rich in Se (37−301 μg kg^{-1}) and that lentils grown in Saskatchewan have the potential to provide an excellent natural source of this essential element. Our analyses gave us a preliminary understanding of the genetic basis of Se uptake in lentil and indicated that any potential strategy for micronutrient biofortification in lentil will require choice of field locations that minimize the spatial variability of soil Se content.

KEYWORDS: Selenium; selenomethionine (SeMet); selenocysteine (SeCys); lentils

INTRODUCTION

Selenium (Se) is an essential micronutrient in human nutrition and is involved in important regulatory and protective mechanisms. The nutritional benefits of Se were first published in 1957 (*1*). Se or, more specifically, selenocysteine (SeCys) is a key component of certain enzymes, for example, in the Se-dependent iodothyronine deiodinases involved in activating thyroid hormone. It also forms the integral parts of glutathione peroxidases and selenoprotein P (*2*), containing one or more atoms of Se per protein molecule. Essential Se-based roles in enzymes, antioxidants, and protective pathways have been discovered and have recently gained importance in cancer suppression, HIV treatment, free radical induced diseases, and protection from toxic heavy metals (*3*−*5*).

Selenium content in the human diet has increasing importance, as the effect of Se deficiency on human health is becoming a topic of interest in public health systems around the world. A recommended dietary allowance (RDA) of 55 μg of Se day^{-1} has been established for regular adults in the United States (*6*), and 60−75 μg of Se day^{-1} has been recommended for regular adults in the United Kingdom (*7*). This requirement is generally met by North Americans; however, large numbers of people in Europe, Asia, Australia, and parts of Africa have intakes of less than the RDA level. Selenium-enriched commercial fertilizers have been recommended in Finland since 1984 to increase Se content in their major food crops. The recommended fertilizer rate for cereals and other crops was 16 mg of Se as sodium selenate (Na_2SeO_4) per kilogram of fertilizer. Since then, the daily Se intake of the Finnish population has increased from 39 μg of Se day^{-1} in 1984 to 110 μg of Se day^{-1} in 1998 (*8*). Low intake of Se (<25 μg day^{-1}) is linked to specific diseases such as arsenicosis in Bangladesh and fatal juvenile cardiomyopathy (Keshan disease) in China. Deficiency is also linked to specific diseases such as poor skeletal muscle strength in older adults (*9*), and even a slight deficiency has now been associated with other disorders including chronic heart failure and prostate and bladder cancers (*10*−*14*). A dietary intake of 55−200 μg of Se per day is now recommended as safe and adequate to reduce the risks of several types of cancer (*15, 16*). Recently, several clinical studies examined the relationship between serum Se levels and the prevalence of diabetes among U.S. adults and suggested that the adverse effects of a high intake of Se may increase primary or secondary diabetes (*12, 17, 18*).

The Se content of the soil from which foods are derived is the major influence on dietary intake of Se. Soil Se is highly variable in distribution and chemical availability. Most soils around the world contain 0.1−2 μg of Se kg^{-1} (*19*). Deficient soils in New Zealand, Australia, Denmark, central Siberia, northeast to south central China, parts of India, and Bangladesh

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Table 1. Market Class, Major Consuming Countries, Protein Content and Cotyledon Color of 19 Lentil Genotypes Grown in Saskatchewan, Canada

market class	seed wt (mg)	major consuming countries	genotype	protein[a] (%)	cotyledon color
extra small red	<30	Bangladesh, Pakistan, Egypt	CDC Robin	26	red
			CDC Rosetown	27	red
			CDC Imperial	27	red
small red	30−50	England, Middle East, Sri Lanka, India, Pakistan	CDC Blaze	27	red
			CDC Impact	27	red
			CDC Redberry	25	red
			CDC Rouleau	22	red
large red	>65	USA, Dubai, Sri Lanka	Red Chief	25	red
large green	>65	Spain, Turkey, Iran, Germany, Algeria	Laird	24	yellow
			CDC Grandora	23	yellow
			CDC Greenland	24	yellow
			CDC Plato	22	yellow
			CDC Sedley	22	yellow
			CDC Sovereign	24	yellow
medium green	50−60	Latin America, Europe	CDC Richlea	20	yellow
			CDC Meteor	23	yellow
small green	<40	Italy, Morocco, Greece, mexico	CDC Viceroy	24	yellow
			CDC Milestone	25	yellow
			Eston	25	yellow

