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ORIGINAL PAPER

Phytic acid and mineral micronutrients in field-grown chickpea (*Cicer arietinum* L.) cultivars from western Canada

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Abstract Zinc (Zn), iron (Fe), magnesium (Mg), and calcium (Ca) in chickpea seed are important constituents in vegetarian diets. The aim was to investigate associations of these nutrients in different chickpea (*Cicer arietinum* L.) cultivars with phytic acid (PA), another naturally occurring constituent of grain that may influence the bioavailability of mineral micronutrients. Chickpea was grown at Saskatoon and Swift Current, SK, in 2002 and 2003, representing dryland production from high-yielding locations in western Canada. Minerals were measured by atomic absorption spectroscopy; PA was measured using high-performance anion-exchange conductivity detection methodology. Seed from 10 genotypes contained from 29 to 52 mg/kg Zn, 77–112 mg/kg Fe, 1,448–2,457 mg/kg Mg, 1,211–2,457 mg/kg Ca, to 3.8–9.0 mg/g PA. Phytic acid, Fe, Mg, and Ca decreased in 2003 from 2002 concentrations. Kabulis had greater Zn, the same Fe, but lower Mg and Ca concentrations than desi genotypes. Large-seeded genotypes had greater or the same Zn, the same Fe and Mg, but lower Ca than small-seeded genotypes. Iron and Ca concentrations positively correlated with PA concentration. Nutrients were affected by environment and genotype, which means that chickpea can be exploited by breeding, in addition to sourcing favorable nutritional profiles by environment, seed size, and market class.

Keywords Chickpea · Phytic acid · Zinc · Iron · Magnesium · Calcium · Cultivar

Abbreviations

AAS	Atomic absorption spectrometry
HPAE	High-performance anion-exchange conductivity detection
HPLC	High-performance liquid chromatography
PA	Phytic acid

Introduction

Micronutrient malnutrition, the hidden hunger, affects more than half of the world's population, especially in Asia, Africa, and Latin America. Populations in these regions depend mainly on cereal diets that are frequently deficient in iron (Fe), zinc (Zn), calcium (Ca), and magnesium (Mg). Traditional methods of addressing micronutrient deficiency such as dietary supplementation, food fortification, and dietary diversification have had limited success in many regions and populations due to the lack of social and economic infrastructure [1]. Therefore, long-term and sustainable solutions are urgently needed to reduce micronutrient malnutrition in developing countries [2, 3]. Biofortification can address micronutrients in diets by improving soil fertility or improving nutritional potential through traditional plant breeding [4]. Variation in seed nutrition may be due to genotype, market class (seed size, color), and the growing environment and may also be enhanced by premarket processing such as size sorting, dehulling, or

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splitting [5, 6], or milling [7, 8]. Significant genetic variation in Fe and Zn concentrations with low concentrations of phytic acid (PA) is being reported in various crops, including pulses [3, 4, 6, 9].

Phytic acid (PA) is an antinutrient present mainly in legumes and cereals. Generally, PA is present at levels of 5–50 mg/g (0.5–5% w/w) in edible legumes, nuts, cereals, and oil seeds [10–13]. Phytic acid has the potential to bind mineral micronutrients (e.g., Fe, Zn, and Cu) in a food matrix and thereby reduce their bioavailability in humans. Recent studies have indicated that PA is an antioxidant, it shows anticarcinogenic/antineoplastic properties, reduces kidney stone formation, and is important in many human physiological functions [14, 15]. However, high concentrations of PA present in animal feed have been demonstrated to limit mineral micronutrient bioavailability, especially Ca and Zn utilization [11]. Therefore, cultivars containing low concentrations of seed PA have been developed for animal feed in maize (*Zea mays*), barley (*Hordeum vulgare*), rice (*Oryza sativa*), and wheat (*Triticum*), and the oil/protein crop soybean (*Glycine max*) [11, 16, 17]. Such cultivars produce 50–95% less PA in their seeds, but long-term dietary effects of the reduction in PA are unknown.

Micronutrient and PA concentrations in the pulse lentil may vary depending on the geographical location, plant genotype, soil factors, temperature, and other growing season conditions [18, 19]. Temperature and soil factors of the growing location such as soil P concentration during grain filling have significant impact on pulse PA concentration [19–21]. Among the pulses, chickpea (*Cicer arietinum* L.) is an excellent source of complex carbohydrates, proteins, dietary fiber, and energy [22–25] and an important ingredient in many Middle Eastern and Indian dishes. Pulses combined with cereals are major dietary components for billions of people, and the potential for micronutrient biofortification of pulses is high. Chickpea is an important source of micronutrients like Fe, Zn, Mg, and Ca in vegetarian diets [24–26]. World chickpea production is approximately 9.8 million metric tonnes, and about 50–60% is grown in India [27]. Canada began producing chickpea in the late 1980s and has many cultivars available to suit production areas and market classes.

