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Oxalate and Phytate Concentrations in Seeds of Soybean Cultivars [*Glycine max* (L.) Merr.]

HARRY T. HORNER,*,† TERESA CERVANTES-MARTINEZ,† ROSANNE HEALY,† MANJU B. REDDY,‡ BETSY L. DEARDORFF,|| THEODORE B. BAILEY,§ ISMAIL AL-WAHSH,|| LINDA K. MASSEY,|| AND REID G. PALMER⊥

Department of Genetics, Development and Cell Biology and Bessey Microscopy Facility, Iowa State University, Ames, Iowa 50011-1020, Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011-1120, Department of Statistics, Iowa State University, Ames, Iowa 50011-1210, Department of Food Science and Human Nutrition, Washington State University, Spokane, Washington 99210-1495, and U.S. Department of Agriculture−Agricultural Research Service CICGR, and Department of Agronomy, Iowa State University, Ames, Iowa 50011-1010

This study analyzed soybean seeds from 116 cultivars for total, insoluble, and soluble oxalate (Ox), phytate ($InsP_6$), calcium (Ca), and magnesium (Mg) because of their potential beneficial or harmful effects on human nutrition. These cultivars were divided into four groups (A−D) on the basis of the year and geographic location where they were grown. Oxalate concentration ranged from about 82 to 285 mg/100 g of dry seed. The $InsP_6$ concentration ranged from 0.22 to 2.22 g/100 g of dry seed. There was no correlation between Ox and $InsP_6$ within or among the four groups of cultivars. There was a significant correlation between total Ox and Ca, but not Mg, in group D cultivars ($r = 0.3705$; $p < 0.0005$). No significant relationship was found in the group A−C cultivars. Eleven group D cultivars had $InsP_6$ less than 500 mg/100 g, but all had total Ox of 130 mg/100 g or greater. Five cultivars from groups A−C had relatively low $InsP_6$ (group B; ≤1.01 g/100 g) and low Ox (<140 mg/100 g). These cultivars could be useful for producing soy foods beneficial to populations at risk for kidney stones and for improved mineral bioavailability. The Ox and $InsP_6$ concentrations of the cultivars indicate that choosing specific parents could generate seeds in succeeding generations with desirable Ox and $InsP_6$ concentrations.

KEYWORDS: Calcium oxalate; cultivar; nutrition; oxalate; phytate; seeds; soybean

INTRODUCTION

The presence of oxalate (Ox) in fungi, animals, humans, and plants has been known for many years (*1*−*4*). Numerous studies have documented the deleterious effects of Ox when consumed by animals and humans. The Ox can be produced in plants as well as in humans from several precursors, including ascorbate (*1*, *5*). Ox occurs as soluble salts of Na^+ and K^+, oxalic acid, and/or as insoluble calcium oxalate [CaOx; crystalline state with two hydration forms, weddellite (dihydrate) and whewellite (monohydrate)] (*1*, *6*). The CaOx crystals are visible in human and animal urine and in various tissues where supersaturation conditions occur. The CaOx crystals are present in a variety of tissues of about 75% of the flowering plants, which include the vast majority of edible foods (*7*, *8*), including edible legumes (*9*).

Humans and animals accidentally or intentionally eat plants high in Ox (spinach, rhubarb, *Dieffenbacchia* and other members of the Araceae, *Halogeton*, and *Chenopodium*). Intake of high levels of Ox can induce hyperoxaluria, potentially leading to kidney and bladder stones, and at the extreme, renal edema and calcification.

Recently, soy foods have been touted as having a variety of positive health components. Soybeans have a high content of unsaturated fats and folate, as well as high protein and dietary fiber (*10*). Soybeans also contain compounds, such as isoflavones, that are known to prevent osteoporosis, cancer, and cardiovascular disease (*10*−*12*). Soybeans may be eaten whole as vegetables or as processed food products (e.g., tofu, soynuts, soy beverage). Several recent publications describe Ox concentrations and locations in soybean seeds and foods prepared from them (*13*−*17*). In healthy non-stone-formers, 2−8% of food Ox is absorbed (*18*). Normal urinary Ox excretion is 10−39 mg/day, and up to half of the human urinary Ox is derived

* Corresponding author: e-mail hth@iastate.edu.
† Department of Genetics, Development and Cell Biology and Bessey Microscopy Facility, Iowa State University.
‡ Department of Food Science and Human Nutrition, Iowa State University.
§ Department of Statistics, Iowa State University.
|| Department of Food Science and Human Nutrition, Washington State University.
⊥ USDA-ARS CICGR and Department of Agronomy, Iowa State University.

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from food Ox rather than endogenous synthesis (*19*). Together, these data indicate that certain food products (*20*−*22*) may contribute significant concentrations of Ox to the human diet, especially if they are consumed every day. On the basis of Ox concentration and absorption, frequent consumption of soy products may increase Ox excretion to more than 40 mg/day (*18*), which is defined as hyperoxaluria and may lead to kidney stone formation. In addition, Ox may also reduce mineral absorption (*23*), so excess consumption raises a potential for mineral deficiencies.

Phytate ($InsP_6$) is a fully phosphorylated form of inositol (*myo*-inositol hexakisphorate), a compound that also exists in seeds, which has both beneficial and detrimental effects on human nutrition and health (*23*, *24*). Similar to Ox, $InsP_6$ can be synthesized by the human body. However, like Ox, the main source for $InsP_6$ for humans is from plant foods. $InsP_6$ has long been considered an antinutrient due to its ability to complex with several metal ions such as zinc (*25*), calcium (*26*), and iron (*27*−*30*), thus reducing their bioavailability. However, to the contrary, $InsP_6$ recently has been proposed to reduce kidney stone formation by inhibiting the formation of CaOx and calcium phosphate crystals (*31*). In vivo effect of $InsP_6$ in reducing renal calcification also was shown in rats that were fed 10.8 mM/L in their liquid diet (*32*). The antioxidant role of $InsP_6$ is of current interest since it has strong chelating ability for minerals, such as iron and zinc, that are involved in oxidative reactions causing damage and resulting in cancer and cardiovascular diseases.

Although there is some information available on the $InsP_6$ content (*33*, *34*) in soybean and soy foods, there are no studies that deal with these two antinutrient factors together. Therefore, the objectives of this study were to (1) determine total, insoluble, and soluble Ox content in seeds of 86 cultivars (cvs) of soybean; (2) analyze $InsP_6$ content in 31 of these cvs and in 30 low-$InsP_6$ cvs and to determine its relationship with total Ox; and (3) analyze total Ca and Mg concentrations in an additional 31 cvs and total Ca in 35 cvs to determine their relationships with total Ox. The final objective was to identify specific cultivars with low Ox and low $InsP_6$, which might be useful for preventing human nutritional deficiencies and diseases.