[a] Protein (%) was calculated on the basis of the total seed nitrogen content ($n = 57$).

produce crops that are very low in Se (*20*). On the other hand, soils of Ireland, Colombia, and Venezuela and of the Great Plains of the United States and Canada are naturally rich in Se (*16*). Our initial research showed that Saskatchewan soils have abundant Se and that lentils grown in Saskatchewan may have the potential to provide a significant natural source of this essential element (*21*). However, we have limited understanding of the potential for genetic improvement of Se uptake in Saskatchewan grown lentils.

Biofortification by enrichment of the nutritional contribution of staple food crops through plant breeding is one option that is now widely discussed in the fields of nutrition, public health, and agriculture at national and international levels. Development of an effective biofortification strategy requires the application of genetics and agronomy to provide a solution to wide-scale nutrition problems (*22*). Studies have demonstrated that there is significant genetic variation for Se uptake in soybean, wheat, and *Brassica* vegetables (*23*−*25*).

Pulses combined with cereals are central to the diets of billions of people, and the potential for Se biofortification of pulses is high because of their relatively high protein content. World lentil production on an annual basis is approximately 4 million metric tonnes, and about 20−25% is grown in Saskatchewan, Canada (*26*). Saskatchewan supplies lentils to consumers in more than 100 countries with concentrated regions of consumption in Europe, the Middle East, and most notably South Asia (**Table 1**). Health problems due to Se deficiency affect over 100 million people around the world, many of them in lentil-consuming countries. Progress has been made in controlling Se deficiency through dietary supplementation, food fortification, and agronomic fertilization, but new approaches such as biofortification of basic foodstuffs are needed. Supplying essential Se through widely consumed meals such as lentils and rice could help increase the intake of dietary Se in regions where Se intake is insufficient (*7*). Research is needed to determine whether significant genetic variation exists in pulse crops for Se uptake to develop appropriate breeding strategies in the future. This also requires an understanding of the Se content of soils. We investigated the potential for biofortification of Se content for Saskatchewan-grown lentils as a means of improving human nutrition. The dual objectives of this study were to (1) measure the total Se content of seeds of 19 lentil genotypes grown in 8 key lentil-growing regions in Saskatchewan, Canada, in 2005 and 2006 and (2) identify the chemical forms of Se in extra small red lentil cultivar CDC Robin grown in Saskatoon in 2005.

Table 2. Experimental Design and Sample Protocol

year	2005, 2006
no. of study locations per year	8
study locations (soil zones)	1. Saskatoon (moist dark brown) 2. Kyle (brown) 3. Swift Current (brown) 4. Wilkie (dark brown) 5. Melfort (black) 6. Hodgeville (brown) 7. Rosthern (thin black) 8. Rouleau (moist dark brown)
no. of soil samples per location	4 ($n = 32$)
no. of lentil genotypes per location	19
no. of replications	3 per genotype
no. of lentil seed samples per location (total Se analysis)	114
total no. of lentil samples tested for total Se content	912

EXPERIMENTAL METHODS

Materials. Se standards and chemicals used for digestion and for total Se measurements were purchased from VWR International (Canada) and Sigma-Aldrich Co. (Canada). High-purity chemicals and solvents for HPLC analysis were purchased from Sigma-Aldrich Co. and were used without further purification.

Soil Samples. Locations of the field research sites in Saskatchewan and sample protocol are listed in **Table 2**. These locations cover the major lentil-growing areas in Saskatchewan. Four soil cores were collected at each site from the 0−30 cm soil layer. They 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 collected in October 2005, about 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 several hours (24 h). The resulting slurry was filtered and made up to 50 mL in deionized water. Measurements of total Se using this modified method were validated using NIST standard reference material 2586 (soils; [Se] = 0.6 ± 0.005 mg kg^{-1}). Soils from the South Saskatchewan River bank ([Se] = 110 mg kg^{-1}), where the Se

hyperaccumulator *Astragalus bisulcatus* grows naturally, were used as a laboratory reference material and measured periodically to ensure consistency in the method. The total Se concentrations of different soils are indicated as the mean of three replicates with standard error.

