JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Oxalate in Grain Amaranth

BRUCE GÉLINAS AND PHILIPPE SEGUIN*

Department of Plant Science, McGill University, Macdonald Campus, 21111 Lakeshore Road, Sainte-Anne-de-Bellevue, QC H9X 3V9, Canada

Grain amaranth (*Amaranthus* spp.) is a widely adaptable C4 pseudo-cereal crop that has interesting nutritional characteristics including high protein and calcium concentrations and a lack of gluten. To date, no antinutrient has been found at problematic levels in grain amaranth; however, oxalate has not been thoroughly studied. Dietary oxalate is a potential risk factor for kidney stone development, and its presence in food lowers calcium and magnesium availability. Oxalate concentration and forms and calcium and magnesium concentrations were determined in 30 field-grown grain amaranth genotypes from the species *A. cruentus*, *A. hybrid*, and *A. hypochondriacus*. The effects of seeding date and fertilization with calcium ammonium nitrate were evaluated in field experiments conducted in multiple environments; the effects of cooking were also evaluated. Mean total oxalate concentration in the 30 genotypes analyzed was 229 mg/100 g, with values ranging between 178 and 278 mg/100 g, the greatest proportion being insoluble (average of 80%). Calcium concentration averaged 186 mg/100 g and ranged between 134 and 370 mg/100 g, whereas magnesium averaged 280 mg/100 g and ranged between 230 and 387 mg/100 g. Fertilization only marginally increased total oxalate concentration and had no effects on other variables. Seeding date had no effects on any of the variables studied. Boiling increased the proportion of soluble oxalate but did not affect total oxalate concentration. Grain amaranth can be considered a high oxalate source, however, as most is in insoluble form, and due to its high calcium and magnesium concentrations, oxalate absorbability could be low. This should be confirmed by bioavailability studies.

KEYWORDS: Grain amaranth; *Amaranthus*; oxalate; calcium; kidney stones; new crops

INTRODUCTION

Grain amaranth (*Amaranthus* spp.) is a C4 dicotyledonous (*1*) pseudo-cereal crop that was widely cultivated in pre-Columbian America (*2*). After a drastic decline of the crop following the Spanish conquest, interest in grain amaranth was revived in the 1970s, and emphasis was put on adapting the crop to mechanized agriculture (*3*). This plant is adaptable to a wide range of environments and has been successfully introduced in several countries of Europe, Asia, and Africa (*3*). Grain amaranth has interesting nutritional properties such as a high content of lysine-rich protein (*4*) and an absence of gluten (*5*). It is estimated that over 2.1 million people in the United States alone are affected by celiac disease, which renders affected people intolerant to gluten (*6*). This represents a great potential market for grain amaranth. Calcium in amaranth was reported to be as high as 308 mg/100 g (*7*), and its grain has thus been proposed as a weaning food ingredient (*8*). Most antinutrients that have been studied to date in grain amaranth appear to be nonproblematic. Levels of tannin and phytic acid are comparable to those observed in grain cereals (*9*), whereas trypsin and chymotrypsin activity were reported to be lower (*10*). The potential for aflatoxin and zearalenone production was also reported to be comparable to or lower than those of other grains (*11*). Saponin content is low, and the saponins produced are of low toxicity (*12*). However, there are concerns regarding grain amaranth oxalate concentration, given that amaranth leaves are known to contain high oxalate concentrations (*13*).

Oxalate is a naturally occurring antinutrient present in several cultivated plants such as spinach (*Spinacia oleracea* L.) and rhubarb (*Rheum rhabarbarum* L.). High oxalate intake can lead to mineral deficiency, most notably calcium and magnesium, as oxalic acid binds these two elements to form insoluble calcium or magnesium oxalate (*14*, *15*). Sodium and potassium oxalate are more soluble and are therefore considered to be more absorbable (*16*). Urinary oxalate excretion, which necessarily follows oxalate absorption, is known to be an important risk factor in the formation of kidney stones (*17*). Calcium oxalate stone formers usually have higher urinary oxalate excretion (*18*) and are advised to avoid oxalate-rich foods (*19*). The amount of urinary oxalate reported to be derived from the diet varies, ranging from 5 to 50%, the remaining being endogenously synthesized (*17*, *18*). Strawberries, chocolate, and soynuts are examples of common foods that have been shown to increase urinary oxalate levels (*16*). Calcium or magnesium ingestion along with oxalate is known to decrease oxalate absorption, as they form insoluble complexes (*20*). Therefore, the oxalate to

* Author to whom correspondence should be addressed [telephone (514) 398-7855; fax (514) 398-7897; e-mail philippe.seguin@mcgill.ca].

