# Inheritance of Morphological Characters and Glycoalkaloids in Potatoes of Somatic Hybrids between Dihaploid *Solanum acaule* and Tetraploid *Solanum tuberosum*

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Steroidal glycoalkaloids occur in potatoes and are reported to impart resistance to phytopathogens including bacteria, fungi, and insects. Because glycoalkaloids can be passed to progenies during breeding programs designed to develop improved potatoes, it is of importance to determine the quality of desired characteristics and the composition of glycoalkaloids of new somatic hybrids. The objective of this study was to determine the appearance, size, and shape (morphological characters) as well as the glycoalkaloid content of potato tubers of somatic hybrids between tetraploid *Solanum tuberosum* cv. Dejima ($2n = 4x = 48$ chromosomes) and the dihaploid clone ATDH-1 ($2n = 2x = 24$ chromosomes) induced by anther culture from *Solanum acuale*-T (acl-T, $2n = 4x = 48$ chromosomes). Tuber size and shape in somatic hybrids were in accord with those of cv. Dejima, whereas the tuber skin color resembled that of ATDH-1. Thin-layer chromatography, high-performance liquid chromatography, and gas−liquid chromatography/mass spectrometry studies showed that the two steroidal glycoalkaloids (α-chaconine and α-solanine) were present in the tubers of *S. tuberosum*, whereas acl-T and ATDH-1 tubers were found to contain α-tomatine and demissine. The concentrations of total glycoalkaloids in both acl-T and ATDH-1 was >100 mg/100 g of fresh weight tuber cortex, much higher than in *S. tuberosum*. All somatic hybrids, except one clone, contained four glycoalkaloids (α-chaconine, α-solanine, α-tomatine, and demissine) derived from the fusion parents. The lack of α-tomatine in the remaining clone may be due to somaclonal variation. The results show that character expression is influenced by ploidy level and that total glycoalkaloid levels in most somatic hybrids were intermediate between those of the fusion parents. The possible significance of these findings for plant breeding and food safety is discussed.

**Keywords:** *Dihaploid Solanum tuberosum; tetraploid Solanum tuberosum; anther diffusion; electrofusion; potato clones; somatic hybrids; morphology; glycoalkaloids; α-chaconine, α-solanine, α-tomatine, demissine*

## INTRODUCTION

Potato tubers are known to contain small quantities of steroidal glycoalkaloids such as α-chaconine and α-solanine (Figure 1). Excessively high glycoalkaloid content imparts bitterness (Maga, 1980). Moreover, ingestion of potatoes with a glycoalkaloid content of >20 mg/100 g of fresh weight of tubers has been associated with outbreaks of human poisoning (Friedman and McDonald, 1997, 1999a,b; Jadhav and Salunkhe, 1975; Keeler, 1986; McMillan and Thompson, 1979).

It is, therefore, important to develop potato varieties with low glycoalkaloid concentrations for human consumption and potato breeding.

Many wild potato species have been used to introduce desirable traits into *Solanum tuberosum* by sexual crosses or protoplast fusion. However, hybrids between *Solanum tuberosum* and wild potato species generally have both useful and undesirable traits from donor plants. Tubers from wild species contain within their tissue higher concentrations of steroidal glycoalkaloids than those of cultivated potatoes. This high glycoalkaloid content is one of the undesirable characteristics of wild species. Mattheij et al. (1992) produced somatic hybrids between *S. tuberosum* and the wild species *Solanum circaeifolium*. Although these tetraploid hybrids were found to be resistant to both *Phytophthora infestans* and *Globdora pallida*, they contained high concentrations of glycoalkaloids. Furthermore, different glycoalkaloids of demissidine glycoside type from both fusion parents were observed in the tubers of somatic hybrids.