MATERIALS AND METHODS

Cultivars for Ox and $InsP_6$ Analyses. Eighty-six soybean [*Glycine max* (L.) Merr.] cultivars (**Tables 1** and **3**) were obtained from R.G.P. at Iowa State University and were analyzed for their seed content of total, insoluble, and soluble Ox. Thirty-one of them were analyzed for $InsP_6$ content and 35 for Ca and Mg. To sample a wide range of genotypes, different maturity groups (different photoperiod responses) were included. Seventy-four low-$InsP_6$ cultivars were obtained from Dr. Jim Wilcox, Purdue University, West Lafayette, IN, and 30 of these (**Table 2**) were analyzed for $InsP_6$ content. Seeds from all of these cultivars were grown at the Bruner Farm near Ames, IA in 2000 (group A) and 2001 (group D), at Savoy, IL in 2000 (group B), or near Raleigh, NC in 2000 (group C) for late maturity groups (see **Table 1**). We also compared seed composition from plants grown at the same location in the same year. Some of the cultivars used in this study are typical commodity-grade soybeans, whereas others were developed for various human food uses (see food uses in **Table 1**). Pedigrees for the nonproprietary cultivars were included in **Tables 1** and **2** to help in interpreting the data.

Identification of CaOx Crystals. Cotyledons from several cultivars were removed from seeds, cleared with a 5% Clorox solution (Clorox Co., Inc., Oakland, CA), and viewed between crossed polarizers with a compound microscope to detect the presence of CaOx crystals (**Figure 1A,B**). Ground seed fragments used for Ox determination were treated with 10 mmol/L buffered ethylenediaminetetracetic acid (EDTA), pH 7.6, to remove the crystals over time. This progression is shown in **Figure 1C**−**E**.

Determination of Ox Concentrations. Duplicate Ox analyses were carried out for each of the 86 cvs in groups A−C (**Tables 1** and **3**). To analyze for total and insoluble Ox in each cultivar, about 3.5 g of seed was ground in a continually cleaned (air-blasted) coffee grinder (Cafémill Grindmaster, Wayne, PA) for approximately 2 min, or until the seeds were reduced to a fine powder. This powder then was filtered through a tea strainer, and any remaining seed fragments (usually seed coat) were reground with a clean pestle and mortar. The resulting powder, containing all parts of the seed, was placed into a small aluminum weighing dish and dried at 60 °C for 3 days.

The dried powder was transferred to a marked glass vial, sealed, and stored in a desiccator. For the enzyme/colorimetric assay (described below), two 100-mg amounts from each cultivar were weighed out: sample A (100 mg) was used to analyze for total Ox, and sample B (100 mg) was used to analyze for insoluble Ox. Duplicate samples were analyzed. Each 100 mg of dried powder was placed into tubes and sterilized for 20 min by autoclaving.

Two milliliters of sterile, deionized (DI) water was added to sample B and the mixture was homogenized at low speed. Sample B then was sonicated for 6 min, after which an additional 2 mL of sterile, DI water was added. The sonication process lysed the cells, freeing the soluble Ox, which was dissolved in the water. Sample B then was spun down in a countertop centrifuge, the liquid was poured off, and the residue was resuspended and centrifuged several times with sterile, DI water. After these washes, a standard water-operated vacuum filtration system was used to finally concentrate the wet powder that was then dried again. Next, 2 mL of an EDTA buffer solution (pH 6.9−7.0) was added to untreated sample A (with total Ox) and to sample B (minus soluble Ox). Both samples were sonicated for 6 min, after which an additional 2 mL of 10 mmol/L buffered EDTA, pH 7.6, was added. The samples were incubated in a water bath at 55 °C for 24 h. This treatment dissolved all of the CaOx crystals in the wetted powder as determined microscopically between crossed polarizers (**Figure 1C**−**E**). Each sample then underwent centrifugation for 5 min at 4000 rpm. Then 4 mL of supernatant from each sample were removed, and 50 μL amounts were taken for analysis.

Samples A and B were analyzed on a spectrophotometer (Genesys 2, Spectronic Instruments, Rochester, NY) according to the modified protocol from the Sigma urinalysis diagnostics kit [procedure 591; reagents A [3-methyl-2-benzothiazolinonehydrazone (MBTH) and 3-(dimethlyamino)benzoic acid (DMAB)] and B (oxalate oxidase), diluent (EDTA + buffer), and standard (0.5 mM/L oxalate) are now all available from Trinity Biotech Plc, IDA Business Park, Bray, County Wicklow, Ireland; 1-800-325-3424 U.S.; 1-800-603-8076 Ireland; www.trinityusa.com]. For each run, a blank (water), a standard (known Ox concentration), and samples A and B were prepared.

The reactions that occurred in samples A and B and standard cuvettes were (1) solublized total or insoluble Ox + O_2 + oxalate oxidase → $2CO_2$ + H_2O_2 and (2) H_2O_2 + MBTH + DMAB-peroxidase → indamine dye (purple color) + H_2O. Indamine dye concentration was measured at 590 nm.

From the absorbance readings, the relative concentrations in milligrams of total and insoluble Ox in 100 g of dried soybean seed powder were calculated. Soluble Ox for each sample was determined by subtracting the obtained value for insoluble Ox from the obtained value for total Ox. The Sigma urinalysis kit information regarding reagents and the modified equations to obtain these results are presented below, as well as modifications to specifically obtain the results with seed material.

The equation to determine milligrams of Ox in 100 g of dried soybean seed is described as follows. The Ox standard contains 0.5 mM Ox/L; (0.5)(90 mg/mM) = 45 mg/L; 50 μL of Ox standard contains (45 mg of Ox/L)(5×10^{-5} μL/L) = 2.25×10^{-3} mg of Ox/50 μL or 2.25 μg/50 μL. To calculate the concentrations of total and insoluble Ox in samples A and B, respectively from the spectrophotometer readings: (sample A or B reading/standard reading) × [2.25 μg of Ox in standard (μg/50 μL)] × [80 (measured volume is $^1/_{80}$ of original volume)] = micrograms in original 100 mg of powder. This latter