10.1021/jf070384d CCC: $37.00
Published on Web 05/19/2007

Table 1. Description of Soils Used for Field Experiments Conducted in Sainte-Anne-de-Bellevue, QC, Canada

field	soil type	pH	P (kg/ha)	K (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	organic matter (%)
A	loamy sand	6.70	335	408	3700	292	4.00
B	sandy clay loam	5.93	210	466	2331	235	3.10
C	sandy clay loam	5.85	195	193	2362	318	2.87
D	sandy clay loam	6.00	96	205	4927	641	4.25

calcium molar ratio of foodstuff is believed to influence the amount of oxalate absorbed (*17*). The proportion of soluble versus insoluble oxalate present in foodstuff is often regarded as an indicator of the bioavailability of oxalate (*16*, *17*), although this theory has been criticized (*18*).

Oxalate levels in a given crop can be controlled by cultivar selection and breeding, field management, and processing (*14*). Variation in oxalate concentrations and forms has been reported among soybean [*Glycine max* (L.) Merr.] cultivars (*21*). The oxalate concentration in spinach was manipulated by varying nitrogen levels and forms (*22*) and in bean leaves (*Phaseolus vulgaris* L.) by varying calcium supply (*23*). Calcium fertilization, however, did not affect oxalate concentration in soybean seeds (*24*). Boiling or steaming was found to decrease oxalate concentration in several vegetables (*25*).

To our knowledge, there has been only one report of grain amaranth oxalate concentration, with concentrations in one cultivar of *A. caudatus* ranging between 228 and 236 mg of total oxalate/100 g, with 35% in the soluble form (*26*). Such levels are similar to those reported for soybean and soy products and could be problematic for calcium oxalate stone formers (*27*). It is already known that oxalate is present in high concentrations in grain amaranth leaves, variation being observed between cultivars (*28*). It is not known whether a similar variability is present in the grain or whether agronomic practices could affect grain amaranth oxalate concentration.

If grain amaranth is to be consumed in appreciable amounts by people affected by celiac disease, by vegetarians looking for alternative protein sources, or due to its use as an ingredient in weaning food, it is important to minimize oxalate concentration in seeds. Therefore, our main objectives were to (i) determine variability in oxalate concentration and forms in seeds of 30 different grain amaranth genotypes representing 3 different species; and evaluate the effects of (ii) calcium ammonium nitrate fertilization, (iii) seeding date, and (iv) cooking on oxalate concentration and forms.

MATERIALS AND METHODS

General Description and Field Conditions. Field experiments were conducted in 2005 and 2006, at the Emile A. Lods Agronomy Research Center in Sainte-Anne-de-Bellevue, QC, Canada (45° 25′ 45″ N latitude, 73° 56′ 00″ W longitude). Soils used are described in **Table 1**. Except for the fertilization experiment, nitrogen, phosphorus, and potassium fertilization was done on the basis of soil tests according to local recommendations for sorghum (*29*), no recommendations existing for grain amaranth. All harvested seeds were dried at 50 °C until the moisture content reached about 9% and were stored at room temperature until analysis. Seeds were then ground through a 1 mm screen using a Tecator Cyclotec sample mill (Tecator Corp., Höganäs, Sweden).

Genotype Evaluation. Thirty grain amaranth genotypes (**Table 2**) were obtained from the U.S. National Genetic Resources Program (*30*). Plants were grown in 2005 in field A in a randomized complete block design with three blocks. Each plot consisted of one 2 m row. Plants were spaced 20 cm in the row and 80 cm between rows. The field was hand-seeded on June 9 and hand-harvested on October 30 (i.e, one week after the first killing frost). All plants were harvested.

Fertilization Experiment. Cultivar 'Plainsman' (Albert Lea Seedhouse, Albert Lea, MN) was grown in a randomized complete block design with three blocks, replicated over two growing seasons with two sites each season. Fields A and B were used in 2005 and fields C and D in 2006 (**Table 1**). Plots were 5 m long with four rows spaced 76 cm apart. Nitrogen was broadcast applied on the day of seeding in the form of calcium ammonium nitrate (27.5% N; 4.6% Ca; 2.4% Mg) at rates of 0, 50, 100, 150, and 200 kg of N ha^{-1}. Depending on the year and site, seeding was done between May 30 and June 1 at a rate of 1.5 kg ha^{-1} using a disk drill (Fabro, Swift Current, SK, Canada). Only the middle two rows of each plot were harvested. Harvesting was done mechanically using a self-propelled harvester (Wintersteiger, Saskatoon, SK, Canada) between October 28 and November 1.

Seeding Date Experiment. Field layout, plot size, seeding method, cultivar, and harvesting were as in the nitrogen fertilization experiment. The experiment was conducted in 2005 at two sites (i.e., fields A and B; **Table 1**). Seeding dates were May 20, May 31, and June 30. Harvesting was done on October 31 in field A and on October 27 in field B.

Cooking Experiment. Seeds of the cultivar 'Plainsman' were used for this experiment because it is currently the most widely grown grain amaranth cultivar in North America (*31*) and therefore the most consumed. Cooking was done by boiling at atmospheric pressure (uncovered) 50 mL of nonground seeds in 100 mL of distilled water until complete water absorption. The resulting porridge was spread as a thin layer on aluminum foil, dried for 3 days at 60 °C, ground through a 1 mm screen using a Tecator Cyclotec sample mill (Tecator Corp.), and stored at room temperature until analysis. The cooking experiment was repeated on two different days, with three replicates on the first day and four on the second.

