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Glycoalkaloids and acclimation capacity of hybrids between *Solanum tuberosum* and the incongruent hardy species *Solanum commersonii*

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Abstract F_1 and backcross hybrids between sexually incompatible species *Solanum commersonii* and *Solanum tuberosum* were characterized for glycoalkaloid content and capacity to cold acclimate. Glycoalkaloid (GA) analysis revealed that F_1 triploids and BC_1 pentaploids contained the glycoalkaloids of both parents. In BC_2 (near) tetraploids the situation was different, in that some hybrids produced the GAs of both parents, whereas others contained only the GAs of *S. tuberosum*. This suggested that the GAs from *S. commersonii* may be lost rapidly, and that they may have a simple genetic control. The total tuber GA content of BC_1 and BC_2 groups averaged quite acceptable levels (165.9 mg/kg in BC_1 and 192.8 mg/kg in BC_2), with six genotypes having a GA content <200 mg/kg fresh weight. The F_1 triploid hybrids expressed a capacity to cold acclimate similar to *S. commersonii*, whereas BC_1 and BC_2 genotypes generally displayed an acclimation capacity higher than the sensitive parent but lower than *S. commersonii*. However, one BC_1 and two BC_2 genotypes with an acclimation capacity as high as *S. commersonii* were identified. The polar lipid fatty acid composition in *S. commersonii* and its hybrid derivatives showed that, following acclimation, there was a significant increase in 18:3. Correlation analysis between the capacity to cold acclimate and the increase in 18:3 was significant, suggesting that the increase in 18:3 can be used as a biochemical marker for the assisted selection of cold-acclimating genotypes in segregating populations.

Keywords Interspecific hybridization · Genomic ratios · Potato · Fatty acids · Ion leakage

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Introduction

Wild tuber-bearing *Solanum* species have desirable genes contributing to the development of new potato varieties possessing stress resistance, as well as improved agronomic and horticultural traits. They also provide allelic diversity and interactions useful to ensure high performance for polygenic traits (Ortiz 1998). The genetic contribution of wild potato germplasm is considered very important in light of the narrow genetic base present in cultivated *Solanum tuberosum* (tbr). Interestingly from the breeding standpoint, the lack of extensive differentiation among species genomes generally allows effective chromosome pairing, normal crossing over and good fertility of interspecific hybrids. All these unique features make *Solanum* species a precious storehouse for potato breeders.

In spite of these noteworthy characteristics, the use of potato genetic resources has been very limited so far (Pavek and Corsini 2001). One of the main limitations in the use of wild species is that, together with the useful traits, they can transfer characteristics that are undesired from the commercial standpoint. In the case of *Solanum* species, traits such as long stolons, deep eyes and undesired quality traits can be transmitted. Therefore, during any potato breeding program undesired traits should be identified early and selected against, so that they are not introduced into the advanced material developed.

Among tuber-bearing species, diploid ($2n = 2x = 24$) *Solanum commersonii* (cmm) is very interesting (Hanneman and Bamberg 1986). It carries several useful traits, such as resistance to abiotic stresses, bacterial wilt, potato

virus X and *Erwinia carotovora*. Particularly attractive for potato breeding are the resistance to low temperatures and the capacity to cold acclimate. These traits are very important given that freezing stress is often a major factor in reducing both tuber yield and the geographical distribution of the potato crop. It should be pointed out that among *Solanum* species, cmm is the one with the highest capacity to cold acclimate. However, the use of cmm in breeding programs is hampered by a number of obstacles. First of all, this species can be potentially hazardous to human health because of the high content of toxic glycoalkaloids (Friedman and McDonald 1997). A concentration of 20 mg/100 g of fresh weight is considered the maximum level by the common current guidelines for potatoes. Solanine and chaconine are the main glycoalkaloids found in cultivated tbr, whereas commersonine, demissine, tomatine, dehydrocommersonine and delta-5-demissine were found in cmm (Vàzquez et al. 1997). Although a number of environmental and post-harvest factors can influence glycoalkaloid content, their production is mainly genetically controlled (Friedman and McDonald 1997).