The object of this paper is (a) to produce somatic hybrids of *S. tuberosum* ($2n = 4x = 48$) and ADTH-1, dihaploid *S. acuale* [$2n = 2x = 24$, resistant to potato virus X(PVX), potato spindle tuber viroid and cyst nematode, and frost tolerant by electrofusion techniques]; (b) to investigate the morphological character

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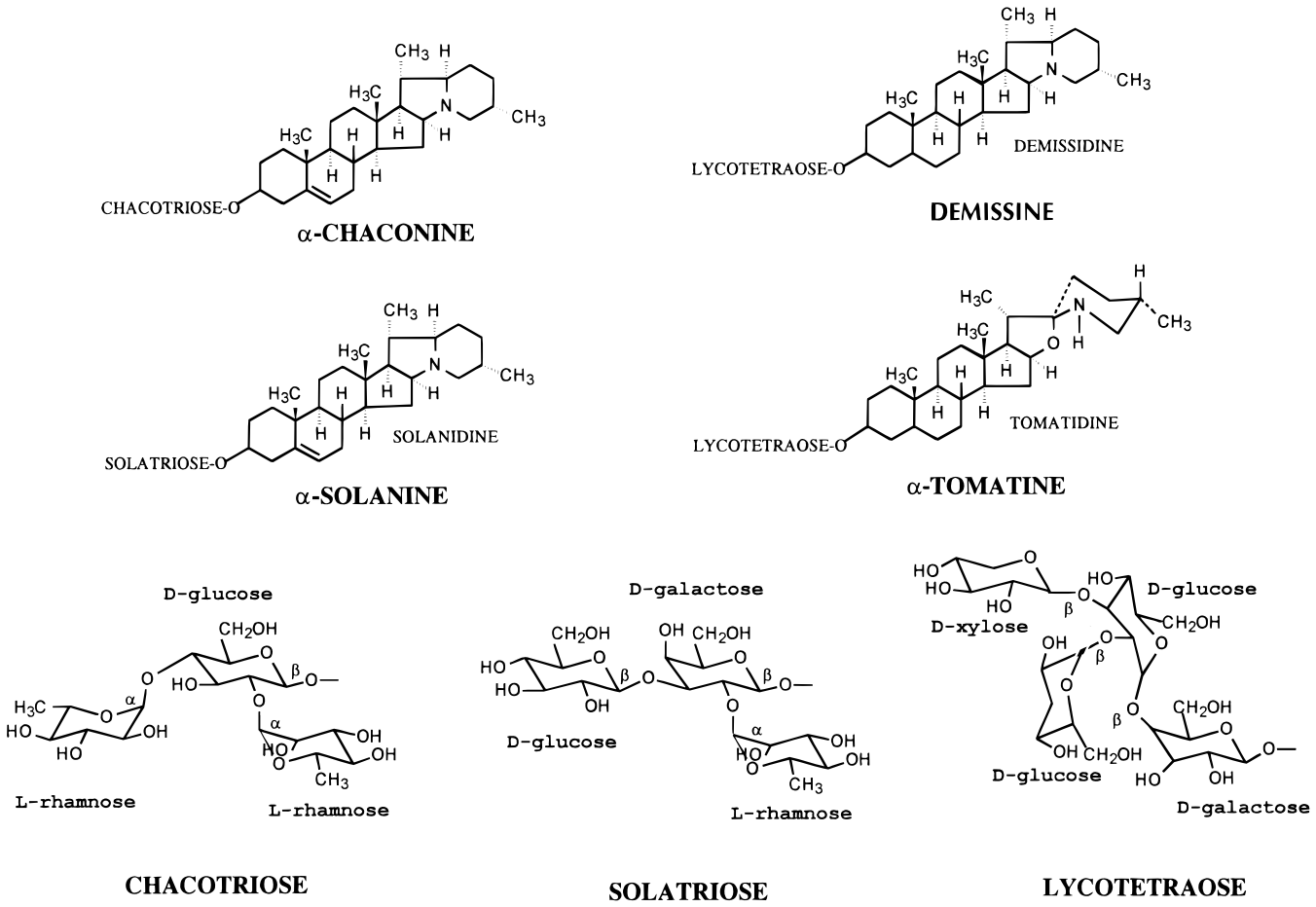


**Figure 1.** Structures of glycoalkaloids evaluated in this study.

of the tubers in somatic hybrids; (c) to separate and determine the steroidal glycoalkaloids in the cortex of somatic hybrid tubers; and (d) to evaluate the potential of these hybrids in practical plant breeding programs.