Table 1. Seed Group, Total, Insoluble, and Soluble Oxalate Concentrations and Their Percentages, and Total Phytate in Seeds of 86 Soybean Cultivars and Their Food Uses and Pedigrees[a]

cultivar	seed group	total Ox (mg/100 g)	insol Ox (mg/100 g)	sol Ox (mg/100 g)	insol Ox (%)	sol Ox (%)	total $InsP_6$ (g/100 g)	food uses	pedigree
Proto	A	82.3	29.4	52.8	35.8	64.2	1.23	high protein, tofu	M70-504 × M69-42
Galena Genetics 003	A	91.6	74.5	17.1	81.5	18.5	1.01	tofu	proprietary
Saturn	B	92.4	37.8	54.5	41.0	59.0	1.66	tofu, vegetable	Hobbit × Jogun from Korea
N6201	C	94.3	42.6	51.7	46.3	53.7	1.41	tofu	Nakasennari × Young
LS201	B	98.5	71.9	26.6	73.6	26.4	2.16	tofu	(HS235 × A79-240011) × A79-240002
Galena Genetics 004	A	101.5	76.4	25.1	75.9	24.0	1.11	tofu	proprietary
low linolenic	A	101.8	75.0	26.8	73.7	26.5	*b*	vegetable oil	unknown
Gardensoy 22	B	104.5	85.1	19.3	82.8	17.2	1.30	vegetable	Century-*lx2* × Disoy
Galena Genetics 001	A	111.4	83.2	28.2	75.2	24.9	*b*	tofu	proprietary
Gardensoy 41	B	111.6	69.9	41.6	63.5	36.6	*b*	vegetable	Disoy × Miyagi Shirome B
Gardensoy 42	B	112.2	75.7	36.5	67.8	32.2	*b*	vegetable	Disoy × Miyagi Shirome A
Verde	A	114.0	51.6	62.5	45.3	54.7	*b*	vegetable	Aoda × A50-7445(Richland × Jogun)
L97-0161	B	117.0	82.3	34.8	72.5	27.5	0.89	vegetable	Osaya × Disoy
Galena Genetics 002	A	119.6	64.4	55.2	56.0	44.0	*b*	tofu	proprietary
IA1008	B	120.4	71.5	48.9	59.4	40.6	*b*	various uses	Northrup King S20-20 × Jack
low saturated	A	121.2	96.1	25.1	79.4	20.6	*b*	vegetable oil	unknown
Gardensoy 31	B	123.6	83.0	40.7	68.5	31.5	*b*	vegetable	Disoy × Miyagi Shirome A
IA4001	B	124.7	105.1	19.6	84.8	15.2	*b*	natto, sprouts	(A3205 × SS202) × SS201
Iroquois	B	126.6	106.3	20.3	84.5	15.5	0.77	commodity grade	LN81-1029 × (DSR252 × LS301)
Norpro	A	127.6	69.6	58.1	55.3	44.7	*b*	high protein	Ozzie × Proto
Cisne	B	127.8	79.8	48.0	62.4	37.6	1.06	commodity grade	Burlison × A3733 (Elf × A3127)
Vinton 81	A	129.4	73.0	56.4	56.4	43.6	*b*	tofu	(Harosoy × Higan) × Vinton (5)
IA2025	B	131.3	63.9	67.4	49.3	50.7	*b*	triple null lipoxygenase	*lx1 lx2 lx3* × IA2020
L99-3640	B	132.4	120.1	12.3	90.9	9.1	*b*	natto, sprouts	Jack × L91-1533[c]
Gardensoy 12	B	133.8	59.0	74.8	44.2	55.8	*b*	vegetable	Prize × Kaohsiung-No. 1, PI 518.758
IA2011	B	134.2	98.9	35.3	74.0	26.0	*b*	lipoxygenase-2 null	(L2-3 × HP203) × HP203
Peking	A	135.4	100.6	34.8	74.2	25.8	*b*	hay type	(PI 17.852 B) from China in 1906
L99-1106	B	135.4	61.7	73.8	45.7	54.3	*b*	vegetable	Disoy × Miyagi Shirome A
IA2042	B	135.9	76.0	59.9	56.1	43.9	*b*	high protein	A89-246013 × A91-613029
N7103	C	137.2	112.3	24.9	83.4	16.6	*b*	natto	NTCPR90-143 × Pearl
Harosoy	A	137.6	112.3	25.4	82.1	17.9	*b*	commodity grade	Mandarin (Ottawa) × AK (Harrow)
Gardensoy 21	B	137.8	60.4	77.4	43.9	56.1	*b*	vegetable	Disoy × Verde
HP204	A	138.3	102.5	35.8	74.3	25.7	*b*	high protein	Vinton 81 × Hardin
IA3006	B	139.1	83.9	55.2	60.3	39.7	0.94	high protein	A87-194013 × (DSR252 × LS301)
L99-1422	B	141.7	63.8	77.9	45.2	54.8	1.49	vegetable	Saturn × L93-5557 (Century-*lx2* × Disoy)
L96-5742	B	142.3	111.2	31.0	78.2	21.8	2.22	natto, sprouts	Jack × IL1[c]
L96-5269	B	146.2	145.2	1.0	99.3	0.7	*b*	natto, sprouts	L87-612[c] × L86K-73[c]
L99-1202	B	147.6	65.0	82.5	44.1	55.9	1.72	vegetable	Tousan 114 × Vinton 81
Gardensoy 24	B	147.9	76.8	71.1	53.8	46.2	*b*	vegetable	Disoy × Verde
L99-1179	B	149.0	115.9	33.2	77.7	22.3	2.19	vegetable	Vinton 81 × Tousan 114, (PI 507.445)
Parker	B	150.3	115.3	35.0	76.2	23.8	1.89	commodity grade	A79-136012 × Dawson
N7102	C	150.7	103.8	46.8	69.1	31.0	1.30	natto	Vance × Jizuka
L99-1241	B	150.8	83.6	67.2	55.5	44.5	1.22	vegetable	Vinton 81 × PI 86.134-1
L99-1132	B	151.1	70.6	80.5	47.3	52.7	*b*	vegetable	Disoy × Miyagi Shirome B
N 7101	C	151.6	110.8	40.8	73.7	26.3	1.30	natto	Vance × Jizuka
L98-6080	B	152.1	91.9	60.1	60.5	39.5	1.50	vegetable	Resnik × Verde
IA2024	B	152.2	119.9	32.3	79.5	20.5	1.66	commodity grade	(NK523-03 × SS204) × SS201
L99-1349	B	153.3	117.4	35.9	76.6	23.4	*b*	vegetable	Williams-*lr* × L93-5557[‡]
Gardensoy 43	B	156.8	69.7	87.1	44.5	55.5	*b*	vegetable	L83-3432[c] × Verde
L99-3831	B	157.1	139.7	17.3	88.8	11.2	*b*	natto, sprouts	Chapman × Mercury
L96-5237	B	157.5	140.6	16.1	88.7	11.3	*b*	natto, sprouts	L87-612[c] × L86K-73[c]
L99-3310	B	159.5	140.2	19.3	88.1	11.9	*b*	natto, sprouts	L86K-73[c] × Nattosan
L91-1552	B	160.9	104.8	56.1	65.4	34.6	*b*	natto, sprouts	lL2 × L87-626
L94-5886	B	161.0	85.7	75.4	53.6	46.4	*b*	vegetable	Resnik × Verde
IA2021	B	162.3	128.6	33.8	79.2	20.8	*b*	commodity grade	Elgin 87 × Marcus
Heilong 35	A	161.5	143.7	17.7	90.2	9.8	*b*	high protein	Chinese cultivar
L99-3380	B	162.6	148.9	13.7	91.6	8.4	*b*	natto, sprouts	L86-K73[c] × Jizuka
L98-7052	B	165.5	138.9	26.5	84.3	15.7	*b*	natto, sprouts	(Century 84 × R81) × 922X
L99-3403	B	165.6	135.6	30.0	82.0	17.9	*b*	natto, sprouts	L86K-73[c] × Jizuka
L95-1116	B	168.4	132.5	35.9	79.8	20.2	*b*	natto, sprouts	L87-612[c] × L86K-73
L99-3329	B	169.4	97.5	71.9	57.5	42.4	*b*	natto, sprouts	L87-612[c] × SH2 (from Japan)
Gardensoy 02	B	170.4	81.8	88.6	48.0	52.0	*b*	vegetable	Parker × IA3006
L96-6381	B	171.1	148.9	22.2	87.4	12.6	*b*	natto, sprouts	Jack × L91-1533[c]
L99-3612	B	171.8	133.5	38.2	78.5	21.5	*b*	natto, sprouts	Jack × L91-1533[c]
L95-1409	B	178.3	152.4	25.8	87.0	13.0	1.50	natto, sprouts	L87-612[c] × Jack
L99-3036	B	178.5	107.7	70.8	60.8	39.2	*b*	natto, sprouts	IL2[c] × L87-626[c]
L98-7220	B	180.3	121.7	58.6	67.3	32.7	*b*	natto, sprouts	IL1[c] × L86K-73[c]
Gardensoy 23	B	181.8	95.9	85.9	52.9	47.1	*b*	vegetable	L86K-73[c] × Magna
L97-1300	B	183.4	155.4	27.9	84.6	15.4	*b*	natto, sprouts	IL1[c] × [Williams 82 × PI 65.549 (wild)]
L99-3907	B	184.9	136.5	48.4	74.0	26.0	*b*	natto, sprouts	L87-612[c] × Mercury
L99-3921	B	185.3	137.3	48.0	74.7	25.3	*b*	natto, sprouts	L87-612[c] × L93-3539[c]
L96-5198	B	186.0	171.4	14.6	92.1	7.9	1.49	natto, sprouts	L87-612[c] × L86K-73[c]
L96-5408	B	187.5	167.9	19.5	89.6	10.4	*b*	natto, sprouts	L87-612[c] × Jack
L99-3591	B	189.6	146.0	43.6	77.1	22.9	*b*	natto, sprouts	Jack × L91-1533[c]
L97-0875	B	191.5	155.8	35.7	81.3	18.6	*b*	natto, sprouts	L87-612[c] × L86K-73[c]
L98-7362	B	192.2	121.1	71.1	63.0	37.0	1.36	natto, sprouts	IL1[c] × Spry
L96-5201	B	194.9	157.3	37.5	81.0	18.9	1.47	natto, sprouts	L87-612[c] × L86K-73[c]
L96-5441	B	196.2	141.1	55.1	72.3	27.7	*b*	natto, sprouts	L87-612[c] × TCPR90-143
L98-6070	B	197.7	108.7	89.0	55.1	45.0	1.27	vegetable	Will-*e2Rsv1m* × Magna
L99-3073	B	198.6	196.6	2.0	99.0	1.0	*b*	natto, sprouts	L87-612[c] × L86K-73[c]
L99-3890	B	200.1	127.6	72.5	64.6	35.4	*b*	natto, sprouts	L87-612[‡] × Mercury
L99-3292	B	203.8	141.3	62.5	69.7	30.3	1.65	natto, sprouts	IL1[‡] × [Williams 82 × PI 65.549 (wild)]
L98-7191	B	208.7	164.7	44.0	79.0	21.0	1.82	natto, sprouts	L87-612[c] × Spry

