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# Influence of Incorporated Wild *Solanum* Genomes on Potato Properties in Terms of Starch Nanostructure and Glycoalkaloid Content

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Interspecific somatic hybrids produced by protoplast fusion between two wild *Solanum* species (*S. acaule*, *acl*; *S. brevidens*, *brd*) and cultivated potato *Solanum tuberosum* (*tbr*) were analyzed in terms of the starch nanometer-range structure and glycoalkaloid (GA) contents. The crystallinity of starch granules, the average size of starch crystallites, and the lamellar distances were obtained from tuber samples using wide-angle and small-angle X-ray scattering methods. These measurements showed that incorporation of wild genomes from either nontuberous (*brd*) or tuberous (*acl*) *Solanum* species caused no significant modifications of the nanostructure of potato starch. In contrast, the GA profiles of the hybrids, which were analyzed by LC-ESI-MS in both tuber and foliage samples, differed considerably from those of cultivated potato. Regardless of the low total tuber GA concentrations (∼9 mg/100 g of fresh weight), the somatic hybrids contained GAs not detected in the parental species. A high proportion of spirotype GAs consisting of 5,6-dihydrogenated aglycons, for example, α-tomatine and tomatidine bound with solatriose, and chacotriose were found in the hybrids. In conclusion, the foliage of interspecific hybrids contained a higher variation in the structures of GAs than did the tubers.

**KEYWORDS: Glycoalkaloid; starch; X-ray scattering; liquid chromatography; electrospray ionization; mass spectrometry; *Solanum*; interspecific hybrid**

## INTRODUCTION

The family Solanaceae is composed of a very diverse range of species including various kinds of herbs, shrubs, vines, and even trees. Most of the 2000 species of the Solanaceae family belong to the genus *Solanum*, which contains all of the tuber-bearing potato species. Cultivated potato (*S. tuberosum* ssp. *tuberosum*) is economically the most important crop plant of the whole family. Global tuber production from cultivated potato was 311 million tons in 2003 (*1*). In the human diet potato tubers are an important source of carbohydrates and proteins. In addition, potatoes are processed for various applications ranging from foods to coatings, paper-making, and animal feed.

The wide range of the *Solanum* species can be utilized for incorporating desirable traits from the wild germplasm into the genetic background of the cultivated potato. These traits include resistance or tolerance to biotic and abiotic stresses, which can have a strong impact on potato production. Although most of the wild *Solanum* species are sexually incompatible with *S. tuberosum* (*2*), interspecific somatic hybrids can be produced via the fusion of somatic cells (*3*). However, as a result of the fusion, many traits important for agronomy and tuber quality exhibit a wide variation in the somatic hybrids and their progenies. These traits include yield and tuber shape (*4*), starch content (*5*), skin and flesh color (*6*), and glycoalkaloid (GA) content (*7*−*9*).

Starch is the major component of the dry matter content of potato tubers, comprising ∼70% of the total solids (*10*). Starch granules (size of ∼1 μm) consist of lamellae (periodicity of ∼9 nm) formed of alternating layers of partially crystalline amylopectin and amylose. Amylopectin side chains form double helixes (interhelix distance of ∼16 Å) that are packed into B-type crystalline structures (*11*). Starch properties can be described by various parameters, such as gelatinization temperature and crystallinity. Gelatinization temperature increases with increasing crystallinity. Crystallinity constrains the swelling of hydrated starch. Wide-angle and small-angle X-ray scattering (WAXS and SAXS) studies have not revealed any significant differences in the starch (nano)structure between *S. tuberosum* cultivars at the same tuber storage age (*12*). However, hybrids between *S. tuberosum* and *S. phureja*, which have lower

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**Table 1.** Recoveries of the Glycoalkaloids and Aglycons from SCX Solid-Phase Extraction Cartridges[a]

| added amount of each compd ($\mu$g/5 mL of extract) | recovery[b] (%) | | | | |
|---|---|---|---|---|---|
| | α-solanine | α-chaconine | α-tomatine | demissidine | solasodine |
| 50 | 70 | >100 | 41 | 70 | 59 |
| 100 | 38 | 61 | 70 | 91 | 39 |
| 150 | 71 | 90 | 89 | 96 | 31 |

[a] The leaf extracts of *S. tuberosum* cv. Pito were spiked with various amounts of GA and aglycone standards. [b] Mean of two determinations for each level and each compound.

susceptibility to low-temperature sweetening, show higher crystallinity and gelatinization temperature (*13*). Because no extensive studies have been conducted on the nanometer-range structure of starch from interspecific potato hybrids, we here compare wild *Solanum* species and their hybrids with cultivated potato in order to investigate whether starch structure is altered as a result of somatic fusion. Starch properties, such as crystalline structure, crystallinity of the tuber sample, average crystallite size, thickness of lamellar stacks, and lamellar distance, were determined from tuber slices by X-ray scattering methods (WAXS and SAXS).

The increased GA concentration in potato tubers is an undesirable characteristic that diminishes their quality, because GAs are potentially toxic to mammals and vertebrates (*14*). On the other hand, the role of the GAs naturally expressed in the *Solanum* species is to protect the host plant from the attack by fungi, bacteria, and insects. Cultivated potato and wild *Solanum* species contain steroidal GAs consisting mainly of solanidane or spirosolane aglycon carrying a sugar moiety (**Figure 1**). The cultivated potato tubers contain only the GAs α-solanine and α-chaconine, whereas several different GAs have been found in wild *Solanum* species (*7*−*9*, *14*, *15*). According to previous studies, interspecific potato hybrids contain GA aglycons from both parental species, although novel GA aglycons are also sometimes expressed (*7*).

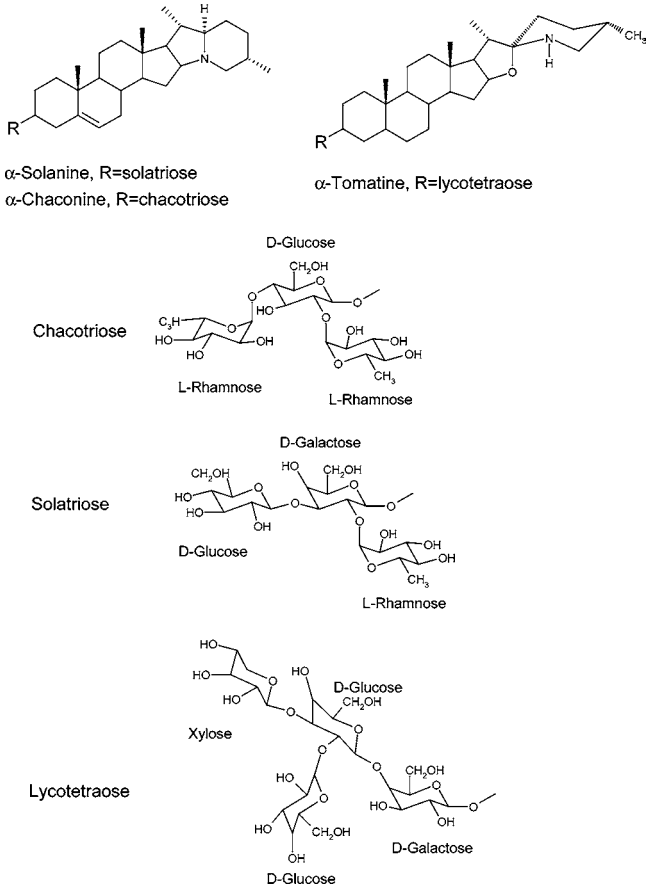



**Figure 1.** Structures of the glycoalkaloids α-solanine, α-chaconine, and α-tomatine.

**Table 2.** Glycoalkaloid Contents of Foliage (Milligrams per 100 g of Fresh Weight ± Standard Deviation) Determined Using LC-ESI-MS

| $[M + H]^+$ | $t_R$ | compd[b] | plant type[a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | 4x *tbr* Pito | 2x*brd* | (6x) 4x*brd* + 2x *tbr* | 4x*acl* | (6x) 4x*acl* + 2x *tbr* |
| 1032.8 | 30.1 | **4** (dehydrotomatine) | – | 12 ± 0.4 | + | – | – |
| 884.7 | 28.2 | **2** (solasonine)/ | 14 ± 1.1 | – | – | – | – |
| | 31.0 | **5** (α-solamarine) | 7.5 ± 1.2 | – | – | – | – |
| 868.9 | 28.3 | **3** (solamargine)/ | 66 ± 2.0 | – | – | + | + |
| | | **6** (β-solamarine) | + | – | - | – | – |
| 1034.8 | 26.7 | **7** (soladulcine B) | – | – | + | 3.7 ± 0.04 | – |
| | 29.6 | | – | + | + | + | – |
| | 30.6 | **10** (α-tomatine) | – | 432 ± 14 | 38 ± 10 | 14.5 ± 0.8 | 52 ± 3.7 |
| 886.7 | 26.7 | **8**, **11** | – | – | + | – | – |
| | 29.5 | | – | | + | – | – |
| | 30.4 | | – | – | 15 ± 3.1 | – | 8.3 ± 3.8 |
| | 31.5 | | – | – | 6.3 ± 1.5 | – | – |
| 870.9 | 26.9 | **9**, **12** | – | – | + | + | + |
| | 30.3 | | – | – | 8.0 ± 1.4 | – | 27 ± 9.1 |
| 1018.7 | 31.1 | **13** (demissine) | – | – | 4.9 ± 2.2 | 4.2 ± 1.2 | 10 ± 2.0 |
| 870.9 | 30.9 | **14** | – | – | 1.6 ± 0.2 | – | 14 ± 1.6 |
| 854.8 | 31.8 | **15** | – | – | 0.4 | + | 31 ± 3.3 |
| 1016.7 | 31.0 | **16** (dehydrodemissine) | – | – | – | – | – |
| 868.7 | 31.2 | **17** (α-solanine) | 233 ± 14 | – | + | + | + |
| 852.8 | 31.4 | **18** (α-chaconine) | 373 ± 33 | – | + | + | 1.6 ± 0.5 |
| total | | | 677 ± 29 | 443 ± 13 | 82 ± 22 | 21 ± 2.7 | 144 ± 19 |

[a] +, detected; –, not detected. [b] Identification is based on the hypothesis that glycosides are solatriose, chacotriose, or lycotetraose. The compound structures are given in **Table 4**.

