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Distribution of Glycoalkaloids in Potato Tubers of 59 Accessions of Two Wild and Five Cultivated *Solanum* Species

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Steroidal glycoalkaloids are naturally occurring, secondary plant metabolites that are found in foods, including potatoes and tomatoes. Their content in plants is controlled by both genetic and environmental factors. Glycoalkaloid profiles can be passed to progenies during breeding and hybridization of wild and cultivated potatoes designed to develop improved potatoes. The most common potato, *Solanum tuberosum*, contains primarily the glycoalkaloids, α-solanine and α-chaconine. However, wild-type potatoes being used for breeding new varieties contain other, less common glycoalkaloids. Because glycoalkaloid composition is a major criterion for the release of new potato cultivars, we used HPLC, TLC, GC, and GC/MS to determine their nature and content in several *Solanum* species widely used in potato breeding and hybridization programs. *Solanum tuberosum*, as well as *S. andigena* and *S. stenotomum*, contained α-solanine and α-chaconine. *S. canasense* was found to contain only dehydrocommersonine. *S. acaule* contained α-tomatine and demissine. *S. juzepczukii* and *S. curtilobum* contained demissine and two previously unidentified glycoalkaloids. We characterized them as demissidine-glucose/rhamnose (1/1 ratio) and demissidine-galactose/glucose/rhamnose (1/1/1 ratio), tentatively named dihydro-β_1-chaconine and dihydrosolanine, respectively. We found extensive variability in the glycoalkaloid profiles in the tested potato varieties. The possible significance of these findings for plant breeding and food safety is discussed.

KEYWORDS: Wild-type potatoes; HPLC; TLC; GC; GC/MS; potato glycoalkaloids; new glycoalkaloids; solanthrene; potato breeding.

INTRODUCTION

Germplasm of wild and cultivated potatoes is now widely used to impart desirable properties to potato tubers by sexual crosses or protoplast fusion. These properties include resistance to phytopathogens (fungi, bacteria, viruses, insects, and worms) and climatic stress (frost and heat) as well as enhancement of nutritional quality and safety (*1, 2*).

Studies on wild and cultivated potatoes, including *Solanum curtilobum*, *Solanum juzepczukii*, *Solanum stenotomum*, *Solanum tuberosum*, and their wild progenitor *Solanum acaule*, indicate the inheritance of the following glycoalkaloids in their progenies: α-chaconine, commersonine, dehydrocommersonine, α-solanine, demissine, dehydrodemissine, α-tomatine, dehydrotomatine, α- and β-solamarines, solamargine, solasonine, and soladulcine as well other known and unknown ones (*3*−*16*). The generally observed increased concentration of glycoalkaloids in the tubers of interspecific hybrids may be an undesirable characteristic. However, this may not always be true because glycoalkaloids have been shown to exhibit both adverse and beneficial effects in cells, animals, and humans, as reviewed in ref *17*.

Previously, we (*18*) determined the morphological character (appearance, size, and shape) as well as glycoalkaloid content of potato tubers of somatic hybrids between *S. tuberosum* and a wild subspecies *S. acaule*. All 19 somatic hybrids, except one clone, contained four glycoalkaloids (α-chaconine, α-solanine, α-tomatine, and demissine) inherited from both parents. The results also showed that character expression is influenced by ploidy level and that total glycoalkaloid content in most somatic hybrids was intermediate between those of the fusion parent clones.

The objectives of the present study were (a) to determine, with the aid of high-performance liquid chromatography (HPLC),

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Table 1. Composition of Glycoalkaloid Acid-Hydrolysates Collected from the HPLC Column and Analyzed by GC and GC/MS

potato species	HPLC peak[a]	product ion (*m*/*z*) of aglycones by mass spectrometry[b]	mole ratio of sugars in acid-hydrolysates of glycoalkaloids by GC/MS[c]	glycoalkaloids identification
S. acaule	1 (13.0)	tomatidine [114 (100), 138 (78.6), 207 (11.9), 387 (13.9), 416 (10.8)]	xylose/glucose/galactose (1:2:1)	α-tomatine
	2 (22.0)	demissidine [150 (100), 204 (35.2), 399 (32.1)]	xylose/glucose/galactose (1:2:1)	demissine
S. andigena	1 (11.5)	solanidine [150 (100), 204 (22.1), 397 (10.0)]	rhamnose/glucose (2:1)	α-chaconine
	2 (24.5	solanidine [150 (100), 204 (15.8), 397 (9.8)]	rhamnose/glucose/galactose (1:1:1)	α-solanine
S. canasense	1 (29.5)	solanidine [150 (100), 204 (39.8), 397 (24.0)]	rhamnose/glucose (1:3)	dehydrocommersonine
S. curtilobum	1 (12.0)	demissidine [150 (100), 204 (35.2), 399 (32.2)]	xylose/glucose/galactose (1:2:1)	dihydro-β_1-chaconine (new)
	2 (22.5)	demissidine [150 (100), 204 (30.5), 399 (29.3)]	rhamnose/glucose/galactose (1:1:1)	demissine
	3 (25.3)	demissidine [150 (100), 204 (43.5), 399 (32.8)]	rhamnose/glucose/galactose (1:1:1)	dihydrosolanine (new)
S. juzepczukii	1 (12.0)	demissidine [150 (100), 204 (40.2), 399 (33.5)]	rhamnose/glucose (1:1)	dihydro-β_1-chaconine (new)
	2 (22.5)	demissidine [150 (100), 204 (25.7), 399 (10.9)]	xylose/glucose/galactose (1:2:1)	demissine
	3 (25.3)	demissidine [150 (100), 204 (48.0), 399 (35.0)]	rhamnose/glucose/galactose (1:1:1)	dihydrosolanine (new)
S. stenotomum	1 (11.5)	solanidine [150 (100), 204 (28.7), 382 (17.9), 397 (18.9)]	rhamnose/glucose (2:1)	α-chaconine
	2 (24.5)	solanidine [150 (100), 204 (26.5), 382 (15.8), 397 (17.5)]	rhamnose/glucose/galactose (1:1:1)	α-solanine
S. tuberosum	1 (11.5)	solanidine [150 (100), 204 (26.5), 397 (18.5)]	rhamnose/glucose (2:1)	α-chaconine
	2 (24.5)	solanidine [150 (100), 204 (16.4), 397 12.2)]	rhamnose/glucose/galactose 1:1:1)	α-solanine

[a] Values in parentheses are elution times in min on HPLC column. [b] Values in parentheses are percent relative % intensities. [c] GC retention times of sugars (in min): α-xylose, 24.8; β-xylose, 27.4; α-rhamnose, 20.5; β-rhamnose, 23.3; α-galactose, 32.6; α-glucose, 33.9; β-galactose, 34.8; and β-glucose, 39.0.

thin-layer chromatography (TLC), gas−liquid chromatography (GC), and GC/mass spectrometry (GC/MS), the structures and distributions of glycoalkaloids present in 59 accessions of two wild and five cultivated *Solanum* species widely used in breeding programs; and (b) to suggest the availability of accessions that may be suitable for breeding improved potatoes with both low amounts and the right kinds of glycoalkaloids.

