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Glycoalkaloid Content and Chemical Composition of Potatoes Improved with Nonconventional Breeding Approaches

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This paper reports the results of chemical analyses performed on two distinct groups of new potato genotypes. The first group contained five clones transformed with the gene *ech42* encoding for an endochitinase. The second included 21 interspecific hybrids between the cultivated potato *Solanum tuberosum* and the wild species *S. commersonii*, obtained either by somatic fusion or by sexual hybridization. Tubers from transgenic plants were analyzed for several morphological and biochemical parameters to ascertain the substantial equivalence between the transgenic genotypes and the original cultivar Désirée. The interspecific hybrids were analyzed for the same parameters in order to identify genotypes with novel improved chemical characteristics and with low levels of glycoalkaloids deriving from the wild species and potentially hazardous to human health. For transgenic tubers, the results provided evidence that indicates the substantial equivalence between the transgenic genotypes and the cultivated control for the considered traits. The results suggest that chitinase gene insertion did not alter other metabolic pathways of potato tubers and did not cause unintentional pleiotropic effects. As far as interspecific hybrids are concerned, wide variability for all of the parameters analyzed was found. For some useful traits (e.g., soluble solids and proteins, dry matter content) the interspecific hybrids performed better than both the cultivated control and the wild species. In a number of genotypes, glycoalkaloid levels were close to or lower than those of the control varieties, suggesting that selection for low glycoalkaloid content is possible. The results also indicated that glycoalkaloids from *S. commersonii* may be lost rapidly. Indeed, some hybrids were found to have the same glycoalkaloid profile as *S. tuberosum*. Finally, the results showed that among the parameters considered, glycoalkaloid content is the most sensitive to variation. Therefore, glycoalkaloid determination should be used for routine control of genotypes produced by interspecific hybridization.

KEYWORDS: Transgenic potatoes; interspecific hybrids; chipping quality; glycoalkaloid; chlorogenic acid; antioxidant activity

INTRODUCTION

The potato (*Solanum tuberosum*, tbr) is a major crop in many parts of the world. It has wide food versatility and a full complement of nutrients (*1*). Because its genetic basis is rather restricted, several strategies are currently being undertaken for the genetic improvement of cultivated genotypes, including widely used techniques such as interspecific hybridization and genetic transformation. The former is based upon the use of wild *Solanum* species as a unique source of useful genes to enlarge the cultivated gene pool, the latter on the molecular manipulation of single important genes and the availability of vectors for the transfer into the plant cell.

Both techniques have been successfully used to transfer resistance traits (*2*−*4*), improved tuber quality (*5*, *6*), and allelic diversity (*7*) to the potato. However, like other breeding technologies, they can cause the appearance of undesired traits and/or changes in the chemical composition of newly produced genotypes. For example, the use of some wild species (e.g., *S. acaule*, *S. commersonii*, and *S. demissum*) may potentially cause a useful increase in the dry matter content of tubers and an undesired increase of glycoalkaloids (GA), which are considered to be undesirable for human consumption at concentration >200 mg/1000 g of total tuber weight (*8*). For this reason several backcrosses with the cultivated genotypes are necessary before a new hybrid genotype is released as a cultivar.

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Similarly, genetic transformation can lead to undesired changes due to the transformation system employed and the interaction between the transgene and the genome of the transgenic plant (*9*, *10*).

The present paper reports the results of chemical analyses performed on two distinct groups of potato genotypes. The first group contains potato plants transformed with the gene *ech42* from the antagonistic fungus *Trichoderma harzianum* encoding for an endochitinase. This gene conferred resistance to different pathogens such as *Alternaria alternata*, *Alternaria solani*, *Botrytis cinerea*, and *Rhizoctonia solani* (*3*). Transformed plants were analyzed to ascertain the substantial equivalence between the transformed genotypes and the original cultivar. It is known that genetic modification could result in unintentional pleiotropic effects that in some species lead to alteration of the amount of inherent plant toxins. In this respect for potatoes the most interesting compounds to be monitored are GA (*11*).

The second group of material includes interspecific hybrids between the cultivated potato and *S. commersonii* (cmm), a noteworthy species with several useful traits (resistance to biotic and abiotic stresses, high dry matter content of tubers). The hybrids were analyzed for several morphological and biochemical parameters, in order to identify genotypes with superior quality traits and to ascertain if, together with the target resistances, GA levels hazardous to human health were transmitted.

MATERIALS AND METHODS

Plant Material. The group of transformed plants included five transformed genotypes of tbr cv. Désirée expressing the *ech42* (endochitinase) transgene and obtained as described by Lorito et al. (*3*). Data on transgene integration and expression are reported in the same paper. The cmm−tbr hybrids derived either from somatic fusion or from sexual hybridization. In the first case, the fusion partners (clone cmm1 of cmm and tbr haploid SVP11), the somatic hybrid SH9A obtained by Cardi et al. (*12*), and 13 backcross hybrids SH9A × tbr (hereafter coded BCSH) were analyzed. The eight sexual hybrids (hereafter called PTH) derived from crosses between a pentaploid cmm−tbr hybrid and tbr varieties and were obtained according to the breeding scheme reported by Carputo et al. (*13*). The cmm clone was the same used for somatic fusion experiments.

Three plants per genotype were grown in a greenhouse under controlled light and humidity. Five tubers from each genotype were randomly selected, washed, cut into small cubes (including skin and cortex), and frozen. The samples were freeze-dried and finely ground. The powders were used for different analyses.

Morphological and Proximal Analyses. At harvest, the skin and flesh color and the eye depth were evaluated. Fresh weight and dry matter content of potato tubers were measured. The soluble solids were measured by a refractometer (Atago), and results were reported as Brix grade at 20 °C.