**Table 3.** Glycoalkaloid Content of Tubers (Milligrams per 100 g of Fresh Weight ± Standard Deviation) Determined Using LC-ESI-MS

| | | | plant type[a] | | | |
|---|---|---|---|---|---|---|
| | | | 2x *tbr* | (4x) | | (6x) |
| $[M + H]^+$ | $t_R$ | compd[b] | White Lady 15. | 1x *brd* + 3x *tbr* | 4x *acl* 7–8 | 2x *acl* + 4x *tbr* |
| 1032.8 | 30.1 | **1**/ | – | – | – | – |
| | | **4** (dehydrotomatine) | – | – | – | 0.05 ± 0.01 |
| 884.7 | 28.2 | **2** (solasonine)/ | – | – | – | – |
| | 31.0 | **5** (α-solamarine) | – | 0.7 ± 0.1 | – | – |
| 868.9 | 28.3 | **3** (solamargine)/ | – | – | – | – |
| | | **6** (β-solamarine) | – | – | – | – |
| 1034.8 | 26.7 | **7** (soladulcine B) | – | – | – | – |
| | 29.6 | | – | – | – | – |
| | 30.6 | **10** (α-tomatine) | – | 0.2 ± 0.04 | 34 ± 0.3 | 0.7 ± 0.1 |
| 886.7 | 26.7 | **8**, **11** | – | – | – | – |
| | 29.5 | | – | – | – | – |
| | 30.4 | | – | 0.2 ± 0.04 | – | 3.4 ± 1.2 |
| | 31.5 | | – | 0.3 ± 0.02 | – | – |
| 870.9 | 26.9 | **9**, **12** | – | 2.6 ± 0.7 | – | 2.9 ± 0.7 |
| | 30.3 | | | | | |
| 1018.7 | 31.1 | **13** (demissine) | – | + | 5.2 ± 0.8 | 0.2 ± 0.1 |
| 870.9 | 30.9 | **14** | – | – | – | 0.4 ± 0.2 |
| 854.8 | 31.8 | **15** | – | 1.3 ± 0.3 | + | 1.8 ± 0.4 |
| 1016.7 | 31.0 | **16** (dehydrodemissine) | – | – | + | + |
| 868.7 | 31.2 | **17** (α-solanine) | 2.2 ± 0.5 | 1.0 ± 0.1 | – | 0.5 |
| 852.8 | 31.4 | **18** (α-chaconine) | 6.5 ± 0.6 | 3.6 ± 0.7 | – | 1.8 ± 0.4 |
| total | | | 8.6 ± 1.1 | 9.1 ± 2.0 | 39 ± 0.6 | 9.3 ± 3.2 |

[a] +, detected; –, not detected. [b] Identification is based on the hypothesis that glycosides are solatriose, chacotriose, or lycotetraose. The compound structures are given in **Table 4**.

The biological activity (or toxicity) of GAs greatly depends on the structure of the aglycon and on the sugar moiety (*16*, *17*, and the references cited therein). The membrane-disruptive effect of the GAs is based on their ability to inhibit acetylcholinesterase and thereby impair neural function in mammals (*14*, *17*). As the aglycons alone are nontoxic, analysis of the intact molecules is necessary. Furthermore, different GAs are also reported to act synergistically (*18*). In this study, GAs expressed in wild *Solanum* species and their interspecific hybrids were analyzed by using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS).

The aim of the present work was to evaluate how severely tuber quality in terms of starch nanostructure and GA content is altered as a result of genetic enhancement when resistance to diseases in cultivated potato is improved through interspecific hybridization (*19*, *20*). Potato hybrids having incorporated genomes from two distinct wild *Solanum* species were included into the study: *S. brevidens*, which is a nontuberous E genome species and remotely related to cultivated *S. tuberosum* (A genome) (*19*), and the tuberizing (A genome) potato species *S. acaule*, which is a close relative of *S. tuberosum* (*20*).

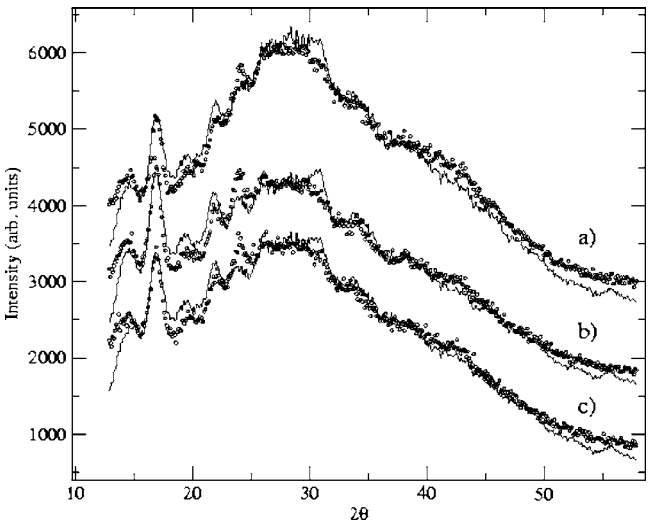



**Figure 2.** WAXS patterns of tuber slices obtained from (**a**) *S. tuberosum*, (**b**) the hybrid 4x *S. acaule* + 2x *S. tuberosum*, and (**c**) *S. acaule*.

## EXPERIMENTAL PROCEDURES

**Plant Materials.** The plant material in the present study consisted of various interspecific somatic hybrids between tetraploid (2n = 4x = 48) and dihaploid (2n = 2x = 24) cultivated potato (*Solanum tuberosum*) (*tbr*) and two wild *Solanum* species, 2x *S. brevidens* (*brd*) CPC 2451 and 4x *S. acaule* (*acl*) PI 4726558 (7–8), including its anther-derived 2x lines. Along with the somatic hybrids, analyses of the corresponding parental species composed of various genotypes were included into the study (**Tables 2** and **3**; **Figures 3–5**). Of the interspecific hybrids analyzed, the nontuberous hexaploid (2n = 6x) *brd* + *tbr* somatic hybrid 0502 was derived from a fusion of two protoplasts of diploid *brd* CPC 2451 and one protoplast of dihaploid *tbr* line Pito 4 (*19*). The genome of the 4x *brd* + 2x *tbr* hybrid 0502 consisted of 42 *brd* chromosomes and 22 *tbr* chromosomes (*21*). The hexaploid 2x *brd* + 4x *tbr* hybrids were derived from somatic hybridization between *brd* CPC 2451 and *tbr* cv. Pentland Dell (*22*). The tetraploid second-generation somatic hybrid was derived from a fusion between a diploid interspecific somatohaploid (1x *brd* + 1x *tbr*) and a dihaploid *tbr* line, which was originated from cv. Van Gogh (unpublished plant material, Veli-Matti Rokka). In addition, three different somatic hybrids, each with a specific genome constitution from fusions between the *acl* and *tbr* lines, were included in the study: (A) hexaploid 2x *acl* + 4x *tbr*, (B) tetraploid 2x *acl* + 2x *tbr*, and (C) hexaploid 4x *acl* + 2x *tbr* hybrids (*20*, *23*).

The plant materials for the starch nanostructure and glycoalkaloid analyses were grown under a photoperiod of 16 h with a diurnal temperature profile of 14 °C (night) and 23 °C (day). After being grown under these conditions for 3–4 months, the tuber-forming potato lines

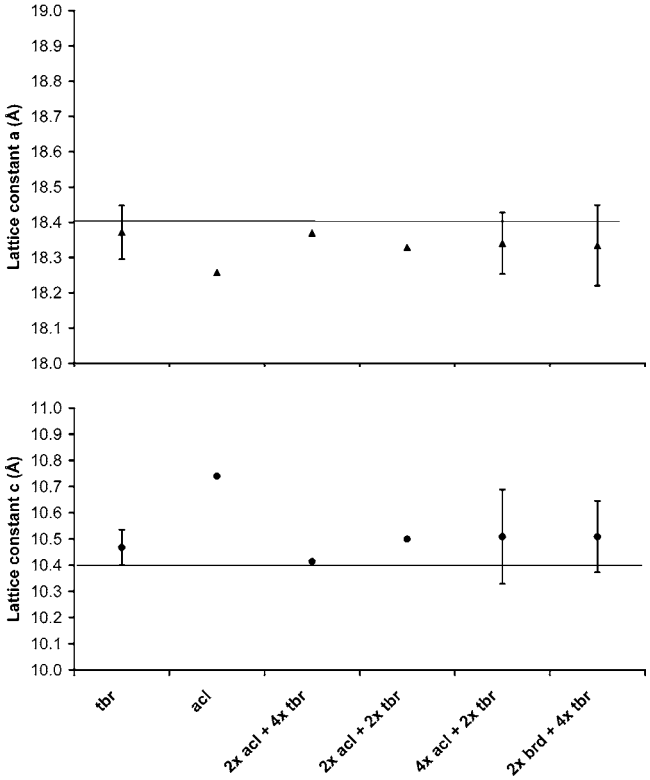



**Figure 3.** Lattice constants *a* and *c* determined by WAXS for tuber slices of parental lines *S. tuberosum* (*tbr*) and *S. acaule* (*acl*) and for various somatic hybrids between *S. acaule* and *S. tuberosum* and between *S. brevidens* (*brd*) and *S. tuberosum*. Values presented are means of three determinations, except which have only one determination. Lines at 18.4 and 10.4 Å mark the average values for the lattice constants obtained from 60 samples of three *S. tuberosum* cultivars as reported earlier by Väänänen et al. (*12*).

