

Variability in the Rate of Cold Acclimation and Deacclimation among Tuber-bearing *Solanum* (Potato) Species

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ABSTRACT. Two major components of frost resistance are freezing tolerance in the nonacclimated state (growing in normal condition) and capacity to cold acclimate (increase in freezing tolerance upon exposure to chilling temperatures). In addition to these two major components, numerous factors contribute to frost survival. Although the rate of cold acclimation and deacclimation have been recognized as important factors contributing to frost survival, very little information about them is available. Our objective was to determine if there is variability in the rate of cold acclimation and deacclimation among tuber-bearing wild potato species: *S. acaule* Bitter, *S. commersonii* Dunal, *S. megistacrolobum* Bitter, *S. multidissectum* Hawkes, *S. polytrichon* Rydb., *S. sanctae-rosae* Hawkes, and *S. megistacrolobum* subsp. *toralapanum* (Cárdenas & Hawkes) Giannattasio&Spooner. Relative freezing tolerance of these species was measured after 0, 3, 6, 9 and 12 days of cold acclimation and after 12 and 24 hours deacclimation. Our results showed there were differences in the rates of cold acclimation and deacclimation among these species. With respect to the rate of acclimation we found these species can be divided into four groups: (i) early; (ii) late acclimators; (iii) progressive acclimators, and (iv) nonacclimators. Likewise, a wide range of cold deacclimation behavior was found. Some species showed as low a loss of 20% of their freezing tolerance, others showed as much as >60% loss after 12 hours of deacclimation. Significant deacclimation was observed in all cold acclimating species after 1 day. These results demonstrate that the rates of cold acclimation and deacclimation were not necessarily related to the cold acclimation capacity of a species. Rapid acclimation in response to low temperatures preceding a frost episode and slow deacclimation in response to unseasonably warm daytime temperatures could be advantageous for plants to survive frost events. Thus, in addition to nonacclimated freezing tolerance and acclimation capacity, it would be very desirable to be able to select for rapid acclimation and slow deacclimation abilities. Results demonstrate that variability for these two traits exists in *Solanum* L. (potato) species.

Freezing temperatures cause major yield losses in potato (*Solanum tuberosum* L.) production in many parts of the world (Estrada, 1978; Li and Palta, 1978; Midmore, 1992; Plaisted and Hoopes, 1989). While in the temperate regions of North America the growing season is limited by frost damage during spring and fall, in the Andean highlands of South America frost can damage the potato crop at any time during the growing season. Unfortunately, only limited success has been achieved by using traditional plant breeding methods for improvement of freezing stress resistance in crop plants (Estrada, 1978; Marshall, 1982; Midmore, 1992; Palta, 1991; Palta and Simon, 1993; Ross and Rowe, 1969; Stushnoff et al., 1984). One of the main reasons is probably because frost and winter survival have been recognized as complex traits, in which many factors may interact (Palta, 1991; Palta and Simon, 1993; Palta and Weiss, 1993; Stushnoff et al., 1984; Teutonico et al., 1993).

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Two major components of freezing stress resistance are freezing tolerance in the nonacclimated state (normal growing condition) and capacity to cold acclimate (increase in freezing tolerance upon exposure to chilling temperatures) among annual and perennial plant species (Levitt, 1966; Li and Palta, 1978; Palta and Simon, 1993; Sakai and Larcher, 1987; Teutonico et al., 1995). *Solanum* species vary greatly in both nonacclimated freezing tolerance and cold acclimation capacity (Chen and Li, 1980; Li and Palta, 1978; Palta and Li, 1979; Vega and Bamberg, 1995). Stone et al. (1993) demonstrated that nonacclimated freezing tolerance and the ability to cold acclimate are under independent genetic control in potato. This has now been confirmed in other plants (Arora et al., 1998; Teutonico et al., 1993; 1995). In addition to these two components of freezing stress resistance, many other environmental and plant factors contribute to survival of a plant from a frost episode. Environmental factors such as lowest temperature reached, freezing (cooling) rate, duration of freezing temperatures, ice nucleation temperature, thawing rate and postthaw conditions should be considered. Two important plant factors are hardening status of the plant and plant health and age (Palta and Weiss, 1993). Likewise, the rate of cold acclimation and deacclimation have been recognized as important factors that contribute to frost and winter survival (Palta, 1994; Palta et al., 1997; Pellet, 1997).