MATERIALS AND METHODS

Reagents. Analytical-grade α-chaconine, α-solanine, α-tomatine, and demissidine were obtained from Sigma Chemical Co. (St. Louis, MO). Solanidine was purchased from MP Biomedicals, Inc. (Columbus, OH). Pure demissine and dehydrocommersonine were extracted and isolated from the tuber cortex of *S. acaule* and *S. canasense*.

Sources of Potatoes and Ploidy Levels. Tubers of two common potatoes [*S. tuberosum* ssp., *Tuberosum* cv. 'Danshaku' and 'May Queen' ($4n = 4x = 48$)] were obtained from commercial sources. Tubers of four cultivated [*S. stenotomum* ($2n = 4x = 24$), *S. tuberosum* ssp. *Andigena* ($2n = 4x = 48$), *S. juzepczukii* ($2n = 3x = 36$), and *S. curtilobum* ($2n = 5x = 60$)] and two wild [*S. canasense* ($2n = 2x = 24$) and *S. acaule* ($2n = 4x = 48$)] potatoes were provided by Dr. Norio Yamamoto of The National Museum of Ethnology, Osaka, Japan.

The seed tubers were planted at the beginning of October in a greenhouse located on the campus of the Faculty of Agriculture, Kobe University, Japan, and harvested in late January. Size 5, 2 L horticultural pots (15 cm in diameter) were used to grow the potatoes. The following amounts of fertilizer were applied: seed cake, 6 g; bone dust, 4 g; chemical fertilizer (N/P/K = 12:12:12%), 2.4 g; magnesium lime, 1.2 g; and phosphate, 3 g. The plants were exposed to natural sunlight at the day-length of about 11 h. The seed tubers were watered once or twice daily. Sufficient water was used to cause leakage from the bottom of the pot. The plants were grown under the following conditions: light intensity, daylight; temperature, 7 °C (minimum) to 29 °C (maximum); and day length, from 10.3 to 12.0 h during cultivation. One plant was harvested for each variety. **Table 1** provides additional information about the sources, ploidy levels, and resistance properties of the evaluated potatoes.

Isolation of Demissine from *S. acaule* and Dehydrocommersonine from *S. canasense* Tubers. Accessions of *S. acaule* (acl-E-4) and *S. canasense* (PI310939) were used for isolation of demissine and dehydrocommersonine, respectively. Each potato periderm (10 g, 1.5−2.0 mm thick) was used for isolation of the glycoalkaloids. The extraction and analysis of glycoalkaloids were carried out as described below. Demissine and dehydrocommersonine fractions were collected from the HPLC column and dried under reduced pressure at 30 °C. After the residue was dissolved with 0.01 N HCl, the solutions were made basic with NH_4OH and extracted into butanol. The butanol was then evaporated, yielding demissine (2.5 mg) and dehydrocommersonine (5.3 mg). For structural determination, each glycoalkaloid was acid-hydrolyzed into sugars and aglycones; the sugars were determined by GC, and the aglycones were determined by GC-MS (*19, 20*). The purities of the glycoalkaloids were also confirmed by TLC, as follows. Whatman thin-layer chromatography LHP-KF High Performance Silica Gel Plates (size, 10 × 10 cm; thickness, 20 μm) were used in the analysis. After separation on the plates by the following two solvents, the chromatographic spots were detected with 1% iodine in methanol: (a) ethyl acetate/pyridine/water (10:4:1, v/v/v) → demissine (Rf = 0.23) and dehydrocommersonine (Rf = 0.15); (b) chloroform/methanol/1% NH_4OH (2:2:1, v/v/v; low layer) → demissine (Rf = 0.28) and dehydrocommersonine (Rf = 0.19). Only one spot for demissine or dehydrocommersonine was detected in each analysis.

Extraction of Glycoalkaloids. The procedure was adapted from previous studies (*21*). Briefly, tubers of approximately equal weight were selected for analysis of glycoalkaloids to minimize variation due to tuber size. Each analysis was repeated three times and was carried out with 4−5 tubers, each weighing 20−30 g. Isolation and determination of glycoalkaloids were carried out according to previously described methods (*18*). Cortex layer (10 g, ~5 mm of peripheral tissue) was collected from the tubers. The cortex was chopped into small pieces with a knife. These were then blended in a homogenizer with 150 mL of chloroform/methanol (2:1, v/v) (Wako Pure Chemical Industry, Osaka, Japan). The mixture was filtered through a Toyo filter paper No. 2 in a Büchner funnel. The residue was washed with the same solvent, and the filtrate was transferred to a 500 mL round-bottom flask and concentrated to 3 mL under reduced pressure at 30 °C. To the concentrate was added 0.2 N HCl (40 mL) followed by sonication for 5 min in an ultrasonic cleaner. The flask was rinsed twice with of 0.2 N HCl (10 mL) and centrifuged at 12 000*g* at 1 °C for 10 min. The supernatant was transferred to a 250 mL Erlenmeyer flask. Concentrated NH_4OH (30 mL) was added to the flask to precipitate the glycoalkaloids. This basic solution was placed into a 65 °C water bath for 50 min and then refrigerated overnight. The precipitate was collected by centrifugation at 12 000*g* at 4 °C for 10 min.

HPLC. HPLC analysis was carried out with the aid of a Hitachi liquid chromatograph model 655A-II with an autosampler model 655-40. The stainless steel chromatographic column [25 cm × 4.0 mm (i.d.)] was packed with Inertsil NH_2 (particle diameter, 5 μM; GL Science, Osaka, Japan). Glycoalkaloids were eluted with tetrahydrofuran/acetonitrile/20 mM KH_2PO_4 (50:30:20, v/v/v) (Kanto Kagaku, Tokyo, Japan) at 20 °C and at a flow rate of 1 mL/min. The UV detector (Hitachi model 655A UV monitor) was set at 208 nm.