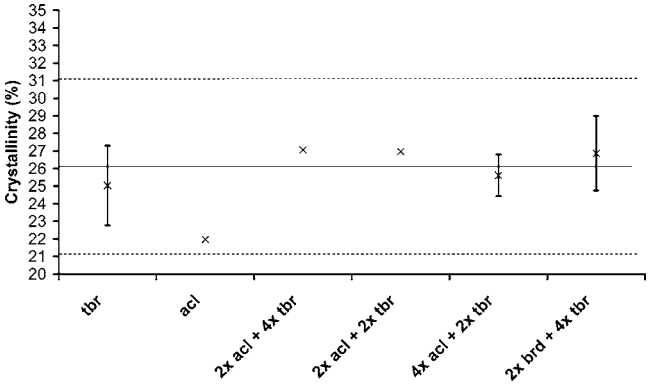



**Figure 4.** Crystallinity (percent) of the samples measured by WAXS from potato tuber slices of parental lines *S. tuberosum* (*tbr*) and *S. acaule* (*acl*) and from various somatic hybrids between *S. acaule* and *S. tuberosum* and between *S. brevidens* (*brd*) and *S. tuberosum.* Values presented are means of three determinations, except those which have only one determination. The line at 26% points to the average value obtained from 60 samples of three *S. tuberosum* cultivars reported earlier by Väänänen et al. (*12*). Dashed lines are the lowest and highest values measured in the data.

were allowed to produce tubers. Tubers of *S. acaule* were produced in a growth chamber at a constant temperature of 15 °C under a photoperiod of 12 h. All of the tubers harvested were subsequently stored in the dark at 4 °C for 3−7 months until they were analyzed. Tubers were not allowed to sprout before the samples for analyses were collected. The tubers were between 1 and 5 cm in diameter except for the tubers of *S. acaule*, which were 0.3−2 cm in diameter.

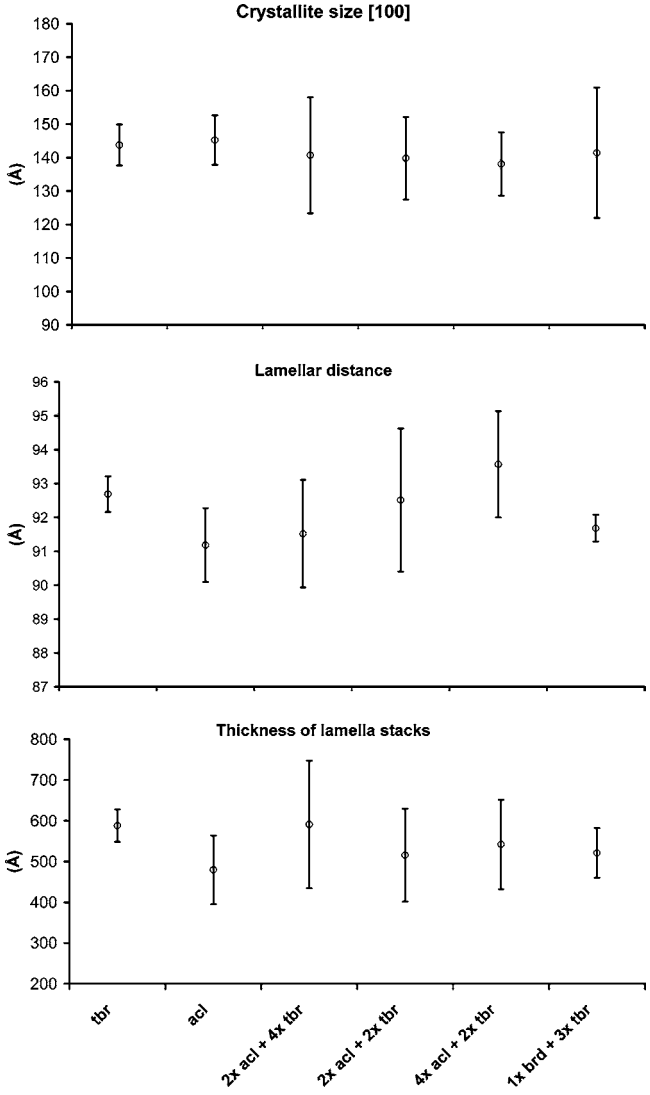



**Figure 5.** Results obtained by SAXS from potato tuber slices of parental lines *S. tuberosum* (*tbr*) and *S.* acaule (*acl*) and from various somatic hybrids between *S. acaule* (*acl*) and *S. tuberosum* and between *S. brevidens* (*brd*) and *S. tuberosum*. Results are means ± standard deviation of three determinations. Differences between the plant materials are not statistically significant ($P = 0.05$) for any of the parameters determined.

**X-ray Scattering Experiments.** Slices with a thickness of 1 mm were taken from raw potato tubers and immediately measured. Three same-sized tubers were used for each determination. The data analysis was carried out as described by Väänänen et al. (*12*). WAXS experiments were carried out with symmetrical transmission geometry with Cu Kα radiation obtained from a sealed X-ray tube operated at 45 kV and 28 mA and monochromatized with a quartz monochromator in the incident beam. The scattered intensities were measured with a scintillation counter. An angular step of 0.1° and a measuring time of 60 s per point were used. SAXS measurements were performed using a sealed fine-focus X-ray tube in the point-focus mode. Cu Kα radiation (wavelength $\lambda = 1.54$ Å) was monochromatized with a Ni filter and a totally reflecting mirror (Huber small-angle chamber 701), and the scattered intensity was measured with a Bruker AXS Hi-Star area detector. The distance between the sample and the detector was 51 cm. The magnitude of the scattering vector was defined as $q = (4\pi/\lambda) \sin \theta$, where $2\theta$ is the scattering angle. The $q$ range used was 0.25−0.47 1/Å. The scattering vector was calibrated using a silver−behenate standard. The intensities were corrected for absorption and nonsample scattering.

**Chemicals and Standard Solutions.** The glycoalkaloids α-solanine (purity of ∼95%), α-chaconine (purity of ∼95%), and α-tomatine (no

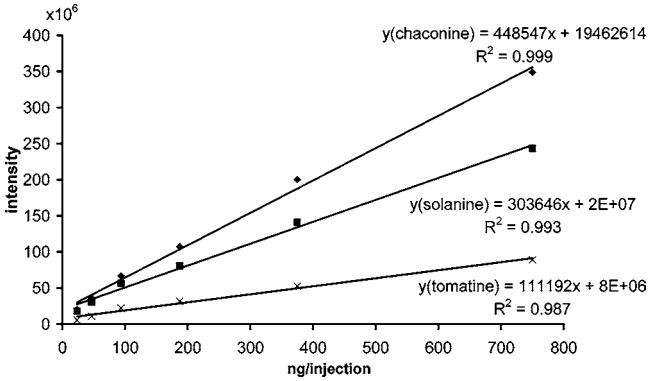



**Figure 6.** LC-ESI-MS standard curves of the glycoalkaloids α-solanine, α-chaconine, and α-tomatine used in the analysis of GAs in plant extracts.

label purity), containing dehydrotomatine, obtained from Sigma (St. Louis, MO), were used as standard compounds. Each compound was dissolved in methanol (HPLC grade, Rathburn Chemicals Ltd., Walkerburn, Scotland) to make stock solutions of 1 mg/mL. Standard solution I (0.75 mg/mL total GA concentration) was obtained by mixing 250 μL of each stock and diluting the mixture to 1 mL. This was further diluted 1:1 v/v to obtain standard solution II (0.375 mg/mL total GA concentration). External standard calibration was carried out by injecting 23.44, 46.88, 93.77, 187.5, 375, and 750 ng of GAs to the LC column. The curves, in which peak areas were plotted against concentrations, were used for quantitative analysis of the compounds in the extracts. The aglycons solanidine, demissidine, tomatidine containing dehydrotomatidine, and solasodine (Sigma) were prepared in the same way as GAs and mixed in a ratio of 1:1:1:1 to obtain a standard solution of 1 mg/mL.

HPLC-grade acetonitrile (Rathburn Chemicals Ltd.) was filtered through a 0.45-μm membrane filter (Millipore, Milford, MA). Triethylammonium acetate (TEAA) buffer in water (1.0 M) was obtained from Fluka Biochemika AG (Buchs, Switzerland). TEAA eluent of 25 mM at pH 6.8 was prepared by diluting 25 mL of 1.0 M TEAA to 1000 mL with MQ-purified water.

**Extraction and Preparation of GA Samples.** Oven-dried and powdered leaf material (500 mg) was suspended in 5% acetic acid (prepared from 99.5% acetic acid, Riedel-deHaen, Seelze, Germany) and homogenized (20000 rpm, Ultra Turrax T25, Janke & Kunkel GmbH & Co., IKA Labortechnik, Staufen, Germany) for 2 min. In the same way, 20 g of nonpeeled freeze-dried and homogenized tuber material was extracted. The suspension was filtered through filter paper (Whatman no. 4) to a volume flask and topped to 100 mL (foliage) or 250 mL (tubers).