Biochemical Analyses. *Glycoalkaloid Analysis.* HPLC grade solvents and water were from Merck and filtered through disposable 0.2 μm filters from Acrodisc (Gelman Sciences). Absorbance was measured using a UV−vis 2100 Shimadzu spectrophotometer. Chlorogenic acid, α-solanine, and α-chaconine were obtained from Sigma Chemical Co.

One gram of freeze-dried potato sample was extracted by 20 mL of 2% of acetic acid for 1 h (*14*). The extract was prepurified by a Sep-Pak column (*15*). ELISA analysis was performed with the potato glycoalkaloid plate kit from Envirologix Inc. (Portland, ME). To calculate the results, the ELISA plate reader was set at 450 nm using a Bio-Rad plate reader. Data are the mean of three replicates and were expressed in milligrams per 100 g of fresh weight. Values were corrected for the determined recovery (70%).

GA identification was performed on a sample of cmm−tbr hybrids by mass spectrometry analysis using an API-100 single-quadrupole mass spectrometer (Perkin-Elmer Sciex Instruments) equipped with an atmospheric pressure ionization source. A probe voltage of 5.0 kV and a declustering potential of 60 V were used. The instrument mass-to-charge ratio scale was calibrated with the ions of the ammonium adducts of polypropylene glycol. GA analysis was performed by injecting the raw extracts obtained from the different tubers directly into the ion source at a flow of 10 μL min^{-1}. Acquisition was made in positive ion mode in the range of 300−1200 amu with a dwell time of 2 ms and a step size of 0.5 amu. Data were processed through the Bio Multi View software (Sciex).

Chlorogenic Acid Content. Chlorogenic acid content was determined by using a spectrophotometric method as previously described by Friedman (*16*).

Antioxidant Activity. The DMPD (*N*,*N*-dimethyl-*p*-phenylenediamine) method (*17*) was used to determine the water-soluble antioxidant activity. Two hundred milligrams of freeze-dried potato sample was added to 2 mL of a solution containing the DMPD radical cation in acetate buffer. The absorbance quenching at 505 nm was compared with that obtained by a standard solution of ascorbic acid. The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method, performed as described by Pellegrini et al. (*18*), was employed to assess the antioxidant activity of the water-insoluble fraction.

Total Soluble Proteins and SDS-PAGE Analysis. Proteins were determined according to the method of Bradford (*19*) using BSA as standard. SDS-PAGE was performed according to the method of Laëmmli (*20*).

Chipping Test. A test to evaluate chipping ability was performed on tubers soon after harvest and after 2 months of storage at 7 °C without reconditioning. The test was performed as previously described (*21*). Peanut oil heated at 180 °C was used. Chip color evaluation was performed using an empirical scale between 1 and 10 (1 = light, 10 = completely dark). Values <4.5 were considered to be an index of good quality.

Statistical Evaluation of Data. Analysis of variance was used to evaluate differences among the four groups of genotypes (i.e., transgenic clones, *S. tuberosum* genotypes, BCSH hybrids, and PTH hybrids) for all of the biochemical parameters tested. Means were compared by the Tukey HSD test. In addition, a *t* test was performed to compare means of transformed genotypes and the control cultivar Désirée for all parameters taken into consideration. All statistical analyses were performed using the SPSS ver. 8 package for Windows.

RESULTS

Morphological and Proximal Analyses. Results of the morphological evaluation of tubers and proximal analyses on dry residues and total solids are reported in **Table 1**. The transformed lines closely resembled cv. Désirée in all traits considered, and the *t* test of means comparison confirmed that there was no effect of the gene *ech42* on tuber characteristics.

As for the hybrid material, high variability was found among the genotypes analyzed. Most of them showed yellow skin (19 of 21) and yellow flesh (13 of 21). The majority of genotypes with white flesh were found within the PTH hybrids. Among BCSH hybrids, shallow eyes were found in one clone and deep ones in the others. Variability was also found in terms of dry matter and soluble solids. The former ranged from 15.7 to 23.9% and from 17.8 to 25.2%, and the latter from 2.4 to 3.8 and from 2.2 to 3.4 in the BCSH and PTH genotypes, respectively. Analysis of variance provided evidence that there were no significant differences between the two hybrid groups and the *S. tuberosum* group.

Biochemical Analyses. *Soluble Sugars and Proteins.* Total sugar and protein contents of the transformed clones were similar to those of the nontransformed control cv. Désirée (**Table 2**). *t* test analyses showed that there was no significant difference for both traits (data not shown). By contrast, the interspecific cmm−tbr hybrids showed high variability for the same traits. Within the BCSH, soluble sugars ranged from 2.5 to 3.4 mg/100 g and soluble proteins from 2.2 to 4.0 mg/100 g. As far as