Knowledge of micronutrient content, environmental influence, and genetic variation could be used in the present to source micronutrient-rich chickpea, and in the future to improve cultivar content. Our objectives were to (1) determine the concentration of PA in a range of chickpea genotypes grown in western Canada, (2) determine the concentrations of Zn, Fe, Mg, and Ca in the same genotypes, and (3) investigate how PA, Zn, Fe, Mg, and Ca concentrations are affected by environment, genotype, market class, and seed size.

Experimental methods

Materials

Standards, chemicals, and high-purity HPLC solvents used for PA extraction, HPLC, and micronutrient analysis (Fe, Zn, Ca, Mg) were purchased from Sigma–Aldrich Co. (St. Louis, MO) and Alfa Aesar—A Johnson Matthey Company and used without further purification.

Chickpea seed samples

Ten chickpea genotypes consisting of nine registered cultivars and one unregistered genotype, adapted to western Canada and the northwestern USA, were chosen with a range of seed size and market class (Table 1). The unregistered genotype from the Crop Development Center (CDC, University of Saskatchewan) breeding program was 9207340 (a desi type; 370 mg seed^{-1}).

Field experiments

Field experiments were conducted in 2002 and 2003 at Saskatoon (52.1′N, 106.41′W) and Swift Current (50.2′N, 107.4′W), Saskatchewan, Canada, under typical chickpea production practices. Seed yields from these four environment years represent a wide range of environmental conditions with low- to high-yielding conditions and typical production management. At Saskatoon, the soil was a Dark Brown Chernozem (Typic Borolls), and at Swift Current, the soil was an Orthic Brown Chernozem (Aridic Haploborolls). In 2002, 10 genotypes were measured; in 2003, eight genotypes of the same set were used. Seed was sown at 45 plants m^{-2} and inoculated with commercial rhizobia inoculant for nitrogen fixation at 50 mm depth with a row spacing of 0.3 m. At both locations, plot size was 8 rows and 2.4 m wide by 4.9 m long. Weeds were controlled using a preseeding application of ethalfluralin plus a preemergence application of imazethapyr at recommended rates and hand weeded during the growing season. One application of chlorothalonil was used at initial flowering, followed by two sprays separated by 10-day intervals of pyraclostrobin at recommended rates to control fungal disease in 2002. In 2003, only one fungicide application was needed.

The weather of 2002 was close to the 30-year climate normals for both Swift Current and Saskatoon crop seasons, but 2003 was drier and warmer (Table 2). The Swift Current 2002 growing season was slightly cool due to a lower maximum temperature and a substantially high cumulative precipitation, and plot yields were above average and the highest in the data set. Saskatoon 2002 was slightly cool with cooler nights but warmer days, almost normal precipitation, and plots yielded above average.

Table 1 Market class, seed size, and protein content of 10 chickpea genotypes grown in Saskatchewan, western Canada

Market class	Genotype	Coat color	Average seed size (mg seed^{-1})	Protein[a] (%)	Seed-size category
Kabuli	CDC Chico	Cream	265	23.1	Small
Kabuli	CDC Diva	Cream	490	23.0	Large
Kabuli	CDC Xena	Cream	470	23.2	Large
Kabuli	CDC Yuma	Cream	410	23.3	Large
Desi	9207340	Tan	370	18.6	Large
Desi	Amit[b]	Tan	265	18.4	Small
Desi	CDC Anna	Tan	220	20.5	Small
Desi	CDC Desiray	Light tan	200	21.7	Small
Desi	Myles	Tan	200	23.4	Small
Desi	CDC Nika	Tan	330	22.1	Large

[a] Protein (% by dry weight at 13% moisture) was calculated from total seed nitrogen content × 6.25

[b] Amit was formerly known as B-90

Table 2 Climate normals (1976–2006, Environment Canada), weather summary, and yield for chickpea grown in 2002 and 2003 at Swift Current and Saskatoon

	May to August				End of season	
	Average minimum temperature (°C)	Average maximum temperature (°C)	Average temperature (°C)	Cumulative precipitation (mm)	Seed yield[a] (kg ha^{-1})	Mean seed size (mg seed^{-1})
Location year						
Swift Current 2002	8.5	21.3	14.9	325.0	2,234[b]a	335 a
Swift Current 2003	9.6	23.7	16.7	117.5	1,409 c	328 a
Saskatoon 2002	8.1	23.3	15.7	198.4	1,708 b	329 a
Saskatoon 2003	9.3	24.7	17.0	113.5	758 d	296 b
Climate normals						
Swift Current	8.5	22.4	15.5	219.0	–	–
Saskatoon	8.9	22.6	15.8	209.4	–	–