Oxalate Quantification. Oxalate was quantified with an enzymatic kit (procedure 591, from Trinity Biotech, Newark, NJ) according to the method of Horner et al. (*21*), which allows quantification of total and insoluble oxalate. Soluble oxalate was determined by subtraction. Fifteen milliliter centrifuge tubes were used to carry out autoclaving and removal of soluble oxalate. A Branson 5510 (Kell-Strom, Wethersfield, CT) sonicator bath was used for sonication. Absorbance at 590 nm was measured using an Ultrospec II spectrophotometer (Biochrom Ltd., Cambridge, U.K.).

Calcium and Magnesium Determination. Ground dry seeds were digested using the method of Parkinson and Allen (*32*). Calcium and magnesium concentrations were determined using a Perkin-Elmer atomic absorption spectrophotometer (Perkin-Elmer, Waltham, MA). Only seeds from the field experiments were analyzed for Ca and Mg concentrations.

Statistical Analyses. All statistical analyses were performed with SAS 9.1 (*33*). Residuals were tested for normality, using the Shapiro−Wilk test in PROC UNIVARIATE. Logarithmic transformation was applied to non-normal data. Heterogeneity of variance was evaluated using the BIC criteria. Statistical analyses for the genotype and the cooking experiments were performed using the PROC GLM (*33*). Multiple comparison tests were performed with Scheffé's procedure. In the genotype experiment, given the objective of finding whether variation exists among genotypes, and due to the large number of entries, multiple comparison tests were not performed. The data from the seeding date and the nitrogen experiments were analyzed using PROC MIXED in SAS (*33*), because block and environment were considered to be random effects. Statistical models were elaborated according to the method of McIntosh (*34*). Regression analysis was performed with the data from the fertilization experiment. Using PROC CORR, Pearson product−moment correlation coefficients were calculated among variables from the genotype experiment. For all analyses, the level of statistical significance was set at 0.05.

RESULTS AND DISCUSSION

Genotypes. Among the 30 genotypes, there were significant differences ($p < 0.05$) in total and insoluble oxalate, as well as in calcium and magnesium concentrations and the oxalate/calcium ratio (**Table 3**). The mean total oxalate concentration of the 30 genotypes analyzed was 229 mg/100 g, with values

Table 2. Description of Grain Amaranth Genotypes Grown in Sainte-Anne-de-Bellevue, QC, Canada

genotype[a]	common descriptive	species	origin[b]	seed color	flower color
PI 451711		*Amaranthus cruentus*	Mexico	black	red
PI 477912	RRC 416	*A. cruentus*	Mexico	white	green
PI 477913	RRC 1011	*A. cruentus*	Mexico	white	green
PI 515959	Montana-3	*A. cruentus*	Montana	white	green
PI 525498	MT-5	*A. cruentus*	Montana	white	green
PI 538255	Amont	*A. cruentus*	Montana	white	green
PI 538319	K266	*A. cruentus*	Pennsylvania	white	green
PI 538320	K283	*A. cruentus*	Pennsylvania	white	pink
PI 538321	K436	*A. cruentus*	Pennsylvania	white	red
PI 538323	K432	*A. hybrid*	Pennsylvania	white	green
PI 538324	K433	*A. hybrid*	Pennsylvania	white	green
PI 538325	K593	*A. hybrid*	Pennsylvania	white	red
PI 538326	D70-1	*A. hybrid*	Pennsylvania	white	red
PI 538327	D136-1	*A. hybrid*	Pennsylvania	white	green
PI 558499	Plainsman	*A. hypochondriacus*	Pennsylvania	white	red
PI 568179	Ames 12991	*A. hybrid*	Iowa	white	red
PI 576447	Ames 13446	*A. cruentus*	Nigeria	brown	green
PI 604666	RRC 1027	*A. cruentus*	Pennsylvania	white	orange
PI 605354	R-158	*A. cruentus*	Pennsylvania	white, translucent	red
PI 606767	Ames 8272	*A. cruentus*	Pennsylvania	white	orange
PI 606797	A200D	*A. cruentus*	Illinois	white	green
PI 606799	RRC 1017	*A. cruentus*	Pennsylvania	white	red and green
PI 618962	Ames 2015	*A. cruentus*	Benin	dark brown	green
PI 619250	Ames 2265	*A. hypochondriacus*	Pennsylvania	golden	red, green, and pink
PI 628780	RRC 423	*A. cruentus*	Mexico	white	purple-red
PI 628781	RRC 444	*A. cruentus*	Mexico	white	green
PI 628782	RRC 446	*A. cruentus*	Mexico	white	green
PI 628783	RRC 776	*A. cruentus*	Mexico	white	red and green
PI 633584	RRC 27	*A. cruentus*	China	dark brown	red
PI 636182	RRC 1386	*A. cruentus*	Argentina	white	dark pink

