SCREENING TUBER-BEARING *SOLANUM* (POTATO) GERMPLASM FOR EFFICIENT ACCUMULATION OF TUBER CALCIUM

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Abstract

Resistance to several pathological and physiological tuber defects has been correlated with calcium level in the peel. In this study, a representative sample of potato species (including cultivated), was grown in the greenhouse. Plants were watered with low and high calcium solutions, and the resulting tubers analyzed. Thus, potato germplasm was screened for ability to accumulate tuber calcium when a control level of calcium is available, and ability to respond when higher levels are supplied. Some wild species significantly exceeded cultivated materials in one or both of these measures. Such species may provide a resource for breeding varieties which are more efficient calcium accumulators.

Compendio

La resistencia a varios defectos patológicos y fisiológicos de los tubérculos han sido correlacionados con el nivel de calcio en la piel. En este estudio, una muestra representativa de especies de papa (incluyendo a las cultivadas), se mantuvo en el invernadero. Las plantas fueron regadas con soluciones de baja y alta concentración de calcio y se analizaron los tubérculos obtenidos. Así, el germoplasma de papa fue evaluado y selecionado para capacidad de acumulación de calcio en los tubérculos cuando se dispone de un nivel de control del calcio y capacidad para responder cuando se proveen niveles más altos. Algunas especies silvestres excedieron significativamente a los materiales cultivados en una o ambas de estas medidas. Tales especies pueden proporcionar un recurso para el mejoramiento de variedades que son acumuladoras más eficientes de calcio.

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Introduction

Increased concentrations of calcium in the peel of potato tubers has been shown to significantly reduce the severity of economically important tuber defects such as soft rot caused by *Erwinia* spp. (8), internal brown spot, and subapical necrosis (13). Increased levels of peel calcium have also been shown to be correlated with improvements in tuber yield and grade (11).

Calcium movement in most soils is limited, and calcium availability to the plant may be particularly limited if the soil's natural calcium content is low, a low pH is maintained to reduce scab disease, the cation exchange capacity is low (11), or if a combination of these factors exists. Calcium movement within the plant is also limited, apparently dependent on xylem transport. Tubers have much lower calcium concentrations than aerial organs, presumably because tubers buried in the soil have low rates of transpiration and thus draw relatively little xylem water (6).

Increased tuber calcium concentrations and its associated benefits has been shown to be attainable by application of water soluble forms of calcium to the tuber zone at bulking (7, 11). This may occur through direct absorption by the tuber, through roots on stolons or on tubers (6).

The ability to accumulate calcium has also been shown to be greater in certain potato cultivars (genotypes) (5, 9, 13), presumably due to genetic differences in the potential for absorption or movement of calcium to the tuber. Thus, it may be possible to increase tuber calcium by both cultural and genetic means.

Solanum tuberosum ssp. *tuberosum* is the only potato species cultivated in the United States, but six other tuber-bearing *Solanum* species are cultivated in Latin America, and over 200 wild species exist (3). These species often exhibit a broader range of resistance to diseases, pests, and stresses than found within ssp. *tuberosum* (1). Fortunately, many of these species can be crossed directly with ssp. *tuberosum*, or through relatively simple ploidy manipulations (4). Over 100 of these species are maintained at the Inter-Regional Potato Introduction Project (IR-1) at Sturgeon Bay, Wisconsin USA (2), and are available for use by potato breeders in the U.S. and worldwide. As a result, there are numerous cases in which wild and primitive cultivated species have been incorporated into U.S. varieties (10). It is difficult to evaluate these species for tuber traits under field conditions in North America, however, since the short days (*i.e.* long nights) these species require for tuberization only occur during winter.

The objective of this study was to survey the variability of calcium accumulation among a broad spectrum of tuber-bearing *Solanum* species under greenhouse conditions, in an attempt to identify promising germplasm for breeding potato cultivars with greater calcium accumulating potential and the associated greater resistance to storage rots and defects.