Another constraint in the use of cmm is the fact that it cannot be normally crossed with tbr haploids ($2n = 2x = 24$) due to post-zygotic barriers (Johnston and Hanneman 1982). To overcome its incompatibility barriers, non-conventional breeding strategies have to be developed. We have recently achieved this goal by the production of ploidy bridges (Carputo et al. 1997). This strategy is based on the concept that when the chromosome number of cmm is doubled, tetraploid cmm produces fertile F_1 triploids if crossed with diploid tbr. Further ploidy manipulations may be carried out if triploids produce 2n gametes and are fertile in $3x \times 4x$ crosses. As part of the breeding program aimed at transferring resistance traits from cmm into tbr through ploidy bridges, we have evaluated the glycoalkaloid content of triploid F_1 and backcross sexual hybrids emphasizing different ploidy levels and wild to cultivated genomic ratios. In this paper we report the glycoalkaloid content in foliage and tuber tissues, and the capacity to cold acclimate of hybrids developed. We also examined if there was any relationship between leaf fatty acid composition and the capacity to cold acclimate, in order to find useful markers for assisted selection.

Materials and methods

Plant material

The development of F_1, BC_1 and BC_2 hybrids used in this study was previously reported (Carputo et al. 1997). In brief, a tetraploid ($2n = 4x = 48$) clone of cmm obtained through tissue culture was crossed with the diploid *Solanum phureja – S. tuberosum* genotype UP88-P5 to obtain triploid F_1 hybrids. The triploids produced 2n eggs, and were successfully used in $3x \times 4x$ crosses with tbr to generate the BC_1 pentaploid generation. Two selected BC_1 fertile genotypes were further used in $5x \times 4x$ and $4x \times 5x$ crosses to generate the BC_2 tetraploid-near tetraploid generation. Genetic material used in this study included the following: parental genotypes cmm (PI 243503) and UP88-P5; three F_1, three BC_1 and six BC_2 hybrids; and four tbr varieties Blondy, Carmine, Spunta and Tollocan. Six tubers of each genotype were planted in 25-diameter pots in a temperature-controlled greenhouse (24–26 °C) in February 2000. Plants were used for foliar and tuber glycoalkaloid analysis, to evaluate the capacity to cold acclimate, and to determine the fatty acid composition of leaves during acclimation. Also planted for tuber glycoalkaloid analysis were 13 BC_3 hybrids obtained from crosses between the BC_2 hybrid PTHF7 and Blondy.

Glycoalkaloid (GA) analysis

The pattern type of GAs present in tuber and leaves was obtained by MALDI-TOF mass spectrometry, while GAs were quantified in tubers by HPLC with UV detection. HPLC-grade solvents and water were from Merck (Germany) and filtered through disposable 0.2 μm filters from Acrodisc (Gelman Sciences, USA); α-solanine and α-chaconine were obtained from the Sigma Chemical Company (St. Louis, Mo.). The standard of commersonine was obtained by HPLC purification from cmm leaves. HPLC separation was carried out using a Shimadzu LC 10 (Shimadzu, Japan) apparatus with a diode array detector. Data were processed by CLASS VP software. MALDI-TOF spectra were recorded using a Kompact MALDI instrument (Kratos Analytical, Shimadzu Group Company, Japan). The instrument was equipped with a nitrogen laser, emitting light at 337 nm and with a pulse width of 3 ns. Positive ions were recorded in a linear mode. For calibration, insulin [$(M + H)^+$, $m/z = 5734.5$, and $(M + 2H)^{2+}$, $m/z = 2867.7$] was used as an external standard. Matrices used consisted of either α-cyanohydroxycinnamic acid (α-CHCA) or synapinic acid. Ions were accelerated by acceleration voltages of 40 kV. Saturated solutions (10 mg of ml^{-1}) of matrices were prepared in acetonitrile: 0.1% TFA in water, 2:3 or 1:1 (v:v). Sample slides were prepared as follows: the matrix was pipetted first, then the sample and again the matrix. At each step, volumes of 0.6 μl were pipetted on the sample plates and slides were dried in a fan heat oven (Kratos Analytical Co., Manchester, UK) for about 30 s between each step, in order to dry solvents. Kompact 1.2 software was used for analyzing data.

For tuber GA analysis, five tubers from each genotype were selected, washed, cut into small cubes (including skin and cortex), freeze-dried and finely ground. Tubers collected had a similar size. As for leaf GA analysis, 10 g of leaves were randomly harvested, lyophilized and finely ground. Three aliquots of each samples were analyzed for GA composition: lyophilized tuber tissue (1 g) and lyophilized leaf tissue (0.5 g) were extracted with 20 ml of 2% acetic acid for 1 h. The extract was pre-purified by a Sep-Pak column and the supernatant was used to determine GA by HPLC, as described by Esposito et al. (2001).