While nonacclimated freezing tolerance and the acclimation capacity of several plant species have been investigated and in some cases used in genetic and breeding studies (Chen and Li, 1980; Chen et al., 1999a; 1999b; Palta and Simon, 1993; Stone et

al., 1993; Teutonico et al., 1995), very little is known about the variation of the rate of cold acclimation and deacclimation and the role these factors might play in frost survival. The ability to gain freezing stress resistance rapidly in response to decreasing air temperatures (cold acclimation) would be advantageous for sudden frost events particularly during late spring, early fall or during the growing season. Cold acclimation can be reversed upon exposure to warm temperatures (cold deacclimation). Therefore, not only the ability to gain freezing tolerance rapidly in response to low temperatures in fall and spring but also being able to deacclimate slowly in response to warm daytime temperatures would be advantageous against spring or fall frosts. Ideally one would want to have rapid acclimation and slow deacclimation incorporated into the same plant. Thus, it would be important to be able to differentiate and select for rapid acclimators and slow deacclimators.

Studies on seasonal changes in the freezing stress resistance of needles of red pine (*Pinus resinosa* Ait.) and Austrian pine (*Pinus nigra* Arnold) have suggested there is genetic variation for the rates of cold acclimation and deacclimation (Sutinen et al., 1992). Similar studies on other woody species such as mock orange (*Philadelphus lewisii* Pursh.) and hydrangea (*Hydrangea paniculata* Sieb.) clones (Suojala and Lindén, 1997), Chinese elm (*Ulmus parvifolia* Jacq.) (Lindstrom and Dirr, 1991), red maple (*Acer rubrum* L.), downy serviceberry (*Amelanchier arborea* Michx. f. Fern.), scarlet oak (*Quercus coccinea* Muenchh.), Florida anise (*Illicium floridanum* Ellis) and small anise (*Illicium parviflorum* Michx.) (Lindstrom and Dirr, 1989) have also found variation for these traits. To our knowledge no such information exists among herbaceous plant species. Our research was aimed at studying for variation in the rates of cold acclimation and deacclimation among wild potato species.

Materials and Methods

PLANT MATERIAL AND GROWING CONDITIONS. We selected seven wild tuber-bearing *Solanum* species with different levels of freezing tolerance and cold acclimation capacity according to previous reports (Li, 1977; Li and Palta, 1978; Palta and Li, 1979; Ross and Rowe, 1969; Stone et al., 1993; Sukumaran and Weiser, 1972; Vega and Bamberg, 1995). The species selected for the present study corresponded to a wide range of altitudes (200 to 4400 m) and countries of origin (Argentina, Bolivia, Mexico, and Peru). The accessions chosen for each species have been characterized previously for resistance to various diseases (Bamberg et al., 1994; Bamberg et al., 1996; Martin et al., 1996).

Seeds of *S. acaule* (PI 472715), *S. commersonii* (PI 243503), *S. megistacrolobum* (PI 500031), *S. multidissectum* (PI 498304), *S. polytrichon* (PI 186545), *S. sanctae-rosae* (PI 230464), and *S. megistacrolobum* subsp. *toralapanum* (PI 546015) were obtained from the Inter-Regional Potato Introduction Station (NRSP-6), Sturgeon Bay, Wis. After treating overnight with gibberellic acid (GA_3) solution ($2 \text{ mg} \cdot \text{L}^{-1}$, w/v), the seeds were surface sterilized with 70% (v/v) ethanol for 2 min and 5% (v/v) sodium hypochlorite for 5 min, then germinated aseptically on a balanced nutrient medium for further clonal propagation (Murashige and Skoog, 1962). Plantlets were grown from single node stem sections in culture tubes for 4 weeks under a continuous photoperiod at $20 \pm 1^\circ \text{C}$ with $70 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of photosynthetically active radiation (PAR) [measured at plant level with a quantum/radiometer/photometer (LI-185A; LI-COR, Lincoln, Nebr.)] from cool-white fluorescent lamps (Sylvania/GTE, Danvers, Mass.). Plant-

lets of each species obtained from culture were potted individually in 2.5 L plastic pots containing 1 peat:1 vermiculite (v/v) (Jiffy Mix N° 901, Jiffy Products of America, West Chicago, Ill.) and grown in a controlled-environment room ($3.7 \times 2.6 \text{ m}$) at the University of Wisconsin-Madison Biotron facility.