Table 1. Morphological and Proximal Analyses on Tubers from Different Potato Genotypes[a]

genotype		skin color[b]	flesh color[b]	eye depth[c]	dry matter[d] (%)	soluble solids[d] (%)
transgenic clone	P10	R	Y	I	17.6 ± 0.9	2.6 ± 0.1
	P24	R	Y	I	17.1 ± 0.9	3.0 ± 0.1
	P13	R	Y	I	15.8 ± 0.8	2.6 ± 0.1
	P11	R	Y	I	16.4 ± 0.8	3.0 ± 0.1
	P15	R	Y	I	16.7 ± 0.7	2.8 ± 0.1
	mean				16.7 a	2.8 a
S. tuberosum	Désirée	R	Y	I	17.5 ± 0.7	2.8 ± 0.1
	Spunta	Y	Y	S	17.8 ± 0.2	3.2 ± 0.1
	SVP11	Y	Y	I	nd	nd
	mean				17.6 ab	3.0 a
BCSH	9ATD8	Y	Y	I	15.7 ± 0.7	3.0 ± 0.2
	9ATD32	Y	Y	I	23.9 ± 0.9	3.2 ± 0.1
	9ATE14	Y	W	I	21.9 ± 0.4	2.8 ± 0.1
	9ATE61	Y	W	I	18.1 ± 0.8	3.3 ± 0.1
	9ATE70	Y	Y	I	21.7 ± 0.1	3.7 ± 0.1
	TC9A4	Y	Y	I	17.0 ± 0.7	3.4 ± 0.1
	TD9A67	Y	Y	I	20.4 ± 0.7	3.8 ± 0.1
	TD9A74	Y	Y	S	17.6 ± 0.4	3.2 ± 0.1
	TD9A50	Y	Y	I	18.6 ± 0.9	3.5 ± 0.1
	TD9A75	Y	Y	I	16.9 ± 0.1	3.2 ± 0.1
	TE9A19	R	Y	I	22.4 ± 0.5	3.1 ± 0.2
	TE9A20	Y	Y	I	17.5 ± 0.5	2.4 ± 0.1
	TE9A65	Y	Y	I	23.3 ± 0.3	3.0 ± 0.1
	mean				19.6 ab	3.2 a
PTH	PTHE10	R	Y	VD	17.8 ± 0.9	3.2 ± 0.1
	PTHA5	Y	Y	I	23.9 ± 0.7	3.2 ± 0.1
	PTHD13	Y	W	VD	20.8 ± 0.1	3.2 ± 0.2
	PTHF7	Y	W	I	19.5 ± 0.2	3.2 ± 0.1
	PTHB2	Y	W	I	18.6 ± 0.5	2.2 ± 0.1
	PTHA4	Y	W	I	20.8 ± 0.5	3.1 ± 0.2
	PTHB8	Y	W	VD	21.3 ± 0.4	3.4 ± 0.1
	PTHD16	Y	W	VD	25.2 ± 0.3	2.9 ± 0.1
	mean				20.9 b	3.0 a
somatic hybrid	SH9A	Y	Y	I	nd	nd
S. commersonii	PI 243503	Y	Y	I	nd	nd

[a] Data from *S. tuberosum* controls, *S. commersonii* and somatic hybrid SH9A [*S. commersonii* (+) *S. tuberosum* SVP11] are also reported. [b] W, white; Y, yellow; R, red. [c] VD, very deep; I, intermediate; S, shallow. [d] Means ± SD. nd, not determined. Within each column, means with the same letter are not significantly different according to the Tukey HSD test ($P = 0.05$).

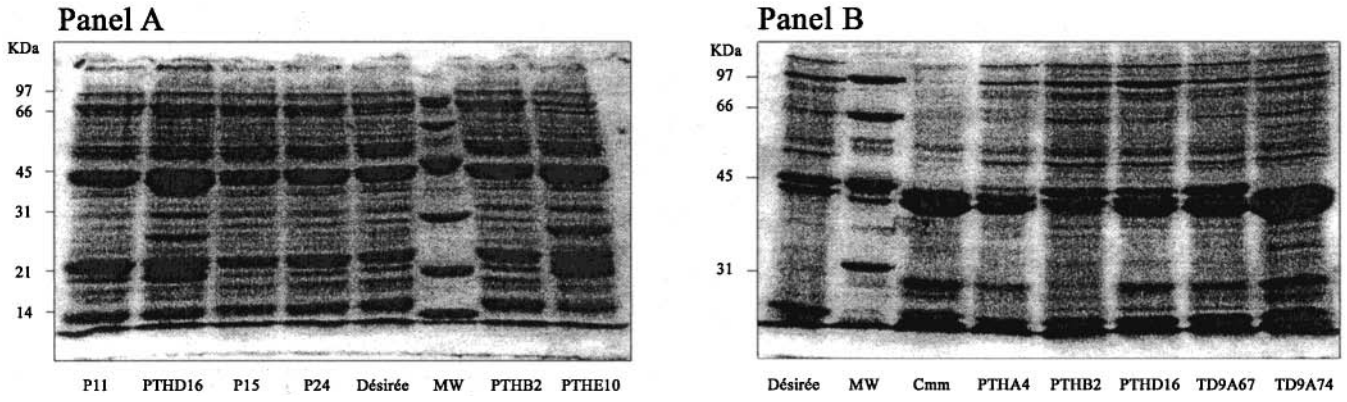

Figure 1. SDS-PAGE analyses of total proteins extracted from improved potatoes: (A) molecular weight from 14 to 100 kDa; (B) molecular weight from 30 to 100 kDa. Refer to **Table 1** for lane identification.

PTH hybrids are concerned, soluble sugars ranged from 1.8 to 3.5 mg/100 g and soluble proteins from 2.0 to 3.3 mg/100 g. The SDS-PAGE revealed that the protein pattern of the transgenic lines P11, P15, and P24 was very similar to that of cv. Désirée (**Figure 1A**). In the hybrids the protein pattern was intermediate between that of the wild species cmm and the cultivated control Désirée (**Figure 1B**). This is particularly evident following a group of high molecular weight proteins (estimated molecular weight of ~100 kDa) that were present in tbr (cv. Désirée) and very faint in cmm and two proteins at 42 and 30 kDa, which were abundant in cmm and almost absent in tbr. Analysis of variance indicated no significant difference among the four groups of genotypes for soluble sugars. As far as the soluble proteins are concerned, no significant differences were found between the two hybrid groups and the *S. tuberosum* group.