Lentil Seed Samples. Lentil seeds 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, market class, and major consuming countries are listed in **Table 1**. For the genotype × environment study, samples of between 10 and 20 g of dry lentil seeds (14% moisture) were collected from each location with three replicates. Each replicated seed sample was prepared by standard HNO_3 H_2O_2 digestion as described previously (*21*). Measurements of total Se concentration using this modified method were validated using NIST standard reference material 1573a (tomato leaves; [Se] = 0.054 ± 0.003 mg kg^{-1}). Total Se was measured by hydride generation flame atomic absorption spectroscopy (HGAAS) on a Varian SpectrAA150 equipped with a hydride generation apparatus (Varian Canada Inc., Mississauga, ON, Canada). Measurements were made on the digested sample solutions outlined above.

Se Speciation. For the Se speciation study, seed samples of 250−500 g were obtained from three replicated plots of the variety CDC Robin (*27*) grown at the Saskatoon location in 2005. The seeds were dehulled in a Satake TM-05 grain-testing mill (Satake Engineering Co. Ltd. Japan) and then carefully separated by hand into seed coat, embryo, and cotyledon fractions. Se species were separated on a BioCAD Sprint perfusion chromatograph fitted with a 100 μL sample loop using an anion-exchange column (Hamilton PRP-X100, Reno, Nevada, NV) and a reverse-phase C18 column (Varian, Lake Forest, CA) using previously developed and reported methods (*28*). Anionic exchange was carried out with 10 mM citric acid, 1 mM potassium hydrogen phthalate (KHP), and 1 μM rubidium nitrate made to pH 4.5 with ammonium hydroxide in water and 2% v/v methanol. Other Se compounds were confirmed using the C18 column with 10 mM triethylamine and 1 mM KHP at pH 9 in water and 2% v/v methanol. Relative concentrations of Se species in natural samples were determined by ICP-MS (Saskatchewan Research Council, Saskatoon, SK, Canada) normalized to rubidium-spiked HPLC solvent.

Lentil samples were ground to a fine powder, and a 250 mg subsample was suspended in 4 mL of Millipore water. Samples were digested by 10 mg of protease XIV (*Streptomyces griseus*) at 38 °C for 90 min, centrifuged, filtered through a 0.5 μm PTFE membrane, and mixed with 3 equiv of HPLC solvent. Standards (SeMet, Se-methylselenocysteine, selenate, selenite) were used after simple dilution to 40 ng Se mL^{-1}. SeCys was prepared from CysSeSeCys by dissolution at pH 11, followed by sodium borohydride reduction. CysSeSeCys was dissolved with 6 M HCl before dilution with solvent.

Statistical Analysis. The experimental design was a randomized complete block design with 3 replicates, at 8 locations for 19 genotypes over 2 years. Subsamples of lentil seeds for the determination of total Se were randomly taken from the entire harvested sample of each of the field plots. Data from both years and 8 locations were combined, and data error variances were tested for homogeneity. Locations, replications, years, and genotypes were considered as random factors. Class variables were year, location, replication, and genotype. Mixed-model analysis of variance was performed using the PROC GLM procedure of SAS version 8.2. Means were separated by Fisher's protected LSD at $P < 0.05$ (*29*). For each location−year data were analyzed separately using the General Linear Model procedure (PROC GLM) of SAS version 8.2 (*29*). Means were separated by Fisher's protected least significant difference (LSD) at $P < 0.05$. The broad sense heritability (H^2) of Se concentration in lentil seeds was calculated from the error mean squares from PROC GLM of SAS version 8.2 (*30*).

