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Lentils (*Lens culinaris* L.), a Rich Source of Folates

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ABSTRACT: The potential for genetic biofortification of U.S.-grown lentils (*Lens culinaris* L.) with bioavailable folate has not been widely studied. The objectives of this study were (1) to determine the folate concentration of 10 commercial lentil cultivars grown in Minot and McLean counties, North Dakota, USA, in 2010 and 2011, (2) to determine the genotype (G) × environmental (E) interactions for folate concentration in lentil cultivars, and (3) to compare the folate concentration of other pulses [field peas (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.)] grown in the United States. Folate concentration in lentil cultivars ranged from 216 to 290 μg/100 g with a mean of 255 μg/100 g. In addition, lentil showed higher folate concentration compared to chickpea (42−125 μg/100 g), yellow field pea (41−55 μg/100 g), and green field pea (50−202 μg/100 g). A 100 g serving of lentils could provide a significant amount of the recommended daily allowance of dietary folates (54−73%) for adults. A significant year × location interaction on lentil folate concentration was observed; this indicates that possible location sourcing may be required for future lentil folate research.

KEYWORDS: *lentils, biofortification, folates, tetrahydrofolate, chickpea, field pea*

INTRODUCTION

Folate deficiency is a global problem affecting millions of people in both developed and developing countries.[1] Inadequate intake of folic acid during pregnancy increases the risks of preterm delivery, low birth weight, fetal growth retardation, and developmental neural tube defects (NTDs). In addition, low folate intake and elevated homocysteine levels are associated with the occurrence of neurodegenerative disorders, cardiovascular diseases, and a range of cancers, while adequate intake of both folates and folic acid in diets decreases total homocysteine levels in plasma.[2−4]

Tetrahydrofolate and derivatives, collectively called folates, are water-soluble B-vitamins. Humans and animals cannot synthesize folates, and therefore they must be supplied from plant-based and animal foods including liver and eggs. Pteroylmonoglutamic acid (folic acid) is the synthetic form of folate used in supplements and food fortification. In 1998, US and Canada mandated folic acid enrichment in all grain products to lower the risk of NTDs. This resulted in a 20−53% decrease in the incidence of NTDs and more than a 38% reduction in the prevalence of anencephaly.[2,5] Currently, the recommended daily intake (RDA) of folate is 400 μg of dietary folate equivalent for adults and 600 μg for pregnant women.[6] Folic acid fortification and supplementation approaches have been adopted in many parts of the world, largely due to folate bioavailability.[2,7] Thus, alternative approaches to supply daily folates through biofortification of staple food crops may provide a sustainable means to provide bioavailable folates to people in many parts of the world.[8]

Most staple food crops, including cereals, potato (*Solanum tuberosum* L.), and banana (*Musa* sp), are poor sources of dietary folates, and diets based on these foods often do not reach the folate RDA of 400 μg/day.[1,6] Generally, leafy vegetables contain more folates (1.5−4.5 nmol/g fresh weight) than roots (0.3 nmol/g fresh weight) and fruits (0.2−0.8 nmol/g fresh weight).[9] The USDA nutrient database shows lentils (*Lens culinaris* L.) and common beans (*Phaseolus vulgaris* L.) are two pulses that are rich in folates.[10]

Lentil is a traditional pulse crop mostly grown in low-rainfall, dryland cropping systems in rotation with cereals, wheat and rice. Annual world lentil production is approximately 4.4 M tons, about 90% of which occurs in five specific regions: Canada (35%-1.53 t); India, Nepal, and Bangladesh (30%-1.23 t); Turkey and Syria (13%-0.55 t); Australia (8%-0.38 t); and the Midwestern region of the USA including North Dakota, South Dakota, and eastern Montana (4%-0.21 t).[11] Lentils are an emerging crop in North Dakota, and Montana, providing economic benefits in addition to the benefits derived from crop

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rotation, nitrogen fixation, and sustainable agriculture.[12] Lentil cultivars are grouped into at least six market classes, including extra small red, small red, small green, medium green, large green, and dark green speckled. This classification is based on the size and color of the seed.[13] Lentils are rich in protein (20−30%), prebiotics (including fructooligosaccharides, galactooligosaccharide, and resistant starch), and minerals, and are naturally low in phytic acid.[14−16] Therefore, the selection and development of lentils cultivars high in bioavailable folates could have large benefits due to the complementarity profiles of other bioactive molecules present in lentils. To our knowledge, this study is the first comprehensive study on USA-grown lentils to assess their potential as a source of folates for future genetic studies on biofortification.