Acid-Heat Treatment of Solanidine and Demissidine. Standard solanidine (1.35 mg) or demissidine (1.02 mg) in a 5 mL vial with a sealed Teflon cap was dissolved in 1 N HCl (1 mL) and then heated at 100 °C for 10, 30, 60 and 120 min. After cooling, the mixture was

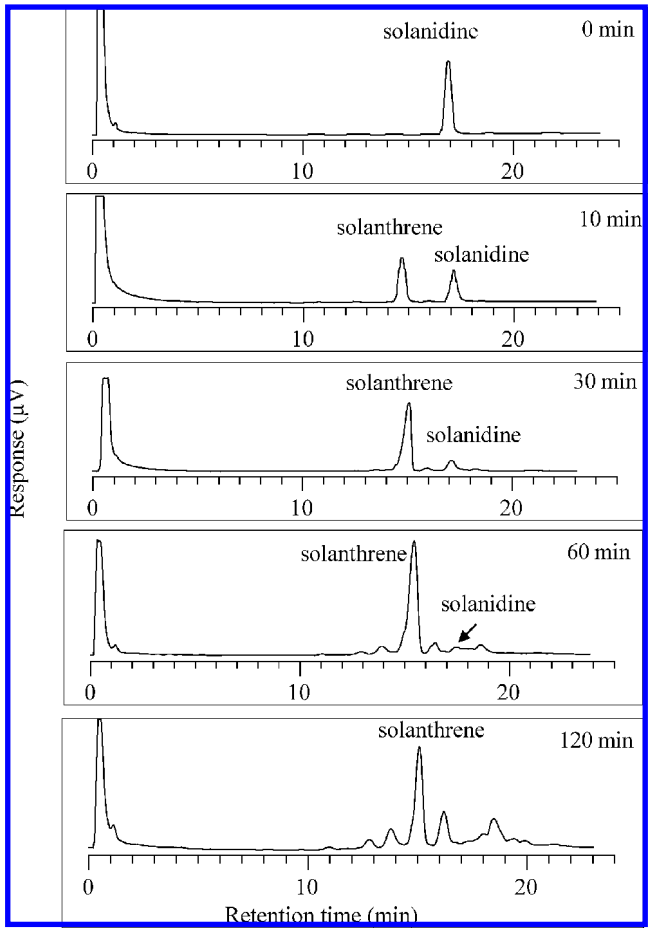

Figure 1. GC chromatograms showing the time-dependent transformation of solanidine to solanthrene at 100 °C. Chromatographic conditions: column temp, 120 °C, programmed to 205 at 2 °C/min.

neutralized with 1 N NH_4OH to pH 7.0−7.2 and then partitioned five times with benzene (2 mL). The combined benzene solutions were washed five times with H_2O (2 mL). The benzene was then evaporated to dryness, and the residue was dissolved in benzene (1 mL). Aliquots of this solution were used for GC-MS (*19, 20*).

Identification of Glycoalkaloids. Two methods were used to identify glycoalkaloids in wild and cultivated potato tubers: (a) Retention times of HPLC peaks of standard α-chaconine, α-solanine, α-tomatine, and pure demissine or dehydrocommersonine isolated from wild potatoes were compared to corresponding peaks from the potato extracts. (b) Multiple samples collected from each HPLC peak were acid-hydrolyzed into sugars and aglycones as previously described (*19*−*21*). The sugars were converted to trimethylsilyl ester derivatives. Individual compositions and molar ratios of sugars were determined by gas−liquid chromatography (GC). Identification of the aglycones was by GC/mass spectrometry (GC/MS), as described in detail in our previous publications (*19*−*21*).

Quantification. Quantification of glycoalkaloids was accomplished with the aid of a Hitachi Chromato-integrator model D-2500 by comparing the integrated HPLC peak areas from the potato extracts to the standard curve constructed with eight concentrations of commercial α-chaconine, α-solanine, and α-tomatine, and with demissine and dehydrocommersonine isolated from wild potatoes.

RESULTS AND DISCUSSION

Heat-Induced Transformation of Solanidine to Solanthrene. During studies of the hydrolysis of the carbohydrate side chain of solanidine-containing glycoalkaloids, we observed that the aglycones eluted as two peaks on the GC column. **Figure 1** shows GC chromatograms of the time-dependent transformation at 100 °C. Mass spectral analysis of the peaks shows that the fragmentation pattern and parent ion peak of the first peak were consistent with the structure for solanidine, and those of the second were consistent with that of solanthrene (**Figure 2**). The transformation, which involves loss of H_2O from solanidine to form a double bond in ring A conjugated with the double of ring B, is ∼50% complete after 10 min, ∼90% complete after 60 min, and 100% complete after 120 min. These results suggest that this aspect should be taken into account in interpreting mass spectral data of solanidine. A similar study with demissidine showed that this aglycone was stable to heat. A possible explanation for this difference is that loss of H_2O from solanidine to form solanthrene results in the formation of a molecule with two conjugated double bonds in ring A and B that has a lower ground-state energy (is more stable) than the starting compound with one double bond. This would not be the case with demissidine. These observations are in agreement with previous observations that solanidine based glycoalkaloids can hydrolyze to solanthrene (*22*).

Glycoalkaloid Identification. Figure 3 depicts the structures of all glycoalkaloids evaluated in the present study, and **Table 1** summarizes the analytical data used to identify the glycoalkaloids and the corresponding aglycones. Additional experimental details for the newly observed glycoalkaloids dihydro-β1-solanine and dihydrosolanine are described in the sections *S. curtilobum* and *S. juzepczukii*.

Inheritance of Glycoalkaloids. Because accessions evaluated in the present study can provide a resource for improving potato nutrition and safety via inheritance, i.e., integrating genes that are beneficial to the potato genome (*23*), to place our findings in proper perspective, we will first briefly mention several reported studies on inheritance that are relevant to the theme of the present study.

Andigena potatoes derived from *S. stenotomum* evaluated in the present study form the primary gene pool for improving worldwide grown potatoes (*24*−*27*). Genetic diversity of the Andean tetraploid cultivated potato was largely brought about from cultivated diploid species and further modified through sexual polyploidization and intervarietal and/or introgressive hybridization and dispersal of potato seeds by humans (*28*). Concentrations of commercially grown cultivars ranged from 1 to 35 mg/100 g of fresh wt and of wild potatoes, from 3.6 to 432 mg/100 g of fresh wt or up to 100 times more than in cultivated varieties (*3*−*6, 12*). Selection for reduced toxicity may have occurred as part of the domestication of the potato (*9*).