Chlorogenic Acid. The analytical procedure adopted gave a recovery of 70%, comparable to those reported in the literature

Table 2. Biochemical Analyses on Tubers from Different Potato Genotypes[a]

genotype		soluble sugars[b] (mg/100 g)	soluble proteins[b] (mg/100 g)	chlorogenic acid[b] (mg/100 g)	antioxidant activity[b] (μg of ascorbic acid/mL of solution)
transgenic clone	P10	2.8 ± 0.2	1.9 ± 0.1	7.8 ± 0.5	4.8 ± 0.2
	P24	2.4 ± 0.2	1.7 ± 0.1	6.8 ± 0.1	4.2 ± 0.1
	P13	2.4 ± 0.2	2.6 ± 0.1	5.9 ± 0.1	4.3 ± 0.3
	P11	2.9 ± 0.1	2.4 ± 0.1	5.4 ± 0.3	3.6 ± 0.4
	P15	2.5 ± 0.1	2.6 ± 0.1	7.6 ± 0.7	4.8 ± 0.3
	mean	2.6 a	2.2 a	6.7 a	4.3 a
S. tuberosum	Désirée	2.5 ± 0.2	2.5 ± 0.2	9.6 ± 0.4	4.7 ± 0.4
	Spunta	2.5 ± 0.1	3.1 ± 0.4	11.2 ± 0.5	4.5 ± 0.2
	SVP11	2.3 ± 0.2	2.4 ± 0.2	13.0 ± 0.1	5.4 ± 0.6
	mean	2.4 a	2.7 ab	11.2 b	4.9 a
BCSH	9ATD8	2.5 ± 0.2	3.0 ± 0.1	9.2 ± 0.1	4.2 ± 0.4
	9ATD32	2.8 ± 0.1	3.9 ± 0.1	7.2 ± 0.1	5.3 ± 0.2
	9ATE14	3.3 ± 0.7	3.5 ± 0.1	12.0 ± 0.9	4.2 ± 0.1
	9ATE61	3.2 ± 0.1	2.2 ± 0.2	8.5 ± 0.6	5.7 ± 0.4
	9ATE70	3.3 ± 0.7	3.8 ± 0.5	8.0 ± 0.4	4.6 ± 0.1
	TC9A4	3.4 ± 0.2	2.8 ± 0.1	5.9 ± 0.4	5.1 ± 0.5
	TD9A67	2.9 ± 0.1	3.7 ± 0.1	7.2 ± 0.4	6.0 ± 0.3
	TD9A74	2.7 ± 0.1	2.8 ± 0.1	4.9 ± 0.4	5.7 ± 0.3
	TD9A50	2.9 ± 0.1	3.2 ± 0.1	7.9 ± 0.2	5.0 ± 0.1
	TD9A75	2.6 ± 0.1	3.0 ± 0.2	9.0 ± 0.4	4.3 ± 0.3
	TE9A19	2.8 ± 0.1	3.1 ± 0.1	11.6 ± 0.2	4.7 ± 0.3
	TE9A20	2.8 ± 0.1	4.0 ± 0.2	10.0 ± 0.3	5.1 ± 0.2
	TE9A65	2.8 ± 0.1	2.6 ± 0.2	12.5 ± 0.2	4.1 ± 0.3
	mean	2.9 a	3.2 b	8.8 ab	4.9 a
PTH	PTHE10	2.9 ± 0.1	2.3 ± 0.1	8.1 ± 0.4	5.4 ± 0.2
	PTHA5	3.5 ± 0.1	3.3 ± 0.1	7.6 ± 0.3	5.3 ± 0.2
	PTHD13	2.0 ± 0.9	2.8 ± 0.1	10.8 ± 0.1	3.0 ± 0.4
	PTHF7	1.9 ± 0.1	2.4 ± 0.1	7.8 ± 0.1	4.5 ± 0.4
	PTHB2	2.4 ± 0.1	2.7 ± 0.1	7.9 ± 0.4	4.3 ± 0.2
	PTHA4	2.7 ± 0.1	3.3 ± 0.1	7.7 ± 0.3	4.5 ± 0.1
	PTHB8	1.8 ± 0.2	2.0 ± 0.4	13.5 ± 0.9	4.0 ± 0.1
	PTHD16	3.0 ± 0.2	2.2 ± 0.2	7.0 ± 0.5	4.0 ± 0.3
	mean	2.5 a	2.6 ab	8.8 ab	4.4 a
somatic hybrid	SH9A	2.3 ± 0.1	2.5 ± 0.2	4.2 ± 0.2	4.8 ± 0.4
S. commersonii	PI 243503	2.2 ± 0.1	2.8 ± 0.2	9.8 ± 0.4	4.5 ± 0.4

[a] Data from *S. tuberosum* controls, *S. commersonii* and somatic hybrid SH9A [*S. commersonii* (+) *S. tuberosum* SVP11] are also reported. [b] Means ± SD. Within each column, means with the same letter are not significantly different according to the Tukey HSD test ($P = 0.05$).

(*22*). The chlorogenic acid content of the different samples is reported in **Table 2**. As in the case of soluble sugars and proteins, the variation between the transformed clones was relatively low (from 5.4 to 7.8 mg/100 g of fresh weight), and values were not statistically different from those of cv. Désirée. By contrast, analysis of variance indicated a significant difference between the group of transgenic clones and that of *S. tuberosum* genotypes. This can be explained considering the high value of chlorogenic acid of genotypes Spunta and SVP11, which resulted in a high mean value for the *S. tuberosum* group (11.2 mg/100 g). Among the cmm−tbr hybrids, the amount of chlorogenic acid varied from a minimum of 4.9 mg/100 g of fresh weight (BCSH hybrid TD9A74) to a maximum of 13.5 mg/100 g of fresh weight (PTH hybrid B8). Analysis of variance showed no significant difference between the mean values of the two hybrid and the *S. tuberosum* groups.