Materials and Methods

Twenty-one wild and cultivated species were chosen from the IR-1 collection. The sample was constructed to represent broad genetic diversity according to current taxonomy (3), while selecting species which were expected to be most easily introgressed into *S. tuberosum*. The IR-1 collection maintains multiple collections (accessions) of the selected species (2). Three accessions from different geographical areas were selected to represent each species. *Solanum tuberosum* ssp. *tuberosum* was represented by two accessions of botanical seed from Chile and by the U.S. cultivar Ontario. Identification of the materials used is given in Table 1.

In mid-September, botanical seeds of each accession were soaked for 24 hours in 2,000 ppm GA_3 to improve germination, rinsed with tap water, sowed on Jiffy Mix in 8 cm clay pots, and covered with a thin layer of vermiculite. When about 3 cm tall, 15 seedlings were transplanted to soil in 6 cm Jiffy pots. About one month after sowing, when plants were about 10 cm tall, the 12 most vigorous plants were transplanted to Promix BX

TABLE 1.—*Identification of potato germplasm evaluated for efficient accumulation of tuber calcium.*

Species (*Solanum* . . .)	Accessions (PI . . .)	Abbreviation
acaule	472661, 473481, 500047	acl
canasense	265863, 310956, 473345	can
chacoense	197760, 275139, 320293	chc
demissum	160208, 230589, 498232	dms
fendleri	275156, 497998, 498004	fen
gourlayi	265579, 473062, 500049	grl
infundibuliforme	265867, 472894, 498351	ifd
kurtzianum	472923, 472941, 498359	ktz
megistacrolobum	265873, 473133, 498383	mga
microdontum	218225, 473171, 500041	mcd
oplocense	435079, 473185, 473190	opl
papita	249929, 283102, 498033	pta
pinnatisectum	184774, 275236, 347766	pnt
polytrichon	184770, 255547, 498039	plt
spegazzinii	205407, 472986, 500053	spg
stenotomum[1]	195204, 230512, 292110	stn
stoloniferum	205510, 283109, 498057	sto
tarijense	195206, 473243, 473336	tar
tuberosum ssp. *andigena*[1]	233994, 258881, 500060	adg
tuberosum ssp. *tuberosum*[1]	Ontario, 209770, 245796	tbr
verrucosum	161173, 275255, 498062	ver

[1]Cultivated species. Others are wild.

in 15 cm clay pots, as were well sprouted tuber pieces of the *tuberosum* cultivar Ontario.

After final transplanting, plants were watered only with the test solutions, while they had previously been watered with tap water. Two nutrient solutions were prepared. The control solution was formulated to approximate ¼ strength Hoagland's solution, previously determined to promote satisfactory growth under similar conditions. Since the solutions were made using tap water which was found to contain about 80 ppm calcium, calcium nitrate in the formula was omitted and the prescribed concentration of nitrogen was restored by addition of urea. The treatment solution was prepared by adding calcium chloride to the control solution to bring it to 800 ppm calcium. Thus, treatment and control solutions were similar except for a 10-fold increase of calcium in the treatment solution. Calcium levels of the solution were monitored throughout the experiment.

Treatment and control solutions were administered as follows: A single greenhouse compartment with two long rows of benches was used. A pair of 3 cm supply hoses was fixed to the middle of each of these two rows. One of each of these pairs carried control solution, the other, treatment solution. Each row of benches represented a block of a Randomized Complete Block design. The 12 seedlings of each accession were divided into four sets of three contiguous pots each, each set being an experimental unit. Two of these units were marked as "treatment," the remaining two as "control". Thus, one experimental unit designated treatment and one designated control from each of the 63 accessions were randomized within each block, and each species was represented by six experimental units within each block. The appropriate solutions were delivered from the supply hoses to the pots by 3 mm plastic capillary watering tubes. Solutions were pumped to the supply hoses from 1000 liter tanks. Prior to initiation of the experiment, the system was tested to ensure that each location along the watering system delivered solutions at approximately the same rate.