A 5-mL aliquot of the filtrate was applied to preconditioned solid-phase extraction (SPE) cartridges (500 mg, Varian, Harbor City, CA), and the GAs were eluted with 4 mL of 2.5% ammonia in methanol (prepared from 25% ammonia, FF-Chemicals, Yli-Ii, Finland, and methanol, Rathburn Chemicals Ltd.) following the procedure described by Väänänen et al. (*24*) The solvent was evaporated to dryness, and the residue was dissolved in 1.0 mL of methanol to obtain an LC sample. All of the samples were filtered through 0.45-μm filters before the LC injections. Each sample was analyzed in triplicate.

Recoveries of the GAs and the aglycons from SPE were measured. Aliquots of 5 mL of an extract were spiked with 50, 100, and 150 μg of each GA, demissidine, and solasodine mixed together. Furthermore, 5-mL aliquots of 5% acetic acid were spiked with 150 μg of each GA and aglycon. Each spiking level was carried out in duplicate.

**LC-ESI-MS Instrumentation and Conditions.** For LC-ESI-MS analysis, an Agilent 1100 series HPLC including autoinjector, heated column compartment, diode array detector, and HP ChemStation Plus A.07.01 software was employed (Agilent Technologies, Palo Alto, CA). The analytical chromatography column was a Luna $C_{18}$-2 (150 × 1 mm, 5 μm, Phenomenex Ltd.). A modified method by Kuronen et al. was applied (*25*). The mobile-phase elution with acetonitrile and the TEAA buffer followed the gradient where the proportion of acetonitrile increased from 20 to 70% in 20 min, and that 70% was kept constant for another 55 min. The column was operated at a constant temperature of 50 °C. The flow rate of the eluent was 50 μL/min. The injection volume of the samples was 2 μL, containing ~0.1−2 μg of GAs or aglycons.

Mass spectrometric analysis was carried out on an Esquire-LC quadrupole ion trap mass spectrometer equipped with an electrospray interface (Bruker Daltonics, Bremen, Germany). The software employed was Esquire-LC NT, version 3.1 (Bruker Daltonics). Electrospray ionization was performed in positive ion mode at the scan range of *m*/*z* 100−1200. A capillary voltage of 2400 V and a trap drive value of 55 were used. The ICC was set on. Multiple-stage MS(n) was carried out using helium as the collision gas.

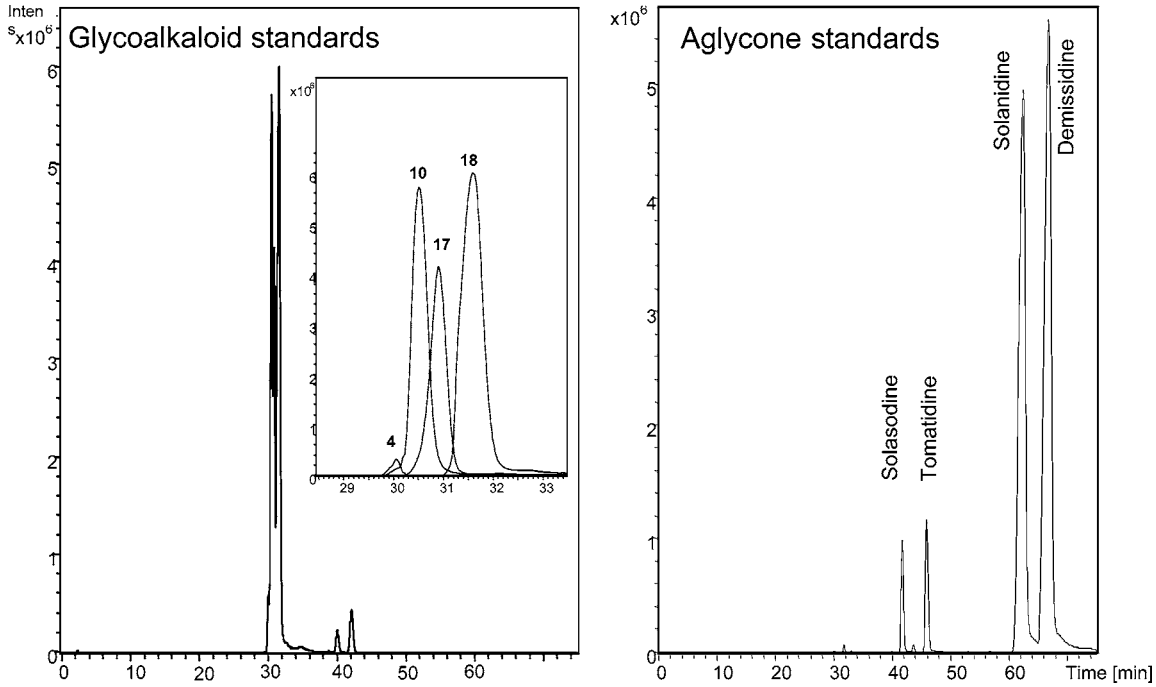



**Figure 7.** LC-ESI-MS chromatograms obtained from glycoalkaloids dehydrotomatine **4**, α-tomatine **10**, α-solanine **17**, and α-chaconine **18** and from aglycons solasodine, tomatidine, solanidine, and demissidine.