Determination of cold acclimation capacity

The acclimation capacity of each genotype was evaluated as the difference between the killing temperature of plants grown in a non-acclimated condition and the killing temperature of plants acclimated for 2 weeks in a cold room (4–2 °C, light/dark) at 100 μmol/m^2 s. To evaluate killing temperatures under each growing condition, the ion leakage procedure was used. This method is well established for the precise determination of freezing tolerance (Stone et al. 1993). Mature expanded leaves were put in culture tubes and submerged in a glycol bath at 0 °C. Three replicates per genotype were used in each temperature treatment. The temperature was lowered 0.5 °C every 30 min. The control treatment consisted of three replicates per genotype kept on ice at 0 °C. After 30 min at the desired freezing temperature, the tubes were placed on ice to thaw overnight. Thawed leaves were then cut, vacuum-infiltrated in 25-ml distilled de-ionized water, and shaken for 1 h at room temperature. Freezing damage was assessed by evaluating ion leakage from thawed leaf samples with a conductivity meter. For each genotype, a freezing curve was constructed by plotting the percent ion leakage (mean ± SD from three separate

measurements) vs freezing temperatures. The maximum conductivity, representing total ion leakage for each sample, was determined after autoclave heat killing. The minimum ion leakage was determined by measuring the ion leakage from the controls held at 0 °C. The freezing tolerance (non-acclimated NAFT; acclimated ACFT) for each genotype was determined by calculating the temperature at 50% of ion leakage (LT_{50}), that is assumed to represent freezing injury to the plant, according to the logistic model described by Janácek and Prásil (1991). The difference between ACFT and NAFT was defined as the acclimation capacity.

Fatty acid composition

Quantitative analysis of fatty acids was carried out by GC-MS using a GC-17A gas chromatography and a QP-5000 mass spectrometer (Shimadzu, Japan). Electronic impact ionization was achieved at 70 kV. Lipids were extracted from fresh leaf tissue collected from the same plants grown and used for acclimation studies. One g of finely ground tissue was extracted for 3 h at room temperature with 24 ml of a 2:1 mixture of chloroform:isopropanol. The suspension was filtered and added to 6 ml of 0.2 M KCl. The water phase was discarded while the organic phase was dried under vacuum and re-suspended in 2 ml of 0.05% BHT in n-exane. Chromatographic separation was carried out on the fatty acid methyl ester derivatives obtained by sulfuric acid and methanol. A 5% diphenyl and 95% dimethylpolysiloxane column (30 m, 0.25 mm internal diameter) from Perkin Elmer (USA) was used. A compound identity was confirmed by the mass spectra library and compared with authentic standards.

Statistical evaluation of data

The analysis of variance was used to evaluate differences among genotypes for glycoalkaloid content. Means were compared by the Tukey HSD test. All statistical analyses were performed using the SPSS ver. 8 package for Windows. As for the results of freezing tests, statistical differences between LT_{50}s of genotypes tested were calculated with the program "LV50" version 2.1 (Janácek and Prásil 1991). The Spearman's rank correlation coefficients of interest were evaluated using the SYSTAT ver. 5.2.1 statistical software package.

Results

GA analysis

Table 1 reports the qualitative analysis of individual GAs in leaves and tubers of parental genotypes, F_1, BC hybrids and tbr controls. Dehydrodemissine, dehydrotomatine and dehydrocommersonine were the GAs present in both tubers and leaves of cmm, whereas solanine and chaconine were those present in tubers and leaves of UP88-P5 and of tbr controls. As for the F_1 triploids, due to the lack of tuberization, GA analysis was performed only on leaves. F_1 hybrids contained the GAs of both parents. A similar trend was found in the BC_1 pentaploid hybrids analyzed, except for MCOP5, that did not contain dehydrocommersonine in the tubers. In the BC_2 tetraploid, near-tetraploid genotypes, the situation was different. In fact, some hybrids (e.g. PTHA1) produced the GAs of both parents, whereas some others (e.g. PTHF7) contained only the GAs of tbr. In PTHC4 there was no correspondence between foliar and tuber GA, in that tubers produced only solanine and chaconine, the GAs of tbr, while leaves contained GAs of both tbr and cmm.

To assess the quantitative levels in which we were working, the individual GA content in tubers of our genotypes was determined (Table 2). Individual GAs were summed to determine the total GA present in each sample. The two parental genotypes, differing qualitatively, also differ in GA content. The cmm was about four times higher in total GAs than the tbr parent UP88-P5 and the cultivated controls (481.3 mg/kg of total fresh weight of cmm vs 138.3 mg/kg of Spunta, 154.3 mg/kg of UP88-P5, 147.9 mg/kg of Blondy, 140.5 mg/kg of Tollocan and 139.4 mg/kg of Carmine).