Growth conditions were $20 \pm 1^\circ \text{C}$ day/ $18 \pm 1^\circ \text{C}$ night temperatures, with a 14 h photoperiod of $400 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR) (measured at plant level with a quantum/radiometer/photometer from cool-white fluorescent lamps. Relative humidity was $70\% \pm 5\%$. Plants were irrigated with half-strength modified Hoagland's solution (Steffen et al., 1989) delivered automatically two times a day for 15 min to ensure that plants would be watered in excess of container capacity to prevent salt accumulation. Cold acclimation treatments were applied when the plants were 5 weeks old after potting. To achieve cold acclimation, air temperatures were lowered to 4°C day/ 2°C night with a 14 h photoperiod of $100 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR) (measured at plant level with a quantum/radiometer/photometer) for 12 d. These conditions were determined previously to be ideal for cold acclimation of potato species (Steffen et al., 1989). After full cold acclimation was achieved, the plants were returned to $20 \pm 1^\circ \text{C}$ day/ $18 \pm 1^\circ \text{C}$ night and a 14h photoperiod for the 24 h of deacclimation experiments or to $18 \pm 1^\circ \text{C}$ in the dark for the 12 h of deacclimation experiments.

DETERMINATION OF RELATIVE FREEZING TOLERANCE (RFT). Freezing tolerance of the plant material was determined by a modification of the protocol of Steffen et al. (1989). After 1, 3, 6, 9, and 12 d of cold acclimation, fully expanded terminal leaflets were collected from the seven *Solanum* species for each of the cold acclimation intervals for freezing tolerance and cold acclimation evaluations. The leaflets were excised at the end of the dark period before the lights came on and placed in covered culture glass tubes ($25 \times 200 \text{ mm}$) (one leaflet per tube) that were submerged in a glycol-containing controlled temperature cooling bath (model 2323, Forma Scientific, Marietta, Ohio) at 0°C . Controls were placed immediately on ice. After 30 min, the temperature in the cooling bath was lowered to -0.5°C , held for 30 min and then lowered to -1.0°C . After 30 min at -1.0°C , ice nucleation was initiated by adding a small piece of ice to each tube. Samples were held at -1.0°C for another 30 min, then the temperature was lowered to -1.5°C and held for 1 h. Further cooling was at a rate of $0.5^\circ \text{C}/30 \text{ min}$ down to -10°C and $1.0^\circ \text{C}/30 \text{ min}$ below -10°C . Tubes containing the frozen leaflets were removed at predetermined temperatures (used to develop an ion leakage curve) and thawed on ice overnight prior to evaluation of injury. Three leaflets were evaluated at each temperature for each of the species.

Freezing injury was assessed by measurement of ion leakage (Flint et al., 1967; Li and Palta, 1978; Murray et al., 1989; Palta et al., 1977, 1982; Wilner, 1960). Ion leakage was expressed as the ratio of electrolyte leakage from freeze-injured tissue to electrolyte leakage from autoclaved tissue. Thawed leaflets were sliced into strips before addition of 25 mL deionized, distilled water at about 22°C . Samples were infiltrated for 5 min at 10 kPa by using a vacuum pump then shaken for 1 h at 220 rpm on agytratory shaker (model G-10; New Brunswick Scientific Co., New Brunswick, N.J.). Electrical conductivity (R_1) was measured with a conductance meter (model 32; Yellow Springs Instrument Co., Yellow Springs, Ohio). Total conductivity (R_2) of each sample was measured following a 24 h cooling period after autoclaving at 121°C for 15 min. Percentage mean ion leakage expressed as $(R_1/R_2) \times 100$ of triplicates was plotted as a

Table 1. Nonacclimated freezing tolerance (RFT), acclimation capacity (Δ RFT), and acclimated freezing tolerance at 4 °C day/2 °C night for six *Solanum* species.

<i>Solanum</i> species	Nonacclimated freezing tolerance (RFT ^{xy} in °C) ^x	Acclimation capacity (Δ RFT in °C) ^z				Acclimated freezing tolerance (°C) ^y
		Days of acclimation				
		3	6	9	12	
<i>S. acaule</i>	-4.4 ± 0.1	0.4	0.7	1.0	1.3	-5.7 ± 0.3
<i>S. commersonii</i>	-4.5 ± 0.1	0.9	2.8	3.1	4.8	-9.3 ± 0.5
<i>S. megistacrolobum</i>	-3.7 ± 0.2	0.8	1.4	2.0	2.6	-6.3 ± 0.3
<i>S. multidissectum</i>	-3.0 ± 0.1	0.8	1.3	1.5	1.4	-4.4 ± 0.2
<i>S. sanctae-rosae</i>	-3.8 ± 0.2	-0.2	0.3	1.8	1.8	-5.6 ± 0.4
<i>S. megistacrolobum</i> subsp. <i>toralapanum</i>	-3.4 ± 0.1	0.9	1.3	1.9	2.2	-5.6 ± 0.2

^zAcclimation capacity (Δ RFT) was assessed as acclimated RFT minus nonacclimated RFT.

