

Sorting Genes Controlling Freezing Stress Resistance¹

Strategy for Moving Desired Traits by Merging Physiological and Genetic Approaches

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Abstract

Factors that contribute to a plant's ability to survive winter or a frost episode include: a) freezing tolerance, i.e. tolerance of stresses associated with extracellular ice; b) freezing avoidance, i.e. ability to prevent ice formation; c) capacity to cold acclimate, i.e. ability to increase freezing tolerance and/or avoidance upon exposure to low (usually non-freezing) temperatures; and d) speeds of acclimation and deacclimation. We have demonstrated that freezing tolerance in the nonacclimated state and capacity to cold acclimate are under independent genetic control. In support of these genetic studies we have found that distinct plasma membrane lipids are associated with freezing tolerance in the nonacclimated state, and these lipids are different than those that are associated with increase in freezing tolerance during cold acclimation. We have also found evidence for genetic variations for the speeds of cold acclimation and deacclimation. In addition we have found that it is possible to independently select for freezing tolerance and freezing avoidance and then recombine them to get desired plants. Our results not only provide insight into molecular mechanisms of freezing stress resistance, but also provide avenues for improving frost/winter survival of crop plants.

Introduction

Freezing stress resistance is regarded as a very complex trait with polygenic inheritance (Marshall, 1982; Stushnoff et al., 1984; Thomashow, 1990). Lack of understanding of this complexity, in part, is due to heavy reliance on field selection for improving frost or winter survival. Field selection has many inherent problems. Ideally one hopes for a 'test frost episode' or a 'test winter' that is severe enough to kill the most sensitive genotypes, cause various degrees of injury to intermediate genotypes, and cause no injury to most resistant genotypes. However, such test frost episodes or test winters are rare.

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Many factors contribute to frost or winter injury in plants. For example injury to plants from a frost episode is influenced by ice nucleation temperature, freezing (cooling) rate, lowest temperature reached, duration of exposure to ice, thawing rate and post-thaw conditions (Palta and Weiss, 1993). Similarly speed of acclimation in fall, variation in snow cover, mid-winter thaw periods followed by very cold temperatures and speed of deacclimation in spring are major factors that contribute to winter survival. To develop a comprehensive approach for improving frost or winter survival we must address the following questions: Are there different physiological/biochemical/molecular parameters associated with different components that contribute to frost or winter survival? Do different components of freezing stress resistance have separate genetic control? How can we individually select for various components of freezing stress resistance?

We have addressed, to some degree, these fundamental questions in our studies. (Stone et al., 1993; Teutonico et al., 1993; Palta and Weiss, 1993; Palta and Simon, 1993). Our results show that three major components of freezing stress resistance namely non-acclimated freezing tolerance, ability to cold acclimate and thereby increase in freezing tolerance, freezing avoidance are under separate genetic control. In addition we have found evidence for the existence of genetic variability for the speeds of acclimation and deacclimation (Sutinen et al., 1992). These results suggest that study of individual components not only provide insight into molecular mechanisms of freezing stress resistance, but also provide avenues for improving frost/winter survival of crop plants. A brief account of our recent research is given here.

Independent Genetic Control for a Non-Acclimated Freezing Tolerance and Capacity to Cold Acclimate

Two major components of freezing stress resistance are (a) freezing tolerance in the non-acclimated state (i.e. normal growing conditions) and (b) capacity to cold acclimate (i.e. increase in freezing tolerance upon exposure to chilling temperatures for few days to few weeks period). Both of these traits are important for frost or winter survival depending on the climatic conditions preceding the frost episode and on the time of season.

The plant will be expected to rely on its nonacclimated tolerance if it is subjected to a sudden frost episode during the growing season or during the late spring and early fall periods. This is because of relatively long chilling period (few days to few weeks) are required for cold acclimation. During early spring or late fall a frost episode will be preceded by a cool period allowing plant to cold acclimate. Thus under these conditions ability to cold acclimate will be expected to play an important role in plant survival.