[a] Mean yield (at 13% moisture) of eight genotypes grown at all four location-years

[b] Means followed by the same letter do not differ at $P < 0.05$

Swift Current and Saskatoon 2003 were record hot dry years, with warmer temperatures, low cumulative precipitation, and yields were well below average. Yield was combine-harvested at physiological maturity from interior rows for each plot. Harvested seed was dried at 60 °C for a week, cleaned, and weighed for seed yields. 2003 seed contained immature seed and was hand sorted to remove immature and aborted seed (seed smaller than 100 mg). The mean seed size from a subsample of 100 seed per plot from the eight genotypes common to both locations and years is listed in Table 2. Despite the removal of immature seed in 2003, seed size averaged over both locations in Table 2 was lower ($P < 0.001$) in 2003 (312 mg) than in 2002 (332 mg). Averaged over years, seed at Swift Current (331 mg) was significantly larger ($P < 0.001$) than at Saskatoon (313 mg). Subsamples of bulk seed per plot were ground (UDY Cyclone Sample Mill, UDY Corporation, Fort Collins, Colorado) to a meal using a 0.5 mm screen size after 1 year of room temperature storage and then stored for 5 years at −18 °C followed by 2 years at room temperature.

Phytic acid analysis

Selected replicated seed samples grown in each plot were prepared using a modified PA extraction method described [6, 19]. One hundred mg each of finely ground samples (<0.5 mm sieve) was extracted in acidic conditions to release phytic acid from the sample matrix and immediately analyzed. A Waters 2695 separation module attached to a Waters 432 conductivity detector was employed for high-performance ion-exchange (HPAE) separation analysis (Waters, Mississauga, ON, Canada). Briefly, extracted PA was separated using an Omnipac Pax-100 anion-exchange column (250 × 4 mm I.D.) connected to an Ominipac Pax-100 (8 µm) guard column (Dionex, Sunnyvale, CA) in a series. A gradient mobile phase using 130 mM sodium hydroxide (A), deionized water-isopropanol (50:50, v/v) (B), and water (C) was applied at 1.0 mL/min similar to previously described gradients [28]. An anion suppressor ASRS® 300 4-mm (Dionex, Sunnyvale, CA) system suppressed the mobile phase conductivities prior to the PA detection [6, 28].

Micronutrient analysis

Subsamples of ground seed for the determination of Fe, Zn, Mg, and Ca concentrations were taken from ground sample of each replicated field plot for each environment year. Each sample was prepared by standard HNO_3–H_2O_2 digestion [29]. All chemicals used were of analytical grade or higher purity. Approximately 200 mg of chickpea meal (<0.5 mm particle size) per sample was placed in a digestion tube with 3 mL of conc (70%) HNO_3. Tubes were heated to 70–80 °C to complete the digestion, and then, 0.5 mL of 30% H_2O_2 was added and vortexed for 2 min. After digestion, the sample solution was diluted with Millipore water to a known volume (25 mL). Measurements of total Fe and Zn concentration were validated using NIST standard reference material 1573a (tomato leaves; $[\mathrm{Fe}] = 368 \pm 21\ \mathrm{mg\ kg^{-1}}$) and $[\mathrm{Zn}] = 30.9 \pm 0.8\ \mathrm{mg\ kg^{-1}}$). Elements were measured by atomic absorption spectroscopy (AAS) using an AJ ANOVA 300 (Lab Synergy, Goshen, NY, USA).

Statistical analysis

The experiment was a randomized complete block design with the genotypes replicated four times for each of four environment years. Analysis of variance was performed for each micronutrient using the mixed and general linear model procedures of SAS [30] with environment and year as a fixed effect. Means separation was done using Fisher's protected least significant difference (LSD) at the 0.05 level of significance, and contrast statements at the 0.05 level of significance were used to test whether micronutrients were affected by market class (desi vs. kabuli) or seed-size category (large vs. small).

Results and discussion

Statistical analyses for each separate environment year demonstrated both genetic variation and the strong effects of weather on growing conditions and seed quality (Table 3). In 2002 for both Swift Current and Saskatoon, PA in seed differed due to genotype and market class. The effect of seed size within an environment year did not have a significant impact on PA measurement with the exception of the high-yielding location of Swift Current (2002). In 2003, yields and PA concentrations in seed were lower across genotypes and so the range of variation was reduced for both locations. In 2003, PA in seed was not affected by genotype, market class, or seed size. Generally, in 2002, the micronutrients Zn, Fe, Mg, and Ca were affected by genotype and market class, with two exceptions (Table 3). Seed-size category only affected Fe at Saskatoon and Mg and Ca at Swift Current in 2002. In 2003, Zn was only affected by genotype, market class, and seed size at Swift Current, not Saskatoon. Iron was relatively constant across genotypes, except for a detectable seed-size effect for Saskatoon only. Magnesium was constant in Swift Current grown seed, but differed according to genotype, market class, and seed size at Saskatoon. Calcium was consistently affected by genotype and market class for each of the four location years. Calcium was also significantly affected by seed-size category at both locations in 2003.