^yRFT was determined from the midpoint of the maximum and minimum (control) ion leakage values obtained from each species (Stone et al., 1993).

^xStandard error calculated from three experiments.

function of freezing temperature. Relative freezing tolerance was determined from the midpoint of the maximum (autoclaved) and minimum (control) ion leakage values obtained for each species (average of the three leaflets) after a specific period of cold acclimation as described previously (Stone et al., 1993; Sutinen et al., 1992). The absolute value of this temperature was defined as the RFT. Acclimation capacity (Δ RFT) was expressed as acclimated RFT minus nonacclimated RFT. The percentage of maximum acclimation observed at various time intervals was calculated relative to the Δ RFT after 12 d of acclimation.

To study the variation in the rate of acclimation of the various species, the RFT of each species was determined after 3, 6, 9, or 12 d of cold acclimation. To evaluate the rate of deacclimation, the RFT of fully cold-acclimated plants was determined after 12 h and 1 d at 20 ± 1 °C day/18 ± 1 °C night.

EXPERIMENTAL DESIGN AND DATA ANALYSIS. Determination of RFT of all the species before, during, and after cold acclimation was repeated on three different occasions (three experiments), each time starting with new material from tissue culture. RFT of each species was evaluated before cold acclimation and after 3, 6, 9, and 12 d of cold acclimation in each experiment. Leaflets of two separate plants of each species were sampled for each RFT evaluation (a total of 10 plants per species were sampled for each experiment).

Likewise, for the cold deacclimation study, determination of RFT of all the species after 12 h and 1 d of deacclimation was repeated on three different occasions (three experiments), each starting with new material from culture. RFT of each species was evaluated before and after 12 d cold acclimation and 12 h and 1 d of deacclimation. Leaflets of two plants of each species were sampled for each RFT evaluation (a total of eight plants per

species were sampled for each experiment).

For all the experiments, pots were placed randomly in the controlled-environment room. Data from three experiments were pooled for statistical analyses. Mean separation was performed using LSD procedures at $P < 0.001$.

Results

VARIABILITY IN THE RATE OF COLD ACCLIMATION AMONG POTATO SPECIES. Table 1 shows the RFT before and after acclimation and the gain in freezing tolerance (Δ RFT) at 3, 6, 9, and 12 d of cold acclimation. The frost tolerance and cold acclimation capacity varied among the potato species, from *S. commersonii* with the highest nonacclimated freezing tolerance and acclimation capacity to *S. polytrichon*, which was chilling sensitive and had no acclimation capacity (data not presented).

The percentage and rate of cold acclimation throughout the hardening period are presented in Table 2. According to the rate and timing of acclimation, four types of acclimators could be observed: 1) early acclimators, species that showed 40% to 50% of acclimation within the first 3 d of cold treatment (days 1 to 3); 2) late acclimators, species that gained the majority of its acclimation capacity after at least 6 d of cold treatment; 3) progressive acclimators, species that showed a steady increase in their percentage of cold acclimation over time; and, 4) nonacclimators. Once the cold acclimation response began, the species also varied in the rate at which they cold acclimated (Fig. 1).

Four distinct cold acclimation responses were observed among the different potato species. As shown in Table 2, *S. multidissectum* and *S. megistacrolobum* subsp. *toralapanum* fell into the first group, early acclimators, with 53% and 41% of acclimation after

Table 2. Percentage of cold acclimation achieved in various intervals and rate of acclimation during 12 d at 4 °C day/2 °C night for six *Solanum* species.