Examination of the patterns of solanidine glycoalkaloid variation in four gene pools of the cultivated potatoes revealed that, in addition to α-chaconine and α-solanine, dehydrocommersonine was present in an accession of *S. canasense* (*13*). Somatic hybrids derived from wild *Solanum* genomes contained glycoalkaloids not selected in the parent species (*14, 18*). LC−MS analysis of seven potato genotypes indicated that glycoalkaloids are a major source of metabolite diversity (*16*). Other cultivars were found to be drought-resistant (*29, 30*). The cited observations indicate that glycoalkaloid content of a specific potato appears to be genetically controlled and that glycoalkaloid composition should be a major criterion for the creation and release of new potato cultivars.

Distribution of Glycoalkaloids in 59 *Solanum* Accessions. Table 2 shows the origin of the evaluated potatoes, and **Figure 4** traces the phylogenetic relation between wild and cultivated potatoes examined in this study. Below, we discuss the nature of the glycoalkaloids and their content (in mg/100 g of fresh wt of the cortex) in the seven *Solanum* subspecies and their accessions in alphabetical order.

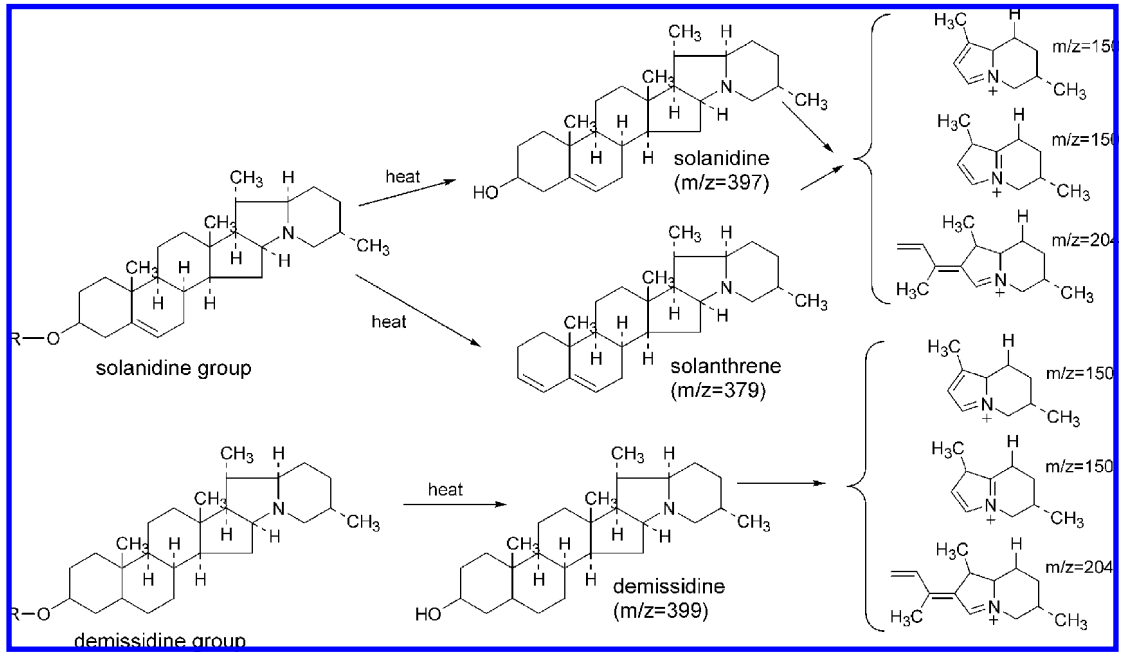

Figure 2. Postulated structures of mass fragments of solanthrene and solanidine from acid-hydrolysates of *S. canasense* (PI 1246533). Mass spectral fragments (*m*/*z*, % intensity): solanidine [150 (100), 204 (36.0), 382 (15.0), 379 (24.8.0)]; solanthrene [150 (100), 204 (16.0), 397 (31.0)].

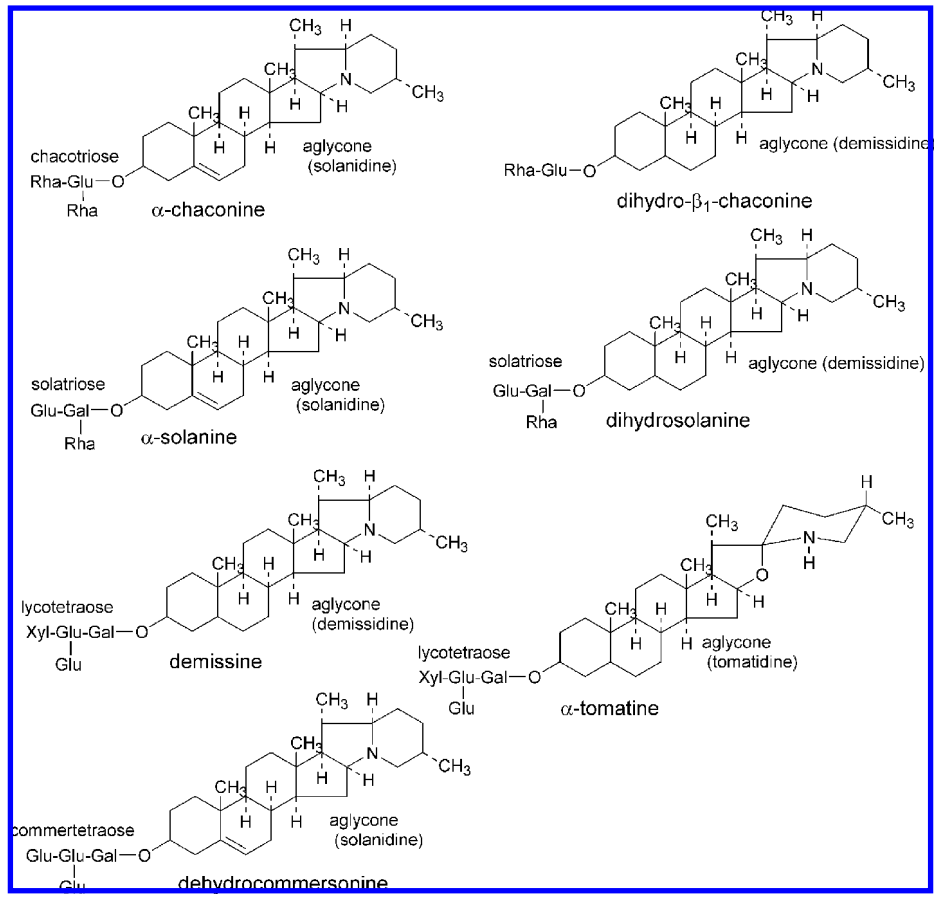

Figure 3. Structures of new (dihydro-β_1-chaconine and dihydrosolanine) and known potato glycoalkaloids evaluated in the present study. Abbreviations: Gal = galactose; Glu = glucose; Rha = rhamnose; and Xyl = xylose.