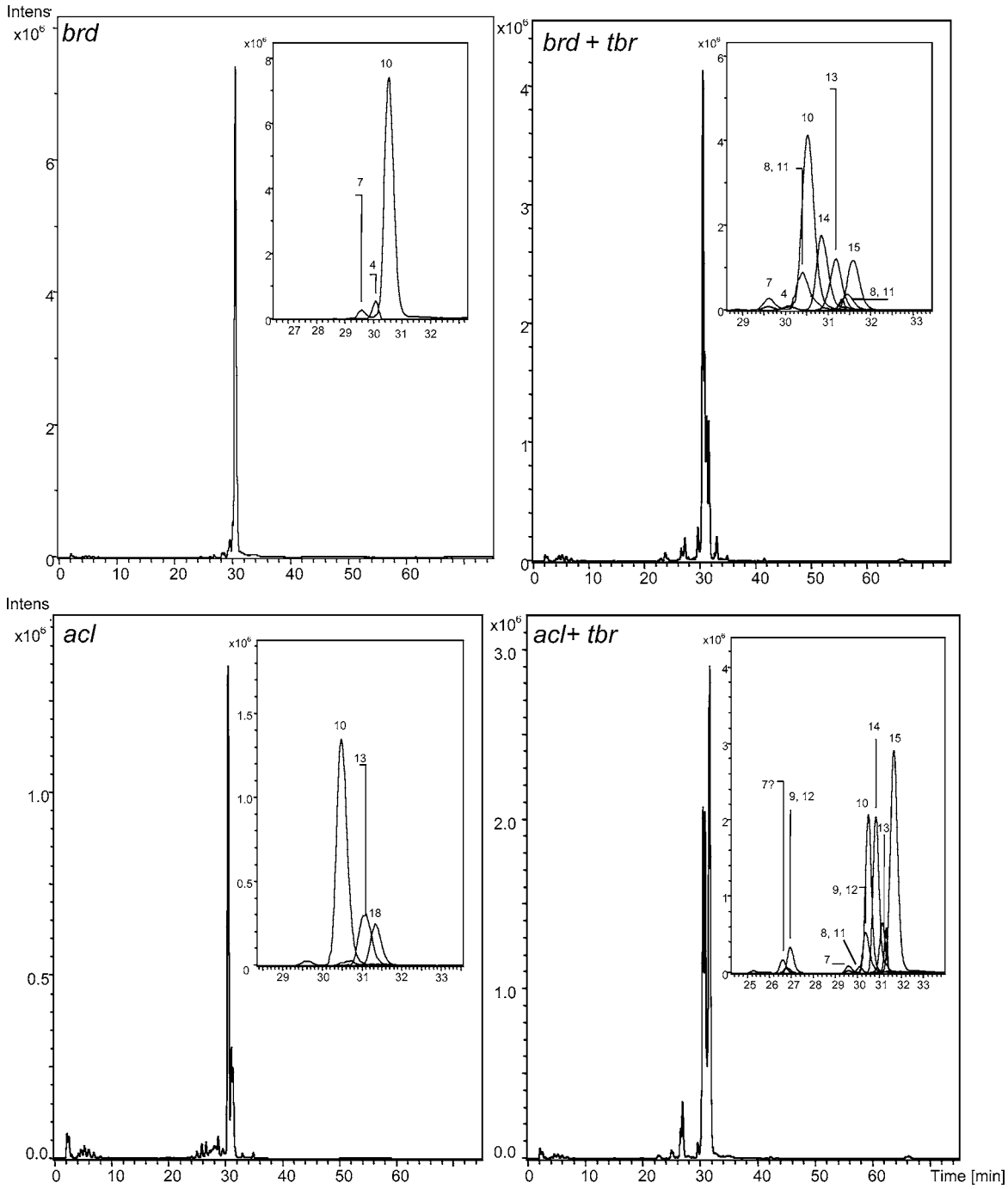


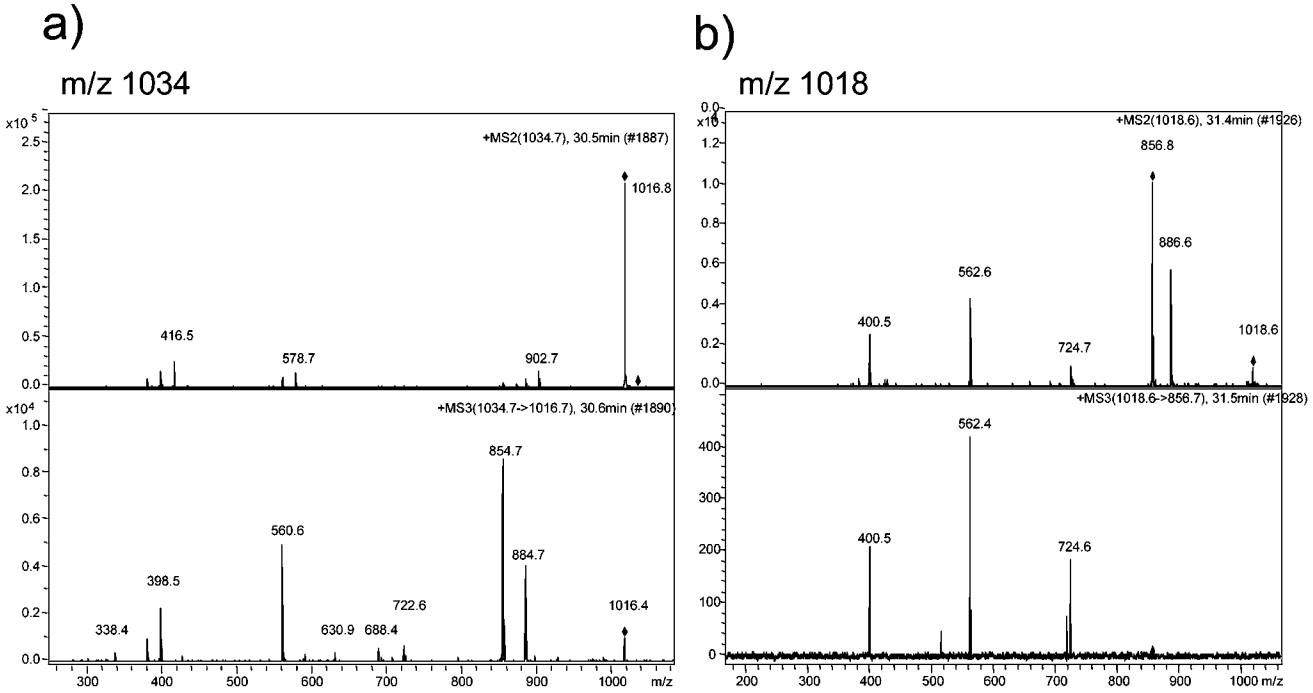

**Figure 8.** LC-ESI-MS chromatograms obtained from foliage extracts of wild species *S. acaule* (*acl*) and *S. brevidens* (*brd*) and their somatic hybrids with *S. tuberosum* (*tbr*). Numbers of the peaks refer to the compounds listed in **Table 4**.

**Dry Matter Content Determinations.** The dry matter content of tubers was determined following the modified procedure of the AOAC (*26*). The dry matter content of foliage was estimated as 30% by weighing the materials before and after oven-drying at 80 °C.

**Statistical Analysis.** The *t* test of Statgraphics Plus 4.0 was used to statistically analyze the differences in starch parameters between the parental species and hybrids with different genome constitutions described in **Figure 5**.

## RESULTS

**Starch Properties.** The WAXS patterns showed that all tubers, even if they were derived from plants with wild *Solanum* genomes, consisted of B-type starch (**Figure 2**). As seen in **Figure 3**, the lattice constants *a* and *c* determined from the wild species *S. acaule* only slightly deviated from the mean value obtained in *S. tuberosum* cultivars in the previous study by Väänänen et al. (*12*) and from the value obtained in interspecific hybrids in the present study, but the differences found were not statistically significant ($P = 0.05$). The cultivated potato (*tbr*), the wild potato species (*acl*), and the somatic hybrids (*acl* + *tbr*, *brd* + *tbr*) analyzed showed crystallinity between 22 and 29% (**Figure 4**). The fitting error in the determination of crystallinity was ~5% according to our previous study, in which the average crystallinity of a number of samples from *tbr* cultivars varied between 21 and 31% (*12*). Because we have observed that physiological aging causes changes in the crystallinity (*12*), we can conclude that the crystallinities in the *tbr* lines, in the wild species *S. acaule*, and in the interspecific somatic hybrids (*acl* + *tbr*, *brd* + *tbr*) are approximately equal.




| Aglycone structure | $[M+H]^+$ | Fragment ions (m/z) | Neutral fragment | Compound nr. |
|---|---|---|---|---|
| 5αH / Δ5 | 1034/1032 | 1016/1014<br>884/882<br>854/852<br>722/720<br>560/558<br>398/396 | 18<br>18, 132<br>18, 162<br>18, 132, 162<br>18, 132, 162, 162<br>18, 132, 162, 162,162 | 7,10 / 1,4 |

| Aglycone structure | $[M+H]^+$ | Fragment ions (m/z) | Neutral fragment | Compound nr. |
|---|---|---|---|---|
| 5αH / Δ5 | 1018/1016 | 886/884<br>856/854<br>724/722<br>562/560<br>400/398 | 132<br>162<br>132, 162<br>132, 162, 162<br>132, 162, 162, 162 | 13 / 16 |

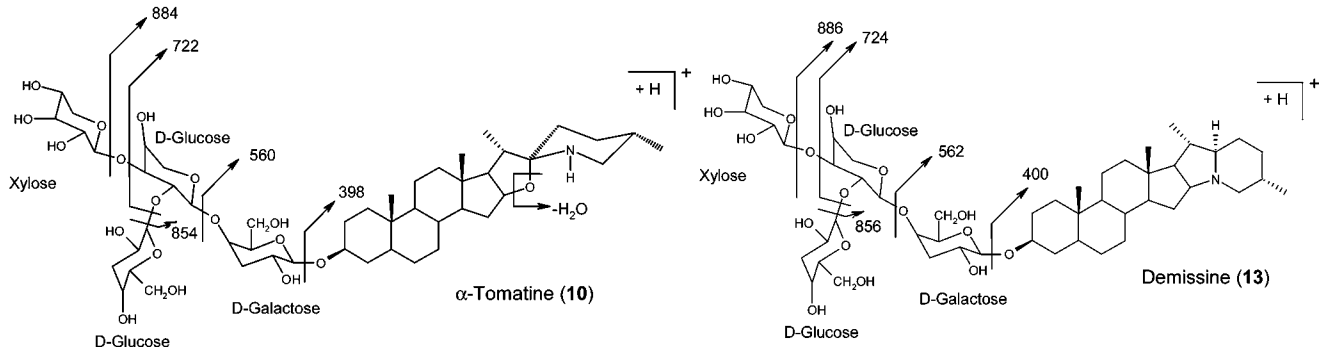
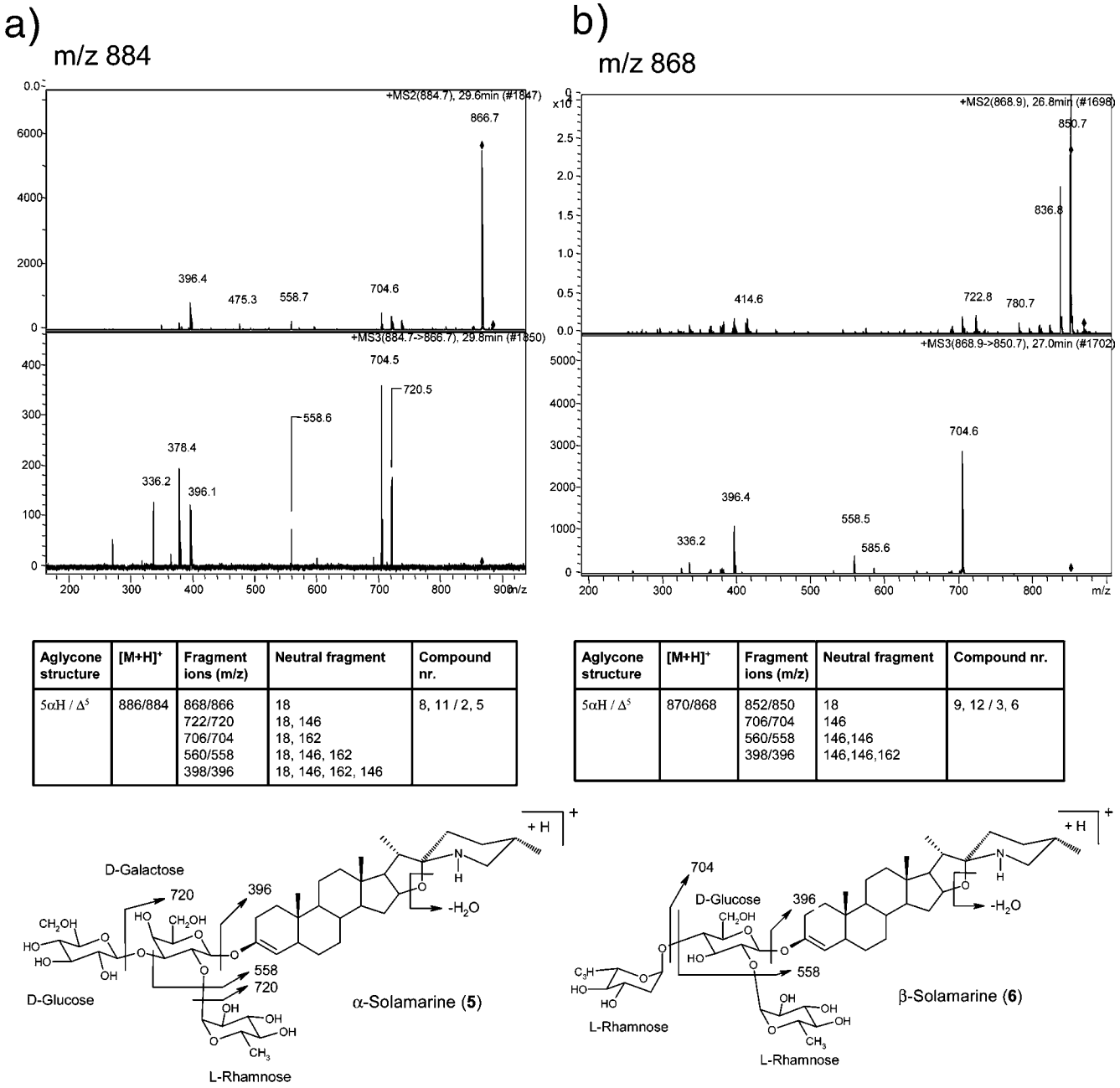