## MATERIALS AND METHODS

**Materials.** Analytical grade α-chaconine, α-solanine, and α-tomatine were obtained from Sigma Chemical Co., St. Louis, MO. Pure demissine was extracted and isolated from the tuber cortex of *S. acaule*-T.

**Fusion Parents.** *S. tuberosum* cv. Dejima ($4n = 4x = 48$) was obtained from Aino Potato Branch, Nagasaki Perfectual Agricultural and Forestry Experiment Station, Japan, and ADTH ($2n = 2x = 24$), a dihaploid clone induced by anther culture from a Peruvian accession of *S. acaule*-T ($2n = 4x = 48$) maintained in our laboratory (Collection of the Expedition of Cultivated Plants in the Andean Areas, Kyoto University, 1971). These fusion parents were grown as in vitro cultures under the following conditions: the temperature was not controlled; cultivated plants from early March to May for 4 weeks at 6° C (minimum) to 26 °C (maximum); light intensity, daylight; photoperiod, day length from 11.5 to 13 h during cultivation.

**Protoplast Isolation, Electrofusion, and Regeneration.** Leaf strips from fusion parents were precultured overnight at 4 °C and digested in an enzyme solution [1% Cellulase, ONOZUKA RS (Yakuruto Corp., Japan), 0.25% Macroenzyme R-10 (Yakult Corp., Japan), 0.5% dextran sulfate potassium salt, 0.5 M mannitol] for 3 h at 28 °C. The isolated protoplasts were aligned into short chains by applying an alternating-current collecting field of 150 V/cm and fused by one 5 $\mu$s direct current square pulse at 1500 V/cm with a somatic hybridizer SSH-I (Shimazu Corp., Japan). After electrofusion, the protoplasts were transferred to a protoplast culture medium (Kikuta et al., 1984) containing 0.4 M mannitol, 0.2% sucrose, 0.5 mL/L zeatin, 4 mg/L naphthaleneacetic acid (NAA), 0.2% coconut water, and 500 mg/L 2-(*N*-morpholine)ethanesulfonic acid (MES) (pH 5.8). The small calli generated were then placed on the callus growth medium (Murashige and Skoog basal medium, MS) with 0.2 M mannitol, 1% sucrose, 0.5 mg/L benzylaminopurine (BAP), 0.1 mg/L NAA, and 0.3% agar. Growing calli were transferred to the regeneration medium [MS containing 0.2 M mannitol, 0.5% sucrose, 1 mg/L zeatin, 0.1 mg/L IAA, 0.1 mg/L gibberellic acid ($GA_3$), and 0.6% agar]. Regenerated plants were recovered after ~50 days and designated DA with callus number. For plants derived from multiple shoots of a single callus, the shoot number was added after the callus number.

**Analysis of Morphological Characters.** To examine the morphological character of 14 somatic hybrids, 3 plants for each genotype were planted in 30 cm clay pots in a glasshouse at Kobe University in the spring. After harvest, the morphological characteristics of the tubers, such as size, skin color, shape, and depth of eyes, were recorded. All tubers were yellowish white in color with the depth of eyes ranging from <2 mm (shallow) to >2 mm (deep).

Tuber weights ranged from 9.6 g/tuber (small) to 45.4 g/tuber (large) with a mean weight per tuber of 15.8 g.