<i>Solanum</i> species	Days of acclimation (% , rate in °C·d ⁻¹)			
	0-3	3-6	6-9	9-12
<i>S. acaule</i>	30.7 c (0.1) ^z	23.1 c (0.1)	23.1 c (0.1)	23.1 b (0.1)
<i>S. commersonii</i>	18.7 d (0.3)	39.6 a (0.6)	6.3 e (0.1)	35.4 a (0.6)
<i>S. megistacrolobum</i>	30.7 c (0.3)	23.1 c (0.2)	23.1 c (0.2)	23.1 b (0.2)
<i>S. multidissectum</i>	53.4 a (0.3)	33.3 b (0.2)	13.3 d (0.1)	0.0 d (0.0)
<i>S. sanctae-rosae</i>	0.0 e (0.0)	16.7 e (0.1)	83.3 a (0.5)	0.0 d (0.0)
<i>S. megistacrolobum</i> subsp. <i>toralapanum</i>	40.9 b (0.3)	18.2 d (0.1)	27.3 b (0.2)	13.6 c (0.1)
LSD ($\alpha < 0.001$) ^z	0.186	0.322	0.300	0.263

^zMean values within the same column that have the same letter are not significantly different.

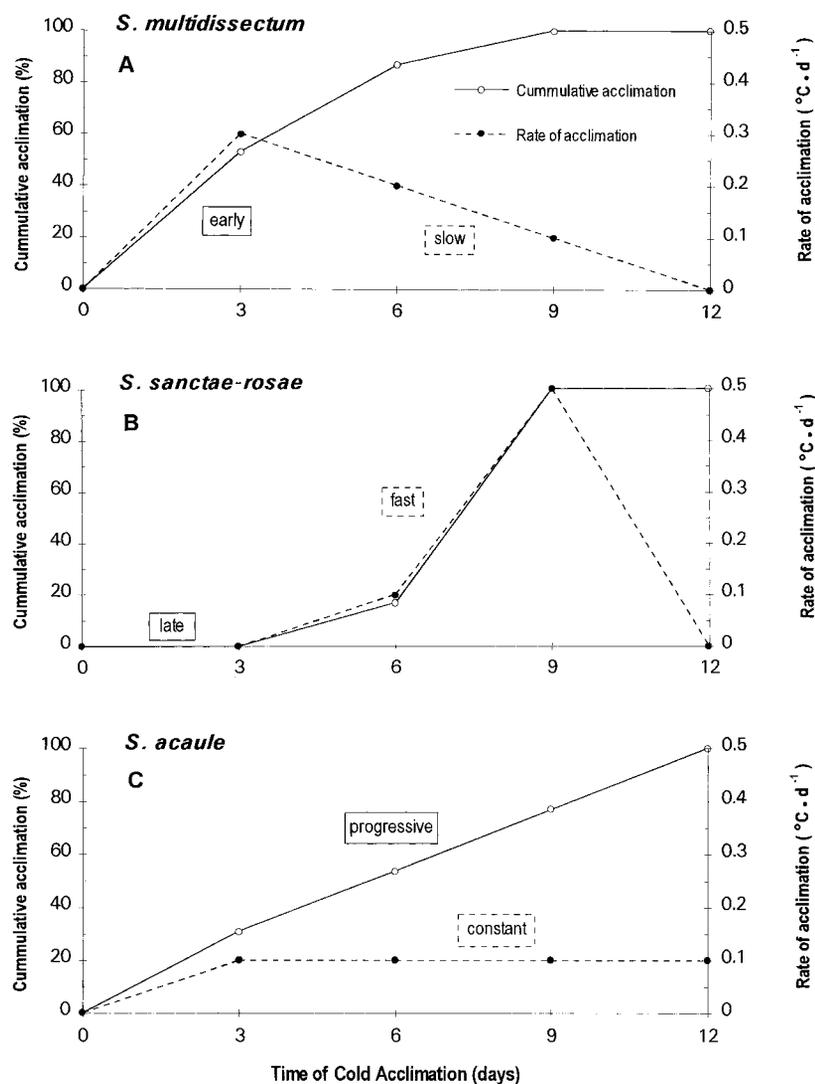


Fig. 1. Cumulative percentage and rate of cold acclimation of (A) an early acclimator, (B) late acclimator, and (C) a progressive acclimator (data taken from Table 2).