Phytic acid

The mean PA concentrations for each of the 10 genotypes tested from 2002 are listed in Table 4. The greatest range in means separation was seen for Swift Current 2002, where CDC Diva had the highest PA concentration followed by CDC Chico, CDC Xena, and CDC Yuma. A similar effect was seen in Saskatoon 2002, with the highest PA concentrations in CDC Xena, CDC Chico, and CDC Diva. Kabuli chickpea also contained more PA (7.9 mg/g) compared with desis (6.8 mg/g) in Swift Current and also in Saskatoon (6.8 mg/g for Kabulis, 5.9 mg/g for desis). Large-seeded genotypes had greater PA concentrations than small-seeded genotypes in Swift Current (7.7 mg/g for large, 7.0 mg/g for small) but not in Saskatoon (6.5 mg/g for large, 6.2 mg/g for small). In contrast to 2002, seed in 2003 had lower mean PA concentrations for Swift Current (4.6 mg/g) and Saskatoon (4.1 mg/g; Table 4). Therefore, in high-yielding environment years (2002) where chickpea plants had opportunity to develop seeds to maximum size and produce good yields, seed PA was increased with location and genotype differences evident. In lower-yielding environment years (2003), plants had less opportunity to produce optimal seed size and yield, and seed PA levels were lower along with reduced variation across genotypes.

Our chickpea PA concentrations ranged from 5.2 to 9 mg/g across genotypes in a cool year for western Canada and 4–5 mg/g for a hot dry year, using HPAE separation technology, similar to 2.5–8 mg/g reported for three chick-

Table 3 Analysis of variance for phytic acid, Zn, Fe, Mg, and Ca concentrations in chickpea seed for 10 genotypes grown in 2002 and eight genotypes grown in 2003 at Swift Current (SC) and Saskatoon (ST)

Variable	Phytic acid (mg/g DW)		Zn (mg/kg DW)		Fe (mg/kg DW)		Mg (mg/kg DW)		Ca (mg/kg DW)	
Location	SC	ST	SC	ST	SC	ST	SC	ST	SC	ST
2002										
Genotype	*	*	NS	**	*	**	***	*	***	*
Kabuli vs. desi	**	**	*	**	*	NS	***	**	*	**
Large vs. small seed	*	NS	NS	NS	NS	**	**	NS	***	NS
2003										
Genotype	NS	NS	**	NS	NS	NS	NS	*	**	**
Kabuli vs. desi	NS	NS	**	NS	NS	NS	NS	**	***	***
Large vs. small seed	NS	NS	**	NS	NS	*	NS	*	**	***

Contrasts for market class (kabuli vs. desi) and seed-size category (large-seeded genotypes vs. small-seeded genotypes) are indicated below genotype. Seed were on a dry weight (DW) basis with 13% moisture content

*, **, *** Significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, NS denotes nonsignificant at $P < 0.05$

Table 4 Mean phytic acid in chickpea seed for 10 genotypes grown at two locations in 2002 and eight genotypes in 2003

Genotype\location	Phytic acid (mg/g dry weight)			
	2002		2003	
	Swift Current	Saskatoon	Swift Current	Saskatoon
9207340	7.2[a]bcd	6.1 abc	–	–
Amit	7.0 bcd	6.5 ab	–	–
CDC Anna	6.3 d	5.2 c	4.4 a	4.0 a
CDC Chico	8.0 ab	7.2 ab	4.3 a	4.1 a
CDC Desiray	6.5 cd	6.3 abc	5.0 a	3.8 a
CDC Diva	9.0 a	7.1 ab	4.8 a	4.4 a
Myles	7.2 bcd	6.0 bc	4.4 a	4.1 a
CDC Nika	6.9 bcd	6.2 abc	4.6 a	3.8 a
CDC Xena	7.8 abc	7.3 a	4.7 a	4.3 a
CDC Yuma	7.8 abc	6.1 abc	4.6 a	3.8 a
Mean	7.36	6.39	4.52	4.04
LSD	0.15	0.12	0.10	0.10
Contrasts				
Desi	6.8 b	5.9 b	4.6 a	3.9 a
Kabuli	7.9 a	6.8 a	4.5 a	4.2 a
Large seeded	7.7 a	6.5 a	4.6 a	4.1 a
Small seeded	7.0 b	6.2 a	4.5 a	4.0 a

Data are the mean of four replicates. Seed were on a dry weight basis with 13% moisture content

[a] Treatments (genotypes) from a group within a column (location), if followed by the same letter, do not differ at $P < 0.05$

pea cultivars in six environment years in Greece [20]. Other researchers report 9–18 mg/g PA for field-grown chickpea in Spain [31, 32], 5.8–10 mg/g for field-grown chickpea in Egypt [22], 8.2 mg/g in Brazil [33], 2.3–6 mg/g for field-grown chickpea in Pakistan [34], 18 mg/g for field-grown chickpea in northern USA [35], and 9.8 mg/g in field-grown chickpea cotyledons from India [8], all from different extraction methodology.