Table 1 (Continued)

cultivar	seed group	total Ox (mg/100 g)	insol Ox (mg/100 g)	sol Ox (mg/100 g)	insol Ox (%)	soluble Ox (%)	total $InsP_6$ (g/100g)	food uses	pedigree
L99-3836	B	209.5	195.9	13.6	93.6	6.4	1.62	natto, sprouts	Jack × Mercury
L98-7439	B	213.6	159.7	53.8	74.8	25.2	1.38	natto, sprouts	L87-612[c] × SH2
Mercury	B	213.6	145.6	68.0	69.1	30.9	1.44	natto, sprouts	T208, YuTae from Korea × Hobbit
std error of mean		19.3	16.1	15.5	9.1	9.1	0.19		
range		82.3–213.6	29.4–196.6	1.0–89.0	35.8–99.4	0.7–64.2	0.77–2.22		

[a] Seeds are from the 2000 harvest in Ames, IA (group A); Savoy, IL (group B); or Raleigh, NC (group C). Cultivars are arranged in order of increasing total Ox concentrations. [b] No data. [c] L91-1533 [from IL2 × L97-561 (Hobbit × PI 82.264 from Korea)]; IL1 (Hobbit × T208, Yu Tae, T208 from Korea); L87-612 (Hobbit × PI 85.505 from Korea); L86K-73 (Corsoy x Williams sib) × L76-0132. (Beeson × Kosamame)]; L93-5557 (Century- *lx2* x Disoy); L83-3432 (from crosses among 12 parental lines: Williams, Essex, Harosoy- *dt1*, Clark-*dt1E1e2*, and eight southern (group VI-VIII) commercial cultivars); IL2 [Hobbit × PI 408.0168B from Korea); L87-626 (Hobbit × PI 157.404 from Korea); L93-3539 (Williams- *I r Rps1-c Rps2 Rsv1*).