RESULTS

Soil Se Concentrations and Conditions. Se availability in soils depends upon soil pH, aeration, organic carbon, and iron levels. In acidic soils, Se is relatively unavailable to plants and occurs mainly as insoluble selenides and elemental forms. This study examined soils from eight different locations in Saskatchewan covering major soil zones where the lentil crop is grown. The total soil Se concentration ranged from 37 to 301 μg of Se kg^{-1}, or equivalent to 0.5−3.8 μmol of Se g^{-1} (**Table 3**). The Saskatoon location showed significantly higher total soil Se concentration than Rouleau or Swift Current. The Wilkie and Melfort locations were the second highest in total soil Se concentration. The most predominant soil texture at these locations was clay loam, and soil pH ranged from 5.9 to 7.9 (**Table 3**). The soil at Rouleau was more alkaline, and at Wilkie it was slightly acidic and poorly aerated.

Table 3. Total Soil Se Concentration, Soil Texture, and pH from Various Locations in Saskatchewan, Canada

location	soil texture	soil pH	total soil Se (μg kg^{-1})	SE[a]
Saskatoon	clay loam	6.3	301	5
Wilkie	clay loam	5.9	262	5
Melfort	clay loam	7.3	213	7
Kyle	clay loam	6.3	75	3
Rosthern	silt loam	6.5	71	5
Hodgeville	clay loam	7.1	70	5
Swift Current	clay loam	6.4	45	5
Rouleau	heavy clay	7.9	37	3

[a] Standard error ($n = 4$).

Table 4. Summary of Combined Analysis of Variance for Total Se Concentration of 19 Lentil Genotypes Grown at Different Locations in Saskatchewan, Canada

source	df	mean square[a]
year	1	1845855*
location	7	19770258*
genotype	18	156536**
replication (year, location)	32	306369*
year × location	7	8191592*
genotype × year	18	101913
genotype × location	126	72551
error	576	32043

[a] Mean square was significantly different at *, $P < 0.05$, and **, $P < 0.1$, respectively.

Total Se Concentration and Se Species (Beneficial Forms) in Lentil Seeds. Combined statistical analysis (mixed model) over the years and locations showed that variation in total Se concentration in lentil seeds was significant ($P < 0.05$ and $P < 0.1$) for years, locations, genotypes, and the interaction between location and year (**Table 4**). As expected with most quantitative traits, the interaction between the genotype and location shows that most of the variation in the total Se concentration in the lentil seeds may have been due to environmental variation such as soil Se content, soil moisture, and other crop management practices. Therefore, data were analyzed and presented in this paper separately for each location−year (**Table 5**). Significant genotypic differences in total Se in lentil seeds were observed at all but two locations: Rosthern and Wilkie in 2005 (**Table 5**). Lentils grown at Saskatoon and Kyle had the greatest mean total Se concentration (643−1884 μg of Se kg^{-1}) compared to those from Swift Current (139−233 μg of Se kg^{-1}), Rosthern (87−305 μg of Se kg^{-1}), and Wilkie (206−392 μg of Se kg^{-1}) (**Table 5**). Furthermore, it was found that the Se concentration of lentil seeds from Wilkie and Melfort was not influenced by soil Se concentration. High levels of seed Se were not observed, despite high soil Se concentration at these locations. The soil moisture conditions, weather patterns, and soil Se available to plants might explain these differences (**Table 3**). Lentils grown at Wilkie may have had lower concentrations of Se due to soil

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

		total Se concn in lentils (μg kg^{-1})			
year	location	min	max	mean (SE)[a]	genotype effect[b]
2005	Saskatoon	900	2104	1324(7)	*
	Kyle	553	851	643(2)	*
	Hodgeville	232	1403	536(3)	*
	Rosthern	162	560	305(3)	NS
	Melfort	108	619	298(3)	*
	Rouleau	149	403	269(1)	*
	Swift Current	137	327	233(1)	*
	Wilkie	76	505	206(3)	NS
2006	Kyle	1236	2609	1884(5)	*
	Saskatoon	510	1662	885(4)	*
	Rouleau	442	990	633(1)	*
	Melfort	160	507	308(2)	*
	Hodgeville	128	380	220(1)	*
	Wilkie	121	614	392(3)	*
	Swift Current	61	254	139(1)	*
	Rosthern	32	185	87(1)	*