**Figure 9.** MS(n) spectra and fragmentations of GAs found in plant extracts: (**a**) α-tomatine; (**b**) demissine.

The SAXS experiments revealed that the average size of starch crystallites calculated from the width of the reflection 100 was comparable in *acl*, interspecific hybrids, and *tbr* (**Figure 5**). The lamellar distance, varying between 91 and 95 Å, was slightly lower than the value of 97 ± 3 Å previously obtained in *tbr* (15), but the difference was not statistically significant ($P = 0.05$). The thickness of the lamellar stack varied between 480 and 590 Å for all of the studied plant materials, which is in accordance with previous work (*12*), where a lamellar stack thickness of 513 ± 6 Å was observed for *tbr* cultivars.

**GA Quantification.** The standard GAs and aglycons were separated in a single chromatographic run using gradient elution. However, to obtain the standard curves, the GA and aglycon standards were run separately. The compounds showed varying ionization efficiencies when analyzed by ESI-MS (**Figure 6**). Standard curves for GAs with six levels were obtained by linear fitting. The standard curves with the three lowest levels were used for analyzing the plant extracts containing compounds too dilute to be quantified using the six-level curves. In the plant extracts, the spirosolanes were quantified as α-tomatine, whereas solanidane GAs, except for α-chaconine, were quantified as α-solanine. Only trace amounts of aglycons were detected in the plant extracts, and they were not quantified. All of the results were calculated from peak areas.

The recoveries of α-solanine, α-chaconine, and α-tomatine from SPE SCX cartridges were 63, 74, and 76%, respectively, whereas for demissidine and solasodine the recoveries were 91 and 36% when the GAs and aglycons were loaded as a 5% acetic acid solution (150 μg/5 mL). In **Table 1**, the recoveries are presented on the basis of a test in which aliquots of a plant extract were spiked with the GAs and aglycons. The recovery of α-tomatine at the lowest addition level (50 μg/5 mL) was poor (41%). However, the higher amounts (100 and 150 μg) added gave rise to better recoveries. As a conclusion, the quantitative determination of α-tomatine (and likely also other spirosolanes, which were not tested) at concentrations lower




| Aglycone structure | [M+H]$^+$ | Fragment ions (m/z) | Neutral fragment | Compound nr. |
|---|---|---|---|---|
| 5αH / Δ$^5$ | 886/884 | 868/866<br>722/720<br>706/704<br>560/558<br>398/396 | 18<br>18, 146<br>18, 162<br>18, 146, 162<br>18, 146, 162, 146 | 8, 11 / 2, 5 |

| Aglycone structure | [M+H]$^+$ | Fragment ions (m/z) | Neutral fragment | Compound nr. |
|---|---|---|---|---|
| 5αH / Δ$^5$ | 870/868 | 852/850<br>706/704<br>560/558<br>398/396 | 18<br>146<br>146,146<br>146,146,162 | 9, 12 / 3, 6 |



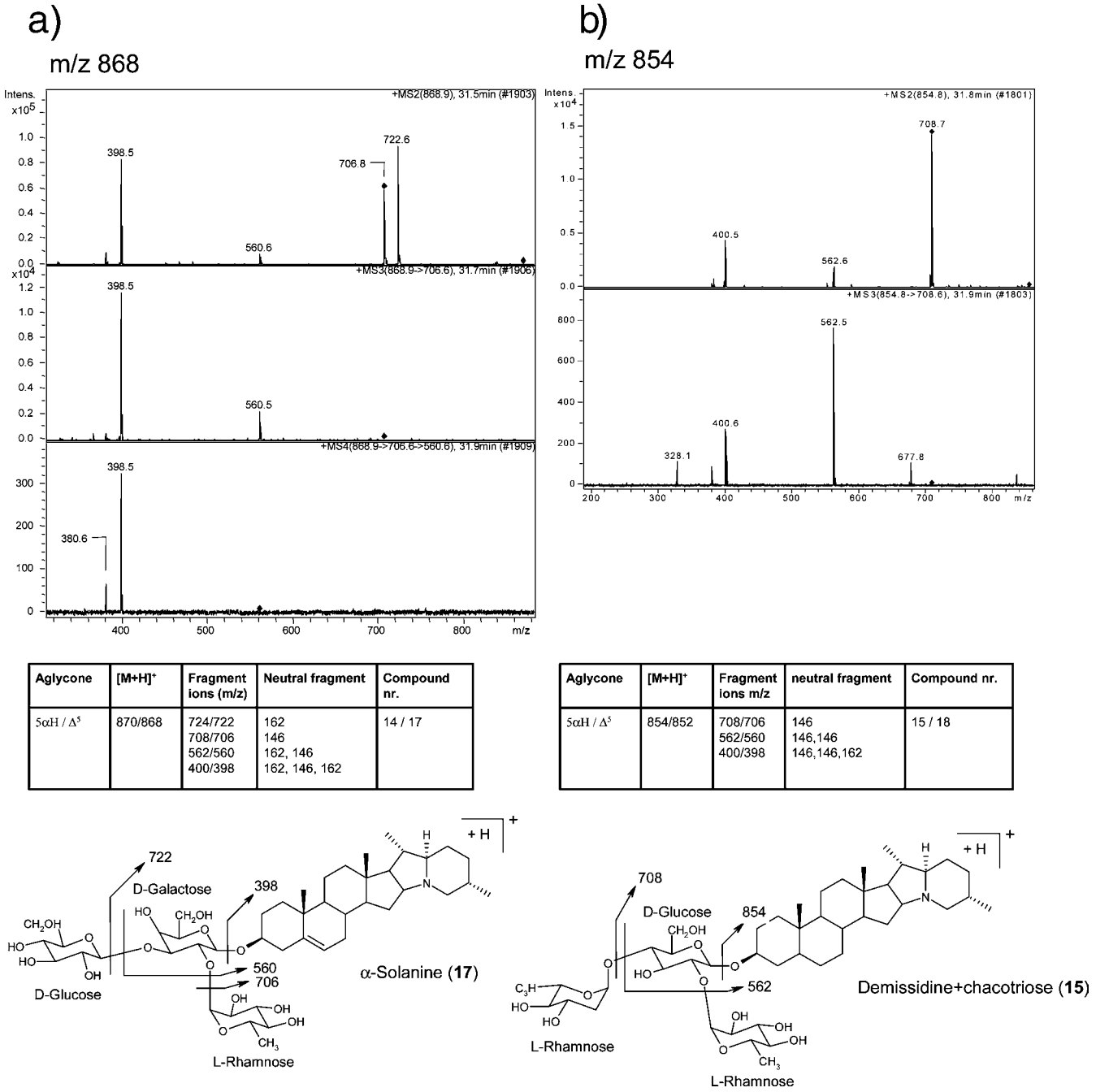

**Figure 10.** MS(n) spectra and fragmentations of GAs found in plant extracts: (**a**) α-solamarine; (**b**) α-solamarine.

than 60 mg/100 g of fresh weight (FW) may have been inaccurate in the present study. On the other hand, even when the total GA concentration (α-solanine, α-chaconine, α-tomatine, and the other GAs detected in the extract) exceeded 700 mg/100 g of FW, the SPE was accurate, meaning that the 500-mg SCX cartridge could be loaded at least with 1.2 mg of the GAs and 0.3 mg of the aglycons in a leaf−acetic acid matrix. In the present study, the total GA concentrations in all of the plant samples were <500 mg/100 g of FW. Demissidine was reliably recovered from the cartridges at each addition level, whereas solasodine showed low recoveries at all levels.

**Analysis of GAs Present in the Plant Extracts.** Complex mixtures of various GAs in the plant extracts were separated in HPLC and showed relative retention values between 13 and 16, tomatine-type structures eluting in front of α-solanine and α-chaconine (**Figures 7** and **8**).

Electrospray ionization produced protonated molecular ions $[M + H]^+$ from the samples of the *Solanum* species and the interspecific hybrids (**Tables 2** and **3**). Traces of sodium and potassium adducts were also formed. The $[M + H]^+$ ions were fragmented at multiple stages [MS(n)]. Spirosolanes showed dehydration at MS2, and they were further fragmented in the third stage of MS. Glycosides from solanidanes were fragmented already in the second stage (**Figures 9**−**12**). Glucose (Glc), galactose (Gal), rhamnose (Rha), and xylose (Xyl) present in the standard GAs gave neutral losses of 162, 162, 146, and 132, respectively. We assume that the neutral loss series of 132/162/162/162, obtained from compounds present in the plant extracts, was lycotetraose (**Figure 1**). Similarly, it was assumed that the series of 146/146/162 and 146/162/162 are chacotriose and solatriose, respectively. The relevant mass spectra of the compounds detected are presented in **Figures 9**−**12**.