Table 2. Total Oxalate, phytate, and Calcium Concentrations and Pedigrees of 30 Soybean Cultivars[a]

Purdue ID no.	total Ox (mg/100 g)	total $InsP_6$ (mg/100 g)	total Ca (mg/100 g)	pedigree
CX1852B-2-3	131	1.06	2.86	A[b]
CX1849C-3-3	139	1.11	2.46	A
CX1849B-2-3	145	0.50	2.68	A
CX1849B-8-2	148	0.44	2.13	A
CX1843I-4-6	170	0.49	2.85	B[c]
CX1850C-3-2	173	0.53	2.81	A
CX1843I-5-1	178	0.22	2.11	B
CX1844B-1-2	185	0.42	3.08	B
CX1844D-3-5	185	0.57	2.90	B
CX1844D-3-1	203	0.30	2.77	B
CX1848C-2-7	205	0.58	3.05	A
CX1843I-4-7	209	0.30	2.49	B
CX1844D-3-2	210	1.98	2.78	B
CX1846C-3-7	210	0.89	2.60	B
CX1847C-8-5	210	0.98	2.75	B
CX1844B-3-1	216	0.98	3.04	B
CX1846B-1-3	220	0.58	2.77	B
CX1850A-5-3	225	0.72	3.11	A
CX1844A-7-1	239	1.75	2.63	B
CX1846F-2-6	239	0.64	3.03	B
CX1846F-2-1	252	0.58	3.07	B
CX1844B-1-4	257	0.47	2.69	B
CX1843H-4-5	260	0.46	2.80	B
CX1845B-8-4	261	1.08	3.19	B
CX1844D-7-8	266	0.63	2.85	B
CX1844B-3-2	269	0.40	3.32	B
CX1845B-3-6	269	0.50	3.53	B
CX1843G-1-3	278	0.38	3.02	B
CX1844D-7-4	283	0.60	3.31	B
CX1843G-1-1	285	0.53	3.21	B
std error of mean	8.29	0.07	0.06	
range	131–285	0.22–1.98	2.11–3.53	

[a] All seeds were from the 2001 harvest in Ames, IA. (group D). Cultivars are arranged in order of increasing total Ox concentration. Correlation of total calcium with total oxalate was 0.615 ($p \leq 0.01$). Correlation of total oxalate with total phytate was –0.087 (p = n.s.). [b] (Savoy × M153-1-4-6-29) × Savoy. [c] (CX1834-1-6) × [(M153-1-4-6-14 × Athow) × Athow].

number was multiplied by 1000 to give the number of milligrams of Ox per 100 g of cultivar.

Total Ox in the 30 group D cvs (**Table 2**) was measured at Washington State University (WSU) by a modified method. A sample of 5 g of each cultivar was placed in a 50 mL polypropylene tube with 20 mL of 2 N HCl. After overnight soaking, each sample was homogenized in the same tube for 1 min by use of a homogenizer (PowerGen 125, Fisher Scientific, Pittsburgh, PA), and centrifuged for 5 min at 3925*g*. The supernatant was collected into a 100 mL graduated cylinder. The pellet was extracted twice more with 20 mL of 2 N HCl. These supernatants were poured into the same cylinder as the first, and the final volume was adjusted to 100 mL with 2.0 mmol of HCl. Some cultivars were analyzed at both laboratories to ascertain potential differences in Ox measurements. Mineral analyses (**Tables 2** and **3**) were carried out by atomic emission spectroscopy (Perkin-Elmer Optima 2000, Boston, MA) at WSU on 65 cvs by use of acid extracts as done for total Ox.

Determination of $InsP_6$ Concentration. Thirty-one of the group A−C cultivars and the 30 group D cvs were analyzed in duplicate for $InsP_6$ content to determine if there was any correlation between $InsP_6$ and Ox content. Group A−C cultivars were chosen to represent the complete range of Ox concentrations. The $InsP_6$ was measured by anion-exchange chromatography and phosphorus analysis by calorimetry (*35*). Briefly, soybean seeds were ground to a powder in a coffee grinder and extracted with 2.4% HCl for 3 h at room temperature followed by centrifugation. The supernatant was filtered and ran through an anion-exchange column. Phytic acid was eluted with 7 M NaCl and subjected to Kjeldahl digestion with concentrated H_2SO_4 and HNO_3. Inorganic P concentration was measured by a calorimetric method with ammonium molybdate and sulfonic acid reagents. Phytic acid concentration was determined on the basis of its P content of 28.2%. Red wheat bran (3% ± 0.3%) obtained from AOAC (Association of Official Analytical Chemists) was used as the control, and the measured values were within the reference values.

Statistical Analyses. We viewed the Ox measurements in group A−C cultivars as being subject to two principal sources of variation in our study, one that was considered larger than the other. The first, and smaller, source of variability was among measurements from duplicate determinations of Ox from the same cultivar grown in a single location, and the second arose from differences among Ox measurements for the same cultivar grown in different locations (fields). For purposes of making statistical tests and calculations of standard errors of means (SEM) of cultivars, we used the variability among locations within a cultivar to estimate experimental error. For $InsP_6$ measurements in all cultivars, we used variability among replicates within a cultivar to estimate experimental error.

The relationship between total Ox and total Ca concentrations was determined separately for group A−C cultivars and low-$InsP_6$ cultivars by a Pearson correlation. A 5% level of significance was used in all analyses.

RESULTS AND DISCUSSION

Whole, cleared, mature seed cotyledons displayed many CaOx monohydrate crystals when viewed between crossed polarizers (**Figure 1A**). Isolated crystals from the cotyledons showed they were twinned prismatic crystals (**Figure 1B**). The modified Sigma analysis kit allowed for the determination of both total and insoluble Ox, and thus soluble Ox. This analysis used EDTA instead of HCl. In two different treatments, ground, aqueous wetted soybean powder was treated with EDTA to digest the total Ox in the powder allowing for the analysis of total Ox. In another aqueous treatment, all soluble Ox was removed, leaving only the insoluble CaOx crystals in the powder seed fragments. EDTA treatment over 24 h digested the insoluble CaOx for insoluble Ox determination (**Figure 1C−E**).

Seeds from all 86 group A−C cvs contained varying amounts of insoluble and soluble Ox (**Table 1**). Soluble Ox was

Table 3. Seed Group, Total and Insoluble Oxalate, Total Phytate ($InsP_6$), Total Divalent Minerals, and Ca/Insoluble Ox Ratios in Seeds of 35 Soybean Cultivars[a]