Mineral micronutrients

In 2002, Zn concentrations of 35 mg/kg were low at Swift Current, with kabuli having 37 mg/kg compared with 33 mg/kg for desi genotypes (Table 5). The same pattern was seen at Saskatoon although Zn concentrations were slightly higher than at Swift Current; Amit and CDC Yuma had the highest concentrations (>42 mg/kg). The literature

Table 5 Mean Zn, Fe, Mg, and Ca concentrations in chickpea seed for 10 genotypes grown at two locations in 2002

Genotype\location	Zn (mg/kg dry weight)		Fe (mg/kg dry weight)		Mg (mg/kg dry weight)		Ca (mg/kg dry weight)	
	Swift Current	Saskatoon	Swift Current	Saskatoon	Swift Current	Saskatoon	Swift Current	Saskatoon
9207340	29[a]a	32 c	87 c	99 de	1,590 cde	1,790 ab	1,860 de	1,710 abc
Amit	36 a	48 a	101 ab	112 ab	1,560 de	1,500 d	1,990 cd	1,450 c
CDC Anna	35 a	32 c	100 ab	105 bcde	1,600 cde	1,550 d	2,100 bc	1,760 abc
CDC Chico	37 a	38 bc	103 ab	115 a	1,640 cd	1,610 bcd	2,340 a	1,610 bc
CDC Desiray	30 a	35 bc	94 bc	106 abcde	1,740 ab	1,770 abc	2,270 ab	1,810 ab
CDC Diva	37 a	38 bc	97 bc	96 e	1,610 cd	1,630 bcd	2,040 cd	1,700 abc
Myles	34 a	36 bc	99 ab	108 abcd	1,820 a	1,860 a	2,460 a	2,010 a
CDC Nika	34 a	31 c	96 bc	102 cde	1,660 bc	1,670 bcd	1,760 c	1,470 c
CDC Xena	37 a	38 bc	93 bc	103 bcde	1,500 e	1,580 cd	1,817 de	1,600 bc
CDC Yuma	38 a	43 ab	109 a	111 abc	1,540 de	1,590 cd	1,740 e	1,490 bc
Mean	34.8	37.0	97.7	105.6	1,625	1,648	2,036	1,660
LSD	10	9	11	10	100	190	220	330
Contrasts								
Desi	33 b	33 b	95 b	104 a	1,680 a	1,720 a	2,090 a	1,750 a
Kabuli	37 a	41 a	100 a	108 a	1,570 b	1,580 b	1,980 b	1,570 b
Large seeded	35 a	36 a	96 a	102 b	1,580 b	1,650 a	1,840 b	1,590 a
Small seeded	34 a	38 a	99 a	109 a	1,670 a	1,650 a	2,230 a	1,730 a

Data are the mean of four replicates. Seed were on a dry weight basis with 13% moisture content

[a] Treatments (genotypes) from a group within a column (location), if followed by the same letter, do not differ at $P < 0.05$

reports Zn concentrations in chickpea as a similar or higher amount of 29.6–43.8 [31], 38.6–44.2 [7], 29.8–60.3 [24], and 73 mg/kg in cooked chickpea [33]. Other western Canada grown chickpea samples, sourced from Saskatchewan and Manitoba processors, contained Zn concentrations of 40.7 mg/kg for desi and 34.0 mg/kg for kabuli [25]. In 2002, kabuli genotypes had higher Fe concentrations in Saskatoon than Swift Current and higher Fe concentrations in the larger seeded genotypes in Saskatoon. Genotypes Amit, CDC Chico, and CDC Yuma had the highest Fe concentrations in both locations (2002). Iron concentrations of chickpea in the literature range from 32.1 to 133 mg/kg [7, 24, 31, 33], with western Canada grown desi having 45.9 mg/kg of Fe and kabuli having 55.0 mg/kg Fe [25].

In 2002, Mg and Ca concentrations in genotypes showed a similar pattern to each other. Desi genotypes had higher concentrations than kabuli genotypes of both elements, and for Swift Current, small-seeded genotypes also had higher concentrations of both than large-seeded genotypes. In general, the genotypes CDC Desiray and Myles exhibited the highest Mg concentrations (>1,730 mg/kg) at either location in 2002 and the same genotypes had the highest Ca concentrations (>2,260 mg/kg in Swift Current; >1,800 mg/kg in Saskatoon) out of the genotypes. Previous reports of Mg concentration in chickpea place this element in the range of 1,650–1,950 mg/kg [7], 1,400–2,400 [24], including 1,690 mg/kg for desi and 1,470 mg/kg for kabuli grown in western Canada [25]. Calcium concentration reported for chickpea ranged from 840 to 3,770 mg/kg [7, 24, 26, 36], with western Canadian desi having 1,650 mg/kg Ca and kabuli having 817 mg/kg Ca [25].