Solanum acaule. *S. acaule* (**Table 4**) contained two glycoalkaloids, α-tomatine and demissine. The average values of the accessions are 27.0 ± 11.2 (n = 17) and range from 7.4 (acl-M-1) to 49.7 (acl-D-3) for tomatine, a 6.7-fold variation from highest to lowest concentrations. The corresponding values for demissine are 118.7 ± 18.8 and ranged from 85.9 (acl-E-4) to 147.9 (acd-D-1), a 1.72-fold variation. Total glycoalkaloid levels (sum of α-tomatine and demissine) ranged from 103.1 (acl-E-5) to 191.7 (acl-D-1), a 1.86-fold variation. The distribution of demissine among the accessions is much narrower than that of tomatine. The indicated ranges suggest that it is possible to select, for breeding and

Table 2. Inventory of Evaluated Andean Cultivated Potato Genera

potato species[a] and accession	cultivar name[b]	2n[c]	source[d]	remarks[e]
		S. acaule		
acl-C-4		48	A	
acl-C-5		48	A	
acl-D-1		48	A	
acl-D-3		48	A	
acl-D-4		48	A	
acl-E-1		48	A	
acl-E-4		48	A	
acl-E-5		48	A	
acl-J-4		48	A	
acl-J-5		48	A	
acl-K-1	PI473439	48	OCH-10112(CIP)	
acl-K-2	PI473439	48	OCH-10112(CIP)	
acl-K-3	PI473439	48	OCH-10112(CIP)	
acl-M-1		48	OCH-15089(CIP)	
acl-M-4		48	OCH-15089(CIP)	
acl-M-5		48	OCH-15089(CIP)	
acl-T-16		48	A	
		S. andigena		
T-AY-46−1	Mantaro	48	A	
T-AY-2−2	unknown	48	A	
T-AY-39	Yana PaPa	48	A	
T-AY-28	Puca Ccolla	48	A	
T-AY-18	Yana Macta	48	A	
T-AY-9	Bore	48	A	
T-AY-11	Compis	48	A	
T-AY-21	Compis	48	A	
T-AY-5	Yana Mactacha	48	A	
T-AY-7	Kkello	48	A	
T-AY-13	Quello Papa	48	A	
T-AY-10	Alca Imilla	48	A	
T-AY-37	Alcai Warmi	48	A	
T-AY-26	Ccushi	48	A	
T-AY-22	Misquilla	48	A	
T-AY-40	Chata	48	A	
T-AY-16	Alca Imilla	48	A	
T-AY-38	Compis	48	A	
T-AY-57	Sunny	48	A	V
T-A-123	unknown	48	A	V
T-AY-35	Renacimiento	48	A	
T-AY-41	Chata Blanca	48	A	
		S. canasense		
PI 310940		24	A	
PI 265864		24	A	
PI 246533		24	A	
PI 310939		24	A	
		S. curtilobum		
T-86-f	unknown	60	CIP	FR
T-86-g	unknown	60	CIP	FR
		S. juzepczukii		
T-109-1	unknown	36	CIP	FR
T-109-2	unknown	36	CIP	FR
T-109-3	unknown	36	CIP	FR
T-109-4	unknown	36	CIP	FR
T-109-5	unknown	36	CIP	FR
T-109-6	unknown	36	CIP	FR
T-109-8	unknown	36	CIP	FR
T-109-10	unknown	36	CIP	FR
		S. stenotomum		
T-AY-1	Suhua Manchachi	24	A	
T-AY-12	Turuna	24	A	V
PI 234015		24	A	
PI 234011		24	A	

[a] The 1st and 2nd numbers denote the cultivars and clones, respectively. [b] The cultivar names are described in Quechua or Aymara language. [c] Blank spaces indicates that the accession has not yet been determined cytologically. [d] A, Collected in Andean highland (Peruvian and Bolivian regions); CIP, supplied from the Centro Internacional de la Papa (Peru). [e] FR, Frost resistant; V, virus diseased.

hybridization studies, *S. acaule* accessions with high or low individual or total amounts of the two glycoalkaloids not present in *S. tuberosum* species. Unlike tomatine in green tomatoes, which is largely degraded during maturation to red tomatoes (*31*), mature potatoes can contain tomatine, presumably because the potatoes lack enzymes that degrade tomatine.

Solanum andigena. We detected the α-chaconine and α-solanine in *S. andigena* (**Table 3**). The average values for α-chaconine are 39.2 ± 37.5 ($n = 22$) and range from 2.2 (T-AY-46−1) to 154.2 (T-AY-41), a 70.1-fold variation from highest to lowest. The corresponding average for α-solanine was 19.2 ± 22.6 with a range from 1.0 (T-AY-46−1) to 84.0 (T-AY-123), an 84.0-fold variation. Total glycoalkaloid levels (sum of α-solanine and α-chaconine) ranged from 3.2 (T-AY-46−1) to 210.4 (T-AY-41), a 65.8-fold variation. α-Chaconine-to-α-solanine ratios ranged from 0.83 (T-AY-123) to 4.01 (T-AY-9). Specifically, the ratios of eight accessions ranged from 0.8 to 1.9; of 9 accessions, ranged from 2.2 to 2.9; and of 5 accessions, ranged from 3.5 to 4.7. Ratio is an important value to track because α-chaconine has been found to be more toxic than α-solanine (*17*).

The cited data indicate extraordinarily large variations in both content and distribution of the two glycoalkaloids among the 22 *S. andigena* accessions. Because adverse and beneficial biological activities of α-chaconine are greater than corresponding activities of α-solanine, and certain combinations of the two can exhibit synergistic biological effects, the different accessions provide a resource, depending on need, for selecting *S. andigena* cultivars with either high or low amounts and/or ratios of widely consumed glycoalkaloids for plant engineering, breeding, and hybridization studies with other cultivars.