only 3 d of cold treatment, respectively. An early acclimator such as *S. multidissectum* gained half of its acclimation capacity within the first 3 d of cold treatment and slowly reached its maximum cold acclimation capacity thereafter, achieving complete cold acclimation after 9 d of cold treatment (Fig. 1A). *Solanum megistacrolobum* subsp. *toralapanum* showed a more gradual response between 3 and 12 d of cold treatment but had a higher acclimation capacity. These results are confirmed by the mean separation analysis using LSD ($P < 0.001$) which showed that even though *S. multidissectum* and *S. megistacrolobum* subsp. *toralapanum* were significantly different from each other, both came at the top of the ranking due to their early cold acclimation abilities (Table 2). Conversely, *S. sanctae-rosae* showed a delayed acclimation response, with very little gain in freezing tolerance during the first 6 d of cold treatment, but with a very rapid acclimation between 6 and 9 d of cold treatment (Fig. 1B). The difference between an early acclimator like *S. multidissectum* and a late acclimator like *S. sanctae-rosae* is even more evident when their percentage of acclimation over time is compared. While *S. multidissectum* reached over half of its acclimation capacity within the first 3 d of cold treatment, *S. sanctae-rosae* started to respond only after the sixth day of cold treatment, yet both species achieved their maximum cold acclimation after 9 d. *Solanum acaule* and *S. megistacrolobum* comprised a third group we called progressive acclimators because they showed a relatively uniform response to the cold treatment over time (Fig. 1C). *Solanum acaule* and *S. megistacrolobum* responded similarly, exhibiting a constant rate and increase in the percentage of acclimation (20% to 30% every 3 d), reaching their maximum acclimation after 12 d of cold treatment. Mean analysis confirmed this observation (Table 2).

The cold-hardest species, *S. commersonii*, reached maximum acclimation in two stages. About 50% of its maximum acclimation capacity was reached within the first 6 d of cold

Table 3. Nonacclimated freezing tolerance (RFT), acclimation capacity (Δ RFT), and loss in freezing tolerance (cold deacclimation) after 12 h of deacclimation at 18 °C (in the dark) for six *Solanum* species.

<i>Solanum</i> species	Freezing tolerance ^{z,y} (RFT) ^x in °C		Acclimation capacity (Δ RFT) ^w	After 12 h deacclimation		
	NA	ACC		RFT °C in °C ^y	Change in RFT (°C)	% Loss in RFT ^v
<i>S. acaule</i>	-4.5 ± 0.1	-5.8 ± 0.3	1.3	-5.3 ± 0.2	0.5	38.5 b
<i>S. commersonii</i>	-4.9 ± 0.1	-9.9 ± 0.5	5.0	-8.0 ± 0.5	1.9	38.0 c
<i>S. megistacrolobum</i>	-4.3 ± 0.2	-6.5 ± 0.3	2.2	-5.9 ± 0.3	0.6	27.3 d
<i>S. multidissectum</i>	-3.8 ± 0.1	-4.6 ± 0.2	0.8	-4.1 ± 0.1	0.5	62.5 a
<i>S. sanctae-rosae</i>	-4.1 ± 0.2	-5.7 ± 0.3	1.6	-5.4 ± 0.2	0.3	18.8 e
<i>S. megistacrolobum</i> subsp. <i>toralapanum</i>	-3.7 ± 0.1	-5.4 ± 0.2	1.7	-5.1 ± 0.2	0.3	17.6 f
LSD ($\alpha = 0.001$)						0.424

^zNA = nonacclimated freezing tolerance, ACC = acclimated freezing tolerance.

^yStandard error calculated from three experiments.

^xRFT was determined from the midpoint of the maximum and minimum (control) ion leakage values obtained from each species (Stone et al., 1993).

^wAcclimation capacity (Δ RFT) was assessed as acclimated RFT minus nonacclimated RFT.

^vPercentage of cold deacclimation observed after 12 h of deacclimation was estimated from the °C of deacclimation observed as a percentage of the 12 d Δ RFT [(°C of deacclimation./ Δ RFT) × 100].

Table 4. Nonacclimated freezing tolerance (RFT), acclimation capacity (Δ RFT), and loss in freezing tolerance (cold deacclimation) after 1 d of deacclimation at 20 ± 1 °C day/ 18 ± 1 °C night (14 h photoperiod, $400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-2}$ PAR) for six *Solanum* species.