In the warmer year of 2003, the highest Zn concentrations were seen in Saskatoon, averaging 45 mg/kg (Table 6). In Swift Current, CDC Yuma and CDC Diva had the highest Zn concentration and CDC Desiray had the lowest, kabuli genotypes and large-seeded genotypes also had higher Zn concentrations than desi and small-seeded genotypes, respectively. In Saskatoon 2003, CDC Yuma had a higher Zn concentration than CDC Desiray, with the other genotypes being intermediate. Therefore, market class and seed-size category had less impact on Zn for this location year. Iron concentration was not significantly different (84 mg/kg) across genotypes for Swift Current but was significantly higher in Myles and CDC Chico than CDC Diva and CDC Xena in Saskatoon. In Saskatoon, desi genotypes had a higher Fe concentration than kabuli genotypes, as did the small seed-size category compared with the large seed size.

In 2003, the lowest Mg concentrations (mean of 1,540 mg/kg) occurred in Swift Current, in contrast to the highest Mg (mean of 1,730 mg/kg) in Saskatoon (Table 6), both higher than 1,340 mg/kg [37] or 1,010 mg/kg [33]. CDC Diva, Myles, and CDC Nika had the highest Mg concentrations in Swift Current, and CDC Desiray, Myles,

Table 6 Mean Zn, Fe, Mg, and Ca concentrations in chickpea seed for eight genotypes grown at two locations in 2003

Genotype\location	Zn (mg/kg dry weight)		Fe (mg/kg dry weight)		Mg (mg/kg dry weight)		Ca (mg/kg dry weight)	
	Swift Current	Saskatoon	Swift Current	Saskatoon	Swift Current	Saskatoon	Swift Current	Saskatoon
CDC Anna	31[a]bc	40 ab	81 a	83 ab	1,470 bc	1,760 ab	1,490 c	1,660 bc
CDC Chico	33 bc	47 ab	83 a	83 a	1,450 c	1,740 abc	1,370 c	1,690 abc
CDC Desiray	29 c	39 b	83 a	80 ab	1,570 abc	1,790 ab	1,790 b	1,970 a
CDC Diva	40 a	43 ab	83 a	77 b	1,640 ab	1,680 abc	1,380 c	1,310 d
Myles	32 bc	41 ab	84 a	84 a	1,600 ab	1,830 a	2,208 a	1,890 ab
CDC Nika	31 bc	47 ab	81 a	83 ab	1,660 a	1,790 ab	1,530 bc	1,650 bc
CDC Xena	36 ab	44 ab	83 a	77 b	1,500 abc	1,600 c	1,300 c	1,210 d
CDC Yuma	40 a	52 a	91 a	81 ab	1,500 abc	1,640 bc	1,350 c	1,440 cd
Mean	33.9	44.3	83.5	81.2	1,540	1,726	1,513	1,591
LSD	5	13	15	6	180	155	300	290
Contrasts								
Desi	31 b	42 a	82 a	83 a	1,570 a	1,790 a	1,670 a	1,780 a
Kabuli	37 a	47 a	85 a	80 b	1,510 a	1,670 b	1,350 b	1,410 b
Large seeded	36 a	47 a	85 a	80 b	1,580 a	1,680 b	1,400 b	1,400 b
Small seeded	31 b	42 a	82 a	83 a	1,500 a	1,780 a	1,630 a	1,790 a

Data are the mean of four replicates. Seed were on a dry weight basis with 13% moisture content

[a] Treatments (genotypes) from a group within a column (location), if followed by the same letter, do not differ at $P < 0.05$

and CDC Nika had the highest in Saskatoon. The reduced range of Mg concentrations in Swift Current was associated with similar market class effects and similar seed-size effects. But in Saskatoon, Mg concentrations were significantly greater for desi compared with kabuli genotypes and significantly greater for small-seeded compared with large-seeded genotypes. Myles had the highest Ca concentration at Swift Current in 2003, and this genotype was also one of several genotypes having high Ca concentration in Saskatoon. In contrast, Xena, a large-seeded kabuli, had consistently low Ca concentration for both locations in 2003. Desi genotypes had significantly greater Ca concentrations (>1,660 mg/kg) than kabulis, and small-seeded genotypes had significantly greater Ca concentrations than large-seeded genotypes. However, mean Ca concentration in 2003, the warm year, was lower than Ca concentration seen in 2002. Presumably, genotypes under heat and drought stress transpire less, resulting in lower seed accumulation of Mg, Ca, and likely Fe. Zinc appeared to be less responsive to environment.