Plants were watered as needed to sustain normal growth. Every two weeks, they were overwatered to flush salts from the medium which might otherwise have accumulated. Pots were placed on wood blocks to preclude cross contamination by puddling on the bench tops. Long days (14 hours) were maintained with artificial illumination added to the end of the natural daylight. When most plants were 30-50 cm tall and were beginning to flower, artificial lighting was discontinued. Thus, natural days declined to a minimum of about 9 hours in mid-December. In mid-January, tuberization was assessed. It was found that all pots contained many tubers with fully developed skins and deteriorating stolons, indicating that tuberization was complete. After a last flushing of the medium with tap water, all watering was discontinued.

All tubers from each experimental unit were collected into one paper bag. They were thoroughly washed and rinsed with distilled water. Fresh weight and number of tubers were recorded for each sample to allow cal-

culation of average tuber size. Tubers were then sliced, dried, ground, ashed, and analyzed for calcium content (ppm of dry weight) by atomic absorption spectrophotometry.

The mean tuber weight (*i.e.*, size) within each experimental unit was calculated. The correlation between tuber size and calcium ppm was computed. Correlations between tuber size and calcium ppm were also done within species and pooled (12). The treatment (T) and control (C) experimental units for each accession within each block were logical pairs. For each of these pairs, the differences in calcium ppm (T-C) was calculated. The (T-C) statistic represented the efficiency of accumulation of supplemental calcium. The (C) statistic represented the ability of tubers to accumulate calcium in an unsupplemented (low) calcium environment. Analysis of variance (ANOVA) was done on (T-C) calcium ppm difference as well as on the control (C) calcium ppm. ANOVA F values were calculated for the effect of blocks, species, and accessions within species. These values were compared to the critical F values for $p = 0.05$ to assess statistically significant differences. The LSD for species means was calculated to differentiate species.

Results and Discussion

The average size of tubers of cultivated species (ssp. *tuberosum*, ssp. *andigena*, *stenotomum*) was 5.8 grams, considerably greater than that of the overall mean tuber size of 2.5 grams. A greater concentration of calcium is found in the peel than in other tissues (7, 9). This could have introduced a bias against large tubers when the entire tuber was tested for calcium ppm, because the proportion of peel : total is less in larger tubers. Thus, larger tubers would be observed to have a lower calcium ppm, even if their peel calcium ppm was equal to that of a smaller tuber.

The overall correlation of tuber size and calcium ppm was calculated at -0.30, which indicates that the association of larger tubers with lower calcium ppm is highly significant. This could have been due to either the decrease in surface area of relatively large tubers as discussed above, or due to a low calcium accumulation potential of particular species (viz., cultivated species), with characteristically large tubers. To differentiate between these two possibilities, correlations within species were calculated and pooled (12). The pooled correlation of tuber size and calcium ppm within species was -0.125 which is not significant at 95% probability. This suggests that the observed decrease in calcium ppm associated with larger tubers is not associated with the decreased proportion of peel tissue in larger tubers, but suggests that some of the species with naturally larger tubers (*e.g.*, cultivated species ssp. *tuberosum*, ssp. *andigena*, *stenotomum*), are also poor calcium accumulators. Therefore, these data of observed ppm calcium from these samples of entire tubers was considered to be an accurate measure of calcium accumulation capacity and were not adjusted to account for tuber size.

Table 2 presents the results of ANOVA. The calculated F values for blocks, species and accessions within species is expressed as percent of the critical F for each source of variation (*i.e.*, ≥100% is significant). Control (C) tuber calcium ppm and the difference between ppm calcium in treatment and control (T-C) tubers revealed that highly significant differences exist among species for their ability to accumulate calcium. Differences among accessions within species were not significant ($p = 0.05$), and the variation among blocks was small. If calcium is a major, easily controlled, indirect measure of resistance to tuber defects, it follows that researchers who perform direct screening would increase precision by standardizing the calcium level of the environment(s) in which tested tubers are grown.