Solanum canasense. We detected only dehydrocommersonine in *S. canasense* (**Table 3**). The average values are 105.4 ± 30.2 ($n = 4$) and range from 76.5 to 134.8. These values are 6−7 times larger than the sums of α-chaconine and α-solanine we determined for the commercial varieties 'Danshaku' and 'May Queen' mentioned below.

Solanum curtilobum. S. curtilobum (**Table 4**) contained demissine (45.7 ± 23.9; $n = 2$) and the two new glycoalkaloids, dihydro-β_1-chaconine (31.2 ± 9.8 α-chaconine equivalents) and dihydrosolanine (57.9 ± 9.9 α-solanine equivalents), which have apparently not been previously characterized. The table shows that demissidine is the common aglycone for both dihydro-β_1-chaconine and dihydrosolanine, that the carbohydrate side chain of dihydro-β_1-chaconine consists of a rhamnose/glucose (1/1 ratio) disaccharide, similar to that of β_1- or β_2-chaconine, and that the side chain for dihydrosolanine consists of rhamnose/glucose/galactose (1/1/1 ratio) trisaccharide, similar to that of α-solanine.

To obtain additional evidence for the correct sequence of the sugars attached to the 3-OH position of demissidine, we carried out additional studies on the release of sugars during acid hydrolysis of the glycoalkaloids after 10, 30, and 60 min. The results show that, for α-chaconine and dihydro-β_1-chaconine, rhamnose was released first followed by glucose; and for α-solanine and dihydrosolanine, the order was rhamnose, glucose, then galactose. The GC chromatogram of the derivatives of the sugars of dihydrosolanine is identical to α-solanine's, suggesting that the sugar in dihydrosolanine is solatriose.

Because dihydro-β_1-chaconine and dihydrosolanine contain the same sugars in the same ratio as β_1-chaconine and α-solanine, respectively, and the release of sugars from dihydro-β_1-chaconine and dihydrosolanine during acid hydrolysis were also similar, these results suggest that dihydro-β_1-chaconine = rha-glu-demissidine and dihydrosolanine = rha-glu-gal-demis-

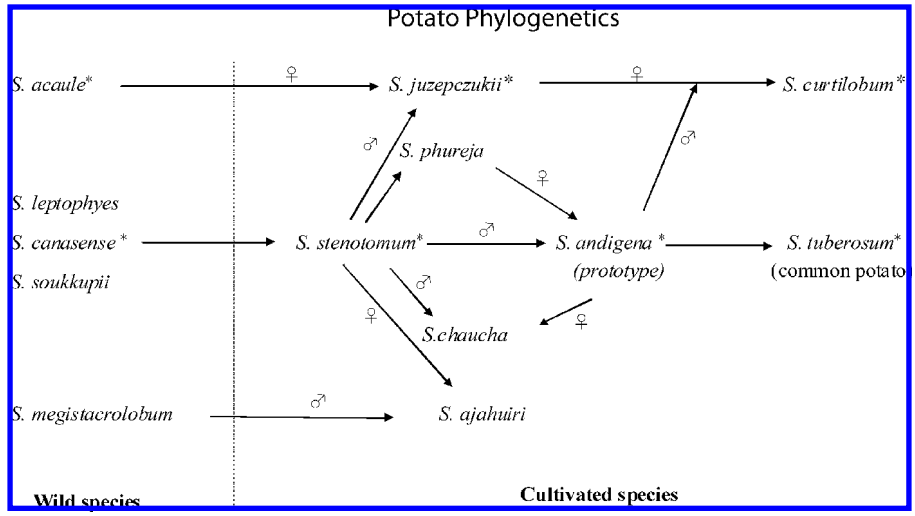

Figure 4. Diagram showing phylogenetic relationships between wild and cultivated potatoes used in the present study. Modification from (*43*). Additional relationships are given in (*24*).

Table 3. Glycoalkaloid Contents of the Cortex of *S. canasense*, *S. stenotomum*, *S. andigena*, and *S. tuberosum*[a]

	mg/100 g fresh wt				
potato species and accession	dehydrocommersonine	α-chaconine (A)	α-solanine (B)	sum	(A)/(B)
		S. andigena			
T-AY-46-1	nd	2.2 ± 1.0	1.0 ± 0.3	3.2	2.2
T-AY-2-2	nd	2.3 ± 0.9	1.7 ± 0.1	4.0	1.4
T-AY-39	nd	7.4 ± 0.9	3.1 ± 1.0	10.5	2.4
T-AY-28	nd	8.8 ± 1.0	3.6 ± 0.4	12.4	2.4
T-AY-18	nd	12.4 ± 1.1	3.3 ± 0.3	15.7	3.8
T-AY-9	nd	13.0 ± 1.1	3.4 ± 0.6	16.4	3.8
T-AY-11	nd	10.1 ± 1.0	4.1 ± 1.0	14.2	2.5
T-AY-21	nd	16.2 ± 1.1	6.2 ± 1.1	22.4	2.6
T-AY-5	nd	17.3 ± 1.2	6.0 ± 1.0	23.3	2.9
T-AY-7	nd	23.4 ± 1.9	6.0 ± 0.6	29.4	3.9
T-AY-13	nd	24.5 ± 0.9	5.2 ± 1.0	29.7	4.7
T-AY-10	nd	21.7 ± 1.2	15.0 ± 1.1	36.7	1.4
T-AY-37	nd	34.8 ± 3.6	9.9 ± 1.1	44.7	3.5
T-AY-26	nd	46.5 ± 3.6	20.9 ± 2.1	67.4	2.2
T-AY-22	nd	50.6 ± 2.4	30.1 ± 1.3	80.7	1.7
T-AY-40	nd	54.0 ± 3.4	27.8 ± 1.1	81.8	1.9
T-AY-16	nd	62.3 ± 2.7	33.6 ± 3.1	95.9	1.9
T-AY-38	nd	59.9 ± 1.9	42.7 ± 1.1	102.6	1.4
T-AY-57	nd	61.8 ± 2.8	44.4 ± 1.4	106.2	1.4
T-AY-123	nd	69.6 ± 1.7	84.0 ± 2.6	153.6	0.8
T-AY-35	nd	108.9 ± 2.9	49.6 ± 1.1	158.5	2.2
T-AY-41	nd	154.2 ± 5.8	56.2 ± 3.3	210.4	2.7
		S. canasense			
PI 310940	76.5 ± 2.4	nd	nd	76.5	
PI 265864	82.2 ± 3.7	nd	nd	82.2	
PI 246533	127.9 ± 6.0	nd	nd	127.9	
PI 310939	134.8 ± 10.6	nd	nd	134.8	
		S. stenotomum			
T-AY-1	nd	4.7 ± 0.8	7.3 ± 0.8	12.0	0.6
T-AY-12	nd	39.5 ± 3.0	18.5 ± 2.0	58.0	2.1
PI 234015	nd	4.5 ± 0.9	1.4 ± 0.4	5.9	3.2
PI 234011	nd	37.6 ± 2.8	37.0 ± 3.3	74.6	1.0
		S. tuberosum			
'Danshaku'	nd	14.0 ± 1.1	7.9 ± 0.9	21.9	1.8
'May Queen'	nd	21.8 ± 0.9	9.4 ± 0.9	31.2	2.3