<i>Solanum</i> species	Freezing tolerance ^{z,y}		Acclimation capacity (Δ RFT ^w)	After 1 d deacclimation		
	(RFT) ^x in °C			RFT °C in °C ^y	Change in RFT (°C)	% Loss in RFT ^v
	NA	ACC				
<i>S. acaule</i>	-4.3 ± 0.1	-6.0 ± 0.3	1.7	-4.9 ± 0.2	1.1	64.7 d
<i>S. commersonii</i>	-4.6 ± 0.1	-10.0 ± 0.5	5.4	-5.5 ± 0.2	4.5	83.3 b
<i>S. megistacrolobum</i>	-4.1 ± 0.2	-6.8 ± 0.3	2.7	-4.2 ± 0.3	2.6	96.3 a
<i>S. multidissectum</i>	-3.7 ± 0.1	-4.7 ± 0.2	1.0	-4.1 ± 0.1	0.6	60.0 f
<i>S. sanctae-rosae</i>	-4.1 ± 0.2	-5.7 ± 0.3	1.6	-4.7 ± 0.3	1.0	62.5 e
<i>S. megistacrolobum</i> subsp. <i>toralapanum</i>	-3.2 ± 0.1	-5.2 ± 0.2	2.0	-3.6 ± 0.1	1.6	80.0 c
LSD ($\alpha = 0.001$)						1.038

^zNA = nonacclimated freezing tolerance, ACC = acclimated freezing tolerance.

^yStandard error calculated from six experiments.

^xRFT was determined from the midpoint of the maximum and minimum (control) ion leakage values obtained from each species (Stone et al., 1993).

^wAcclimation capacity (Δ RFT) was assessed as acclimated RFT minus nonacclimated RFT.

^vPercentage of cold deacclimation observed after 1 d of deacclimation was estimated from the °C of deacclimation observed as a percentage of the 12 d Δ RFT [(°C of deacclimation./ Δ RFT) × 100].

treatment, and the highest rates of cold acclimation (compared to all the other species) were recorded between 3 to 6 and 9 to 12 d of cold treatment (Table 2). Nevertheless, we consider this species in the group of the progressive acclimators since it exhibited a continuous increase in freezing tolerance over the 12 d of cold treatment.

Solanum polytrichon showed no change in its ability to withstand cold even after 12 d of cold treatment (data not presented). In fact, by 9 d under cold treatment, chilling injuries were evident in most of the leaves. For that reason we considered this species to be in a separate group: nonacclimator.

VARIABILITY IN THE RATE OF COLD DEACCLIMATION AMONG POTATO SPECIES. The percentage of deacclimation was estimated from the relative change in RFT as compared to the acclimation capacity measured after 12 d of cold acclimation (Tables 3 and 4). The extent of deacclimation during 12 h at 18 °C varied from low (about 20%) in slow deacclimators like *S. sanctae rosae* (19%) and *S. megistacrolobum* subsp. *toralapanum* (18%) to high (over 60%) in rapid deacclimators, as observed in *S. multidissectum* (63%). The rest of the species (*S. acaule*, *S. commersonii*, and *S. megistacrolobum*) lost close to or more than one-third of their hardiness after 12 h at warm temperatures (Table 3). After 24 h of deacclimation, most of the species showed a dramatic change in RFT (Table 4). While some species lost more than half of their hardiness (i.e., *S. multidissectum*, 60%; *S. sanctae-rosae*, 63%; and *S. acaule*, 65%), others lost almost all of their hardiness (*S. megistacrolobum*, 96%; *S. commersonii*, 83%; and *S. megistacrolobum* subsp. *toralapanum*, 80%) (Table 4). Even though *S. multidissectum* lost more than half of its hardiness within 12 h at warmer temperatures, it did not lose additional hardiness during the following 12 h. Most of the species exhibiting slower deacclimation within 12 h compared to *S. multidissectum* ended up deacclimating more after 24 h at warmer temperatures. This is also evident by mean separation. While *S. multidissectum* ranked first after 12 h of deacclimation, this species was ranked last after 1 d of deacclimation (Tables 3 and 4).

Discussion

Results herein provide evidence for the existence of variability for the rate of cold acclimation and deacclimation among potato

species. To our knowledge this is the first study of this kind in an herbaceous plant species. To date, only few studies have suggested genetic variation for the rate of cold acclimation and deacclimation of woody species (Litzow and Pellet, 1980; Lindstrom and Dirr, 1989, 1991; Sutinen et al., 1992; Suojala and Lindén, 1997).

With respect to the rate of cold acclimation, we found that the potato species used in our study can be divided into four groups: 1) early acclimators (*S. multidissectum* and *S. megistacrolobum* subsp. *toralapanum*); 2) late acclimators (*S. sanctae-rosae*); 3) progressive acclimators (*S. acaule*, *S. megistacrolobum*, and *S. commersonii*); and 4) nonacclimators (*S. polytrichon*). Our results also suggest that there are differences in the rate of deacclimation among these species. After 12 h of deacclimation, some species showed <20% of deacclimation (i.e., *S. megistacrolobum* subsp. *toralapanum*) while others showed as high as >60% (i.e., *S. multidissectum*). After 1 d the hardened plant species deacclimated close to their preacclimation level, which is in agreement with previous studies conducted on several wild potato species (Chen and Li, 1980).