The objectives of this study were (1) to determine the folate concentration of 10 commercial lentil cultivars grown in Minot and McLean counties, North Dakota, USA in 2010 and 2011, (2) to determine the genotype x environment interactions for folate concentration in lentil cultivars and (3) to compare the folate concentration of other pulses [field peas (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.)] grown in the USA.

MATERIALS AND METHODS

Materials. Standards, reagents, and high-purity solvents used for high-performance liquid chromatographic (HPLC) analyses and enzymatic assays were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Folate standards were freshly prepared each day. Water, distilled and deionized (ddH_2O) to a resistance of ≥18.2 MΩ (Milli-Q Water System, Millipore, Milford, MA, USA), was used for sample extractions and preparation.

Seed Samples. The following commercial lentil cultivars were included: CDC Redberry, CDC Red Rider, CDC Lemay, CDC Greenland, CDC Rouleau, CDC Richlea, Riveland, CDC Rosetown, CDC Viceroy, and Pennell.[16] An approximately 250−300 g subsample of seeds was collected. These seed samples were randomly taken from an entire harvested plot with three replications, two locations, and two years (2010 and 2011; total number of samples = 120). Two selected counties were Ward (48° 23′ 25′′ N, 101° 29′ 58′′ W, 27.2 cm average rainfall, and 17.2 °C mean growing season temperature) and McLean (47° 57′ 74′′ N, 101° 239′ 60′′ W, 36.3 cm average rainfall, and 17.2 °C mean growing season temperature), North Dakota. Samples were hand-cleaned of debris, air-dried (40 °C), and ground to pass through a sieve size of 0.25 mm using a top-loading UD grinder (Unholtz Dickie Corp., Wallingford, CT, USA). Samples of 10−20 g of ground seed (7.3% moisture) were stored at −40 °C until analysis. The moisture contents of these ground lentil seeds were measured using AACC method 44-15A.[17]

In addition, three yellow field pea (DS Admiral, CDC Meadow, and Spider), five green field pea (CDC Striker, Shamrock, SGDP, K2, and Arcadia), and eight chickpea (CDC Frontier, Sierra, Dylan, Dwelley, Bronic, Billy Bean, Troy, and Sawyer) commercial seed samples were collected from the 2012 Pulse Quality Survey.[18] A total of 16 seed samples were collected from North Dakota, Idaho, and Washington. An approximately 500−1000 g subsample of seeds was collected from the 2012 Pulse Quality Survey conducted at the NDSU Pulse Quality and Nutrition Laboratory. Field pea and chickpea samples followed the same processing method as previously described for lentils.

Sample Preparation and Analysis. *Homogenization.* A finely ground sample of 0.25 g was weighed and dispersed in 12.5 mL of extraction buffer solution [75 mM potassium phosphate buffer (pH 6.0) containing 52 mM sodium ascorbate and 0.1% (v/v) 2-mercaptoethanol].[19] The mixture was homogenized for 30 s using a vortex mixer. This procedure was done using amber-colored vials under minimum light conditions.