[a] Listed values are averages ± standard deviation (SD); $n = 3$.

sidine. **Table 1** shows the mass spectra derived from these two glycoalkaloids. These glycoalkaloids do not seem to correspond to any of the glycoalkaloids found to be present in wild and cultivated potatoes (*16*).

Solanum juzepczukii. *S. juzepczukii* (**Table 4**) contained demissine (85.6 ± 40.3), dihydro-β_1-chaconine (24.9 ± 10.9; $n = 8$), and dihydrosolanine (45.3 ± 13.2), identical to those present in *S. curtilobum*. The amounts range as follows: demissine, from 30.2 (T-109−6) to 150.8 (T-109−3), a 5.0-fold variation from highest to lowest; dihydro-β_1-chaconine, from 14.0 to (T-109−10) to 44.3 (T-109−6), a 3.2-fold variation; dihydrosolanine, from 31.9 (T-109−10) to 60.5 (T-109−4), 1.9-fold variation; total, from 123.0 (T-109−5) to 214.8 (T-109−3), a 1.7-fold variation.

Table 4. Glycoalkaloid Content of the Cortex of *S. acaule*, *S. juzepczukii*, and *S. curtilobum*[a]

species and accession no.	mg/100 g of fresh weight				
	α-tomatine	dihydro-β_1-chaconine	demissine	dihydrosolanine	sum
		S. acaule			
acl-C-4	25.1 ± 1.7	nd	113.8 ± 9.0	nd	138.9
acl-C-5	33.9 ± 1.3	nd	116.3 ± 5.4	nd	150.2
acl-D-1	43.8 ± 2.1	nd	147.9 ± 5.7	nd	191.7
acl-D-3	49.7 ± 5.4	nd	134.8 ± 7.5	nd	184.5
acl-D-4	38.2 ± 3.4	nd	107.9 ± 4.0	nd	146.1
acl-E-1	13.8 ± 1.1	nd	101.6 ± 5.8	nd	115.4
acl-E-4	35.5 ± 2.4	nd	85.9 ± 4.7	nd	121.4
acl-E-5	15.5 ± 2.1	nd	87.6 ± 8.8	nd	103.1
acl-J-4	21.1 ± 2.2	nd	136.5 ± 11.0	nd	157.6
acl-J-5	23.0 ± 1.4	nd	138.8 ± 6.1	nd	161.8
acl-K-1	23.5 ± 2.9	nd	123.7 ± 6.4	nd	147.2
acl-K-2	18.4 ± 2.0	nd	120.6 ± 6.0	nd	139.0
acl-K-3	21.4 ± 1.9	nd	145.2 ± 7.1	nd	166.6
acl-M-1	7.4 ± 1.0	nd	99.9 ± 6.0	nd	107.3
acl-M-4	24.0 ± 1.1	nd	115.7 ± 6.6	nd	139.7
acl-M-5	29.2 ± 1.8	nd	131.9 ± 8.0	nd	161.1
acl-T-16	35.5 ± 2.4	nd	110.4 ± 7.1	nd	145.9
		S. curtilobum			
T-86-f	nd	38.2 ± 2.1	62.6 ± 3.3	50.9 ± 2.2	151.7
T-86-g	nd	24.3 ± 2.4	28.9 ± 2.2	64.9 ± 3.7	118.1
		S. juzepczukii			
T-109-1	nd	17.1 ± 2.6	103.1 ± 6.9	34.0 ± 2.7	154.2
T-109-2	nd	39.1 ± 3.1	44.0 ± 3.9	67.1 ± 5.3	150.2
T-109-3	nd	17.8 ± 2.1	150.8 ± 5.5	46.2 ± 2.3	214.8
T-109-4	nd	20.2 ± 2.3	82.6 ± 4.8	60.5 ± 4.9	163.3
T-109-5	nd	21.9 ± 1.1	63.4 ± 4.9	37.7 ± 4.2	123.0
T-109-6	nd	44.3 ± 4.1	30.2 ± 2.2	50.6 ± 3.8	125.1
T-109-8	nd	25.0 ± 3.1	124.3 ± 5.6	34.7 ± 3.7	184.0
T-109-10	nd	14.0 ± 1.8	86.1 ± 3.9	31.9 ± 1.2	132.0

[a] Listed valued are averages ± SD; $n = 3$. Dihydro-β1-chaconine is expressed as α-chaconine equivalents; dihydrosolanine is expressed as α-solanine equivalents.

The nature and distribution of the glycoalkaloids in *S. juzepczukii* are similar to those observed with *S. curtilobum*, which is not surprising considering that the two potatoes are closely related genetically (**Figure 4**). The total glycoalkaloids levels in the cortex of these two varieties are much higher than those in *S. tuberosum*.

Solanum stenotomum. We detected only α-solanine (16.1 ± 15.7 g/100 g; $n = 4$) and α-chaconine (21.6 ± 19.6) in S. *stenotomum* (**Table 3**). The values for α-chaconine range from 4.5 (PI 2304015) to 39.5 (T-AY-12), an 8.8-fold variation from highest to lowest. The corresponding values for α-solanine range from 1.4 (PI 2304015) to 37.0 (PI 2304011), a 26.4-fold variation. Total glycoalkaloid levels (sum of α-solanine and α-chaconine) range from 5.82 (PI 2304015) to 74.6 (PI 2304011), a 12.6-fold variation. α-Chaconine-to-α-solanine ratios range from 0.7 (T-AY-1) to 3.2 (PI 2304015), a 4.6-fold variation.