Suggestions for the structures of GAs detected are given in **Table 4**. Compounds with different retention times but with the same molecular weight were detected. The $[M + H]^+$ ions of these compounds were similarly fragmented. One explanation



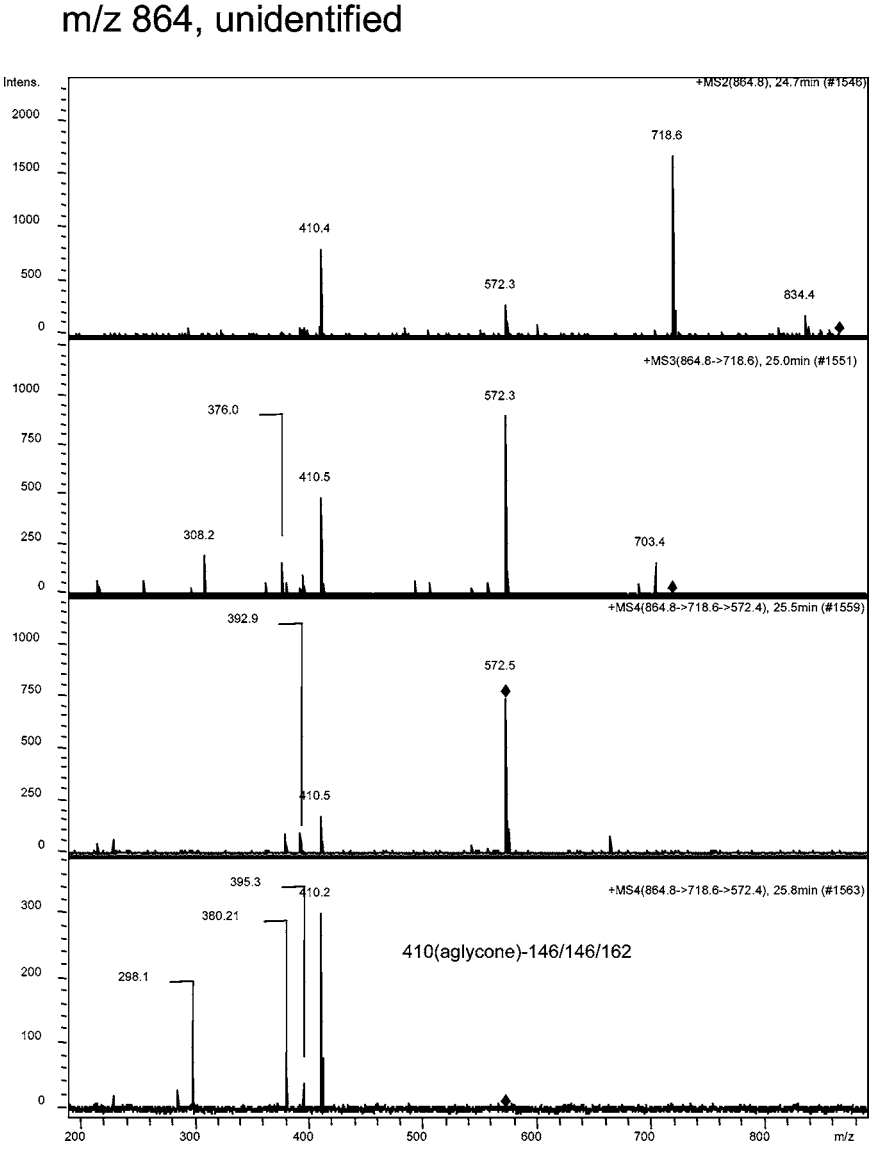

| Aglycone | [M+H]+ | Fragment ions (m/z) | Neutral fragment | Compound nr. |
|---|---|---|---|---|
| 5αH / Δ5 | 870/868 | 724/722<br>708/706<br>562/560<br>400/398 | 162<br>146<br>162, 146<br>162, 146, 162 | 14 / 17 |

| Aglycone | [M+H]+ | Fragment ions m/z | neutral fragment | Compound nr. |
|---|---|---|---|---|
| 5αH / Δ5 | 854/852 | 708/706<br>562/560<br>400/398 | 146<br>146,146<br>146,146,162 | 15 / 18 |

**Figure 11.** MS(n) spectra and fragmentations of GAs found in plant extracts: (**a**) α-solanine; (**b**) demissidine + chacotriose.

is that these GAs consist of a similar sugar moiety but their aglycons are diastereoisomers, such as in soladulcine **7** and α-tomatine **10** (*m*/*z* 1034), both giving the same *m*/*z* ratio (**Table 4**). Other possible diastereoisomeric pairs, such as the compounds **9** and **12** with *m*/*z* 870, were also detected. An additional reason for similar mass spectra could be that the structures of glycosides are different from those assumed (lycotetraose, solatriose, or chacotriose).

The tubers of the 2x *tbr* line (White Lady 15) contained only α-solanine **17** and α-chaconine **18**, whereas the foliage GA profile of 4x *tbr* cv. Pito also included solasodine or dehydrotomatidine bonded with solatriose and chacotriose (compounds **2**, **3** and/or **5**, **6**). The common feature in the GA profiles of the hybrids *acl* + *tbr* and *brd* + *tbr* was that they both contained GAs from their parental species along with new combinations between aglycons and sugar moieties. The GAs α-solanine and α-chaconine originated from *tbr* lines were expressed only as minor compounds in foliage of the hybrids 4x *brd* + 2x *tbr* and 4x *acl* + 2x *tbr*. The proportions of these GAs in the tubers of 1x *brd* + 3x *tbr* and 2x *acl* + 4x *tbr* were approximately 50 and 25%, respectively (**Figure 13**). The high levels of the GA α-tomatine **10**, detected in the parental species *brd* and *acl* foliage samples, were also expressed in the hybrid foliage. In addition, demissine **13** was also detected in the hybrids studied. In the hybrid *acl* + *tbr*, demissine apparently originated from the parental species *acl*, whereas the parental species *brd* contained no demissidine-based GAs at all. In the *brd* + *tbr* hybrid, demissine may have been formed by hydrogenation of solanidine and by combining with lycotetraose moiety, which is the tetraose moiety present also in α-tomatine. Other novel GAs, not detected in the parental species but expressed in the hybrids, were compounds **11** and **12** (or **8** and **9**), in which the aglycon tomatidine (or soladulcidine) was bonded with a solatriose or chacotriose moiety, respectively. These compounds




| Aglycone | $[M+H]^+$ | Fragment ions (m/z) | neutral fragment | Compound nr. |
|---|---|---|---|---|
| 412/410 | 866/864 | 836/834<br>720/718<br>574/572<br>412/410 | 30<br>146<br>146, 146<br>146, 146, 162 | unidentified |

**Figure 12.** MS(n) spectra and fragmentations of GAs found in plant extracts: an unidentified GA.

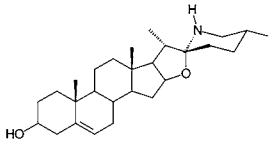
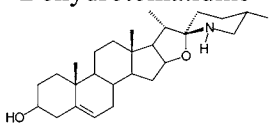
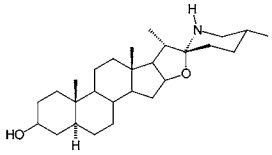
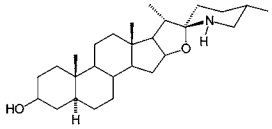
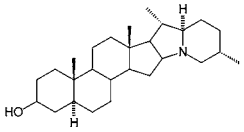
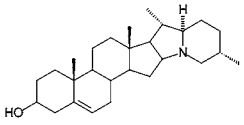
may have been formed by enzymatic hydrogenation of **2**, **3** and/or **5**, **6** inherited from the *tbr* parental line. In the same manner, demissidine was bonded with solatriose and chacotriose, forming compounds **14** and **15**, which may originate from α-solanine **17** and α-chaconine **18** by hydrogenation.

Generally, the foliar GA concentration in *tbr* can be even >10 times higher than that detected in tubers. In contrast, *acl* wild species showed a rather low total GA concentration of the leaves (21 mg/100 g) but a rather high concentration in the tubers (39 mg/100 g of FW) in the present study. The reason for the high GA level in *S. acaule* may be the small size of tubers (0.5−2 cm in diameter) or a characteristically high GA concentration in *acl* (8). Trace amounts of compounds that are not listed in **Table 4** also emerged in the foliage samples. Compounds with *m*/*z* 850 and 866 found in the *tbr* foliage samples have also been detected in transgenic potato tubers (*27*, *28*). They were identified as dehydrosolanine and dehydrochaconine, both having dehydrosolanidine as the aglycon. In the hybrid between *brd* and *tbr*, the compound of *m*/*z* 864, consisting of chacotriose and an aglycone of *m*/*z* 410, remained unidentified.

