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Fine Screening Potato (*Solanum*) Species Germplasm for Tuber Calcium

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ABSTRACT

A representative sample of the US potato germplasm collection was screened in the greenhouse for ability to accumulate tuber calcium. Germplasm with very high and very low calcium accumulation capacity has been identified by progressively screening for extremes among species, among populations (accessions), and finally among genotypes (i.e., fine screening). Among accessions, the best *S. gourlayi* and *microdontum* populations averaged more than six-fold greater tuber calcium than the poorest *S. kurtzianum* population. When screening was performed at the genotype level, the best individuals of *gourlayi* and *microdontum* had over eight-fold greater calcium than the poorest *kurtzianum* individual. When grown in the field, calcium levels of *S. microdontum* selections were confirmed to be significantly higher than *tuberosum* cultivars. When selected genotypes were intercrossed and their progeny were compared to their original populations, mean tuber calcium was significantly changed in the intended direction of selection for *S. kurtzianum* (made lower) and *S. microdontum* (made higher). These stocks are expected to be useful tools for study of the genetic and physiological bases of tuber calcium accumulation, refining screening methods, and breeding improved cultivars.

INTRODUCTION

Resistance to several pathological and physiological tuber defects, and improvement in tuber yield and grade have been correlated with calcium level of tubers (McGuire and Kelman 1984, Palta 1996, Serquen and Peloquin 1996, Simmons and Kelling 1987). In addition, it has been demonstrated that calcium can mitigate heat stress injury to potatoes (Palta 1996, Tawfik *et al.* 1996). Previous work has suggested that increase in tuber calcium is influenced both by calcium applications (Kratzke and Palta 1985, Kratzke and Palta 1986, Simmons and Kelling 1987) and the genotype tested (Bartz *et al.* 1992, Kratzke 1988, McGuire and Kelman 1986, Tawfik *et al.* 1996, Tzeng *et al.* 1990). Potato tubers accumulate calcium primarily from the surrounding soil independently from the calcium accumulation in the leaves (Kratzke and Palta 1985, Kratzke and Palta 1986). Thus, useful genetic diversity for this trait could manifest in the ability to accumulate calcium under conditions of low available soil calcium, and/or ability to efficiently accumulate calcium applied as soil amendments. The breadth of genetic diversity for many traits among the over 200 wild and cultivated *Solanum* species is considerably greater than among US cultivars (Hanneman 1989, Hanneman and Bamberg 1986). With this in mind, we sought to identify germplasm with very high and very low calcium accumulation capacity by progressively screening for extremes for this trait first among species, then among populations, and finally among genotypes (i.e., fine screening).

Measurement of tuber calcium content, although not difficult technically, is labor-intensive and time consuming. Results can vary substantially depending upon environmental conditions, cultivar tested, and other factors. Identification of *Solanum* genotypes with different capacities to accumulate calcium could provide both potential breeding lines and standard clones for gauging the influence of environmental and other factors on the uptake of calcium by tubers We previously identified three *Solanum* species which when grown under regimens of high and low calcium exhibited wider ranges in tuber calcium accumulation than *Solanum tuberosum* (Bamberg *et al.* 1993).

Our previous work screening a representative sample of species identified *Solanum gourlayi* (grl) as a species with high calcium accumulation when watered with a solution low in calcium, and a great increase when watered with a solution high in calcium (Bamberg *et al.* 1993). In these studies we also found that *S. microdontum* (mcd) had only moderate calcium when given low calcium solution, but the highest increase when given high calcium solution. *S. kurtzianum* (ktz) was lowest in both solutions.

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The present work reports testing and selection among populations within the above species, then among genotypes within those populations selected. A preliminary field test of some of these selected genotypes was done and the progeny of selected genotypes were compared to a random sample of individuals in the base population from which they originated.

MATERIALS AND METHODS

Greenhouse tests were conducted on plants in ProMix BX (Premier Brands, Red Hill, PA), in 1.5 liter clay pots in a winter greenhouse at Sturgeon Bay, WI. Plants were watered with either of two nutrient solutions. The control watering solution was 1/4 strength Hoagland's solution (Hoagland and Snyder 1933) made with tap water. This was about 80 ppm calcium. The treatment solution was made by augmenting the control solution to 800 ppm calcium with calcium nitrate (thus a 10-fold increase of calcium concentration). The same amount of nitrogen added to the treatment solution as calcium nitrate was added to the control solution in the form of urea. Tuber tissue produced was processed and assayed for overall ppm calcium on a dry weight basis on whole tubers by atomic absorption spectrophotometry. All tubers from each experimental unit were dried and ground, and a sample taken from the homogenated powder. Other details of methods were the same as in previous screening among species by Bamberg *et al.*, 1993.