cultivar	seed group	total Ox (mg/100 g)	insol Ox (mg/100 g)	insol Ox (mmol/100 g)	total $InsP_6$ (g/100 g)	total Ca (g/100 g)	total Ca (mmol/100 g)	total Mg (g/100 g)	total Mg (mmol/100 g)	total Ca + Mg (mmol/100 g)	Ca:insol Ox ratio (mmol/mmol)
Proto	A	82.3	29.4	0.33	1.23	2.32	5.79	1.79	7.35	13.14	34.7
Galena Genetics 003	A	91.6	74.5	0.85	1.01	1.65	4.11	1.53	6.27	10.38	9.7
Saturn	B	92.4	37.8	0.43	1.66	1.31	3.27	1.58	6.47	9.74	15.2
Galena Genetics 004	A	101.5	76.4	0.87	1.11	1.98	4.94	1.66	6.82	11.76	11.4
low linolenic	A	101.8	75.0	0.85	*b*	1.80	4.48	1.68	6.88	11.36	10.5
Gardensoy 22	B	104.5	85.1	0.97	1.30	2.38	5.94	2.05	8.39	14.33	12.3
Galena Genetics 001	A	111.4	83.2	0.95	*b*	1.64	4.09	1.62	6.64	10.73	8.7
Galena Genetics 002	A	119.4	64.4	0.73	*b*	1.62	4.05	1.49	6.12	10.17	9.3
IA1008	B	120.4	71.5	0.81	*b*	2.37	5.92	1.99	8.16	14.08	14.6
low saturated	A	121.2	96.1	1.09	*b*	1.45	3.63	1.39	5.70	9.33	6.6
IA4001	B	124.7	105.1	1.20	*b*	2.14	5.34	2.06	8.43	13.77	8.9
Cisne	B	127.8	79.7	0.91	1.06	1.99	4.97	1.96	8.01	12.98	11.0
Vinton 81	A	129.4	73.0	0.89	*b*	1.87	4.66	1.85	7.60	12.26	12.3
IA2025	B	131.3	63.9	0.73	*b*	1.58	3.93	1.73	7.11	11.04	11.2
Gardensoy 12	B	133.8	59.0	0.67	*b*	2.09	5.22	1.95	7.98	13.20	15.6
IA2011	B	134.2	98.9	1.12	*b*	1.61	4.00	1.85	7.57	11.57	7.1
N7103	C	137.2	112.3	1.28	*b*	2.03	5.07	1.78	7.30	12.37	7.9
Gardensoy 21	B	137.8	60.4	0.69	*b*	1.64	4.09	1.62	6.63	10.72	11.9
IA3006	B	139.1	87.3	0.99	*b*	2.29	5.72	2.08	8.53	14.25	11.5
Gardensoy 24	B	147.9	76.8	0.87	*b*	2.04	5.10	1.84	7.53	12.63	11.7
L99-1349	B	153.3	117.4	1.33	*b*	2.06	5.13	1.79	7.35	12.48	7.7
L95-1116	B	168.4	132.5	1.51	*b*	1.83	4.58	1.88	7.69	12.27	6.1
Gardensoy 02	B	170.4	81.8	0.92	*b*	1.43	3.58	1.58	6.46	10.04	9.8
L95-1409	B	178.3	152.4	1.73	1.50	1.93	4.82	1.58	6.48	11.30	5.6
Gardensoy 23	B	181.8	95.9	1.09	*b*	2.34	5.85	2.15	8.81	14.66	10.7
L99-3921	B	185.3	137.3	1.56	*b*	1.64	4.08	1.69	6.94	11.02	5.2
L96-5198	B	186.0	171.4	1.95	1.40	2.16	5.39	1.88	7.71	13.10	5.5
L99-3591	B	189.6	146.0	1.66	*b*	1.89	4.72	1.81	7.41	12.13	5.7
L98-7362	B	192.2	121.1	1.38	1.36	1.90	4.75	1.85	7.58	12.33	6.9
L96-5201	B	194.9	157.3	1.79	1.47	2.33	5.82	1.87	7.66	13.48	6.5
L96-5441	B	196.2	141.1	1.60	*b*	1.90	4.75	1.60	6.55	11.30	5.9
L98-6070	B	197.7	108.7	1.24	1.27	1.75	4.38	1.77	7.26	11.64	7.1
L99-3073	B	198.6	196.6	2.23	*b*	2.54	6.34	1.66	6.81	13.15	5.7
L99-3890	B	200.1	127.6	1.45	*b*	1.26	3.13	1.61	6.59	9.72	4.3
L98-7191	B	208.7	164.7	1.87	1.82	2.13	5.32	1.83	7.50	12.82	5.7
std error of mean		6.4	6.6	0.08	0.07	0.06	0.14	0.03	0.12	0.24	0.9
range		82.3–208.7	29.4–196.6	0.33–2.23	1.01–1.82	1.26–2.54	3.13–6.34	1.39–2.15	5.70–8.81	9.33–14.66	4.3–34.7

[a] Cultivars are from groups A–C (**Table 1**) and are arranged in order of increasing total Ox concentration. Correlation of insoluble oxalate with calcium was 0.289 ($0.05 < p < 0.10$). Correlation of insoluble oxalate with calcium + magnesium was 0.186 (p = n.s.). Correlation of insoluble oxalate with magnesium was 0.030 (p = n.s.).[b] No data.

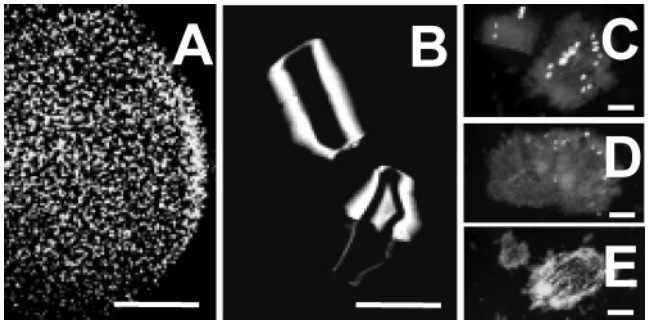

Figure 1. Cleared soybean seed tissues visualized microscopically between crossed polarizers. (**A**) Portion of one cotyledon from a near mature soybean seed. It is filled with many CaOx prismatic crystals. Bar equals 200 μm. (**B**) Two prismatic crystals isolated from cotyledon. One crystal is totally bright while the other is half bright, identifying the twinned nature of crystals. Bar equals 25 μm. (**C**) Portion of untreated ground seed powder. Crystals are present in tissue fragments. (**D**) Portion of 12-h EDTA-treated ground seed powder. Some crystals have been partially dissolved. (**E**) Portion of 24-h EDTA-treated ground seed powder. All crystals have been dissolved. Bars equal 150 μm in **C**–**E**.

determined by subtracting insoluble Ox from total Ox. Other studies (*17*, *18*) have measured total Ox by using HCl as the Ox digesting reagent. These group A−C cultivars contained total Ox ranging from 82.3 to 213.6 mg/100 g of dry seed weight, while the 30 group D cvs were somewhat higher, 131−285 mg/100 g (**Table 2**). In the group A−C cultivars that were grown in two different fields at the same location in the same year, there was no statistical difference in the resulting concentrations, so the data were combined.

Table 1 lists the 86 group A−C cvs from lowest to highest total Ox content, identifies insoluble and soluble Ox concentrations and their percentages, food uses, and pedigrees. Seven of these latter cultivars contained greater than 90.0% insoluble Ox (<10.0% soluble Ox); 17 were between 89.9% and 80.0%; 24 were between 79.9% and 70.0%; 14 were between 69.9% and 60.0%; 11 were between 59.9% and 50.0%; 12 were between 49.9% and 40.0%; and one was between 39.9% and 30.0%.