Antioxidative Ability (AA). This ability was measured on the hydrophilic and lipophilic parts separately using two different methodologies. Lipophilic AA is very low, below the detection limit of the ABTS method. The value of hydrophilic AA is reported in **Table 2**. Again, among the transformed clones the variability was low, from 3.6 to 4.8 μg of ascorbic acid/mL of solution, and differences from the values shown by *S. tuberosum* cultivars were not statistically significant. The cmm−tbr hybrids showed a much higher variation, from 3.0 to 6.0 μg/mL. In particular, BCSH hybrids 9ATE61, TD9A74, and TD9A67 possessed the highest AA (5.7 and 6.0 μg of ascorbic acid/mL of solution, respectively), whereas among PTH the highest values were recovered in E10 and A5 (5.4 and 5.3 μg of ascorbic acid/mL of solution, respectively). Nevertheless, analysis of variance carried out on group means showed no significant differences among the four groups of genotypes.

Glycoalkaloid. Data on the two main GA, α-solanine and α-chaconine, and on total GA content are reported in **Table 3**. Total steroidal GA content of transformed clones did not differ from that of the control, ranging from 225 to 279 ppm. Similarly, these genotypes showed no significant differences in the content of single GA determined by HPLC. α-Solanine content ranged from 47 to 64 ppm and that of α-chaconine from 112 to 160. In the hybrid genotypes the total GA content ranged between 152 and values as high as that of cmm, which had a GA content of 453 ppm. α-Chaconine ranged between 78 ppm for TE9A20 and 258 ppm for 9ATD32, and α-solanine ranged between 20 ppm for 9ATE70 and 190 ppm for PTHA4. Interestingly, the somatic hybrid SH9A had a much lower GA level than that of cmm, and some BCSH hybrids obtained by backcrossing SH9A with tbr showed a drastic reduction in the average level of GA (e.g., TD9A50, with 152 ppm of GA). In contrast, genotypes such as TD9A67 and 9ATD32 (among BCSH) and PTHA4 (among PTH) had quite high GA contents, making them difficult

Table 3. Glycoalkaloid Content of the Tubers from Different Potato Genotypes[a]

genotype		α-solanine[b,d] (ppm)	α-chaconine[b,d] (ppm)	total glycoalkaloids[c,d] (ppm)
transgenic clone	P10	50 ± 1	130 ± 2	260 ± 3
	P24	54 ± 1	160 ± 0	233 ± 6
	P13	64 ± 1	125 ± 0	279 ± 2
	P11	47 ± 0	141 ± 1	268 ± 2
	P15	51 ± 0	112 ± 1	225 ± 8
	mean	53.2 a	133.6 a	253.0 a
S. tuberosum	Désirée	39 ± 1	126 ± 2	213 ± 7
	Spunta	56 ± 1	129 ± 1	298 ± 1
	SVP11	59 ± 0	122 ± 0	nd
	mean	51.3 a	125.7 a	255.5 a
BCSH	9ATD8	161 ± 0	146 ± 0	nd
	9ATD32	156 ± 1	258 ± 1	352 ± 4
	9ATE14	76 ± 0	153 ± 0	nd
	9ATE61	89 ± 1	162 ± 1	353 ± 2
	9ATE70	20 ± 2	136 ± 1	198 ± 4
	TC9A4	67 ± 1	102 ± 1	332 ± 1
	TD9A67	137 ± 0	215 ± 2	411 ± 5
	TD9A74	55 ± 1	96 ± 2	nd
	TD9A50	62 ± 1	157 ± 2	152 ± 11
	TD9A75	81 ± 2	109 ± 1	193 ± 3
	TE9A19	47 ± 1	96 ± 0	305 ± 3
	TE9A20	57 ± 1	78 ± 1	nd
	TE9A65	113 ± 1	125 ± 1	341 ± 7
	mean	86.2 a	141.0 b	293.0 a
PTH	PTHE10	73 ± 1	174 ± 2	335 ± 10
	PTHA5	102 ± 1	174 ± 0	298 ± 3
	PTHD13	123 ± 0	165 ± 1	nd
	PTHF7	76 ± 1	84 ± 1	301 ± 2
	PTHB2	96 ± 0	132 ± 0	312 ± 11
	PTHA4	190 ± 1	210 ± 1	435 ± 1
	PTHB8	67 ± 2	158 ± 1	258 ± 9
	PTHD16	73 ± 0	98 ± 1	300 ± 4
	mean	100.0 a	149.4 b	319.8 a
somatic hybrid	SH9A	109 ± 1	162 ± 1	305 ± 12
S. commersonii	PI 243503	0	0	453 ± 3

[a] Data from *S. tuberosum* controls, *S. commersonii* and somatic hybrid SH9A [*S. commersonii* (+) *S. tuberosum* SVP11] are also reported. [b] Determined by HPLC. [c] Determined by ELISA. [d] Means ± SD. nd, not determined. Within each column, means with the same letter are not significantly different according to the Tukey HSD test ($P = 0.05$).