**Extraction of Glycoalkaloids in the Tubers of the Fusion Parents and Somatic Hybrids.** 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 four to five tubers, each weighing 17−22 g. Isolation and determination of glycoalkaloids were carried out according to the methods Friedman et al. (1998a) and Kozukue et al. (1994). Ten grams of cortex layer (~5 mm of peripheral tissue) was accurately collected from the stored tubers. The cortex was

**Table 1. Morphological Characters of Tubers in Somatic Hybrids**

| genotype | size | shape | skin color | depth of eyes on skin |
|---|---|---|---|---|
| *S. acuale*-T | small | oval | brown | shallow[a] |
| ATDH-1 | small | oval | brown | shallow |
| Dejima | large | round | light brown | shallow |
| DA6 | large | round | brown | shallow |
| DA8-1 | large | round | brown | deep[b] |
| DA8-2 | large | round | brown | deep |
| DA10-1 | large | round | brown | shallow |
| DA10-2 | large | round | brown | shallow |
| DA12-1 | large | round | brown | deep |
| DA12-2 | large | round | brown | deep |
| DA12-3 | large | round | brown | deep |
| DA17 | large | round | brown | shallow |
| DA18 | large | round | brown | shallow |
| DA21 | small | round | brown | shallow |
| DA22 | small | round | brown | shallow |
| DA25 | large | round | brown | deep |
| DA28 | large | round | brown | shallow |

[a] <2 mm. [b] >2 mm.

chopped well by a knife and blended in a homogenizer with 150 mL of mixed chloroform/methanol solvent (2:1 v/v). 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. Forty milliliters of 0.2 N HCl was added to the concentrate. This was followed by sonification for 5 min in an ultrasonic cleaner. The flask was rinsed twice with 10 mL of 0.2 N HCl and centrifuged at 12000 rpm at 1 °C for 10 min. The supernatant was transferred to a 250 mL Erlenmeyer flask, and 30 mL of concentrated $NH_4OH$ was added to precipitate the glycoalkaloids. This basic solution was placed in a 65 °C water bath for 50 min and then refrigerated overnight. The precipitate was collected by centrifugation at 12000 rpm for 10 min at 4 °C. The supernatant (50 $\mu$L) was subjected to HPLC.

**HPLC Analysis.** HPLC analysis was carried out with the aid of a Hitachi liquid chromatograph Model 655A-11 with an autosampler Model 655-40. Two stainless steel chromatographic columns [25 cm × 4.0 mm (i.d.)] connected in series were packed with Nucleosil $NH_2$ (particle diameter = 10 $\mu$M; Nagel). Glycoalkaloids were eluted with tetrahydrofuran/acetonitrile/0.025 M $KH_2PO_4$ (50:30:20, v/v/v) at the flow rate of 1 mL/min. The UV detector (Hitachi, Model 655A UV monitor) was set 208 nm.

**Identification of Glycoalkaloids.** The glycoalkaloids obtained from the tubers of the fusion parents and somatic hybrids were identified by thin-layer chromatography (TLC) on samples of each peak collected from the high-performance liquid chromatography (HPLC) column. The reported values were plotted against a standard calibration curve for each glycoalkaloid analyzed. To confirm these compounds, each peak collected by HPLC was hydrolyzed into sugars and aglycon by acid hydrolysis (Sinden et al., 1978). Sugars were converted into trimethylsilyl ester derivatives, and individual compositions and molar ratios of sugars and aglycons were determined by GLC/mass spectrometry (GLC/MS) (Friedman et al., 1997, 1998a,b; Kozukue et al., 1994).

## RESULTS

**Morphological Characters.** The morphological characters (tuber size, shape, skin color, and depth of eyes) of somatic hybrids are shown in Table 1. The tuber size in somatic hybrids, except DA22, was similar to that of cv. Dejima's and the shape, like Dejima's, was round. The DA22 set had a few small tubers. However, the tuber skin color of somatic hybrids was brown as in ATDH-1. The fusion parents and some somatic hybrids had shallow eyes. However, deep eyes were found in the tubers of six somatic hybrids (DA8-1, DA8-2, DA12-1, DA12-2, DA12-3, DA25).