Trienzyme Treatments. The homogenized seed samples were treated with enzymes according to the method described by Hefni et al.[19] The seed samples were incubated with 1 mL of α-amylase (3000 U/mL) from *Aspergillus oryzae* (EC 3.2.1.1) for 1 h, followed by submersion in a boiling water bath (75 °C) for 12 min, and then cooled on ice. When the samples were cool, 2 mL of protease (5 mg/mL) from *Streptococcus griseus* (EC 3.4.24.31) was added to each, incubated at 37 °C for 1.5 h, and inactivated by submersion in a boiling water bath for 5 min. Finally, 0.2 mL of conjugase from rat serum was added to each sample, and the samples were incubated at 37 °C for 2.5 h.[19] The enzymes were deactivated by placing the sample in a boiling water bath for 5 min and then cooling it on ice. The samples were centrifuged at 4000 rpm for 15 min, and 1 mL of supernatant was collected in amber-colored bottles (minimum light condition) to reduce the breakdown of isolated folates. Then, these samples were immediately analyzed on a reversed phase high-performance liquid chromatograph (RP-HPLC).[20] Seed folate concentration was measured by HPLC (Agilent 1260, Agilent Technologies, Santa Clara, CA, USA) with a fluorescence detector at excitation and emission wavelengths 290 and 360 nm, respectively. Folates were separated on a C18 column (Prodigy 5 μm, 250 × 4.6 mm C18 column, Phenomenex, Torrance, CA, USA), with a guard column (Prodigy 5 μm, 30 × 4.6 mm, Phenomenex). The column temperature was maintained at room temperature, 25 ± 1 °C, during the experiment. The mobile phase was acetonitrile and a 30 mM potassium phosphate buffer (pH 2.3) at a flow rate of 0.4 mL/min. The gradient was initiated at 5% acetonitrile and kept for 5 min and then linearly increased to 25% over 20 min. The gradient was kept at 25% acetonitrile for another 6 min. The concentration of the samples was quantified as micrograms of tetrahydrofolic acid (THF) equivalents per 100 g of dry sample. The concentrations of those analyzed THFs were detected within a linear range of 0.1−2.5 μg/g (r^2 > 0.99). The minimal detectable limit was 0.01 μg/g. An external laboratory reference, CDC Redberry, was also used daily to ensure the accuracy, sensitivity, and reproducibility of detection. High-resolution mass spectrometry was used to confirm the samples and THF standards using a Bruker Daltonics BioTOF (mode, positive; dry gas temperature, 200 °C; capillary, 4500 V; ionization source, ESI; data reported, m/z) at the NDSU Core Synthesis and Analytical Service Facility, Fargo, ND, USA.

Statistical Analysis. The experimental design was a randomized complete block design with three replicates of 10 commercial lentil genotypes grown at two locations over two years (n = 120). For combined analysis, the General Linear Model procedure (PROC GLM) of SAS version 9.3[21] was used to perform analysis of variance with replicates, locations, and genotypes considered as random factors. A separate analysis of variance was performed for each year using SAS PROC GLM. Means were separated by Fisher's protected least significant difference (LSD) at $p < 0.05$. Lentil folate concentrations were subjected to dissimilarity coefficient analysis using NTSYSpc ver. 2.2.[22] A dendrogram was constructed following an unweighted pair group method with arithmetic average (UPGMA) based on a dissimilarity matrix using NTSYSpc ver. 2.2.[22]

RESULTS

Analysis of Variance Components. In combined analysis of variance, genotype effects were not statistically significant ($p < 0.05$) (Table 1). However, individual location and year-specific ANOVA showed that genotypic effects and genotype (G) × environment (E) interactions were significant ($p < 0.05$), with the exception of McClean County in 2011 (Table 2). Partitioning of variance further indicated that year × location and year × location × genotype interaction effects were statistically significant ($p < 0.05$) (Table 1). For 2010, total folate concentration ranged from 196 to 329 μg/100 g with an average of 263 μg/100 g over two locations (Table 2). For 2011, total folate concentration ranged from 187 to 310 μg/100 g with an average of 249 μg/100 g over two locations (Table 2). In this paper, the total folate concentration in lentils was quantified as tetrahydrofolate (THF).

Table 1. Pooled Analysis of Variance for Folate Concentration of 10 Lentil Varieties Grown in North Dakota, USA, in 2010 and 2011

source	df[a]	mean square[b]
genotype	9	9220
location	1	1904
year	1	8467
year × location	1	399746**
year × genotype	9	2545
location × genotype	9	13758
year × location × genotype	9	6880**
error	72	2037

[a]Degrees of freedom based on three replicates. [b]Mean square was significantly different at $p < 0.05$ (**).