Solanum tuberosum. In the two widely consumed commercial varieties of *S. tuberosum* listed in **Table 3**, we detected α-solanine (8.6 ± 1.1 g/100 g; $n = 2$) and α-chaconine (17.9 ± 5.5). The values for α-chaconine for the 'Danshaku' variety (14.0 and 7.9; sum, 21.9; ratio, 1.8) are about two-thirds of the corresponding values for the 'May Queen' variety (α-chaconine, 21.8; α-solanine, 9.4; sum, 31.2; ratio 2.3).

Safety Guidelines for Human Consumption of Potato Glycoalkaloids. Because some of the accessions with a low-glycoalkaloid content described in the present study can serve as a resource for the development of improved potatoes, it is relevant to briefly mention some of the factors that govern the safety of glycoalkaloids in commercial potatoes, reviewed in refs (*17, 32*). The major toxic effects of glycoalkaloids are cell membrane disruption and acetylcholinesterase (AChE) inhibition. Other possible effects are liver damage, teratogenicity, and embryotoxicity. In overall toxicity, α-chaconine is the most toxic of the potato glycoalkaloids. It exhibits the strongest cell disruption, as well as causing inhibition of AChE, organ damage, and teratogenicity in embryos. Although α-solanine is a strong inhibitor of AChE, similar in potency to α-chaconine, it is less teratogenic to embryos than α-chaconine and has little or no lytic effect. This would also seem to indicate that it is probably less damaging to organs. The intermediate hydrolysis products of α-chaconine and α-solanine lose overall toxicity as they lose sugar groups. The aglycone solanidine is the least toxic in all effects. The solanidanes—solanine and chaconine—also appear to be more toxic than their corresponding spirosolanes—solamargine, solasonine, and solasodine. Orally consumed tomatine appears to be nontoxic (*8, 33*).

As critically discussed elsewhere (*32*), the accepted guideline limiting glycoalkaloid content of potatoes to less than 20 mg/100 g of fresh weight, an official requirement in many countries but not in the United States, may be too high. The reasons for his conclusion are that this level only relates to acute and/or subacute effects and not to possible chronic effects. The guideline should be considered a minimum requirement until adequate acceptable levels have been established.

We believe that the guidelines may need to be further revised if findings about the synergism between α-chaconine and α-solanine in inducing adverse effects (*34, 35*) turn out to be a general phenomenon. Because of synergy, it may not be possible to predict the toxicity of a mixture of two or more glycoalkaloids using the results of the individual compounds or of differing ratios present in different potato varieties. Mixtures can vary in their adverse effects depending on the ratio used. Glycoal-

kaloids may be either synergistic or additive at one concentration ratio, whereas the interaction may differ in others.

The situated is even more complicated. Thus, although the safety of glycoalkaloids is still being debated (*36*), we discovered that 17 glycoalkaloids and metabolites (hydrolysis products) and glycoalkaloid-containing potato extracts inhibited the growth of human cancer cells (*37, 38*). The following are some other reported beneficial effects of glycoalkaloids: (a) orally fed tomatine protected rainbow trout against carcinogen-induced liver and stomach tumors (*39*); (b) tomatine reduced plasma cholesterol and triglyceride levels in hamsters (*33*) and stimulated the immune system in mice (*40*); and (c) orally fed potato glycoalkaloids exhibited antibiotic effects against *Salmonella typhimurium* in mice (*41*).

These considerations suggest that we may have reached a watershed in research on biological effects of glycoalkaloids. Newly developed potato varieties should be evaluated not only for enhanced resistance to environmental effects and phytopathogens, but also for both adverse and beneficial biological effects in animals and humans. We are challenged to find an acceptable balance between desirable and undesirable traits of glycoalkaloid-containing potatoes.

Significance for Breeding of Improved Potatoes. In the present study, we found extensive variability in the glycoalkaloid profiles in the tested potato varieties. Although there were broad ranges in levels within a subspecies, the type of glycoalkaloid produced remained the same. Potatoes that are more closely related genetically as shown in **Figure 4** have similar glycoalkaloid profiles. *S. acaule*, *S. juzepczukii*, and *S. curtilobum*, all on the same branch of inheritance, contain demissine and no α-chaconine or α-solanine. The closely related potatoes *S. stenotomum*, *S. andigena*, and *S. tuberosum* all contain only α-chaconine and α-solanine. When demissine and dehydrocommersonine are present, they tend to be present at higher levels than α-chaconine and α-solanine in other potato accessions. When dihydro-β_1-chaconine and dihydrosolanine are present, they are present instead of and at similar levels to α-chaconine and α-solanine. There was no apparent correlation between ploidy and glycoalkaloid levels. The glycoalkaloids appeared to align themselves more with family history than ploidy level. We were surprised to find no α-chaconine or α-solanine in *S. canasense*, the predecessor to *S. tuberosum*. A previous study (*13*) found that α-chaconine and α-solanine were present in *S. canasense*, in addition to a large amount of dehydrocommersonine similar to the amount we observed. Perhaps this is a case in which the cultivars of the same subspecies do have different profiles.

The apparent associations of glycoalkaloids found in tubers with the potato subspecies imply that interspecific crosses can be used to select for specific glycoalkaloids. However, because of the large variation within the subspecies, levels of glycoalkaloids need to be controlled by selecting specific cultivars, with high levels of nontoxic and beneficial tomatine and low levels of toxic α-chaconine plus α-solanine.

The cited safety considerations imply that breeders should attempt to cross accessions of *S. acaule* with the highest tomatine content (in mg/100 g), acl-D-1 (43.8) and acl-D-3 (49.7) (**Table 4**) with accessions containing low total amounts and low ratios of α-chaconine to α-solanine. These include (in mg/100 g; ratio), *S. andigena* T-AY-46-1 (3.2; 2.2) and T-AY-2-2 (4.0; 1.4). Additional varieties to consider include *S. stenotomum* PI 234013, with a low total amount (5.9) but high ratio (3.2), and T-AY-1, with a higher total amount (11.9) but lowest ratio (0.7) (**Table 3**). Finally, because disaccharide glycoalkaloids such as β_1-chaconine are less toxic than trisaccharide ones such as α-chaconine (*42*), it may also be worthwhile to include in breeding programs accessions of *S. curtilobum* and *S. juzepczukii* that contain the disaccharide dihydro-β_1-chaconine.

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