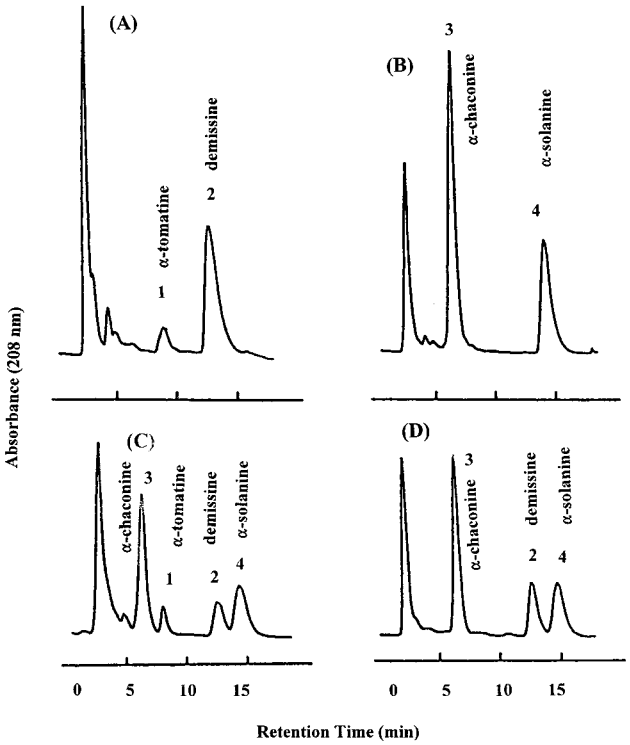


**Figure 2.** HPLC chromatograms of glycoalkaloids from the tuber cortex of *S. acaule*-T, the fusion parents (dihaploid *S. acaule*, ATDH-1, and *S. tuberosum* cv. Dejima), and somatic hybrids: (A) *S. acaule*-T and dihaploid *S. acaule*, ATDH-1; (B) tetraploid *S. tuberosum* cv. Dejima; (C) DA-6; (D) DA8-2. Solvent front; peak 1, α-tomatine; peak 2, demissine; peak 3, α-chaconine; peak 4, α-solanine.

**Table 2. $R_f$ Values of Four Standard Glycoalkaloids on TLC[a] and Individual Peaks on HPLC Isolated from the Tubers of Somatic Hybrids**

| | $R_f$ values on TLC plates with the indicated solvents | | | |
|---|---|---|---|---|
| compound | A[b] | B[c] | C[d] | D[e] |
| α-tomatine | 0.31 | 0.21 | 0.89 | 0.72 |
| demissine | 0.26 | 0.16 | 0.66 | 0.54 |
| α-chaconine | 0.41 | 0.26 | 0.54 | 0.67 |
| α-solanine | 0.23 | 0.13 | 0.22 | 0.45 |
| peak on HPLC | | | | |
| 1 | 0.33 | 0.22 | 0.88 | 0.71 |
| 2 | 0.27 | 0.15 | 0.66 | 0.55 |
| 3 | 0.42 | 0.26 | 0.54 | 0.67 |
| 4 | 0.23 | 0.15 | 0.21 | 0.44 |

[a] TLC was performed on Merck precoated silica gel 60 plates, 0.25 mm thick. [b] Chloroform/methanol/1% $NH_4OH$ (2:2:1, v/v/v, bottom layer). [c] Chloroform/methanol/2% $NH_4OH$ (14:6:1, v/v/v). [d] Chloroform/methanol/1% $NH_4OH$ (65:35:5, v/v/v). [e] Chloroform/methanol/1% $NH_4OH$ (5:5:1, v/v/v).