In 1992, populations from *Solanum gourlayi*, *microdontum* and *kurtzianum* were chosen from the genebank based on diversity of natural geographic origins. Each experimental unit consisted of six randomly chosen seedlings in a randomized complete block (RCB) design with two blocks. From this test, one outstanding population from each species was selected for genotypic screening. This was done by judging a combination of the control and treatment calcium response and, in some cases, thriftiness of the plants. The populations selected are indicated in Table 1 by an arrow (←).

The outstanding population selected for ktz had among the poorest tuber calcium considering both control and treatment. The grl and mcd populations chosen had tubers with among the highest calcium levels in both watering solutions.

In 1993, fifteen random seedlings from each of the three selected outstanding populations were clonally replicated (by nodal cuttings) and evaluated in the same manner as with populations in 1992, except that each experimental unit consisted of three plants (of identical genotype) in an RCB design with two blocks. Three outstanding individual genotypes were selected from each population on the same basis as population selection in 1992. These genotypes were crossed with each other (within their respective species) to create a selected population.

In 1994, two of the three genotypes selected from each species in the 1993 genotype screening were grown in three-hill field plots replicated four times in a completely random design (CRD) with *tuberosum* cultivars Superior and Russet Burbank. Black plastic shading was used to simulate short days, since wild species typically do not tuberize well in the long summer days at Sturgeon Bay, WI. The soil was Bark River silt loam with a calcium content of 1,744 ppm when assayed using the tuber tissue method. Entire tubers were analyzed. Superior peel (about 1 mm deep) and tissue were also analyzed separately.

In 1995, progeny obtained from crossing selected genotypes within species (intermates), were compared to a random sample of individuals from the original populations (the populations from which intermates had been derived). This was to test whether this selection protocol could be used effectively to change population means. The intermates and original populations were compared in the same manner as populations in 1992, except that each experimental unit consisted of twelve seedlings replicated twice in a completely random design.

RESULTS

In 1992, large differences within species were observed. Under control conditions, populations of ktz ranged from 125-231 ppm, barely overlapping with those of grl which were 200-587 ppm. Thus, the best grl showed over four-fold increase over the poorest ktz (Table 1). The average for grl populations was over double that of ktz populations.

Under treatment conditions, mcd and grl populations were 1250-3060 ppm and 775-2708 ppm respectively, and ktz was 444-2164 ppm (Table 1). Thus, the best grl and mcd had about a six-fold increase over the poorest ktz. While these ranges overlapped, the means of mcd and grl populations were again about double that of ktz.

Error was high and the populations were not well distinguished in a statistical sense. Variance in treatment was significantly higher than control, invalidating a pooled ANOVA, thus a separate LSD was calculated for treatment and control. Details of average tuber calcium levels, germplasm selected and significance are given in Table 1.

In 1993, when genotypes within selected populations were tested, their average calcium levels were much lower than the corresponding populations tested in bulk in 1992

TABLE 1.—*Calcium content of tubers of selected populations of three* Solanum *species generated on plants watered with 80 ppm Ca (control) or 800 ppm Ca (treatment), 1992.*

Population	Tuber Calcium (ppm) Control 80 ppm watering solution	Treatment 800 ppm watering solution
Solanum gourlayi (grl)		
PI 473002	200*	775 *
PI 458339	225*	1091 *
PI 458341	487	1142 *
PI 472992	262*	1257 *
PI 442673	250*	1424
PI 210038	425	1518
PI 442669	493	1599
PI 435073	268*	1600
PI 473001	512	1618
PI 442670	262*	1624
PI 458340	387	1711
PI 442671	268*	1761
PI 472993	337*	1846
PI 442672	406	1903
PI 473062	587	2203
PI 320322	406	2205
PI 265579 ←	511	2475
PI 435074	394	2708
mean	371	1692
species[1]	743	2219
Solanum kurtzianum (ktz)		
PI 442678	169	444
PI 472941	168	599
PI 472923 ←	163	624
PI 472924	156	637
PI 320271	162	700
PI 442680	125	768
PI 442679	144	798
PI 442681	187	1137
PI 458328	231	1380
PI 458327	187	2164 *
mean	169	925
species[1]	160	634
Solanum microdontum (mcd)		
PI 265575	175	1250 *
PI 218223	256	1261 *
PI 218222	350	1472 *
PI 208866	232	1494 *
PI 218226	225	2034
PI 218224	256	2087
PI 265881	269	2335
PI 218225 ←	312	3060
mean	259	1874
species[1]	383	2212

[1]Average in species-based screening previously published (Bamberg *et al.* 1993). Populations marked with * are significantly different from the mean of ones selected (←) at p<=0.05. LSD for control = 148, for treatment = 1124.