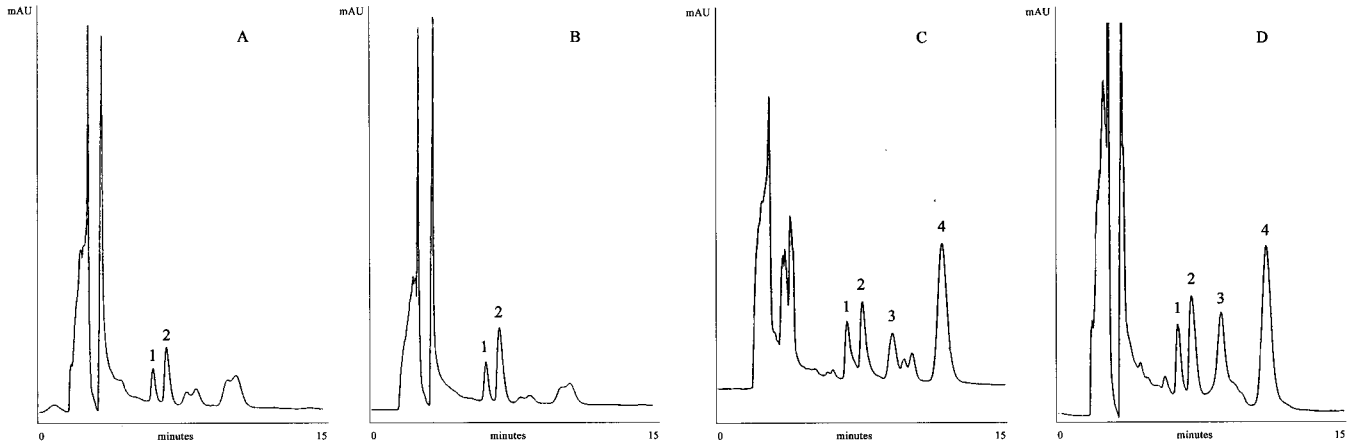

Figure 2. HPLC chromatograms of the GA mixture present in the different tubers: (A) Désirée; (B) transgenic line; (C) *S. commersonii*–*S. tuberosum* hybrid PTHF7; (D) *S. commersonii*–*S. tuberosum* hybrid 9ATE61. Peaks: (1) α-solanine; (2) α-chaconine; (3, 4) not identified.

to use for future breeding efforts. Analysis of variance showed no significant difference among the four groups of genotypes for α-solanine and total GA content. By contrast, significant differences existed between the hybrid and the *S. tuberosum* groups for α-chaconine content.

Besides the quantification of known GA (solanine and chaconine) in all genotypes, HPLC analyses of the hybrids (**Figure 2**) provided evidence that most of the PTH and BCSH cmm−tbr hybrids analyzed had other chromatographic peaks in the same region, thus suggesting that other GA were present. Identification of the main GA present in the extracts was achieved by electrospray mass spectrometry. As shown in the different panels of **Figure 3**, only solanine (MH^+ 868) and chaconine (MH^+ 852) are present in tbr, whereas two different