To demonstrate potential associations between micronutrients, pairwise correlations are listed in Table 7. Phytic acid was moderately and inversely correlated with Zn ($r = -0.26$), had no significant association with Mg, but was strongly and positively associated with both Fe ($r = 0.60$) and Ca. The relationships between PA and Fe (Fig. 1) and, to a lesser extent, between PA and Ca (Fig. 2) were stronger than the other associations explored. Zinc was also moderately and inversely associated with Ca concentration, meaning that as Ca increased, Zn would decrease. Iron and Mg concentrations were moderately and positively associated with Ca concentration, meaning that as Ca increased, these other elements would increase. Any growing conditions that therefore positively influenced seed Ca concentration also increased Fe and Mg concentrations.

Table 7 Correlation coefficients for phytic acid, Zn, Fe, Mg, and Ca concentrations in chickpea seed for eight genotypes across four location years

	Phytic acid	Zn	Fe	Mg
Zn	−0.26*			
Fe	0.60*	−0.08		
Mg	−0.11	0.12	−0.01	
Ca	0.40*	−0.21*	0.25*	0.33*

Seed were on a dry weight basis with 13% moisture content. $N = 115$

* Significant at $P < 0.05$

Implications

Micronutrient malnutrition affects more than half of global populations. Whole food-based solutions have a greater promise of delivering micronutrients to population levels than that of food fortification, dietary supplementation, and diversification. Biofortification of commonly eaten foods with Fe, Zn, Ca, and Mg could have significant positive

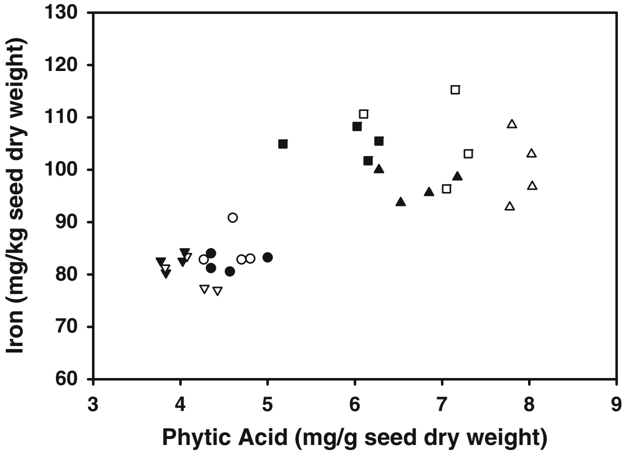

Fig. 1 *Scatter graph* illustrating the relationship between iron and phytic acid concentration in seed meal from each of eight genotypes of chickpea grown at four location years. *Symbol legend* Swift Current 2002 *triangle up*; Saskatoon 2002 *square*; Swift Current 2003 *circle*; Saskatoon 2003 *triangle down*; *solid black symbols* denote desi and *open symbols* denote kabuli. Data are the mean of four replicates. The correlation coefficient *r* is 0.60

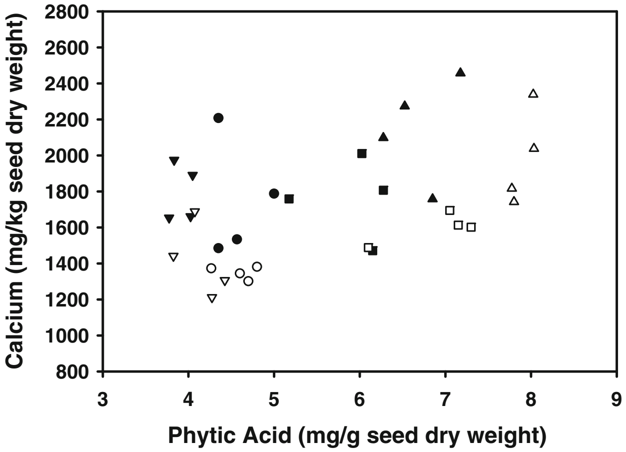

Fig. 2 *Scatter graph* illustrating the relationship between calcium and phytic acid concentration in seed meal from each of eight genotypes of chickpea grown at four location years. *Symbol legend* Swift Current 2002 *triangle up*; Saskatoon 2002 *square*; Swift Current 2003 *circle*; Saskatoon 2003 *triangle down*; *solid black symbols* denote desi and *open symbols* denote kabuli. Data are the mean of four replicates. The correlation coefficient *r* is 0.40

health benefits to the most vulnerable populations around the world. Our results clearly show that chickpea could be a potential crop for micronutrient biofortification. Lower PA in food legumes leads to increased mineral bioavailabilities [6, 16, 31, 37, 38], so a low PA chickpea would likely have a greater bioavailability of mineral micronutrients than high PA chickpea. The identification of suitable chickpea genotypes with low PA and high mineral composition is being used to deliver higher-quality protein and trace element composition in infant foods [31]. But low PA chickpea would need to be selected without lowering key trace elements in seed. We found that Fe and Ca concentrations positively correlated with PA concentration across a range of genotypes, so physiological mechanisms associated with trace element deposition in seed would need to be changed somewhat independently of phytic acid deposition.