(Table 2). The reduction was 30-40% for grl and ktz under control conditions, about 60% for mcd. In control conditions, genotypes of ktz ranged 60-266 ppm, those of grl 232-382 ppm. Thus, the best grl had over a six-fold increase compared to the poorest ktz. Only one ktz individual overlapped into the grl range. The average for grl genotypes was 305 ppm, versus 117 ppm for ktz genotypes.

Under treatment conditions, the grl and mcd genotypes ranged from 955-2,121 ppm and 600-2,198 ppm respectively, while ktz ranged 255-573 ppm. The means of mcd and grl populations were three to four times that of ktz. The best mcd and grl genotypes had an over eight-fold greater calcium compared to the lowest ktz (Table 2).

Error was again relatively high. The mean of the three outstanding genotypes selected within species were not significantly different from many of the other genotypes not selected. The four grl genotypes missing from Table 2 did not tuberize sufficiently for analysis.

In 1994 comparisons of selected genotypes to cultivated *tuberosum* varieties in the field, *tuberosum* cultivars had the lowest calcium, averaging 311 ppm. The ktz selected genotypes (selected for low calcium) were next highest with an average of 451 ppm. The highest calcium was found in mcd selected genotypes (selected for high calcium), having an average of 624 ppm. The grl selections failed to produce sufficient tubers for analysis. Peel of the cultivar Superior averaged 659 ppm calcium, whereas the flesh was 243 ppm. The above noted species differences are each significant at p<=0.05 (Table 3).

In 1995, progeny of intermated selected genotypes were compared to their original populations. Averages for both control and treatment tubers were reduced in the ktz selected populations and increased in the mcd selected populations as compared to their unselected populations. These differences were only significant for mcd and ktz plants grown in high calcium treatment solution.

DISCUSSION

This work indicates that extremes for tuber calcium accumulation can be identified through progressive screening at the species, population and individual level (fine screening). High tuber calcium accumulation capacity observed in the greenhouse is also apparent in field conditions and may be improved even further through selection. While *trends* among species and selected genotypes were fairly consistent, absolute average tuber calcium levels of the same germplasm was highly variable, especially across years

TABLE 2.—*Calcium content of tubers of selected genotypes of three* Solanum *species generated on plants watered with 80 ppm (control) or 800 ppm (treatment) solutions, 1993.*

Genotype	Tuber Calcium (ppm)[1]	
	Control 80 ppm watering solution	Treatment 800 ppm watering solution
Solanum gourlayi (grl) PI265579		
G 9	317	955 *
G 4	306	989 *
G 1	300	1176 *
G 7	251	1326 *
G14	368	1493
G 2	232	1493
G10	264	1552
G 8	323	1592
G11←	277	2010
G 5←	382	2119
G15←	331	2121
mean	305	1530
population 1992[2]	511	2475
population 1995[3]	501	1405
Solanum kurtzianum (ktz) PI 472923		
K 8←	80	255
K 9←	83	270
K12←	60	284
K15	110	300
K 6	83	325
K13	89	328
K 1	266*	340
K 7	95	355
K 2	131	395
K11	91	398
K 5	128	398
K14	83	417
K10	108	423
K 3	166	473
K 4	185*	573
mean	117	369
population 1992[2]	163	624
population 1995[3]	176	922
Solanum microdontum (mcd) PI 218225		
M 2	78	600 *
M 4	113	837 *
M 9	127	848 *
M 6	148	900 *
M 7	109	938 *
M 5	94	968 *
M 8	195	1022 *
M11	75	1057
M12	178	1062
M 1	115	1191
M13	185	1264
M10	140	1307
M 3←	203	1434
M14←	144	1542
M15←	160	2198
mean	138	1145
population 1992[2]	312	3060
population 1995[3]	226	1471

[1]Genotypes marked with * are significantly different from the mean of ones selected (←) at $p<=0.05$. LSD for control = 106, for treatment = 670.
[2]average of random genotypes of the same population tested in 1992
[3]average of random genotypes of the same population tested in 1995

(Table 1, 2). If factors causing this variation could be identified and controlled, the efficiency of selection might be improved. The extreme types identified in this work may be useful as standards to help detect such factors.

In the 1992 experiment to select populations within species, the one selected population was not well distinguished from its peers statistically. One might assume that experimental error resulted from failure to control factors which caused germplasm to accumulate *less* calcium than its genetic potential. This is supported by the larger variance of plants grown in high calcium treatment conditions both in this experiment and the 1993 test of genotypes within these populations. Thus, when selecting for high calcium, selecting the germplasm with the greatest experimental unit value might be advantageous. Previous work suggested that the outstanding feature of mcd is its ability to make use of the calcium supplied in the high calcium treatment. In this regard, the population selected, PI 218225, not only had the highest treatment mean, but also the highest value of any experimental unit. The outstanding feature of grl, according to past work, is its ability to produce relatively high calcium tubers in both treatment and control calcium supply environments. The population selected, while having nearly the greatest treatment and control averages, did not represent the highest observations. Thus, in the case of grl, population PI 473062 might have been a better selection, since it had the highest treatment observation and second-highest control observation. Selection of PI 265579 could not have been too detrimental, however, since the average of its three best genotypes surpassed that of the three best genotypes of the selected mcd population in 1993.