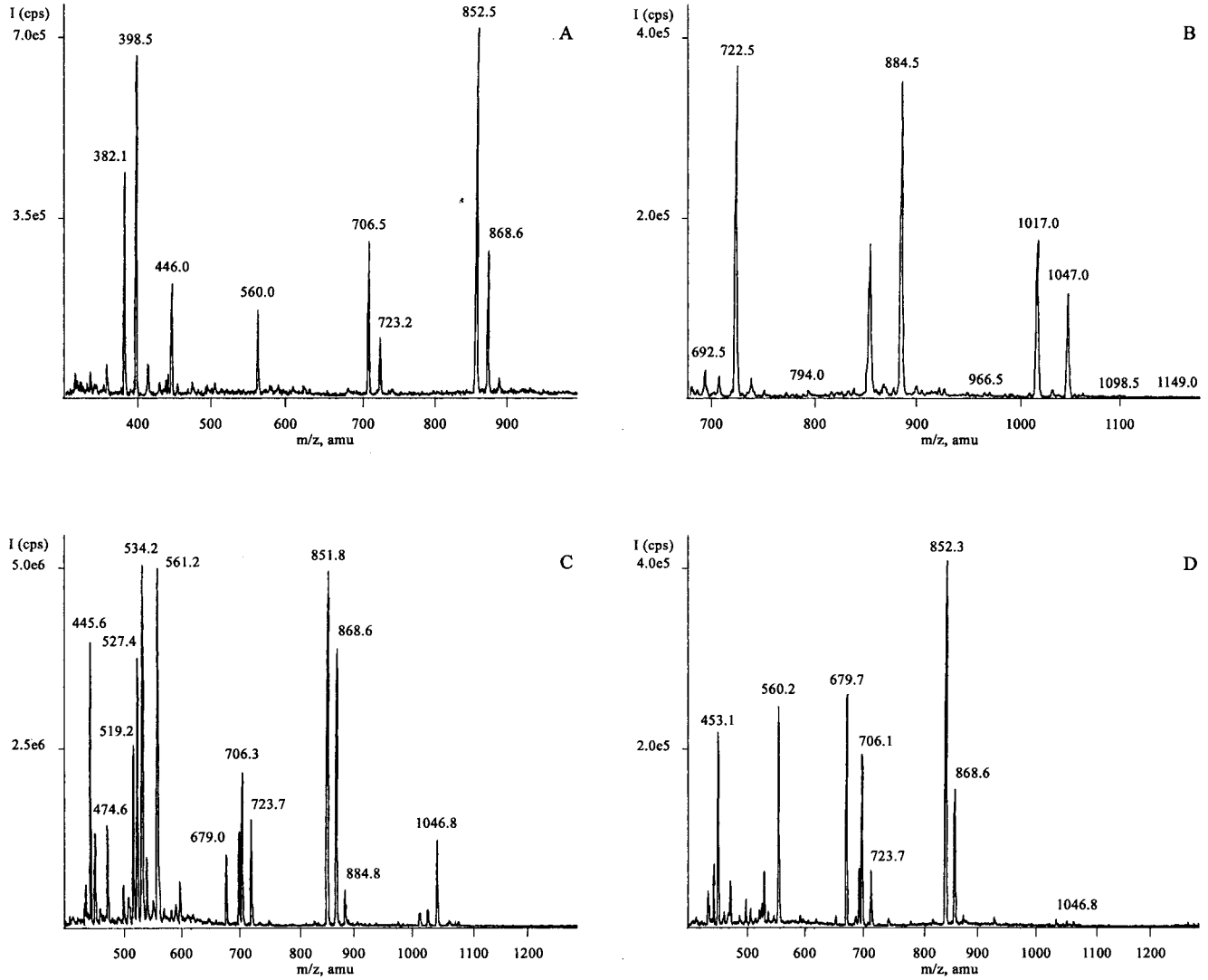

Figure 3. Mass spectra of the GA mixture present in the different tubers: (A) Désirée; (B) *S. commersonii*; (C) *S. commersonii–S. tuberosum* somatic hybrid SH9A; (D) *S. commersonii–S. tuberosum* hybrid TE9A19. Peak identification is highlighted in the figure. Peaks at M – 162 and M – 324 uma are due to losses of one and two hexose units, respectively.

GA, commersonine (MH^+ 1047) and demissidine (MH^+ 1017), are present in cmm. The amounts of these GAs calculated by HPLC were 352 and 231 ppm, respectively. In the somatic hybrid SH9A, solanine, chaconine, and demissidine are still present. Interestingly, the BCSH hybrid TE9A19 completely lost the GA derived from cmm, which also held for the PTHD13 hybrid (data not shown).

Chipping Quality. **Table 4** reports the results from the frying tests. Within the transformed genotypes, the chipping ability index ranged from 4 to 6 at harvest and from 5.5 to 6 after 2 months of storage. *t* test analyses showed that there were no significant differences between the control cv. Désirée and transgenic lines. As for the cmm−tbr hybrids, genotypes with good chipping quality were identified. In particular, at harvest 16 hybrids showed a chipping ability index <4.5. After 2 months of storage, 5 genotypes had a chipping ability index <4.5.

Analysis of variance showed no difference among the means of the four groups of genotypes.

DISCUSSION

Previous studies have demonstrated that the methodologies employed to produce the material used in this study were efficient. Indeed, the potato genotypes obtained through genetic transformation produced fungal chitinase in a range between 0.01 and 0.5% of the total proteins and showed a substantial reduction in disease symptoms when infected with *A. alternata* and *R. solani* (*3*). Similarly, the tbr−cmm obtained through either somatic fusion or sexual hybridization displayed introgression of useful traits from the wild parent cmm. The somatic hybrid SH9A was resistant to potato virus X, and BCSH and PTH hybrids were resistant/tolerant to *Erwinia carotovora* and to low temperatures and also gave high tuber yields (*23*−*25*).

In the present research, we studied the chemical composition of tubers of this newly produced material to add new information to those already available. In the case of transformed plants, the analyses showed that there is a negligible difference in the traits analyzed between the new genotypes and the cultivated control Désirée. By contrast, Hashimoto et al. (*26*) observed that the GA content of potato transformed with the soy protein glycinin increased considerably with respect to the control (between 20 and 100%). Our results appear to be interesting given that the substantial equivalence is a prerequisite to consider commercialization of any genetically modified food product and that concern is growing worldwide on the use of such food products (*11*). Further detailed analyses are necessary

Table 4. Chipping Quality of the Tubers from Different Potato Genotypes[a]

genotype		test 1	test 2
transgenic clone	P10	6.0 ± 0.1	6.0 ± 0.1
	P24	6.0 ± 0.1	6.0 ± 0.2
	P13	4.5 ± 0.1	5.2 ± 0.1
	P11	4.0 ± 0.2	5.5 ± 0.2
	P15	4.0 ± 0.2	6.0 ± 0.2
	mean	4.9 a	5.7 a
S. tuberosum	Désirée	5.5 ± 0.1	5.5 ± 0.2
	Spunta	4.0 ± 0.2	6.0 ± 0.1
	mean	4.7 a	5.8 a
BCSH	9ATD8	3.0 ± 0.1	4.0 ± 0.1
	9ATD32	5.0 ± 0.1	5.0 ± 0.1
	9ATE14	5.0 ± 0.2	5.5 ± 0.1
	9ATE61	5.0 ± 0.1	5.5 ± 0.1
	9ATE70	4.5 ± 0.1	4.5 ± 0.1
	TC9A4	3.5 ± 0.2	6.0 ± 0.1
	TD9A67	4.0 ± 0.1	5.0 ± 0.2
	TD9A74	3.5 ± 0.1	7.0 ± 0.2
	TD9A50	2.5 ± 0.2	7.0 ± 0.1
	TD9A75	3.2 ± 0.1	6.0 ± 0.3
	TE9A19	3.0 ± 0.1	6.0 ± 0.1
	TE9A20	3.7 ± 0.1	5.5 ± 0.1
	TE9A65	4.0 ± 0.1	6.0 ± 0.1
	mean	3.8 a	5.6 a
PTH	PTHE10	6.0 ± 0.1	4.0 ± 0.2
	PTHA5	4.0 ± 0.1	4.0 ± 0.1
	PTHD13	4.5 ± 0.1	5.2 ± 0.1
	PTHF7	5.5 ± 0.2	5.0 ± 0.1
	PTHB2	3.7 ± 0.2	4.0 ± 0.1
	PTHA4	3.7 ± 0.2	4.7 ± 0.1
	PTHB8	3.7 ± 0.1	5.0 ± 0.2
	PTHD16	4.0 ± 0.1	7.2 ± 0.3
	mean	4.7 a	4.9 a

[a] Test 1 was performed at harvest; test 2 after 2 months of storage without reconditioning. Data (means ± SD) are on a scale from 1 (light) to 10 (completely dark). Genotypes with values of <4.5 were considered to be acceptable. Within each column, means with the same letter are not significantly different according to the Tukey HSD test ($P = 0.05$).

to assess the substantial equivalence of these genotypes also for other traits and to confirm that the minor differences observed are the result of nongenetic factors.