Table 1 Qualitative analysis of glycoalkaloid content in leaf/tuber tissues of F_1, BC_1 and BC_2 *S. commersonii – S. tuberosum* interspecific hybrids and their parents *S. commersonii* and UP88-P5. (+) = presence; (–) = absence

Genotype	Chromosomes, #	Pedigree	Chaconine	Solanine	Dehydro-demissine	Dehydro-tomatine	Dehydro-commersonine
F_1							
MCA1	36	cmm × UP88-P5	+/nd[a]	+/nd	+/nd	+/nd	+/nd
MCB1	36	cmm × UP88-P5	+/nd	+/nd	+/nd	+/nd	+/nd
MCB10	36	cmm × UP88-P5	+/nd	+/nd	+/nd	+/nd	+/nd
BC_1							
MCOP5	60	MCB10 × tbr	+/+	+/+	+/+	+/+	+/–
MCPH5	58	MCA1 × tbr	+/+	+/+	+/+	+/+	+/+
MCPH3	60	MCB10 × tbr	+/+	+/+	+/+	+/+	+/+
BC_2							
PTHA1	48	MCOP5 × tbr	+/+	+/+	+/+	+/+	+/+
PTHC4	51	MCPH3 × tbr	+/+	+/+	+/–	+/–	+/–
PTHE3	52	tbr × MCPH3	+/+	+/+	+/+	+/+	+/+
PTHE10	57	tbr × MCPH3	+/+	+/+	–/–	–/–	–/–
PTHF5	54	MCOP5 × tbr	+/+	+/+	+/+	+/+	+/+
PTHF7	48	MCOP5 × tbr	+/+	+/+	–/–	–/–	–/–
PI243503	24	cmm	–/–	–/–	+/+	+/+	+/+
Spunta	48	tbr	+/+	+/+	–/–	–/–	–/–
UP88-P5	24	phu × tbr	+/+	+/+	–/–	–/–	–/–

[a] nd = not detected

Table 2 Quantitative analysis of glycoalkaloid content of BC_1 and BC_2 *S. commersonii* – *S. tuberosum* hybrids, *S. commersonii* (cmm), and *S. tuberosum* controls Spunta, Blondy, Carmine, Tollocan, UP88-P5. In each column, the numbers followed by the same letters are not statistically different at $P < 0.05$ according to the Tukey HSD test

Genotype	Glycoalkaloid (mg/kg of tuber fresh weight)					
	Solanine	Chaconine	Dehydro-demissine	Dehydro-tomatine	Dehydro-commersonine	Total
BC_1						
MCOP5	70.7 ± 2.6 b	119.3 ± 2.0 b	Traces	76.8 ± 2.1 b	–[a]	266.8 ± 6.7 c
MCPH5	Traces	43.0 ± 1.0 h	50.0 ± 1.8 c	Traces	25.2 ± 2.0 c	118.1 ± 4.9 g
MCPH3	Traces	73.6 ± 2.3 f	39.4 ± 0.4 d	Traces	Traces	113.0 ± 2.5 g
BC_2						
PTHA1	Traces	64.2 ± 3.5 g	102.0 ± 1.3 b	Traces	Traces	166.3 ± 2.2 e
PTHC4	31.6 ± 1.8 d	87.8 ± 4.1 e	–	–	–	119.4 ± 6.0 g
PTHE3	38.1 ± 1.1 c	33.4 ± 0.4 i	Traces	73.5 ± 0.3 c	30.6 ± 2.1 b	175.6 ± 3.5 de
PTHE10	136.8 ± 4.1 a	180.2 ± 7.2 a	–	–	–	317.0 ± 6.3 b
PTHF7	70.0 ± 1.1 b	115.4 ± 1.2 bc	–	–	–	185.5 ± 2.3 d
cmm	–	–	199.2 ± 4.5 a	91.0 ± 1.2 a	191.1 ± 3.5 a	481.3 ± 9.2 a
Spunta	37.8 ± 1.9 c	100.5 ± 0.9 d	–	–	–	138.3 ± 2.8 f
Blondy	31.3 ± 0.1 d	116.7 ± 0.9 bc	–	–	–	147.9 ± 0.8 f
Carmine	28.7 ± 0.4 d	110.7 ± 0.4 c	–	–	–	139.4 ± 0.8 f
Tollocan	31.0 ± 0.9 d	109.5 ± 0.2 c	–	–	–	140.5 ± 1.0 f
UP88-P5	33.8 ± 0.7 cd	120.5 ± 0.8 b	–	–	–	154.3 ± 1.3 ef