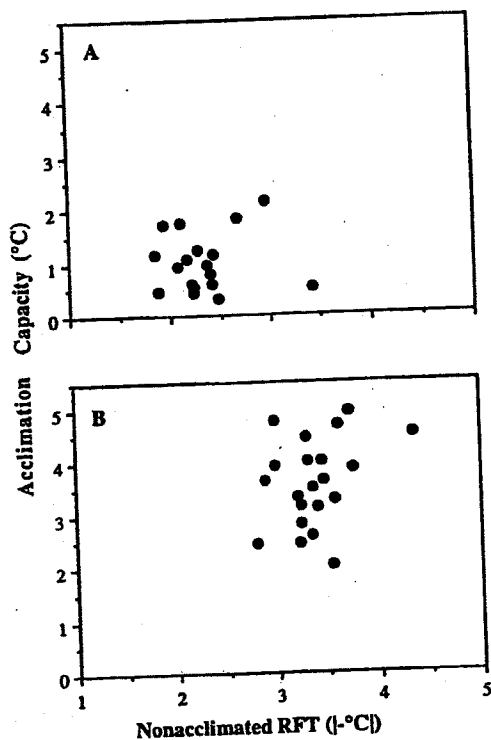


Figure 1. Correlation between nonacclimated freezing tolerance (RFT) and acclimation capacity (increase in RFT following cold acclimation) of individuals in segregating backcross populations of two diploid potato species *S. commersonii* (cmm) and *S. cardiophyllum* (cph). (A) F₁ x cph backcross progeny ($r = -0.066$, $P > 0.5$). (B) F₁ x cmm backcross progeny ($r = -0.317$, $P > 0.2$). Source: Stone et al.(1993).

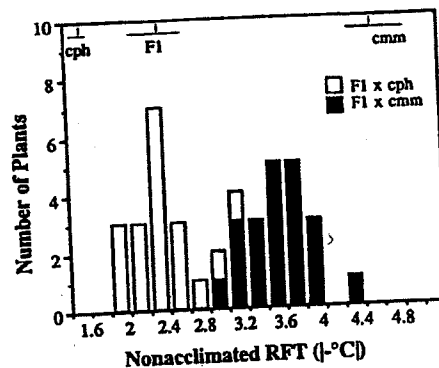


Figure 2. Distribution of nonacclimated RFTs of the backcross progenies (see Figure 1 legend for details). The range and mean RFT values for both parents (cmm and cph) and F₁ (cmm x cph) are indicated at the top of the graph. Source: Stone et al. (1993).

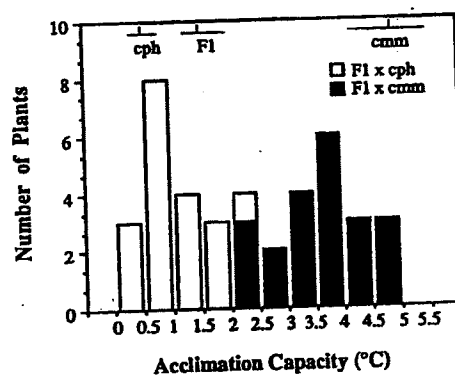


Figure 3. Distribution of acclimation capacity of backcross progenies (see Figure 1 legend for details). The range and mean acclimation capacity values for both parents (cmm and cph) and the F₁ (cmm x cph) are indicated at the top of the graph. Source: Stone et al. (1993).

This trait will also be important in winter survival since sufficient time is usually available for cold acclimation before plants are subjected to very cold winter temperatures.