Table 2. Mean Concentration of Folate and Genotype Effect by Year and Location

year	location	folate[a] (μg/100 g)	genotype effect
2010	McLean	196 (92) a	significant at $p < 0.05$
	Ward	329 (82) b	significant at $p < 0.1$
	mean	263	
	SE[b]	14	
2011	McLean	310 (88) a	not significant
	Ward	187 (66) b	significant at $p < 0.05$
	mean	249	
	SE	13	

[a]Means (standard deviation; $n = 30$) within a column followed by different letters are significantly different at $p < 0.05$ ($n = 60$). [b]SE, standard error of combined data ($n = 60$).

Total Folate Density among Lentil Cultivars. The total folate concentration of lentil cultivars ranged from 216 to 290 μg/100 g with an average of 255 μg/100 g (Table 3). A small red cultivar, CDC Rouleau, showed the highest concentration of 290 μg/100 g, and a large green cultivar, CDC Greenland, showed the lowest (216 μg/100 g). Percent recommended dietary intake (%RDA) of folates is 400 μg/day. Therefore, a single serving of 100 g of lentil on a dry weight basis can supply on average 64% of RDA. Percent contribution to the folate RDA varies from 54% (CDC Greenland) to 73% (CDC Rouleau) from a single serving of 100 g of lentils (Table 3).

Table 3. Mean Folate Concentration and Percent Recommended Daily Allowance (%RDA) of Folates from 10 Lentil Varieties Grown in North Dakota, USA, in 2010 and 2011

market class	cultivar	folate[a] (μg/100 g)	%RDA from 100 g serving[b]
small red	CDC Red Rider	252 (124) a	63
	CDC Redberry	219 (61) b	55
	CDC Rouleau	290 (47) a	73
medium green	CDC Richlea	287 (87) a	72
extra small red	CDC Rosetown	269 (128) a	67
Large green	Pennell	262 (62) a	66
	CDC Greenland	216 (95) b	54
	Riveland	222 (157) a	56
small green	CDC Viceroy	244 (86) a	61
dark green speckled	CDC Lemay	228 (92) b	57
mean		255	64
SE[c]		13	

[a]Means (standard deviation) within a column followed by different letters are significantly different at $p < 0.05$. [b]The %RDA for folates (400 μg per day for adults) was calculated on the basis of a 100 g serving of lentils.[4] [c]SE, standard error of combined data ($n = 120$).

Cluster Analysis Based on Folate Least-Squares Means. Ten lentil cultivars were grouped into three clusters based on the mean values generated from unweighted pair group mean average method of analysis (Figure 1). The two cultivars in cluster I, CDC Rouleau and CDC Richlea (287–290 μg/100 g of folate), had the highest level of folate (Figure 1). Cultivars CDC Rosetown, Pennell, CDC Red Rider, and CDC Viceroy were classified as cluster II with a moderate level of folate (244–269 μg/100 g) (Figure 1). Cluster III consisted of CDC Lemay, Riveland, CDC Redberry, and CDC Greenland with a comparatively lower level of folate (216–228 μg/100 g) (Figure 1).

Comparison with Other Food Legumes. The total folate concentration in yellow field peas ranged from 41 to 55 μg/100 g with an average of 50 μg/100 g, and green field pea folate concentration ranged from 50 to 202 μg/100 g with an average of 105 μg/100 g (Table 4). Chickpea cultivars had folate concentrations ranging from 42 to 125 μg/100 g with an average of 65 μg/100 g (Table 4). A 100 g of serving of yellow field peas, green field peas, and chickpeas can supply 12, 26, and 16% of the daily folate intake requirement, respectively (Table 4).