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

acidity, lower soil aeration, and high soil iron concentrations. Comparison of Se concentrations of Saskatoon and Kyle reflected the influence of soil moisture. In 2005, Saskatoon had higher precipitation than in 2006, whereas Kyle experienced greater precipitation in 2006 compared to 2005 (*31*). Between years, seed Se levels at a particular location varied up to 3-fold. The year-to-year variation in Se levels at any specific location can be explained by both soil and weather factors that influence the uptake of Se during grain filling. At each location, the trial fields follow a particular crop rotation and soil properties from field to field can vary substantially in soils derived from glacial till. The weather patterns, particularly temperature and precipitation, are extremely variable in a continental climate and can have a large influence on the availability of soil Se at any time during the growing season.

Se concentration in the lentil seeds varied 4−5-fold across the locations, and on average, seeds of some genotypes had 40−50% more than others (**Table 6**). The extra small red lentil genotype (CDC Robin) and two of the large green lentil genotypes (CDC Sedley and CDC Grandora) had the highest total Se concentrations (612−672 μg of Se kg^{-1}). The small green lentil genotype, Eston, had the lowest (**Table 6**). We calculated that a 35 g serving of CDC Robin lentil (95th percentile of lentil intake per person) grown in Saskatchewan could supply 42% of the current RDA in the United States (55 μg of Se day^{-1}).

Our elemental analysis from seed fractions of CDC Robin lentil from a high Se location (Saskatoon) had mean total Se concentrations as follows: embryo axis, 3600 μg of Se kg^{-1} (45.6 μmol of Se kg^{-1}); cotyledon, 2800 μg of Se kg^{-1} (35.5 μmol of Se kg^{-1}); and seed coat, 2600 μg of Se kg^{-1} (32.9 μmol of Se kg^{-1}). Our previous experiments (*21*) demonstrated that whole seeds of CDC Robin from the Saskatoon location (0.72 μg of Se kg^{-1}; 9.1 nmol of Se kg^{-1}) and those from Swift Current (0.16 μg of Se kg^{-1}; 2 nmol of Se kg^{-1}) showed the greatest range of total Se concentration of the locations tested.

The relative content of Se chemical forms in the lentil seeds was determined by various HPLC-ICP-MS techniques. CDC Robin is a commercially grown cultivar in Saskatchewan because of its early maturity, disease resistance, high yield, and consumer preference. In addition, it was found that lentil seeds from CDC Robin had the highest Se concentration compared to a wide rage of lentil genotypes grown in Saskatchewan. Furthermore, many South Asian consumers (specifically Bangladesh) prefer red cotyledon, extra small seed size (>30 mg) cultivars such as CDC Robin. On the basis of these factors, we chose CDC Robin to study the Se speciation. More than 70% of the Se in the whole lentil sample was present as organic Se with a small fraction (<20%) as inorganic Se (**Table 7**). Small fractions (7%) of SeCys and γ-glutamylselenocysteine were present in the whole lentil seeds, and the concentrations of the other Se species (selenomethionine, dimethylselenoxide, and Se-methylselenocysteine) were not significant. In the embryonic axis, >80% of the Se was present as organic Se with a small fraction (20%) as inorganic Se. SeMet (73%) and selenate (27%) were the major chemical forms of Se present in CDC Robin cotyledon, and inorganic Se (94%) was the major chemical form of Se present in the lentil seed coat (**Table 7**). Our results clearly indicated that the field-grown CDC Robin lentils contained predominately organic Se (80%) as SeMet and SeCys with a minor component of inorganic Se (20%).