[a] Absent

Table 3 Killing temperature in non-acclimated (NAC) and acclimated (AC) conditions, acclimation capacity (ΔT), and relative acclimation capacity of F_1, BC_1 and BC_2 *S. commersonii* – *S. tuberosum* hybrids, *S. commersonii* (cmm), and *S. tuberosum* controls Blondy, Spunta and UP88-P5

Genotype	LT50 (°C)		ΔT[b] (°C)	Relative acclimation capacity[c] %
	NAC[a]	AC[a]		
F_1				
MCA1	–4.42 bcd	–7.29 ab	2.9	116
MCB1	–3.27 gh	–6.41 bcd	3.1	129
MCB10	–4.77 b	–7.46 ab	2.7	112
BC_1				
MCOP5	–3.80 c	–4.12 h	0.3	16
MCPH5	–3.50 efg	–5.72 cde	2.2	92
MCPH3	–3.45 efgh	–5.12 e	1.7	71
BC_2				
PTHA1	–3.65 def	–4.33 gh	0.7	29
PTHC4	–2.97 i	–4.57 efgh	1.6	67
PTHE3	–3.35 gh	–4.98 ef	1.6	67
PTHF5	–2.71 l	–5.15 e	2.4	100
PTHE10	–3.33 fgh	–6.37 bc	3.0	125
PTHF7	–3.36 fgh	–4.28 h	0.9	38
cmm	–5.82 a	–8.29 a	2.5	–
UP88-P5	–3.38 efgh	–4.48 fgh	1.1	46
Blondy	–2.42 l	–3.33 i	0.9	38
Spunta	–3.12 hi	–3.36 i	0.2	13

[a] Means followed by same letter are not significantly different at $P < 0.05$
[b] ΔT = AC killing temperature–NAC killing temperature
[c] Calculated by the following formula [(ΔT hybrids)/(ΔT cmm)] × 100

Total tuber GA content of the BC_1 and BC_2 groups averaged quite acceptable levels (165.9 mg/kg in BC_1 and 192.8 mg/kg in BC_2). In particular, the total GA content ranged from 317 mg/kg (BC_2 clone PTHE10) to 113 mg/kg (BC_1 clone MCPH3). Out of eight genotypes tested, six showed a GA content <200 mg/kg of fresh weight. All individuals produced solanine and chaconine. However, in BC_1 clones MCPH5 and MCPH3, and BC_2 clone PTHA1, solanine was present only in traces. As for the other GAs derived from the wild parent, they were very often undetectable (e.g. dehydrocommersonine and dehydrotomatine in BC_1 clone MCPH3 and in BC_2 clone PTHA1; dehydrodemissine in BC_1 clone MCOP5 and BC_2 clone PTHE3). Interestingly, in three out of five BC_2 genotypes tested (PTHC4, PTHE10 and PTHF7) the GAs from cmm were completely absent. BC_3 hybrids coming from the PTHF7 × Blondy crosses confirmed the loss of GAs from cmm. Solanine content ranged from 24.8 mg/kg to 154.9 mg/kg, and chaconine from 27.8 mg/kg to 90.6 mg/kg (data not shown).

Because chaconine is more toxic than other GAs, the ratio between chaconine and the other detectable GAs was calculated. It was generally high in the tbr population, ranging from 2.7 (Spunta) to 3.8 (Carmine). By contrast, high variability was found within BC_1 and BC_2 hybrids, where the ratio ranged from 0.2 (PTHE3) to 2.8 (PTHC4).

Acclimation capacity

Results on the capacity to cold acclimate of genotypes tested are shown in Table 3. Cold acclimation treatment resulted in an increased freezing tolerance of 2.5 °C in cmm. By contrast, both UP88-P5 and the other tbr used as controls resulted only in a slight change of freezing tolerance. In fact, their capacity to cold acclimate was around 1 °C, which is within the range of non-acclimating genotypes. The F_1 triploid hybrids expressed a capacity to acclimate similar to cmm, as previous results have already indicated (Carputo et al. 2000). In particular, the acclimation capacity of MCA1, MCB1 and MCB10 was 2.9 °C, 3.1 °C and 2.7 °C, respectively. The relative acclimation capacity of F_1 triploids ranged from 112% to 129%, confirming that the level of resistance in the hybrids was close to the hardy parent.