Previous studies have shown that some wild potato species possess a high degree of nonacclimated frost tolerance as well as high cold acclimation capacity (Chen and Li, 1980; Li, 1977; Li and Palta, 1978; Palta and Li, 1979; Ross and Rowe, 1969; Stone et al., 1993; Sukumaran and Weiser, 1972; Vega and Bamberg, 1995). The RFT values obtained for each species before and after complete cold acclimation were in agreement with previous studies (Li, 1977; Li and Palta, 1978; Palta and Li, 1979; Ross and Rowe, 1969). Recently, it was shown that these two components are independent genetic traits (Stone et al., 1993), suggesting that improvement in frost hardiness of potatoes can be made by selecting individually for these traits and recombining them to get the desired genotype (Palta et al., 1997). Results of the present study show variation in the rates of cold acclimation and deacclimation, suggesting that selection for these traits may be feasible. Furthermore, results of the present study also show that the rates of cold acclimation and deacclimation are not related necessarily to the acclimation capacity of the species. This has also been observed in cold hardiness studies on several cultivars of the woody species *Ulmus parvifolia* Jacq. (Chinese elm) conducted by Lindstrom and Dirr (1991). Their study found that

taxa exhibiting the greatest midwinter cold hardiness do not necessarily have the greatest cold hardiness earlier in the fall and/or later in the spring. We believe our findings have important implications in breeding studies aimed at the improvement of freezing tolerance in plant species. Until now, breeding strategies for improvement of freezing stress resistance of cultivated plant species have often been developed based on the use of the hardiest available related plant species. In potatoes, for instance, *S. commersonii* has been one of the wild potato species used widely for the improvement of freezing stress resistance of cultivated potatoes (Chen et al., 1999a, 1999b; Estrada, 1978; Marshall, 1982; Midmore, 1992) because it is one of the hardiest potato species and has very high cold acclimation capacity. However, *S. commersonii* was not the earliest acclimator among the species that were evaluated in the present study. While this species only reached about 20% of its total acclimation capacity within the first 3d of cold treatment, other species (*S. multidissectum* and *S. megistacrolobum* subsp. *toralapanum*) had already reached about half their cold acclimation capacities during this period. Likewise, *S. commersonii* deacclimated two times faster than two other species evaluated in this study (*S. sanctae-rosae* and *S. megistacrolobum* subsp. *toralapanum*) after 12 h of warm temperatures following cold treatment. As mentioned previously, it is not only the absolute gain in freezing tolerance that determines frost survival of a plant, but also the ability to gain freezing tolerance rapidly in response to decreasing air temperatures and deacclimate slowly in response to warm daytime temperatures. Ideally, one would want to have rapid acclimation and slow deacclimation incorporated into the same plant. It has been estimated that an increase in freezing tolerance of cultivated potatoes by about 2 °C could ensure a successful crop in most cases (Li et al., 1981; Li and Fennell, 1985; Li and Palta 1978). According to our results, *S. megistacrolobum* subsp. *toralapanum*, an early acclimator and slow deacclimator, would be a good choice to accomplish this objective in potato.

Results of this study may also have implications in breeding studies designed for improving winter survival in perennial species. Many factors contribute to winter survival in plants. These include rate of acclimation in fall, variation in snow cover, lowest temperature, midwinter thaw periods followed by very cold temperatures, and rate of deacclimation in spring (Palta and Weiss, 1993). Since factors that limit survival are unique for each winter, one may be selecting for different components of winter hardiness by using field evaluations. This may be, in part, the reason for the lack of success in the improvement of winter hardiness by traditional approaches. The ability to acclimate rapidly during early fall and to deacclimate slowly in response to midwinter thaw is of great importance for winter survival. However, it is impossible to select for these traits under field conditions because they are one of several factors that contribute to winter survival (Palta, 1994). Results of our study suggest that it might be possible to improve winter survival by selecting rapid acclimators and slow deacclimators.

The plant material and the precise evaluation used in the present study have provided important and useful information about the cold acclimation and deacclimation process in potatoes. Our results show that variability in the rate of cold acclimation and deacclimation exists in potato. This variability could possibly be exploited through breeding.

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