[a] PI numbers refer to plant introduction numbers from the U.S. National Genetic Resources Program. [b] Origin is that of the donor to the U.S. National Genetic Resources Program.

ranging between 178 and 278 mg/100 g (**Table 2**), representing a 56% variation. The mean total oxalate concentration observed is comparable to the value of 232 mg/100 g reported previously for one genotype of *A. caudatus* (*26*). Insoluble oxalate averaged 182 mg/100 g (80% of total oxalate) and ranged between 151 and 224 mg/100 g, representing a 48% variation. Soluble oxalate averaged 47 mg/100 g and ranged between 26 and 82 mg/100 g, but differences between genotypes were not significant ($p > 0.05$). These values are comparable to values reported for buckwheat (*Fagopyrum esculentum* Moench.) and quinoa (*Chenopodium quinoa* Willd.) (*26*, *35*), two pseudo-cereals also recommended as gluten-free alternatives to cereals (*5*). However, buckwheat and quinoa have a higher proportion of soluble oxalate than grain amaranth, thus representing potentially a greater risk of kidney stone formation. 'Plainsman', the most widely grown grain amaranth cultivar in the United States (*36*), had 203 mg of total oxalate/100 g with 156 mg of insoluble oxalate/100 g. If amaranth is to be consumed on a regular basis by people affected by celiac disease, or those seeking an alternative protein source, such oxalate concentrations might be problematic, as foods with >10 mg of oxalate per 125 mL serving are considered to be high-oxalate foods (*37*); 125 mL of dry grain amaranth weighs approximatively 100 g and would contain 203 mg of oxalate per serving. However, grain amaranth has much lower oxalate concentrations than vegetable amaranth, which has been reported to contain between 8.57 and 9.57 g of total oxalate/100 g on a dry matter basis (*13*), and most is found as insoluble oxalate (i.e., average of 80% of the total oxalate).

Calcium concentrations averaged 186 mg/100 g and ranged between 134 and 370 mg/100 g, whereas magnesium concentrations averaged 280 mg/100 g and ranged between 230 and 387 mg/100 g. The oxalate/calcium molar ratio averaged 0.59 and ranged between 0.30 and 0.77. Finally, the oxalate/magnesium molar ratio averaged 0.23 and ranged between 0.19 and 0.27. Mean calcium and magnesium concentrations were almost identical to those previously reported in grain amaranth by Becker et al. (*38*), but slightly inferior to those reported by Bressani et al. (*7*). When the results from the present study are compared to those reported elsewhere for grains and legumes (*35*), grain amaranth contains roughly 4−5 times more total oxalate than conventional cereals and legumes, the grains for which amaranth can be used as an alternative. However, due to the high calcium and magnesium concentrations of grain amaranth (**Table 3**), oxalate absorbability might be relatively low. Ingestion of calcium or magnesium along with oxalate-rich food is known to reduce oxalate absorption, as these minerals bind to free oxalic acid and produce insoluble oxalate complexes (*20*). The average oxalate/calcium ratio reported here is much lower than that of spinach, which was reported to be around 1.08 and to have only 5% of the total Ca available (*39*). Grain amaranth can probably be viewed as a good source of calcium, because on a molar basis there is nearly twice as much calcium as oxalate. We are not aware of any study evaluating the bioavailability of calcium in grain amaranth.

Several significant correlations were observed between variables measured (**Table 4**). Total oxalate was positively correlated with both calcium ($r = 0.44$, $p < 0.0001$) and magnesium ($r = 0.51$, $p < 0.0001$) concentrations. As shown in **Figure 1**, although total oxalate is positively correlated to calcium, the oxalate/calcium ratio decreases with increasing calcium. This suggests that breeding for higher calcium could be beneficial, because although oxalate increases with increasing calcium, proportionally more calcium is present to bind oxalate in genotypes with higher calcium concentration.

Variability observed in oxalate, calcium, and magnesium concentrations as well as for the oxalate/calcium ratio suggests

Table 3. Oxalate, Calcium, and Magnesium Concentrations of 30 Grain Amaranth Genotypes Grown in Sainte-Anne-de-Bellevue, QC, Canada ($n = 3$)