The spectrum of genetic variability in wild related species has been shown to be much broader than within the North American cultivated species, *S. tuberosum* ssp. *tuberosum*, for many traits (1). This makes the use of exotic germplasm for cultivar breeding attractive. The results of this investigation concerning tuber calcium accumulation are encouraging. Some species accumulated significantly more calcium than any cultivated species in the low calcium control (C) environment. Furthermore, some species also appear to accumulate calcium much more efficiently than ssp. *tuberosum* when more calcium is provided (T-C). In this respect, all species except *S. kurtzianum* accumulated more calcium than did the cultivated species.

The performance of *S. kurtzianum* is noteworthy, since it was the poorest calcium accumulator in control (C), and accumulated the least additional calcium in treatment environment (T-C). This species may be useful for investigating the genetic and physiological basis of calcium accumulation.

Solanum gourlayi appears to be outstanding for both parameters evaluated. It ranked first for calcium accumulation in the control (80 ppm) environment (C), exhibiting calcium accumulating more than double that of ssp. *tuberosum*. This species also ranked second in additional accumulation in the treatment (800 ppm) environment (T-C), exhibiting a response to supplemented calcium of three times that of ssp. *tuberosum*. Another

TABLE 2.—*ANOVA F values as percent of* $F_{critical 0.05}$ *for potato germplasm evaluated for efficient accumulation of tuber calcium.*

Source of variation	ppm tuber calcium	
	Control	Treatment-Control
Blocks	14% ns	1% ns
Species	332% *	238% *
Accessions within species	38% ns	64% ns

*Significantly different at p=0.05; ns = not significantly different.

TABLE 3.—*Tuber calcium of species grown in control environment and increase when grown in treatment environment.*

Control (80 ppm) environment (C)			Increase in treatment (800 ppm) over control (80 ppm) environment (T-C)		
species[1]	ppm	significance[2]	species	ppm	significance
ktz	160	a	ktz	474	a
chc	239	ab	tbr[3]	498	a
fen	285	abc	adg[3]	714	ab
tbr[3]	294	abcd	stn[3]	739	ab
pta	360	bcde	tar	774	abc
mcd	383	bcde	acl	832	abcd
sto	391	cde	pnt	914	abcde
pnt	421	cdef	dms	976	bcde
tar	437	def	opl	979	bcde
spg	439	def	chc	1058	bcdef
mga	441	def	pta	1145	bcdef
ifd	446	ef	ver	1146	bcdef
adg[3]	466	efg	sto	1188	cdef
acl	479	efg	spg	1201	cdef
dms	545	fg	can	1231	def
ver	550	fg	mga	1299	ef
stn[3]	551	fg	plt	1319	ef
can	568	fg	fen	1326	ef
plt	572	fg	ifd	1459	fg
opl	608	gh	grl	1476	fg
grl	743	h	mcd	1829	g
$LSD_{0.05}$ = 151			$LSD_{0.05}$ = 440		

[1]See Table 1 for full species names.
[2]Species not followed by the same letter are significantly different.
[3]Cultivated species. Others are wild.

noteworthy species is *S. microdontum*. This species exhibited only average calcium accumulation in the control environment (C), but had the highest level of increase in calcium when grown in the treatment environment (T-C).

Both wild species, *S. gourlayi* and *S. microdontum* are in the taxonomic series Tuberosa, the same as cultivated species. The former has both diploid and tetraploid forms, while the latter is diploid. One would expect to find no chromosome differentiation between these species, and little difficulty in making hybrids with *S. tuberosum*. Thus, genes from these species may provide an avenue for improvement of the potential of the cultivated potato to accumulate tuber calcium, and thereby resist losses due to storage rots and other tuber defects. Cultivars with the characteristic of efficient accumulation of supplemental calcium (T-C), would also increase the effectiveness and profitability of the farmer's investment in calcium fertilizer applications.

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