**Glycoalkaloids in the Tuber Cortex of *S. acaule*-T, the Fusion Parents, and Somatic Hybrids.** Figure 2 shows the HPLC chromatogram of glycoalkaloids from the tuber cortex of *S. acaule*-T, the fusion parents, and two representative somatic hybrids. Individual peaks on chromatograms were collected and identified by TLC (Table 2) and by GLC and GLC/MS (Table 3). From these results it was found that *S. acaule*-T and ATDH-1 contained α-tomatine and demissine (Figure 2A). On the other hand, both α-chaconine and α-solanine were detected from *S. tuberosum*

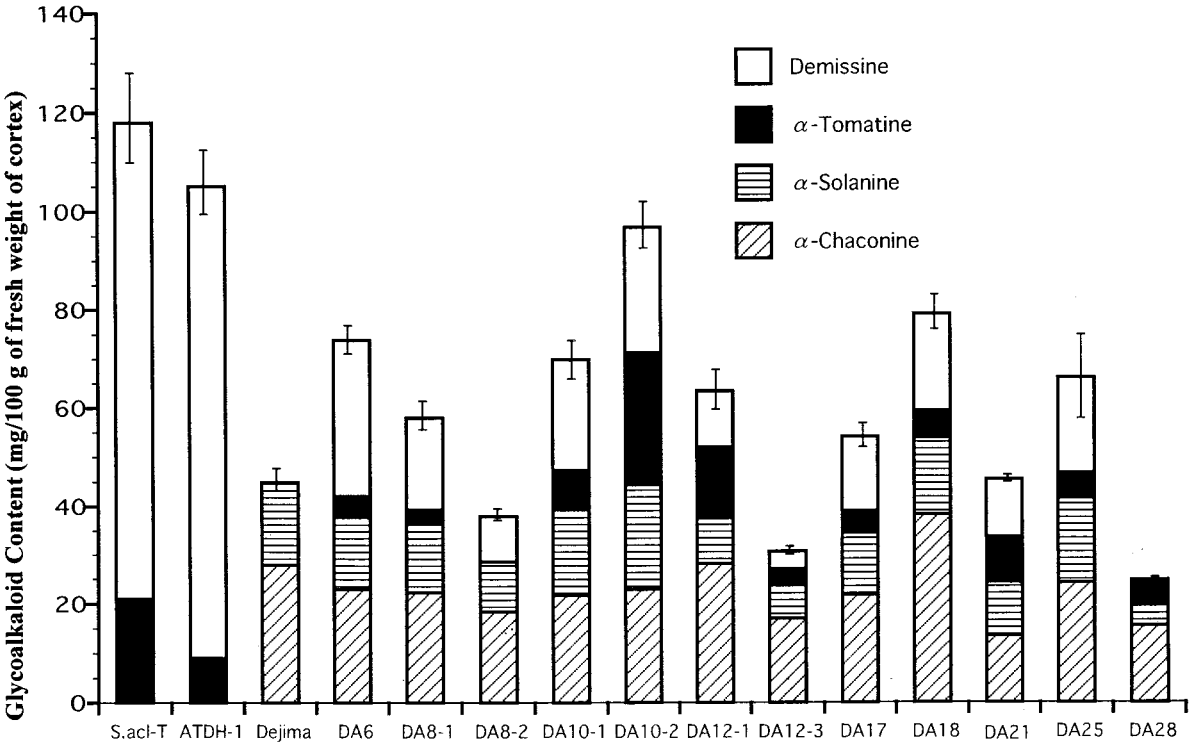


**Figure 3.** Constituent glycoalkaloids from the tuber of cortex of *S. acaule*-T, the fusion parents (dihaploid *S. acaule*, ATDH-1, and *S. tuberosum* cv. Dejima), and 12 somatic hybrids. Vertical bars indicate standard errors (SE) for three separate determinations of the sums of individual glycoalkaloids.