genotype[a]	total oxalate (mg/100 g)	insoluble oxalate (mg/100 g)	soluble oxalate (mg/100 g)	% insoluble oxalate	% soluble oxalate	Ca (mg/100 g)	Mg (mg/100 g)	oxalate/Ca (molar ratio)	oxalate/Mg (molar ratio)
PI 451711	247	191	56	78	22	370	301	0.30	0.23
PI 477912	197	162	35	82	18	157	249	0.58	0.22
PI 477913	234	179	56	76	24	167	277	0.64	0.23
PI 515959	202	164	39	81	19	134	247	0.70	0.23
PI 525498	227	191	37	84	16	188	288	0.55	0.22
PI 538255	214	171	43	80	20	144	264	0.70	0.23
PI 538319	218	153	65	71	29	165	254	0.62	0.24
PI 538320	178	152	26	86	14	140	264	0.58	0.19
PI 538321	234	190	45	82	18	159	280	0.68	0.23
PI 538323	216	186	30	86	14	168	300	0.59	0.20
PI 538324	251	206	46	82	18	191	289	0.64	0.26
PI 538325	262	206	57	78	22	156	289	0.77	0.26
PI 538326	266	224	43	84	16	158	271	0.77	0.27
PI 538327	247	201	47	81	19	185	276	0.61	0.25
PI 558499	203	156	47	77	23	165	242	0.56	0.23
PI 568179	241	195	46	81	19	208	317	0.53	0.21
PI 576447	278	213	65	78	22	349	387	0.36	0.20
PI 604666	247	208	40	84	16	193	281	0.59	0.24
PI 605354	216	177	39	82	18	154	230	0.64	0.26
PI 606767	236	189	48	80	20	199	301	0.54	0.22
PI 606797	199	165	34	83	17	149	244	0.61	0.23
PI 606799	206	161	45	79	21	156	244	0.61	0.23
PI 618962	248	194	54	78	22	252	340	0.45	0.20
PI 619250	258	206	52	81	19	160	272	0.73	0.26
PI 628780	207	168	39	81	19	184	291	0.52	0.20
PI 628781	215	177	39	82	18	168	246	0.58	0.24
PI 628782	209	166	43	80	20	165	267	0.58	0.22
PI 628783	229	151	79	66	34	170	255	0.62	0.25
PI 633584	256	175	82	69	31	244	344	0.48	0.21
PI 636182	211	173	38	83	17	172	275	0.56	0.21
SEM	19.8	14.3	12.9	4.5	4.5	15.5	18.5	0.052	0.019
p value	0.0457	0.0090	0.4880	0.4482	0.4482	<0.0001	<0.0001	<0.0001	0.1605
mean	229	182	47	80	20	186	280	0.59	0.23
range	178–278	151–224	26–82	66–86	14–34	134–370	230–387	0.30–0.77	0.19–0.27

[a] PI numbers refer to plant introduction numbers from the U.S. National Genetic Resources Program.

Table 4. Correlation Coefficients[a] of Oxalate, Calcium, and Magnesium Concentrations and Their Molar Ratios in 30 Grain Amaranth Genotypes Grown in Sainte-Anne-de-Bellevue, QC, Canada ($n = 90$)

	total oxalate	insoluble oxalate	soluble oxalate	Ca	Mg	oxalate/Ca	oxalate/Mg
total oxalate	1.00	0.80***	0.63***	0.44***	0.51***	0.20*	0.55***
insoluble oxalate		1.00	0.05ns	0.33**	0.41***	0.20ns	0.44***
soluble oxalate			1.00	0.31**	0.32**	0.09ns	0.35***
Ca				1.00	0.70***	–0.74***	–0.20ns
Mg					1.00	–0.46***	–0.43***
oxalate/Ca						1.00	0.66***
oxalate/Mg							1.00

[a] ns, not significant ($p > 0.05$). Correlation significant at the (*) 0.05, (**) 0.01, and (***) 0.001 levels.

that breeding for these traits might be possible. The mode of inheritance of oxalate in grain amaranth is, however, currently unknown. In soybean, it was suggested that breeding for low oxalate would be possible (*21*). It must be noted that only 1% of the nearly 3000 grain amaranth genotypes available (*30*) were evaluated in the present study and that more extreme values would most likely be found in a more thorough screening. The recent isolation of *Medicago truncatula* mutants showing altered oxalate crystal patterns and oxalate concentrations has shown that oxalate concentration in plants is at least partially genetically controlled (*40*). Some important genetic tools, such as male sterility (*41*) and nonshattering traits (*42*), are likely to contribute to the development of the next, high-yielding generation of grain amaranth cultivars. Oxalate bioavailability studies are required before the initiation of new grain amaranth breeding programs to determine whether oxalate concentration should be a consideration.

Fertilization and Seeding Date. Averaged over four environments (i.e., two years and two sites), there was a significant ($p < 0.05$) effect of fertilization with calcium ammonium nitrate on total oxalate concentration ($y = 304 + 0.07x$). However, with a regression coefficient of only 0.07, this means that only a slight decrease in oxalate concentration could be achieved by considerably reducing calcium ammonium nitrate fertilization, which however in turn would reduce grain yield (*36*). Adapting calcium ammonium nitrate fertilization levels is thus not a viable means of reducing oxalate concentration in grain amaranth. Other components measured (i.e., insoluble oxalate, soluble