DISCUSSION

A folate concentration of 255 μg/100 g (on average) makes lentil a promising whole food source of folates. To our knowledge, this is the first study to quantify total folate levels in lentils in a replicated field study and the first to analyze variance components in a multiyear and multilocation experiment for folate concentration. Nutritional quality traits of most staple food crops including micronutrient and prebiotic concentrations are mostly influenced by genotype (G) × environment (E) interaction.[23,24] In 2010, total folate concentration ranged between 196 and 329 μg/100 g over two locations. In 2011, total folate concentration varied from 187 to 310 μg/100 g over the locations. In May 2011, the Federal Emergency Management Agency declared both Ward and McLean counties as officially affected by flood damage, and both counties were eligible for public assistance.[25] This major meteorological difference between the years contributed to the high year × location and year × location × genotype variance components. This effect of an interaction component influencing total variances for folate concentration is comparable to the results of several previous studies involving micronutrients, prebiotics, and phenolics in lentil.[14,16,26] The predominance of G × E interaction effects indicates the necessity to include soil fertility analysis of the experimental site particularly before and after the experiment; this analysis will help to evaluate the genetic potential of a genotype for folate concentration more accurately.

The grouping of cultivars based on folate concentration will assist in further genetic and agronomic studies for selection and breeding within these lentil market classes. Chickpea and field pea are other cool-season food legumes that are grown extensively in the temperate areas of the world; however, the

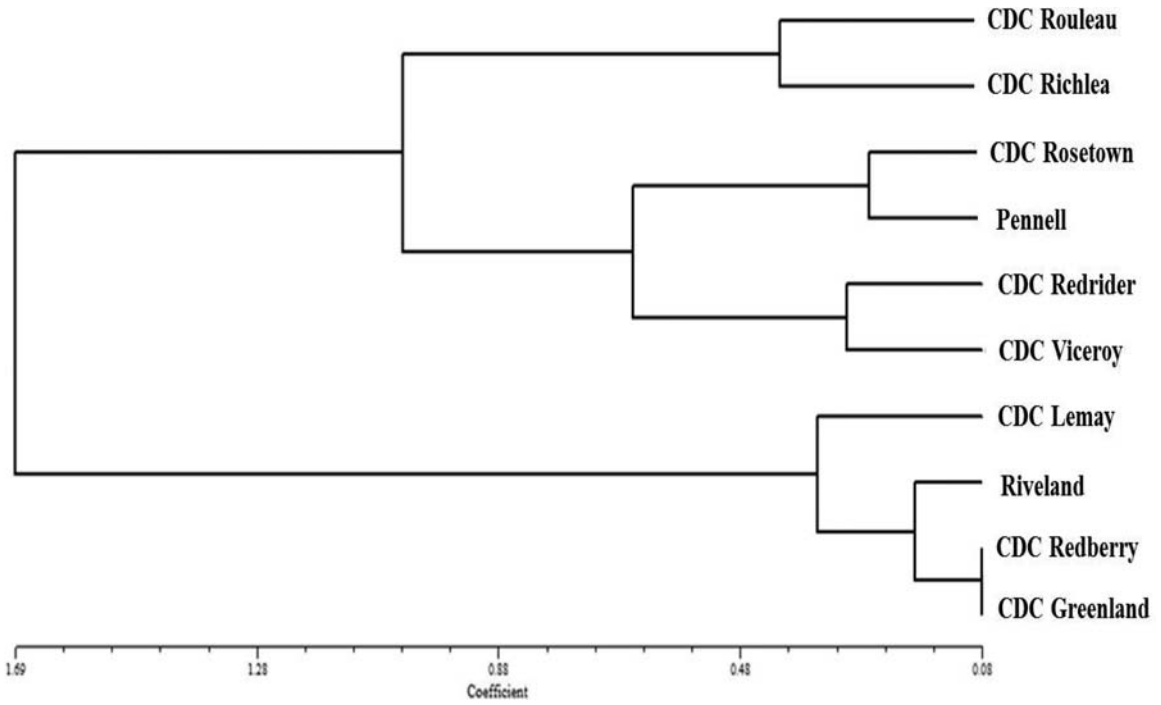

Figure 1. Dendogram based on dissimilarity matrix data of lentil folate concentrations following unweighted pair group mean average method. The ten lentil genotypes are grouped into three distinct clusters based on folate concentration.