Chickpea seed size has several implications as follows: consumer preference, cooking qualities, nutrient concentrations. While kabuli chickpea had greater Zn concentration, seed size had less effect on Zn. Iron concentration tended to be less affected by market class, although small-seeded chickpea contained more Fe than large seeded in half the environments. Desi chickpea had greater Mg and Ca, and small-seeded chickpea also contained more Mg in half the environments and always more Ca. These features of market class and seed size have opportunity for chickpea production specifically suited to consumer preferences while providing sufficient nutrients. Small-seeded chickpea and the desi market class would, therefore, be the best source of Ca among the current cultivars in western Canada. Small-seeded chickpea has a different surface-to-mass ratio than large-seeded chickpea, and for a fixed soil availability of calcium and water, small seeds from a range of genetic material have greater Ca concentrations [26]. Despite the seed-size effect on Ca concentration, chickpea can be bred for higher Ca because genes for seed-size segregate independently of high Ca concentration [26]. Our chickpea Ca concentrations ranged from 1,450 to 2,460 mg/kg across genotypes and environment years and were in the midrange reported for Ca in chickpea genotypes and breeding lines grown in Israel [26]. So Ca concentration could be further increased in Canadian cultivars. Therefore, in chickpea, seed with favorable nutritional profiles could be sourced by production region, market class, seed size, and even from cultivars specifically bred for superior nutrition.

Canada is currently one of the world major exporters of chickpea. Favorable soil micronutrient profiles in Canadian soils and cooler climatic factors could be exploited for producing chickpea with rich micronutrient profiles. For example, previous Canadian grown lentils are a good source of Fe, Zn [19], and Se [18, 29] compared with lentil grown under long-term temperature regimes typical of Lucknow, India [19]. Along the same lines, Canadian grown chickpea might have favorable conditions to synthesize lower PA concentration than warmer climatic zones around the world, particularly those that constrain seed size and seed metabolism via drought or excessive heat.

Contrasting our four Canadian environments, we found lower PA in field-grown chickpea in the hot dry year of 2003 compared with 2002 seed PA concentrations. Cultivar, location, and year effects on chickpea PA concentration, where two out of three cultivars at one location had PA lowered in a high rainfall year, have also been reported

[20]. This study [20] concluded that chickpea with lower antinutrients could be sourced through choice of genetics, seed size, and cultivation area. Our lower PA concentrations, along with lower yields and smaller seed size in 2003, suggest that PA metabolism and accumulation in seed are moderated by many environmental factors. In heat and drought, seed size would also be reduced, and the link between actual seed size, PA, and other micronutrients in chickpea requires further investigation.

Conclusions

In field-grown chickpea, PA concentration varied considerably across genotypes, locations, and years. The year effect was the strongest factor in driving variation in PA concentration, demonstrating that PA in seed is influenced by temperature and rainfall during the growing season. Chickpea PA can be exploited by breeding or by sourcing favorable nutritional profiles by environment, market class, and possibly by seed size. Chickpea seed had 6–9 mg/g PA concentration when grown in wetter environments, particularly for kabuli or large-seeded genotypes. When chickpea was grown in a hot dry year, the drought and heat reduced the range of PA to <5 mg/g.

One gram of chickpea meal contained from 34 to 45 μg of Zn and 82–106 μg of Fe across environment years. Slight cultivar variation in Zn was seen in 2002, and kabulis also tended to contain more Zn. As Zn concentration increased, PA concentration decreased. In contrast, Fe showed a strong significant positive correlation with PA, implying that genotypes with greater Fe content have greater PA content and may not necessarily have greater bioavailability or easily digestible forms of Fe.

Magnesium (1,540–1,730 mg/kg) and Ca (1,510–2,035 mg/kg) are elements associated with the ability of plants to supply seed with water. Seed had greater concentrations of Mg and Ca in the wetter year, and heat lowered Ca concentrations significantly. Desi and small-seeded chickpea tended to contain more Ca within an environment. As seed Ca concentration increased, PA concentration increased. Until chickpea seed is selected so that physiological mechanisms responsible for depositing key mineral elements are more independent from phytic acid deposition, sourcing a more nutritious chickpea with greater Ca concentration would mean seed would also contain greater concentrations of PA.

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