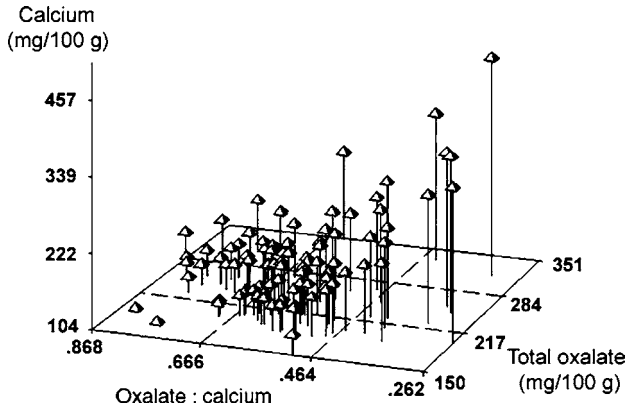

Figure 1. Correlations between total oxalate and calcium concentrations and their molar ratios in 30 genotypes of grain amaranth grown in Sainte-Anne-de-Bellevue, QC, Canada ($n = 90$). Correlation coefficients were as follows: calcium and total oxalate ($r = 0.44$, $p < 0.001$); calcium and oxalate/calcium ($r = -0.74$, $p < 0.001$); oxalate/calcium and oxalate ($r = 0.2$, $p = 0.05$).

Table 5. Effects of Boiling at Atmospheric Pressure on the Oxalate Concentration of Grain Amaranth ($n = 7$)

treatment	total oxalate (mg/100 g)	insoluble oxalate (mg/100 g)	soluble oxalate (mg/100 g)	% insoluble oxalate	% soluble oxalate
raw	268	235	33	87.8	12.2
cooked	271	215	56	79.4	20.6
SEM	4.1	3.1	3.3	1.0	1.0
p value	0.6731	0.0007	0.005	0.0001	0.0001

oxalate, percents of insoluble and soluble oxalate, calcium, magnesium, and oxalate/calcium ratio) all remained unaffected by fertilization ($p > 0.05$). Finally, seeding date had no effect on any variable measured ($p > 0.05$, data not presented).

Increased oxalate concentration was reported in spinach with increases in both calcium and nitrogen fertilization (*14*). Also in spinach, nitrate was reported to increase oxalate more than ammonium (*22*). In the present experiment, we used calcium ammonium nitrate, which supplied half the nitrogen as nitrate and half as ammonium and also provided some calcium and magnesium. We therefore simultaneously varied levels of nitrogen (0−200 kg of N ha^{-1}), calcium (0−33 kg of Ca ha^{-1}), and magnesium (0−17 kg of Mg ha^{-1}). Because we applied all of the fertilizer once at seeding, it is reasonable to assume that most of the applied ammonium had been oxidized to nitrate before uptake. However, we observed only a weak increase in total oxalate concentration with increasing fertilization, which could be attributed to (i) the high calcium levels prior to experimentation of the soils used (**Table 1**), with further calcium fertilization not affecting plants; (ii) nitrogen leaching (although this is unlikely because a grain yield response was observed, data not shown); or (iii) the fact that grain amaranth may not be as responsive as spinach to fertilization. It is also possible that the oxalate concentration of seeds is less affected by environmental factors than that of leaves or vegetative material. As pointed out by Libert and Franceschi (*14*), oxalate formation is not simply a crystallization process, but involves the development of specialized membranes or cells. Plants therefore exert a certain degree of control over the amount of oxalate and calcium accumulated. Another explanation for the weak response to fertilization we observed in grain amaranth is a possible interaction between nutrients. Libert and Franceschi (*14*) indeed reported that nitrogen increased oxalate concentration in spinach under low phosphorus status, but had no effect under higher phosphorus status.

Cooking. Boiling amaranth seeds at atmospheric pressure in a 2:1 water to seeds ratio until complete absorption changed the form but not the total concentration of oxalate (**Table 5**), the soluble oxalate proportion being greater after cooking ($p < 0.05$). The absence of a decrease in total oxalate concentration following boiling is in contradiction with results observed in a range of vegetables (*25*), legumes (*43*), and cereals (*44*). Differences might be attributed to the fact that in our study, the cooking water was completely absorbed by the grains; therefore, any solubilized material was kept and detected in analyses. Beetroot (*Beta vulgaris* L.) was reported to have a greater proportion of soluble oxalate after pressure cooking for 45 min at 15 psi (*45*). Cooking methods for grain amaranth found in the popular literature often recommend (with water to grain ratios varying slightly) boiling until complete absorption of water. If soluble oxalate is, as previously reported, more absorbable than insoluble oxalate (*17*), this method of cooking could increase the total amount of oxalate absorbed.

Results presented herein underscore the need for conducting grain amaranth oxalate absorbability studies. Grain amaranth has high total oxalate concentrations; however, most is found as insoluble oxalate. If oxalate absorbability is low, then substituting amaranth for regular cereals could be done with fewer concerns for those people with predisposition to kidney stone development. If absorbability is high, selection for low-oxalate genotypes will be necessary. Heritability studies for oxalate concentration and forms will also be required. On the basis of our results, management appears not to represent an effective way of manipulating oxalate concentration and forms in grain amaranth. Because of the low oxalate/calcium ratio and the high calcium concentration of grain amaranth, we suggest that it might represent a good source of dietary calcium that could benefit vegetarians, although it should be confirmed by bioavailability studies.