Table 4. Folate Concentrations and %RDA of Other Pulse Crops Grown in the United States

market class	cultivar	state	folate (μg/100 g)	%RDA from 100 g serving
yellow field pea	DS Admiral	North Dakota	54	14
	CDC Meadows	North Dakota	41	10
	Spider	North Dakota	55	14
	mean		50	12
	SE		4	
green field pea	CDC Striker	North Dakota	50	12
	Shamrock	North Dakota	63	16
	SGDP	North Dakota	202	51
	K2	North Dakota	53	13
	Arcadia	North Dakota	156	39
	mean		105	26
	SE		35	
kabuli chickpea	CDC Frontier	North Dakota	125	31
	Sierra	Washington	66	17
	Dylan	Washington	54	14
	Dwelley	Idaho	54	14
	Bronic	Idaho	59	15
	Billy Bean	Idaho	42	11
	Troy	Idaho	70	18
	Sawyer	Idaho	48	12
	mean		65	16
	SE		8	

average concentration of folate (255 μg/100 g) in lentil is higher than in chickpeas and in yellow and green field peas. This study also indicates that the range of variability within the species is comparatively lower in lentil (216−290 μg/100 g) compared to other food legumes (in the case of *kabuli* chickpea and field peas folate ranged from 42 to 125 μg/100 g and from 41 to 202 μg/100 g, respectively) (Table 4). The USDA Nutrition Database indicated that total dietary folate equivalences for raw lentils, field peas, and chickpeas are as follows: 479 μg/100 g for lentils, 557 μg/100 g for field peas, and 65 μg/100 g for chickpeas.[10] Goyer et al. reported that the folate concentration of 12 different common beans grown in different locations of the Unites States ranged from 202 to 257 μg/100 g, and both of these results are similar to the results reported in this study.[27]

Food folate levels have been measured using different analytical methods including HPLC and microbial assays.[19,27−29] These assays include a microbiological method using *Lactobacillus rhamnosus*[28,29] and HPLC-MS methods.[19,29] Liquid chromatography− mass spectrometry (LC-MS) enables the simultaneous identification and quantification of different folates. In the present study 5-methyl-THF and 10-formyl-THF forms were qualitatively identified to determine the presence of different folate forms. An exhaustive analysis by different excitation/emission by fluorescence detection and use of LC-MS would have provided a range of other folate presence due to the analytical capabilities of those methods. No attempts were made in this study as high-resolution mass spectrometry analysis may not be a feasible high-throughput screening tool due to the time and cost constraints. Therefore, HPLC may be a rapid screening tool when a large number of lentil samples are selected for breeding purposes.

Research on folate bioavailability in staple food crops is limited. Food folates are converted to monoglutamyl tetrahydrofolate before absorption in the jejunum. Many

factors affect folate bioavailability including folate form, host background, quantity of folate ingested, and nutrient status.[2] There have been contradictory reports regarding the bioavailability of different folate forms or folic acid. For example, a few studies have suggested that folic acid is more bioavailable than other forms.[30] However, other studies reported that there are no significant differences in terms of bioavailability of folic acid and other folate forms.[30] Because most of the folate in legumes remains as THF, the estimates of THF are appropriate as a measure of folate concentrations in lentils.[29]

Global biofortification efforts for increased levels of micronutrients in lentils have been limited to a few research groups.[14,16] A few studies have been reported in which staple crops have been determined to have a useful level of genetic variability for micronutrients including iron, zinc, pro-vitamin A, and carotenoids.[24,31,32] On the basis of these initial observations, more detailed future study is suggested to determine the range of genetic diversity that exists in lentil germplasm. This would be helpful to generate data for the entire range of existing genetic variability in this crop species and its close relatives. Furthermore, any future study should also take into consideration the environment and its interaction on genotype effects.

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Notes

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