Although there were widely varying concentrations of Ox in all of the cultivars tested, all of the cultivars have more total Ox per 100 g of powdered soybean seed than the acceptable level of less than a 10 mg serving per day (*36*). Since 2−8% of dietary oxalate is absorbed, a serving with this range of seed Ox concentration could raise the urinary Ox excretion. If soy products made with high-Ox-containing seeds are consumed regularly by humans, the Ox intake could then be of concern for the population at risk for kidney stones. Two published human feeding studies carried out at WSU (*16*, *18*) indicated that all of the soybean seed and commercial soy food products fed to human subjects raised the level of Ox excretion. The

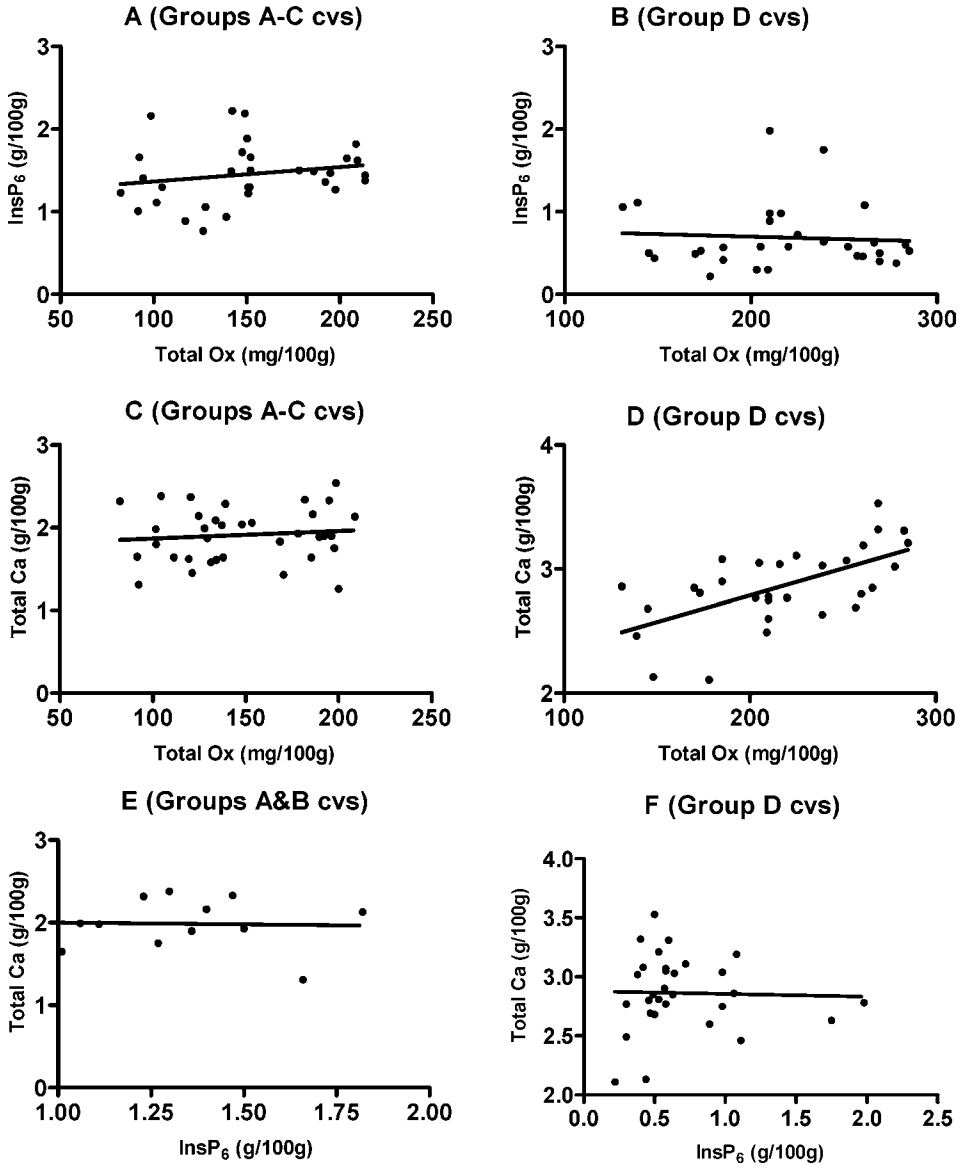

Figure 2. Correlation between total oxalate (Ox), total phytate ($InsP_6$), and total calcium (Ca): total Ox versus $InsP_6$ for groups A–C (**A**) and group D (**B**) cultivars; total Ox and total Ca in groups A–C (**C**) and group D (**D**) cultivars; and $InsP_6$ versus total Ca in groups A–C (**E**) and group D (**F**) cultivars. No significant associations were found except with total Ox and Ca in group D cultivars ($r = 0.6087$; $p < 0.0005$).

unknown factors still needing to be tested are the degree of absorption of both soluble and insoluble Ox; whether the ratios of insoluble to soluble Ox in seeds or food products are significant; and whether certain individuals are less susceptible than others to the potential of forming kidney stones and other Ox-related diseases when soybeans or soy foods are consumed on a daily basis.

Comparisons of Ratios of Insoluble and Soluble Ox to Total Ox. The amount of soluble Ox is important in foods (*1*) as it is the form most likely to be absorbed. Therefore, it seemed reasonable to develop an Ox determination method that analyzed the insoluble Ox, as well as the total Ox. Modification of the Sigma oxalate urinalysis procedure presented in this study allowed for the determination of both, as well as the soluble Ox.

The extreme ratios of high insoluble Ox and low soluble Ox, and conversely low insoluble Ox to high soluble Ox, are represented by L99-3073 [196.6 mg of insoluble Ox (99.0%), 2.0 mg of soluble Ox (1.0%)] and Proto [29.4 mg of insoluble Ox (35.8%), 52.8 mg of soluble Ox (64.2%)], respectively (**Table 1**). The remaining 84 cvs show intermediate ratios of insoluble to soluble Ox. Traditional wisdom states that only soluble Ox is readily absorbed by the gut whereas insoluble CaOx is not absorbed. However, a recent study in rats (*37*) suggested that 2% of CaOx is absorbed intact.

Comparison of $InsP_6$ and Ox Concentrations. Tables 1 and **2** show the $InsP_6$ and Ox concentrations in 31 group A−C cvs, selected from the 86 cvs for the range of Ox content, and 30 low-$InsP_6$ group D cvs, selected for the range of $InsP_6$ content. In the cultivars listed in **Table 1**, the $InsP_6$ varied from 0.77 to 2.22 g/100 g with an average of 1.45 g/100 g. These values are in the range reported by Anderson and Wolf (1−2.3 g/100 g) (*38*) and Raboy et al. (1.39−2.3 g/100 g with a mean of 1.76 g/100 g) (*34*) for soybean. Similarly, in the low-$InsP_6$ cultivars that are listed in **Table 2**, the $InsP_6$ concentration ranged from 0.22 to 1.98 with an average of 0.69 g/100 g, which is similar to the range of 0.63−1.7 g/100 g (mean of 0.97 g/100 g) reported by Wilcox et al. (*33*).