ACKNOWLEDGMENT

We thank David Brenner (North Central Regional Plant Introduction Station, Ames, IA) for providing seeds of the 30 genotypes used in this project.

LITERATURE CITED

(1) Johnson, H. S.; Hatch, M. D. Distribution of the C4-dicarboxylic acid pathway of photosynthesis and its occurrence in dicotyledonous plants. *Phytochemistry* **1968**, *7*, 375−380.
(2) Sauer, J. D. The grain amaranths: a survey of their history and classification. *Ann. Mo. Bot. Gard.* **1950**, *37*, 561−632.
(3) National Academy of Science. *Amaranth: Modern Prospects for an Ancient Crop*; Washington, DC, 1984; p 81.
(4) Downton, W. J. S. *Amaranthus edulis*: a high lysine grain amaranth. *World Crop* **1973**, *25*, 20.
(5) Petr, J.; Michalik, I.; Tlaskalova, H.; Capouchova, I.; Famera, O.; Urminska, D.; Tukova, L.; Knoblochova, H. Extension of the spectra of plant products for the diet in coeliac disease. *Czech J. Food Sci.* **2003**, *21*, 59−70.
(6) Celiac Spruce Association. *Celiac Disease Defined*; 2006; available at http://www.csaceliacs.org (accessed Sept 26, 2006).
(7) Bressani, R.; Gonzales, J. M.; Zuniga, J.; Breuner, M.; Elias, L. G. Yield, selected chemical composition and nutritive value of 14 selections of amaranth grain representing four species. *J. Sci. Food Agric.* **1987**, *38*, 347−356.
(8) Rathod, P.; Udipi, S. A. The nutritional quality and acceptability of weaning foods incorporating amaranth. *Food Nutr. Bull.* **1991**, *13*, 58.