As for the BC_1 and BC_2 genotypes, the average acclimation capacity was 1.4 °C in BC_1 and 1.7 °C in BC_2. Most of the hybrids displayed an acclimation capacity higher than the sensitive parent but lower than cmm. Interestingly, one BC_1 genotype (MCPH5) and two BC_2 genotypes (PTHE10 and PTHF5) showed an acclimation capacity as high as cmm (2.2 °C, 3.0 °C and 2.4 °C, respectively). In these hybrids, the relative acclimation capacity level was 92% (MCPH5), 100% (PTHF5) and 125% (PTHE10). No significant correlation was found between GA content and the acclimation capacity (r = 0.377), NAFT (r = 0.158), and ACFT (r = 0.158).

Fatty acid composition

Following acclimation, variability in changes of 16:0 18:0, 18:1, 18:2 and 18:3 was observed between genotypes (data not shown). The most interesting result was that the cold acclimating cmm showed a large increase in 18:3 (7.1%, from 51.7% to 58.8%), with a simultaneous decrease in 18:2 (5.7%, from 20.1% to 14.4%). By contrast, UP88-P5 and the tbr controls had a different behavior, with no significant change in 18:3 (from 52.5% to 50.6% and from 50.1% to 50.4% in UP88-P5 and Spunta, respectively). Interestingly, F_1, BC_1 and BC_2 hybrids displayed a trend similar to cmm. The average increase of 18:3 was 5.1%, 4.6% and 3.8% in F_1, BC_1 and BC_2, respectively, whereas the average decrease of 18:2 was 1.8%, 2.8% and 3.1% in F_1, BC_1 and BC_2, respectively. Figure 1 shows the scatter diagrams of the genotypes analyzed based on acclimation capacity and changes in 16:0 18:0, 18:1, 18:2 and 18:3. Correlation

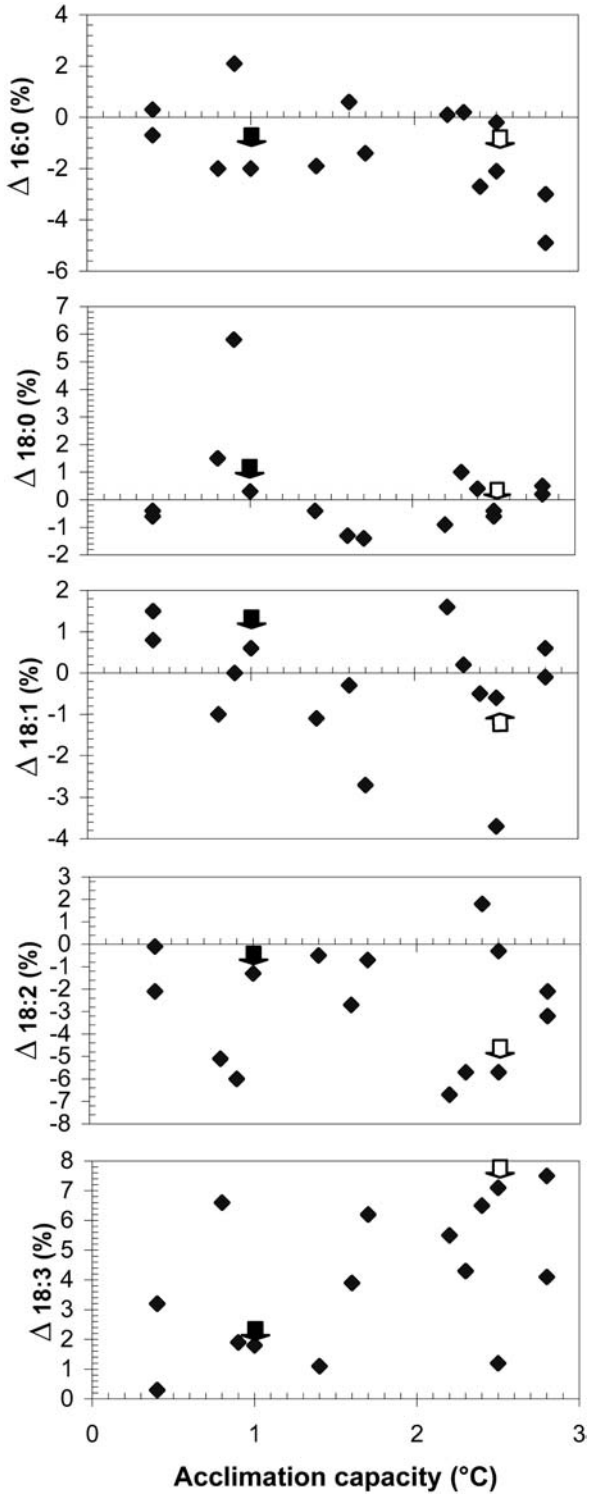

Fig. 1 Scatter diagram of 12 *S. tuberosum* – *S. commersonii* hybrids and the parental genotypes *S. commersonii* (*white arrow*) and UP88-P5 (*black arrow*) for the acclimation capacity (°C), and the % change (Δ) in foliar fatty acid composition following acclimation for 2 weeks at 4 °C