## DISCUSSION

Wild *Solanum* species related to cultivated potato have an excellent, but not very extensively exploited, genetic potential for improving the resistance or tolerance to a diverse range of diseases and pests causing serious harm in potato cultivation. The current low level of utilization of the available wild genetic resources can be derived from concerns that interspecific hybridization may bring undesirable alterations to previously acceptable tuber properties. These changes in quality may result

**Table 4.** Glycoalkaloids Formed by Combining the Main Aglycons and Glycosides Found in *Solanum* Species and the Somatic Hybrids by LC-ESI-MS[a]

| Nr. | Compound name | CAS number | Aglycone | Carbohydrate moiety | Mw |
|---|---|---|---|---|---|
| **1** | (not named) | 90366-10-2 | Solasodine  | Lycotetraose | 1032.2 |
| **2** | Solasonine | 19121-58-5 | | Solatriose | 884.1 |
| **3** | Solamargine | 20311-51-7 | | Chacotriose | 868.1 |
| | | | | - | 413.6 |
| **4** | Dehydrotomatine | 157604-98-3 | Dehydrotomatidine  | Lycotetraose | 1032.2 |
| **5** | α-Solamarine | 20318-30-3 | | Solatriose | 884.1 |
| **6** | β-Solamarine | 3671-38-3 | | Chacotriose | 868.1 |
| | | | | - | 413.6 |
| **7** | Soladulcine B | 90366-11-3 | Soladulcidine  | Lycotetraose | 1034.2 |
| **8** | β-Soladulcine | 11093-43-9 | | Solatriose | 886.1 |
| **9** | Soladulcine A | 156555-52-1 | | Chacotriose | 870.1 |
| | | | | - | 415.7 |
| **10** | α-Tomatine | 17406-45-0 | Tomatidine  | Lycotetraose | 1034.2 |
| **11** | (not named) | | | Solatriose | 886.1 |
| **12** | Dihydro-β-solamarine | 108019-37-0 | | Chacotriose | 870.1 |
| | | | | - | 415.7 |
| **13** | Demissine | 6077-69-6 | Demissidine  | Lycotetraose | 1018.7 |
| **14** | (not named) | 74918-26-6 | | Solatriose | 870.1 |
| **15** | (not named) | 74918-27-7 | | Chacotriose | 854.1 |
| | | | | - | 399.7 |
| **16** | Dehydrodemissine | 195433-57-9 | Solanidine  | Lycotetraose | 1016.7 |
| **17** | α-Solanine | 20562-02-1 | | Solatriose | 868.1 |
| **18** | α-Chaconine | 20562-03-2 | | Chacotriose | 852.1 |
| | | | | - | 397.6 |

[a] Molecular weights (Mw) are from the literature.

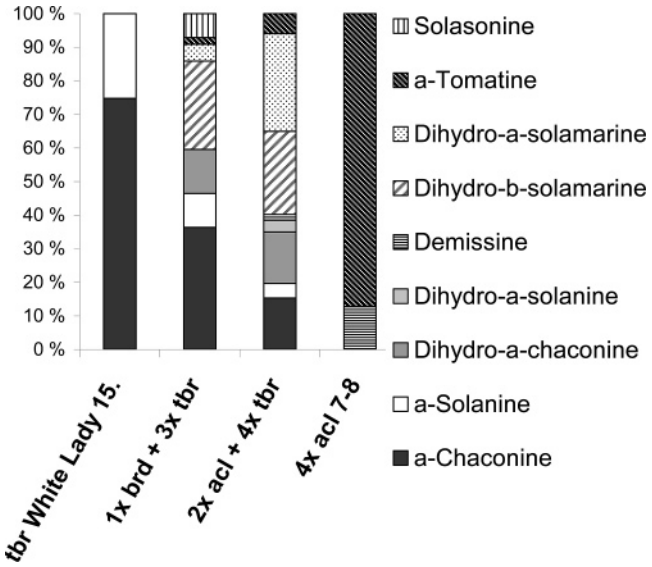



**Figure 13.** Proportions of different GAs detected in the tubers *S. tuberosum* White Lady 15 (*tbr*), *S. acaule* (*acl*), the somatic hybrid of *S. brevidens* (*brd* + *tbr*), and *S. acaule* (*acl* + *tbr*). Additional trace compounds are listed in **Table 3**. Total GA concentrations were 8.6 ± 11, 9.1 ± 2.0, 9.3 ± 3.2, and 39 ± 0.6 mg/100 g of FW, respectively.

from the introduction of additional flanking genes together with the traits of interest into the recipient crop plant. Therefore, the aim of the present study was to analyze the influence of genetic enhancement efforts on two features important for potato tuber quality: starch nanostructure and glycoalkaloid content. The test material comprised two genetically diverse wild *Solanum* species: the nontuberous 1EBN (endosperm balance number) *S. brevidens* (E genome species) and the tuberous 2EBN *S. acaule* (A genome species), which cannot be hybridized sexually with tetraploid 4EBN *S. tuberosum* cultivars.

According to the results obtained in the present study by the X-ray scattering methods WAXS and SAXS, tubers of each potato line consisted of B-type starch. Regardless of whether the interspecific potato hybrids were composed of incorporated genomes from tuberous (*acl*) or nontuberous (*brd*) wild *Solanum* species, the starch nanostructure was basically similar to that of cultivated potato tubers. We found that the starch structure of the hybrids did not remarkably differ from *tbr* tubers even if large portions of wild genomes were present. Even the genetically fairly distant E genome, which is derived from a wild species that does not produce tubers, did not make a large difference in the nanostructure of tuber starch. However, it must be stressed that in addition to the nanostructure, phosphate content and lipids also have an impact on the properties of starch, although these factors could not be analyzed by using our methods. Furthermore, textural properties and other tuber quality characteristics are certainly linked not only to genotype but also to growing conditions (e.g., soil type, fertilization, rainfall, irrigation, temperature) as well as to the influence of postharvest handling. The results clearly showed that wild species can be utilized for the genetic enhancement of potato without affecting the starch structure.

In contrast to the observations on starch nanostructure, the tuber glycoalkaloid profiles of the hybrids differed considerably from those of cultivated potato. On the basis of the total GA concentrations, cultivated potato (2x *tbr* White Lady 15) tubers showed lower GA levels than the tubers of *acl* wild potato species, as expected. *S. acaule* also contained different GAs from those of *tbr*, which, together with the high total levels, may be associated with the tolerance to cold seasons and frosts that is expressed in *S. acaule* (*2*). A low total GA concentration was found not only in *tbr* but also in the tubers of interspecific (*acl* + *tbr* and *brd* + *tbr*) somatic hybrids consistent with suppression of GA synthesis being a dominant trait (*29*). Regardless of their reduced total GA levels, the interspecific hybrids expressed very wide and complex profiles of GAs. Tubers of the 1x *brd* + 3x *tbr* hybrid contained mainly α-solanine and α-chaconine as well as their 5,6-hydrogenated counterparts (compounds **14** and **15**), which have earlier been detected only in the wild potato *S. chacoense* (*30*). In tubers of the 2x *acl* + 4x *tbr* hybrids, high proportions of spirosolanes, especially hydrogenated α-solamarine **11** and *β*-solamarine **12** (or *β*-soladulcine **8** and soladulcine A **9**) were detected. Kozukue et al. found only the GAs of fusion partners, for example, α-tomatine, demissine, α-solanine, and α-chaconine, in the hybrids of *acl* and *tbr* (*8*). We assume that the main reason for this difference in the results is that we used the LC-MS method, which can be used to identify unknown compounds rather easily. Previously, hyphenated MS methods have been successfully applied to GA analyses (*27*, *28*, *31*, *32*), and our studies showed that LC-ESI-MS is a powerful tool to resolve complex GA mixtures.

In sum, the tubers of the somatic hybrids showed a wider variation of GAs than the parental *Solanum* species studied. In the tubers, however, there was less variation in the types of GA structures expressed than in the foliage. Approximately 50% of the foliage GA profile of *acl* + *tbr* and 80% of *brd* + *tbr* hybrids consisted of α-tomatine and other spirosolanes, which have previously been identified also in tomato and *S. dulcamara* (*33*, *34*). These GAs were mainly composed of hydrogenated (no 5,6-double bond in the aglycon) counterparts.

When starch is used only for nonfood applications, the high GA levels or complex GA content expressed in tubers is not necessarily a problem. However, GAs present in foods or feed may be a risk. The safety limit of 20 mg of GAs/100 g of FW of tubers for human consumption may have to be re-evaluated if alien GAs are present in the potato hybrids, because toxicity levels vary with GA structure and there are also synergistic effects between the different GAs (*14*). Fortunately, our hybrid tubers contained high proportions of 5,6-dihydrogenated spirostructures (no double bond in the aglycon). These compounds are potentially less toxic than α-solanine and α-chaconine, because the solanidanes are more toxic than spirosolanes, and the existence of a 5,6-double bond apparently increases teratogenicity of GAs (*17*).

In this context, we must emphasize that the hybrids studied here are not yet commercial potato varieties and that backcrosses or additional back-fusions are needed to improve their agronomic value, such as in terms of tuber yield. In the hybrids, the proportion of α-solanine and α-chaconine can be increased by back-crossing, but alien GAs may still be present (*35*). It must also be pointed out that spirosolanes may alternatively have a positive role in the human diet (*36*), and therefore their increased proportions could become a goal in potato breeding. In addition, novel GAs (bio)synthesized in potato plants could also be used for medical applications (*37*, *38*).

## ACKNOWLEDGMENT

Prof. Eija Pehu and Dr. Into Laakso at the University of Helsinki and Dr. Jaana Laurila at MTT are acknowledged for valuable cooperation and very useful ideas during the course of the project. Kirsti Risunen is gratefully thanked for dry matter content determinations. Prof. Alan Schulman is kindly acknowledged for a critical review of the manuscript.

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