(9) Lorenz, K.; Wright, B. Phytate and tannin content of amaranth. *Food Chem.* **1984**, *14*, 27−34.
(10) Bressani, R. Composition and nutritional properties of amaranth. In *Amaranth: Biology, Chemistry and Technology*; Paredes-Lopez, Ed.; CRC Press: Irapuato, Mexico; 1994; pp 185−205.
(11) Bresler, G.; Vaamonde, G.; Degrossi, C.; Pinto, V. F. Amaranth grain as substrate for aflatoxin and zearalenone production at different water activity levels. *Int. J. Food Microbiol.* **1998**, *42*, 57−61.
(12) Oleszek, W.; Junkuszew, M.; Stochmal, A. Determination and toxicity of saponins from *Amaranthus cruentus* seeds. *J. Agric. Food Chem.* **1999**, *47*, 3685−3687.
(13) Vityakon, P.; Standal, B. R. Oxalate in vegetable amaranth (*Amaranthus gangeticus*)—forms, contents, and their possible implications for human health. *J. Sci. Food Agric.* **1989**, *48*, 469−474.
(14) Libert, B.; Franceschi, V. R. Oxalate in crop plants. *J. Agric. Food Chem.* **1987**, *35*, 926−938.
(15) Bohn, T.; Davidsson, L.; Walczyk, T.; Hurrell, R. F. Fractional magnesium absorption is significantly lower in human subjects from a meal served with an oxalate-rich vegetable, spinach, as compared with a meal served with kale, a vegetable with a low oxalate content. *Br. J. Nutr.* **2004**, *91*, 601−606.
(16) Grentz, L.; Massey, L. Contribution of dietary oxalate to urinary oxalate in health and disease. *Top. Clin. Nutr.* **2002**, *17*, 60−70.
(17) Jaegger, P.; Robertson, W. G. Role of dietary intake and intestinal absorption of oxalate in calcium stone formation. *Nephron Physiol.* **2004**, *98*, 64−71.
(18) Holmes, R. P.; Assimos, D. G. The impact of dietary oxalate on kidney stone formation. *Urol. Res.* **2004**, *32*, 311−316.
(19) Zimmermann, D. J.; Hesse, A.; von Unruh, G. E. Influence of a high-oxalate diet on intestinal oxalate absorption. *World J. Urol.* **2005**, *23*, 324−329.
(20) Liebman, M.; Costa, G. Effects of calcium and magnesium on urinary oxalate excretion after oxalate loads. *J. Urol.* **2000**, *163*, 1565−1569.
(21) Horner, H. T.; Cervantes-Martinez, T.; Healy, R.; Reddy, M. B.; Deardorff, B. L.; Bailey, T. B.; Al-Wahsh, I.; Massey, L. K.; Palmer, R. G. Oxalate and phytate concentrations in seeds of soybean cultivars [*Glycine max* (L.) Merr.]. *J. Agric. Food Chem.* **2005**, *53*, 7870−7877.
(22) Zhang, Y. P.; Lin, X. Y.; Zhang, Y. S.; Zheng, S. J.; Du, S. T. Effects of nitrogen levels and nitrate/ammonium ratios on oxalate concentrations of different forms in edible parts of spinach. *J. Plant Nutr.* **2005**, *28*, 2011−2025.
(23) Zindler-Frank, E.; Honow, R.; Hesse, A. Calcium and oxalate content of the leaves of *Phaseolus vulgaris* at different calcium supply in relation to calcium oxalate crystal formation. *J. Plant Physiol.* **2001**, *158*, 139−144.
(24) Streeter, J. G. Effects of nitrogen and calcium supply on the accumulation of oxalate in soybean seeds. *Crop Sci.* **2005**, *45*, 1464−1468.
(25) Chai, W. W.; Liebman, M. Effect of different cooking methods on vegetable oxalate content. *J. Sci. Food Agric.* **2005**, *53*, 3027−3030.
(26) Siener, R.; Honow, R.; Seidler, A.; Voss, S.; Hesse, A. Oxalate contents of species of the polygonaceae, amaranthaceae and chenopodiaceae families. *Food Chem.* **2006**, *98*, 220−224.
(27) Massey, L. K.; Palmer, R. G.; Horner, H. T. Oxalate content of soybean seeds (*Glycine max*: leguminosae), soyfoods, and other edible legumes. *J. Agric. Food Chem.* **2001**, *49*, 4262.
(28) Prakash, D.; Pal, M. Nutritional and antinutritional composition of vegetable and grain amaranth leaves. *J. Sci. Food Agric.* **1991**, *57*, 573−583.
(29) CRAAQ (Centre de référence en fertilisation), In *Guide de Référence en Fertilisation*, 1st ed.; Centre de référence en agriculture et agroalimentaire du Québec: Sainte-Foy, QC, Canada, 2003.
(30) USDA-ARS. *National Genetic Resources Program. Germplasm Resources Information Network (GRIN)*; National Germplasm Resources Laboratory: Beltsville, MD; available at http://www.ars-grin.gov.
(31) The Jefferson Institute. *Grain Amaranth, a Lost Crop of the Americas*; available at http://www.jeffersoninstitute.org/pubs/amaranth.shtml (accessed Sept 20, 2006).
(32) Parkinson, J. A.; Allen, S. E. Wet oxidation procedure suitable for determination of nitrogen and mineral nutrients in biological-material. *Commun. Soil Sci. Plant Anal.* **1975**, 1−11.
(33) SAS Institute Inc. *SAS*; Cary, NC, 2003.
(34) McIntosh, M. S. Analysis of combined experiments. *Agron. J.* **1983**, *75*, 153−155.
(35) Chai, W. W.; Liebman, M. Oxalate content of legumes, nuts, and grain-based flours. *J. Food Compos. Anal.* **2005**, *18*, 723−729.
(36) Myers, R. L. Nitrogen fertilizer effect on grain amaranth. *Agron. J.* **1998**, *90*, 597−602.
(37) Chicago Dietetic Association. *Manual of Clinical Dietetics*; American Dietetic Association: Chicago, IL, 2000.
(38) Becker, R.; Wheeler, E. L.; Lorenz, K.; Stafford, A. E.; Grosjean, O. K.; Betschart, A. A.; Saunders, R. M. A compositional study of amaranth grain. *J. Food Sci.* **1981**, *46*, 1175−1180.
(39) Heaney, R. P.; Weaver, C. M.; Recker, R. R. Calcium absorbability from spinach. *Am. J. Clin. Nutr.* **1988**, *47*, 707−709.
(40) Nakata, P. A.; McConn, M. M. Isolation of *Medicago truncatula* mutants defective in calcium oxalate crystal formation. *Plant Physiol.* **2000**, *124*, 1097−1104.
(41) Gudu, S.; Gupta, V. K. Male-sterility in the grain amaranth (*Amaranthus hypochondriacus* Ex-Nepal) variety Jumla. *Euphytica* **1988**, *37*, 23−26.
(42) Brenner, D. M. Non-shattering grain amaranth populations. In *Trends in New Crops and New Uses*; Janick, J., Whipkey, A., Eds.; ASHS Press: Alexandria, VA, 2002; pp 104−106.
(43) Quinteros, A.; Farre, R.; Lagarda, M. J. Effect of cooking on oxalate content of pulses using an enzymatic procedure. *Int. J. Food Sci. Nutr.* **2003**, *54*, 373−377.
(44) Judprasong, K.; Charoenkiatkul, S.; Sungpuag, P.; Vasanachitt, K.; Nakjamanong, Y. Total and soluble oxalate contents in Thai vegetables, cereal grains and legume seeds and their changes after cooking. *J. Food Compos. Anal.* **2006**, *19*, 340−347.
(45) Savage, G. P.; Vanhanen, L.; Mason, S. M.; Ross, A. B. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *J. Food Compos. Anal.* **2000**, *13*, 201−206.

Received for review February 9, 2007. Revised manuscript received April 5, 2007. Accepted April 9, 2007. This work was financially supported by the Fédération des Producteurs de Cultures Commerciales du Québec (FPCCQ) and the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT).

JF070384D