analysis between the capacity to cold acclimate and the changes in fatty acids provided evidence that the increase in 18:3 was significant ($r = 0.497$, $P < 0.05$). By contrast, no significant correlation was found between the acclimation capacity and changes in 16:0 ($r = –3.14$), 18:0 ($r = –0.073$), 18:1 ($r = –0.18$) and 18:2 ($r = –0.10$).

Discussion

Several wild tuber-bearing *Solanum* species used in potato breeding programs as sources of desirable genes have a high GA content. Deahl et al. (1993) detected 12 major GAs in leaves of 70 species belonging to a different series, and found a wide intra- and inter-specific variability in the amounts and types of GAs. Thus, interspecific hybridization may cause the transmission of high levels of known and unknown GAs to newly developed potato hybrids. Grassert and Lellbach (1987) found that large amounts of GA can persist in interspecific progenies even after the fourth backcross. Also indicative is the case of cv Lenape, derived from a breeding program involving the wild species *S. chacoense*. Due to the high GA level of tubers, it had to be withdrawn from the market even though it had several noteworthy traits. Since cmm is in the group of species with the highest GA content, it was of great importance to investigate the potential for different types and large amounts of GAs also in the hybrid material developed from this wild species. In the leaves of all F_1 hybrids five different GAs were determined: solanine and chaconine deriving from UP88-P5, dehydrodemissine, dehydrotomatine and dehydrocommersonine deriving from the wild parent. Due to the lack of tuberization, the GA present in the tubers of the F_1 genotypes could not be ascertained. Qualitative data on BC_1 and BC_2 genotypes indicated that generally GAs present in leaves were also present in tubers. Only in few exceptions (dehydrocommersonine in the BC_1 genotype MCOP5; dehydrocommersonine, dehydrotomatine, dehydrodemissine in the BC_2 genotype PTHC4) was there no correspondence between GA types in leaves and tubers. Studies in *S. chacoense* and its hybrid-derivatives demonstrated that for other GAs (i.e. leptine and leptinine) the opposite is true, in that they are synthesized only in leaves (Sanford et al. 1998). Our results suggested that although there is little or no transport of GAs between different parts of the plants (Friedman and McDonald 1997), the five GAs studied here are synthetized both in the aerial part and in the tubers. Since most studies have demonstrated that GA synthesis is higher in organs with great metabolic activity (Maga 1994; Valkonen et al. 1996), it is expected that the GA quantity in leaves of our hybrids reaches higher concentrations than tubers.

Interesting results were obtained from the quantitative detection of GAs in the tubers of BC tbr-cmm hybrids. Total GA content ranged from 113 mg/kg of tuber fresh weight to 317 mg/kg of tuber fresh weight. These values are much lower than that of the wild parent cmm. In addition, in most of the genotypes GA content was lower than 200 mg/kg of tuber fresh weight, which is considered the maximum level for human consumption. This is particularly important in light of the fact that (1) whole tubers (peel, cortex and flesh) were used for GA determination, and (2) tubers analyzed were produced in pots, and had a relatively small size. It is known that peel has a much higher GA content than flesh (Maga 1994) and that the use of small tubers increases the surface area/volume ratio and thus the GA content. Despite a relatively small sample size, our results provided evidence that selection for the acceptable GA content is possible, as found in hybrids involving other species (Kozukue et al. 1999; Sarquis et al. 2000).

Little is known about the inheritance of the GA content in potato, especially in wild Solanums. Sanford and Sinden (1972) suggested that this trait is polygenic, and recent molecular studies by Yencho et al. (1998) allowed identification of several QTLs contributing to GA production in the segregating *S. tuberosum – Solanum berthaultii* hybrids. The finding of several hybrids with acceptable GA levels supports results by Ross (1966) and Sanford et al. (1995), who proposed that cultivated tbr has a dominant effect for low GA synthesis. It is worth noting that the different ploidy level between BC_1 and BC_2 hybrids, as well as the different chromosome number within BC_2 hybrids, did not affect the GA content. By contrast, Kozukue et al. (1999) suggested that the amount of tomatine may depend on the ploidy level in tbr-*Solanum acaule* somatic hybrids.