Screening at the genotype level in 1993 revealed much variation within populations. Although this might suggest that the genetic variation between different sets of six random seedlings used to replicate populations in 1992 introduced unaccounted variation in population means, CV for both experiments was nearly equal at about 31%. Apparently, unknown factors in the growing conditions of these two test years were different and had a significant influence on tuber calcium levels. It would have been ideal to assess this by testing many clonally replicated individuals within many populations in a single experiment, but greenhouse space and watering apparatus were limiting. Such work might be accomplished at a location where daylengths allow tuberization of these materials in the field during a normal growing season. Alternately, one might undertake the considerable effort to cross many individuals with a standard cultivar and screen the resulting progeny in the field. Despite these limitations, genotypes were identified which were significantly different from at least some others in their populations, particularly in the case of mcd.

The potential of mcd selections to have high tuber calcium was verified in field conditions. Surprisingly, the ktz genotypes selected for very low calcium also had significantly higher calcium than cultivars. Since tuber peel contains much more calcium than the interior flesh, there is a bias against large tubers when overall calcium is compared (Bamberg *et al.* 1993). Based on the approximate size and shape of the tubers, a 5:1 flesh:peel volume ratio was expected for cultivars. Calculations based on observed proportion of peel and flesh sample calcium predict 312 ppm for the entire tubers—close to the observed 325 ppm for Superior. Observed calcium levels of entire tubers can also be adjusted so they are comparable to the small tubers of species (where peel:flesh ratio is about 1:1) by multiplying by about 1.44. If this is done, ktz selections are no longer significantly higher than cultivars, but mcd selections still are (Table 3).

In 1995, comparisons of progeny of intermated genotype selections and their original populations provided some evidence that populations could be changed by recurrent selection. This is at least true for response of species mcd (selected family improved) and ktz (selected family declined). It might be expected that grl would not respond to selection, since the range of its genotypes was observed to be much less than ktz or mcd in 1993. Also, it is a tetraploid, while mcd and ktz are diploids. This evidence of the possibility of improvement through selection is encouraging. Families with more extreme means may be a source of even more outstanding individuals.

Continued fine screening and recurrent selection may be able to produce populations uniformly extreme, i.e., homozygous, for as many as possible of the genes that contribute to extreme tuber calcium. Breeders could use any individual from these populations knowing all the genetics for high calcium would be transmitted to the F_1. Individuals from these families would also provide good models for studies of the genetic and physiological bases of tuber calcium accumulation and its associated beneficial effects.

TABLE 3.—*Calcium content of field grown tubers of selected genotypes of wild* Solanum *species and cultivar checks, 1994.*

Genotype[1]	Tuber calcium[2] (ppm)
Russet Burbank	297 a
Superior	325 a
Russet Burbank (adj)[3]	428 b
Superior (adj)[3]	468 b
S. kurtzianum K 9	417 b
S. kurtzianum K 8	484 b
S. microdontum M 15	605 c
S. microdontum M 14	643 c

[1]*S. gourlayi* selections did not tuberize; *S. kurtzianum* genotypes are from PI 472923; *S. microdontum* from PI 218225.
[2]Means followed by the same letter are not significantly different at p<=0.05. LSD = 84
[3]Calculated overall tuber calcium adjusted to normalize peel/flesh proportion of large cultivar tubers (1:5) with smaller wild species tubers (1:1) = large tuber ppm x 1.44.

TABLE 4.—*Tuber calcium content of selected and unselected* Solanum *species populations when watered with control (80 ppm) and treatment (800 ppm) calcium solution, 1995.*

Material	Tuber Calcium (ppm)[1] Control	Treatment
<u>*S. kurtzianum* PI 472923</u>		
unselected population	176 a	922 b
selected population[2]	164 a (7% less)	726 a (21% less)
<u>*S. gourlayi* PI 265579</u>		
unselected population	501 b	1405 c
selected population	491 b (2% less)	1388 c (1% less)
<u>*S. microdontum* PI 218225</u>		
unselected population	226 a	1471 c
selected population	321 a (42% more)	1647 d (12% more)

[1]Means followed by the same letters are not significantly different at p<=0.05. LSD = 157. Remarks compare selected population to unselected source population.
[2]See Table 2 for selected genotypes (←) which were intercrossed to make the selected populations.

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