**Table 3. Characterization of Acid Hydrolysis Products of Four Standard Glycoalkaloids and Each Peak on HPLC Isolated from the Tubers of Somatic Hybrids**

| | compound detected by GC and GC/MS | | |
|---|---|---|---|
| compound | aglycon | carbohydrate (mole ratio) | identification of glycoalkaloid |
| α-tomatine | tomatidine ($M^+$ 416) | xylose/glucose/galactose (1:2:1) | |
| demissine | demissidine ($M^+$ 399) | xylose/glucose/galactose (1:2:1) | |
| α-chaconine | solanidine ($M^+$ 397) | rhamnose/glucose (2:1) | |
| α-solanine | solanidine ($M^+$ 397) | rhamnose/glucose/galactose (l:1:1) | |
| peak on HPLC | | | |
| 1 | tomatidine ($M^+$ 416) | xylose/glucose/galactose (1:2:1) | α-tomatine |
| 2 | demissidine ($M^+$ 399) | xylose/glucose/galactose (1:2:1) | demissine |
| 3 | solanidine ($M^+$ 397) | rhamnose/glucose (2:1) | α-chaconine |
| 4 | solanidine ($M^+$ 397) | rhamnose/glucose/galactose (l:1:1) | α-solanine |

cv. Dejima (Figure 2B). All hybrids, except DA8-2, contained the four glycoalkaloids found in the parents (α-chaconine, α-solanine, α-tomatine, and demissine) (Figure 2C). Although DA8−2 has α-chaconine, α-solanine, and demissine, this clone was found to lack α-tomatine (Figure 2D).

Figure 3 shows the constituent glycoalkaloids in the cortex of *S. acaule*-T, the fusion parents, and 12 somatic hybrids. The concentration levels of total glycoalkaloids in *S. acaule*-T and ATDH-1 were >100 mg/100 g of fresh cortex weight. However, Dejima contained only 45 mg/100 g of fresh cortex weight, whereas the somatic hybrids were found to contain glycoalkaloids ranging from 25 to 90 mg/100 g of fresh cortex. The ratio of α-chaconine to α-solanine ranged from 1.40 to 3.88, with an average value of 2.40.

## DISCUSSION

Most morphological characters of ATDH-1 resembled those of its tetraploid donor plant (*S. acaule*-T) (Table 1). However, the tuber sizes in ATDH-1 were smaller than in *S. acaule*-T. Camadro et al. (1991) reported similar results between dihaploid and tetraploid *S. acaule*-T. Somatic hybrids containing the genome of the wild species often did not set the same large tubers as the recipient parent, *S. tuberosum* (Austin et al., 1986; Laurila et al., 1996; Novy and Helgeson, 1994). In this study, the sizes of the tubers in somatic hybrids were generally similar to those of cv. Dejima. Tuber size of somatic hybrids may be dependent on the proportion of the genome of *S. tuberosum* contained in somatic hybrids. Somatic hybrids resembled Dejima in their round tubers, whereas *S. acaule*-T and ATDH-1 set ovate tubers. This result indicated that round shape may be dominant over oval shape. Other researchers (De Jong and Rowe, 1972; Mollers and Wenzel, 1992; Schick, 1956) have also suggested that round tuber shape is dominant over either oval or long oval shape. Concerning the eye depth of tubers, both fusion parents ATDH-1 and Dejima set tubers with shallow eyes. However, some somatic hybrid clones formed tubers with deep eyes. We do not know the reasons for these results.