The determination of individual glycoalkaloids indicated that solanine and chaconine, the GAs of tbr, were always present in BC hybrids. The presence of trace spots of solanine can be due to genotypic or environmental factors affecting the expression of genes controlling solanine synthesis (McCollum and Sinden 1979). Our results also suggested that the glycoalkaloids from cmm may be lost rapidly, with obvious advantages in terms of breeding efforts. It can be hypothesized that their pathways are simply inherited, or that there might be tbr genes that can break the pathways. In their review on potato GAs, Valkonen et al. (1996) suggested that inheritance of certain GAs from some wild species may be monogenic, and thus it is possible to find absence of these GAs after only one or two backcrosses. The fact that in the hybrids analyzed we found no newly formed GAs is also important. The formation of new types of GAs is a major concern of interspecific hybridization, due to possible recombination events between different types of sugar aglycones. Laurila et al. (1996), for example, found that GAs not produced by the parents were present in tbr-*Solanum brevidens* somatic hybrids.

Several morphological, physiological and quality traits should be considered in newly developed genotypes to make them commercially acceptable. Thus, in any breeding program it is important to ascertain the association between noteworthy traits. In the literature, scant information is available on the association between GA content and resistance to low temperatures. We did not

find any significant positive correlation between either GAs and NAFT or GAs and ACFT, suggesting that the traits are not associated. Thus, if one backcrosses with selection for hardiness, there is not much chance that GA could resist de-selection. Our data also confirmed that NAFT and ACFT are independent traits and that in the potato ACFT is easier to introgress (Stone et al. 1993). Some hybrids exhibited an acclimation capacity higher than 2 °C, which is the hardiness level of some pure hardy species (Chen et al. 1999a). Hybrids with high acclimation capacity are very important for potato breeding in the Mediterranean area. Here potato is planted starting from late fall, and frost episodes are normally preceded by cool days which causes acclimation. The relative resistance of the F_1 triploids was very high, and lower in BC_1 pentaploids and the BC_2 aneuploid-tetraploids. Thus, having more genomes from the hardy parent cmm increased the acclimation capacity. The indication that the genomic ratio plays a key role in the expression of the resistance to low temperatures has already been reported in interspecific sexual and somatic potato hybrids (Chen et al. 1999b) as well as in the Triticeae (Limin et al. 1989). Limin et al. (1995) hypothesized that in cereals competition among the different regulatory elements of parental genomes may cause a low expression of genes from cold acclimating diploid species. In our material it is also possible that, due to backcrossing, the presence of freezing-sensitive genes from tbr increased. However, in larger backcross populations it may well be possible to identify genotypes with an acclimation capacity comparable to that of the hardy parent.

It has been suggested that since cold acclimation results in increased resistance to freeze-thaw stress, changes in lipid composition may improve membrane fluidity and thus may be involved in the acclimation process. In their comprehensive work on the identification of lipids associated with genetic variability in the cold acclimation of cmm and tbr, Palta et al. (1993) were first able to differentiate specific lipid changes associated to increased freezing tolerance during the acclimation process from those resulting from metabolic changes to low, non-freezing temperatures. They found that the increased unsaturation level was due to an increase in 18:2. Our results confirmed that the acclimation capacity was positively correlated with an increase in the level of unsaturated fatty acid. However, according to our analysis the increase in unsaturation level is mainly due to an increase in 18:3 and not in 18:2, as also reported in *Secale cereale* by Uemura et al. (1984). Our results can be explained by the fact that different tissues were used for lipid analysis. We used whole tissues (leaves), whereas Palta et al. (1993) determined the lipid composition in plasma membranes. From our data it may be inferred that the increase in 18:3 can be used as a biochemical marker for the assisted selection of cmm-tbr hybrids with the capacity to cold acclimate. The test based on ion leakage is very well correlated with field response, but it is time consuming and only a limited number of genotypes can be analyzed each time. By contrast, analysis of leaf fatty acid composition is much easier and may be used in the screening of large segregating populations.

Analysis on GA content and acclimation capacity provided evidence that, even in a relatively small sample size, cmm-tbr hybrids that are attractive for further breeding can be identified. This is interesting also in the light of other noteworthy traits found in BC_2 hybrids previously analyzed (Esposito et al. 2001).

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