It is interesting to note that both Désirée and the transformed clones had a GA level >200 mg/1000 g. Similarly, high GA levels in whole tubers of tbr and tbr−*S. acule* somatic hybrids were reported by Kozukue et al. (*27*).

These results can be explained by two factors: first, whole tubers (peel, cortex, and flesh) were used for GA determination. It is known that peel has a much higher GA content than flesh (*27*−*29*) and that peeling removes from 35 to 90% of GA present in the tubers (*16*). Second, tubers analyzed were produced in pots and had a relatively small size; this increases the surface area/volume ratio and thus the GA content. These two factors should be taken into account when the values of the new genotypes analyzed in this study are compared with either the *S. tuberosum* controls or with other genotypes.

The same morphological and biochemical analyses were performed on BCSH and PTH hybrids. They provided strong evidence for broad variability in all of the parameters analyzed. The high within-group variability also explains why analysis of variance mostly gave no statistical difference between the hybrid groups BCSH and PTH and the *S. tuberosum* group. The presence of this high within-group variability is thought to be very important, because the success of plant breeding depends on the variability available. For example, a high dry matter content of tubers is necessary for processing potatoes; it also gives greater nutritional value to the tubers. Within the variability present in the interspecific hybrids, we found a number of genotypes with a dry matter content close to 25%, which is very high and rarely found in cultivated genotypes. Also remarkable is the fact that for some useful traits (e.g., soluble solids and proteins, dry matter content) the interspecific hybrids performed better than both the tbr control varieties and the wild cmm. The results from frying tests are also interesting: the browning during frying tests is related to the amount of reducing carbohydrates. Starch hydrolysis, which occurs during storage at low temperature, leads to the formation of brown chips (*21*). One of the main goals of the breeding programs is to avoid this, obtaining genotypes that can be used immediately after cold storage. Some hybrids, such as 9ATD, 9ATE70, PTHE10, PTHA5, and PTHB2, gave moderate browning also after 2 months of storage. This is important from the breeding standpoint given that only a limited number of varieties can be processed directly from cold storage without reconditioning.

As already pointed out, wild species often transmit undesired traits to tbr. Cmm, the species used in this work, has quite a high level of chlorogenic acid, which causes postcooking blackening after reacting with ferric ions on cooking (*1*). It is also reported to have high levels of GA. In the tubers of the cmm accession used, we find that two GA were present, demissidine and commersonine. By contrast, Vazquez (*30*) reported that five glycoalkaloids are present in the aerial part of this wild species (commersonine, demissine, tomatine, dehydrocommersonine, and δ-5-demissine). Thus, our results suggest that in tubers of cmm the level of GA is not as high as in leaves, and this should facilitate breeding efforts. In fact, GA are quantitatively inherited, have high heritability, and thus can be found in offspring coming from crosses in which one parent has a high glycoalkaloid level (*31*).

The chlorogenic acid content of some of the hybrids was quite high. This can be positive for the antioxidant intake but at the same time should speed potato blackening (*8*, *32*). In some hybrids, such as TD9A50, a low level of chlorogenic acid parallels a low GA content, so these clones were selected for further breeding efforts. In some others (e.g., TE9A19, TE9A65, and 9ATE61) the opposite was true, and these clones require further selection before their possible use for human consumption.

The tbr−cmm hybrids we analyzed showed various GA levels. In a number of genotypes GA levels were close to or even lower than that of tbr, suggesting that selection for low GA content is possible. This also indicates that the gene pool of cmm may be suitable not only for improving resistance traits of tbr but also for creating genotypes that are safe for consumption. Similar results were obtained by analyzing hybrids between tbr and *S. circaeifolium* (*33*), *S. brevidens* (*34*), *S. acaule* (*27*), *S. demissum*, and *S. iopetalum* (*35*). Also in these cases variability for GA content was found, and it was possible to select genotypes associating acceptable GA level and resistance traits.

Laurila et al. (*36*) reported that GA not produced by the parents may be present in the offspring. In some BCSH and PTH hybrids, HPLC analyses did show that other GA, besides solanine and chaconine, were present. Further detailed analyses are necessary to confirm whether these peaks represent new GA or GA inherited from the wild parent cmm. Our results also suggest that the GA from cmm may be lost rapidly, with obvious advantages in terms of breeding efforts to reduce the GA level of interspecific hybrids. Indeed, through mass spectrometry

analyses, we found that only one GA from cmm was still present in the somatic hybrid SH9A, whereas in two hybrids the same GA profile of tbr was recovered.

In conclusion, the compositional analyses of potato tubers obtained from transgenic plants provided evidence that negligible differences existed between them and the control cultivar Désirée. Results also indicated that interspecific hybridization between tbr and cmm creates novel genetic variability where breeders can find not only resistance but also high quality traits. Interestingly, results showed that among the parameters considered, the GA content is by far the most sensitive to variation. Therefore, GA determination should be used for routine control of new genotypes produced by various breeding approaches.

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