Table 6. Comparison of Total Se Concentration in 19 Lentil Genotypes Grown in Saskatchewan, Canada, in 2005 and 2006

	total Se concn (μg kg^{-1})			%RDA[a] (100 g of lentil)	
genotype	Saskatoon (2005)	Kyle (2006)	mean[b] (8 locations, 2 years)	North America (55 μg day^{-1})	Europe (65 μg day^{-1})
CDC Robin	2104 a	2012 bcd	672 a	122	103
CDC Sedley	1446 abcd	2127 abc	612 a	111	94
CDC Grandora	1694 abc	2351 ab	612 a	111	94
Laird	1232 cd	1970 bcde	593 abc	108	91
CDC Greenland	1064 cd	2609 a	544 abcd	99	84
CDC Imperial	1246 cd	1884 bcdef	538 bcde	98	83
CDC Redberry	1947 ab	1583 defg	533 cde	97	82
CDC Sovereign	1503 abcd	2364 ab	533 cde	97	82
CDC Plato	1178 cd	2035 bcd	532 cde	97	82
CDC Meteor	1483 abcd	1470 efg	510 def	93	78
CDC Blaze	1413 bcd	2119 abc	509 def	93	78
CDC Rosetown	1005 d	1942 bcdef	505 def	92	78
CDC Richlea	900 d	2008 bcd	498 defg	91	77
CDC Impact	1136 cd	1844 bcdef	491 defgh	89	76
Red Chief	1429 abcd	1421 fg	472 efgh	86	73
CDC Viceroy	1009 cd	1685 cdefg	471 efgh	86	72
CDC Milestone	1186 cd	1236 g	457 fgh	83	70
CDC Rouleau	1271 cd	1585 defg	431 gh	78	66
Eston	901 d	1555 defg	425 h	77	65
SE[c]	7	5	6		

[a] %RDA was calculated on the basis of the mean total Se 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 mean calculated from mean square of ANOVA for each location ($n = 57$) and mean of eight locations ($n = 912$).

DISCUSSION

The biological importance of Se and its roles in human health have recently become of great interest in the international community. There is a great necessity for food systems to provide at least 55 μg per day for maximal expression of Se enzymes, and large populations in some parts of the world are Se deficient. Se deficiency compromises the health of developing children and reduces the ability to combat the effects of heavy metals in the human diet (*32*). As a common, universal, and quick-cooking nutritious food source, lentils have the potential to deliver

Table 7. Total Se Concentration and Percentage Composition of Se Species for CDC Robin (Saskatoon, 2005)

			percentage of Se species present in lentil seeds as[a]				
			organic Se			inorganic Se	
seed fraction[b]	contribution to total seed wt (%)	total Se (SE)[c] (μg kg^{-1})	SeMet (±SE)	selenocysteines (±SE)[d]	γ-glutamylselenocysteine (±SE)	selenate (±SE)	selenite (±SE)
CDC Robin whole seed	100	2104	69 ± 2	7 ± 1	2 ± 1	10 ± 1	9 ± 1
CDC Robin embryo	5	3600	19 ± 1	53 ± 2	8 ± 1	3 ± 1	17 ± 1
CDC Robin cotyledon	88	2800	73 ± 2	nd[e]	nd	27 ± 2	nd
CDC Robin seed coat	7	2600	nd	nd	6 ± 1	80 ± 2	14 ± 1

[a] Se speciation as determined using LC and ICP-MS. [b] Lentils were collected from Saskatoon location, 2005 (n = 12). [c] SE, standard error. [d] Selenocysteine and selenocystine. [e] nd, not detected within the limits of quantitation (1.6 parts per trillion in HPLC fraction).

beneficial Se to those who need it. Lentil-growing regions with adequate soil Se play a fundamental role in this mass distribution. We have shown that Saskatchewan-grown lentils contain 425−673 μg of Se kg^{-1} depending upon location, soil characteristics, and growing conditions. This potentially provides 80−120% of the minimum recommended daily Se intake in only 100 g of dry lentils. Our data are derived from small-plot field trials. The Se concentration available in commercial lentil shipments would likely reflect a blended average across many fields in multiple locations.