There was no relationship between $InsP_6$ and total Ox concentration among the cultivars studied (**Figure 2A, B**). This lack of correlation suggests that specific cultivars that contain high or low concentrations of $InsP_6$ and low concentrations of

Ox will need to be tested individually, until some other method is developed, for food use targeted for human consumption.

Association of Total Ox with Ca Concentrations. In the 35 group A−C cvs, no significant relationship was found between total Ox and Ca (**Figure 2C**). However, in the group D cultivars, the relationship was significant ($r = 0.3705$; $p < 0.005$) (**Figure 2D**). Overall, there was a mean molar ratio of Ca:insoluble Ox of 9.8, with a range of 4.32−34.65, so Ca is in excess in all cultivars tested. Although the insoluble form of Ox is likely to be mainly Ca salts, total Ca cannot be equated with insoluble Ox.

An unanswered question is what is the chemical nature of insoluble Ox and its relationship to Ca? It has been assumed that insoluble Ox is equivalent to CaOx, especially the crystalline form (**Figure 1A,B**). Indeed, in **Figure 2D**, total Ca correlates significantly with total Ox in these group D cultivars. However, MgOx is also insoluble in water, although its extractability with water in the soluble Ox assay has never been tested. The Mg concentration (**Table 3**, no graph) was not significantly related to either total or insoluble Ox. The Ca was marginally related ($p = 0.12$) to insoluble Ox. This supports the idea that insoluble Ox is CaOx only and that Mg is not bound to Ox in soybean. The majority of Ca in soybean is not bound to Ox. Therefore total Ca cannot be used as a surrogate for Ox in any single cultivar. There was no association found with total Ca and $InsP_6$ (**Figure 2E,F**) in any of the four seed groups.

Cultivars Low in Ox and Low or High in $InsP_6$. **Table 1** identifies 5 cvs (Galena Genetics 003, L97-0161, IA3006, Iroquois, and Cisne) categorized as low (<140 mg/100 g) in Ox content that are relatively low in $InsP_6$ (≤1.01 g/100 g). The remaining cultivars show a variation in both concentrations of both Ox and $InsP_6$ that are higher in total Ox. **Table 1** also identifies 2 cvs (Saturn and LS201) as low in Ox and relatively high in $InsP_6$. The remaining cultivars show a variation in both concentrations of Ox and $InsP_6$. There are no low-Ox cultivars in the 30 group D cvs sampled (**Table 2**), although $InsP_6$ concentrations were mostly lower than those of the group B cultivars. Since the comparison of Ox to $InsP_6$ among the 61 cvs analyzed showed no apparent correlation, breeding for a cultivar low in Ox and low in $InsP_6$ should be possible, based on the pedigree of the parents and their Ox and $InsP_6$ traits.

Pedigrees to Consider When Progeny Are Selected for Certain Ox and/or $InsP_6$ Concentrations. Oltmans et al. (*39*) used reciprocal crosses and reported a phenotypic ratio of 15:1 (normal:low $InsP_6$) in soybean. Low seed $InsP_6$ was controlled by recessive alleles designated *pha1* and *pha2* at two independent loci that exhibited duplicate, dominant epistasis. The inheritance of Ox in soybean has not been reported.

Table 1 identifies parents that have a genetic influence on total Ox. Six cultivars (Proto, Saturn, Verde, Vinton 81, Parker, and Mercury) were used as parents in 16 progeny cvs that we analyzed. Three cultivars (Proto, Saturn, and Verde) with low total Ox gave progeny cultivars with higher total Ox. Cultivar Verde (114 mg/100 g) was a parent that resulted in 5 progeny cvs ranging from 137.8 to 161.0 mg/100 g. Cultivar Vinton 81 (129.4 mg/100 g) was a parent that resulted in 4 progeny cvs ranging from 138.3 to 150.8 mg/100 g. Cultivar Mercury, which had the highest total Ox (213.6 mg/100 g), was a parent that resulted in 4 progeny cvs, all with high total Ox (157.1, 184.9, 200.1, and 209.5 mg/100 g).

Reciprocal cross-pollination of cultivars Vinton 81 and Tousan 114 gave progeny cultivars of 147.6 and 149.0 mg/100 g, which indicated no maternal or cytoplasmic effect on total Ox (see **Table 1**). However, large differences were noticed for insoluble and soluble Ox. Cultivars L87-612 × L86k-73 had 7 progeny cvs that varied from 146.2 to 196.6 mg/100 g total Ox. Two progeny cultivars from this cross combination had the lowest soluble Ox of all of the cultivars analyzed, and one of these, L99-3073, had the highest insoluble Ox concentration.

Our results suggest that an individual parent may have influence by itself on total Ox or $InsP_6$ concentration. Choice of both parents, based on Ox and $InsP_6$ concentration, may allow a plant breeder to choose which parents will result in segregating progenies with low Ox and low $InsP_6$. This approach, based on the present and past data, needs to be tested further by analyzing the various parents in this study where Ox and $InsP_6$ concentrations seem to show an association and by using untested, additional cultivars as parents in reciprocal crosses. Growing conditions and geographical locations also need to be considered as they may influence Ox and $InsP_6$ concentrations or agronomic performance.

The primary purpose of this preliminary study was to determine total Ox and $InsP_6$ concentrations in available cultivars selected for Ox and $InsP_6$ contents. A secondary objective was to determine the association of minerals with the insoluble Ox content. To do this, we developed an Ox determination technique that could measure not only total Ox but also insoluble and soluble Ox. Previous techniques generally measured only total Ox. Being able to calculate total, insoluble, and soluble Ox versus just total Ox may eventually give insight as to how much insoluble Ox may be absorbed by the human gut versus just total Ox. Adding environmental conditions (soil composition and pH, growing temperature, and latitude) with pedigree data may provide better information of how to breed and select optimal cultivars for Ox and $InsP_6$ for reducing the risk of kidney stones and preventing mineral deficiencies.

In conclusion, there are no studies that we are aware of where genes for both Ox and $InsP_6$ have been described for their control of Ox or $InsP_6$ concentrations. Our projection is that as plant genomes are completed and all of the genes are identified as to their functions, the genes controlling Ox and $InsP_6$ biosynthesis will be found. When this occurs, there will be the opportunity to screen seed stocks and to design parental combinations to produce seeds that are nutritionally suited to the prevention of human and animal kidney stones by having both relatively low Ox and $InsP_6$ in soybean seeds and soy food products.

ABBREVIATIONS

CaOx, calcium oxalate; cvs, cultivars; Ox, oxalate; $InsP_6$, phytate.

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