In all somatic hybrids except for DA8-2, four different glycoalkaloids (α-chaconine, α-solanine, α-tomatine, and

demissine) were determined (Figure 2C). Only α-tomatine was not detected in DA-8-2 at the detection limit of our method of ~1 μg/g (ppm) (Figure 2D). DA8-1 was found to have 72 chromosomes, as did four other clones (DA6, DA8-2, DA12-1, and DA17) (data not shown). As these four clones contained four glycoalkaloids, the lack of α-tomatine in DA8-2 may have been caused by somaclonal variation. Except for DA10-2, DA12-1, and DA21, the concentrations of α-tomatine and demissine in somatic hybrids were less than in ATDH-1. For α-tomatine, it was on average 42.5% less and for demissine, 82.7%. A small quantity of α-tomatine and a larger amount of demissine were determined in ATDH-1 (Figure 2A). Approximately 100 mg of demissine was present per 100 g of cortex of ATDH-1 (Figure 3). Because ATDH-1 has only about half as much α-tomatine as *S. acaule*-T (Figure 3), these observations suggest that the amount of α-tomatine, but not demissine, may depend on the ploidy level. The possible reasons for the much greater variation of α-tomatine than of α-chaconine in the somatic hybrids are not known.

Cv. Dejima tubers were found to contain two steroidal glycoalkaloids (α-chaconine and α-solanine) (Figure 2B). Total glycoalkaloid contents of 45 mg/100 g of tuber cortex were determined in Dejima (Figure 3). A concentration of 20 mg/100 g of *total* tuber weight is considered to be undesirable for human consumption. We estimate that the glycoalkaloid content of Dejima is much less than 20 mg/100 g of tuber weight, because the cortex (peel) usually has a much higher glycoalkaloid content than the tuber flesh after peeling. In addition, because glycoalkaloids are largely synthesized in the outer part (cortex) of the tuber and then migrate into the flesh of the potato, the relative amounts of glycoalkaloids in the flesh in different potato hybrids may differ from that in the cortex. This suggestion is supported by the observed ratio of glycoalkaloid content of flesh to peel of 1:5 to 1:10 for four U.S. domestic potato varieties (Friedman and McDonald, 1999a).

The total glycoalkaloid content ranged from 25 to 95 mg/100 g of tuber cortex (Figure 3). These values indicated that the glycoalkaloid contents of the somatic hybrids were intermediate between values of total content in the fusion parents. Similar results were reported in the leaves of somatic hybrids between other potato somatic hybrids (Roddick and Melchers, 1985). We did not measure the glycoalkaloid content of the leaves. Mattheij et al. (1992) observed a new component, demissidine glycoside, in the tubers of somatic hybrids between *S. tuberosum* and *S. circaeifolium*. In the current study, however, peaks different from those of the fusion parents were not detected in somatic hybrids by HPLC analysis.

It is generally thought that glycoalkaloids are involved in resistance to the Colorado beetle (Stürckow and Löw, 1961) and to the spores of *Fusarium caerulum* (McKee, 1959). Somatic hybrids used in this study have some resistance to PVX derived from ATDH-1. Moreover, these clones set tubers as large as those of the cv. Dejima. However, higher glycoalkaloid contents than in Dejima were found in the tubers of somatic hybrids; these hybrids are thus unacceptable as immediate cultivars. Therefore, backcrossings are necessary to recover generally acceptable agronomic characters without losing a target character such as resistance to PVX and various phytopathogens. The availability of a new ELISA kit for the analysis of glycoalkaloids (Friedman et al., 1998b) and of a recommended sampling technique to measure foliar glycoalkaloids in growing potato plants (Brown et al., 1999) may facilitate such studies.

It is also relevant to note that studies on the safety of structurally different glycoalkaloids consumed by animals described in detail elsewhere show that the relative potencies vary widely (Friedman and McDonald, 1997). Because the ratios of α-chaconine to α-solanine ranged from 1.40 to 3.88 for the somatic hybrids evaluated in this study, these considerations imply that both relative concentrations of each glycoalkaloid in a new cultivar and the total amount should guide decisions made about the safety of the tubers and their resistance to pathogens (Rayburn et al., 1995; Roddick et al., 1988). The average value of the ratio of 2.4 is similar to that reported for commercial potatoes (Friedman and Dao, 1992). Additional studies are also needed to determine the levels of glycoalkaloids in somatic hybrids in plants grown under field conditions where they would be exposed to a range of environmental stresses.

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