There is unique potential for Se-rich lentil and other pulse crops to be grown in western Canada without soil supplementation. We conducted a preliminary analysis of the Se content of lentil seeds grown in some other regions of the world (U.S. Pacific Northwest, >50 samples; Australia, >40 samples; Syria, 7 samples; Bangladesh, 12 samples; India, 10 samples; and Nepal, 5 samples). All samples had very low Se concentration, on average, <5% of the Se content of the lowest Se content lentils from Saskatchewan (Swift Current). Many samples from Syria, Bangladesh, India, and Nepal had no detectable Se (<20 ppb) (data not shown).

The chemical species distribution in seeds of Se is important in terms of nutritional benefits. It has long been understood that certain forms of Se are critical to development and self-regulation, whereas others are potential poisons. The biological fate of Se is also determined by the original form and the transformation that occurs during digestion and absorption. The amino acid SeMet is readily incorporated into protein masses, but SeCys, which is found in key regulatory proteins, is tightly controlled and is catabolized into hydrogen selenide. Inorganic forms, such as selenate and selenite, have been studied for their involvement in the treatment of arsenicosis and excretion of mercury (*32*).

The presence of Se in plants grown on soils containing available Se has been reported in many studies. Seleniferous green onion (*Allium cepa* L.) predominantly contained SeMet and small amounts of SeCys (*33*). SeMet and SeCys were the major organic selenides found in sour clover (*Melilotus indica* L.) and alfalfa (*Medicago sativa* L.) grown in seleniferous soils in California (*34*). SeMet is the major organic form of Se found in wheat, common bean, mushroom, and yeast (*35*). Our findings for Se in lentils seed are similar to those reported for seeds of seleniferous wheat (*Triticum aestivum* L.), common bean (*Phaseolus vulgaris* L.), alfalfa, and sour clover, which contain mainly SeMet with a smaller fraction of SeCys (*34, 35*).

HPLC-ICP-MS analysis of the Se species in whole lentils revealed that most of Se was present as SeMet with small amounts of selenate and very small amounts of selenocysteines, selenite, and other selenooligopeptides such as γ-glutamylselenocysteine (gGSeCys) as outlined in **Table 7**. This supports our previous experiments using synchrotron X-ray spectroscopy to identify Se species in lentil seeds and seed tissues (*21*). Synchrotron techniques offer a unique advantage in that samples can be run intact with no pretreatment, but it is difficult to differentiate between chemically similar species such as SeMet and Se-methylselenocysteine (Se-MeSeCys) or to reliably detect smaller components when one type is in great excess. HPLC-ICP-MS methods for Se quantitation can be used to differentiate the forms that X-ray techniques cannot. Conversely, the overlap of other chemical species in the HPLC methods can be differentiated in the related synchrotron experiments. Both methods used in conjunction are sufficient to determine the complete set of Se forms present in seeds.

Our analysis of Se speciation in CDC Robin lentil provided an indication that Se species may vary according to the seed component. The seed coat has a unique Se species profile, largely inorganic. The embryonic axis is enriched for SeCys in comparison to cotyledon tissue. Red lentils are usually decorticated prior to cooking in whole or split form. In terms of Se speciation, split lentils may have lower SeCys content because the embryo fraction is often collected as a byproduct for use in animal feed. In some countries, for example, in Bangladesh, consumers have a distinct preference for decorticated unsplit lentils, which may be beneficial for human nutrition.

Other factors, such as cooking, grinding, and digestion that may affect or transform Se speciation, have been investigated. We found that cooking the lentils in boiling water did not change the total Se content (data not shown). There is a migration of Se from the lentils to the liquid broth, but provided the lentils and broth are consumed as a whole food source, the Se concentration and speciation remain intact. However, we would expect a nearly 50% reduction in Se for lentils that are thermally processed in brine (canned) and consumed after the canning brine is discarded (*36*).

In many parts of the world, lentils with adequate beneficial Se concentration could be considered a natural, whole food source for Se, and a possible solution to Se deficiency-related arsenicosis in Bangladesh and juvenile cardiomyopathy (Keshan disease) in China (*32*). Supplementation of 200 μg per day may help to prevent certain cancers, such as bladder, prostate, liver, colorectal, and lung cancers (*16*). Efforts to optimize the Se in food sources must consider not only the overall concentration but the amounts of the various beneficial forms.

Quantitative traits generally depend on the collective interaction of many genes. The expression of quantitative genes is also influenced by the environment. The phenotypic variance calculation is influenced by the number of years, locations, and replicates used in the experiment, therefore, plant breeders commonly use heritability estimates to distinguish the proportion of total phenotypic variation due to genotype and environmental influences. This estimate is then used to design appropriate genetic improvement strategies. In this study, the estimate of

genetic variance was 1135 and that of phenotypic variance was 2877. The broad sense heritability estimate was 0.4, which indicates that Se content in lentil is in the midrange of heritability. An appropriate genetic improvement strategy for increasing Se content lentil would require that environmental influence be kept to a minimum by careful selection of environments with low spatial variability for soil Se content combined with appropriate replication.

Breeding for enhanced Se accumulation and selective speciation may be an effective strategy to help overcome global Se deficiencies. Some studies have suggested the possibility of genetic improvement for Se uptake in *Brassica* vegetables, wheat and soybean (*23*−*25*). By specifically controlling the Se variability in soil Se content, it would be possible to reduce environmental effects as part of our biofortification approach on lentil genetic improvement. It may be possible to screen lentil genotypes for increased Se uptake ability using atomic absorption spectroscopy techniques or possibly using marker-assisted genetic selection. On the basis of our results, we suggest that it may be possible to cost effectively breed lentil cultivars for enhanced Se uptake for specific regions of the world with soils that have lower levels of Se. In regions where Se is highly deficient, it may be necessary to combine this approach with agronomic biofortification using fertilizer with Se additives. This may be particularly important for regions where the rapidly increasing cost of rice may induce further reduction in the land area devoted to pulse production. Further studies are being performed on diverse genotypes, including wild relatives of cultivated lentil, modern commercial cultivars, and genotypes adapted to different geographic locations in Europe, Asia, Africa, and North America.

Se must be available in the soil for uptake and transformation. In general, soil Se is unevenly distributed and varied in availability, ranging from <0.1 to >100 ppm, and most commonly from 1 to 1.5 ppm (*19*). Ultimately, the total Se in the soil depends on the minerals in the rocks from which the soil was derived. Soils of the Northwest, Southeast, and Great Lakes regions of the United States were derived from volcanic deposits and have low soil Se content (<0.05 ppm) (*37*). Soils originating from cretaceous shales, such as those found in South Dakota and Montana, tend to have concentrations upward of 10 ppm (*37*). However, the availability of the Se is greatly dependent on aeration, water availability, pH, and soil texture and composition. In poorly aerated soils, Se is relatively unavailable to plants and occurs mainly as insoluble selenides and elemental forms. Furthermore, wetter soils with alkaline pH have lower Se concentration due to leaching of mobile selenate (*20*).

Our study indicates that uptake of Se in lentil seeds is affected by soil and environmental conditions such as moisture, soil texture, aeration, and soil fertility and irrigation (*7*). The higher Se concentration observed from the Saskatoon area may be due to higher spatial variability in the soil combined with wet weather conditions, which would increase availability of soil Se. Soils at Rouleau had the lowest Se concentration (**Table 3**) and the highest pH. The Rouleau soil was a moist heavy clay with poor aeration, thus reducing the amount of Se that is available to plants. We found that the total soil Se concentration was not the best indicator of plant Se availability, although it is the most commonly used method of reporting Se availability in the literature (*38*). A complete understanding of the biochemistry of Se in soil and lentil plants will require more in-depth studies of plant biochemistry, agronomy, and physiology.

In summary, our present study shows that Saskatchewan soils are naturally rich in Se and that lentils grown in them have great potential as a quick-cooking, Se-rich, natural food product. Significant genotypic differences for Se concentration were observed across the locations. In addition, genotype × environment analysis of the concentration of Se in the lentils indicated that good potential exists for genetic improvement of the concentration of this essential element in lentil. The Se content and the chemical forms of Se within the seed may be altered by conventional plant breeding approaches or by optimizing agricultural production conditions.

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