We have recently demonstrated that nonacclimated tolerance and capacity to cold acclimate have distinct genetic control (Stone et al., 1993). In these studies we utilized two wild diploid potato species, *Solanum commersonii* and *Solanum cardiophyllum* as parents. These two species represent the extremes of non-acclimated freezing tolerance and acclimation capacity. *Solanum commersonii* is a frost tolerant species (nonacclimated tolerance -4.5°C) which is able to acclimate (acclimated freezing tolerance -9.6°C). *Solanum cardiophyllum* on the other hand is a frost sensitive species (nonacclimated tolerance -1.6°C) and is unable to cold acclimate. The F_1 of these parents was backcrossed to the parents to develop *Solanum* populations segregating for both the traits. Precise assessment of these two traits allowed small but significant differences among genotypes. Relative freezing tolerance (RFT) of these populations were assessed, using electrolyte leakage method, before and after cold acclimation. No correlation was found between nonacclimated RFT and acclimation capacity (Figure 1) suggesting that the two traits have independent genetic control. These results from the *Solanum* populations have been confirmed in rapeseed cultivars where no relationship between nonacclimated RFT and acclimation capacity was found (Teutonico et al., 1993).

Analyses of generation means indicated that all the variance for acclimation capacity and a major proportion of the variance for nonacclimated freezing tolerance can be best explained by an additive dominance model with both traits being partially recessive (Stone et al., 1993). The partial recessiveness of these two traits in *Solanum* populations is also evident from the fact that the F_1 was closer to the sensitive parent for both traits (Figures 2 and 3).

Only Limited Number of Genes Confer Cold Acclimation Ability in Potato

The fact that we were able to recover both parental phenotypes for acclimation ability in a limited population (Figure 3) suggests that cold acclimation ability is controlled by relatively few genes. The generation mean analyses also showed that cold acclimation ability can be explained by a simple additive-dominance model (Stone et al., 1993). Thus our studies suggest that the cold acclimation trait is not as genetically complex as has been thought.

Implications for Improving Frost Tolerance of Cultivated Potatoes

Frost impacts potato production in several ways. In the temperate regions such as Northern USA and Canada, frost damage in spring and fall to the potato crop can limit the growing season. In the Andean

highlands of South America where potato is the staple food, frost can damage the potato crop at any time during the growing season, thus limiting the cultivation of high yielding sensitive varieties. For example, low temperature of -2 to -4°C can reduce yield by about 30% (Estrada, 1987). Our results (Stone et al., 1993) show that we can improve frost survival of potato by independently selecting for nonacclimated tolerance and for capacity to cold acclimate. Once selected the desired genotypes could be intermated for moving both these traits to high yielding varieties. Having high acclimation capacity will ensure frost survival during spring and fall periods whereas a high degree of nonacclimated tolerance will help protect potato plants from frost episodes during the summer period.

Independent selection for freezing tolerance and freezing avoidance and combining these traits to alleviate frost problems in carrots

In addition to tolerating extracellular ice, plants are capable of surviving freezing temperatures by simply avoiding the formation of ice. Freezing avoidance has been found to be important for the survival of overwintering seeds, buds and xylem ray parenchyma cells of many woody species (see reviews of Burke et al., 1976; Sakai and Larcher, 1987; Li and Sakai, 1978, 1982). These tissues avoid ice formation by supercooling and preventing nucleation. There is also evidence that some tissues in herbaceous plants are capable of surviving by freezing avoidance (Sakai and Larcher, 1987).

Different parts of the same plant can survive freezing temperatures by separate mechanisms. For example, in overwintering woody plants, the wood and buds are known to survive by freezing avoidance (supercooling) whereas bark tissue actually freezes and survives by tolerance of ice and tolerance of other stresses associated with freeze-thaw process (Sakai and Larcher, 1987). Such separation of different survival mechanisms for various plant parts within the same plant have not been distinguished in herbaceous plants. It is possible that some of the crown tissue in alfalfa (*Medicago sativa* L.) and winter cereals have potential to survive by freezing avoidance whereas the roots of these plants invariably freeze and thus must survive by freezing tolerance. It is important to know whether root and crown survival during winter in these plants, is controlled by separate genes. Because if they are, these traits should be selected separately and then recombined.

We have recently demonstrated that separate selection for both freezing tolerance and freezing avoidance is needed to improve frost survival of carrots (Palta and Simon, 1993). In various parts of France, Great Britain and the Netherlands, mature carrots are left in the field during fall and the winter months for fresh market. Where they are subjected to periodic frosts. The injury to the plants by freeze-thaw stress is manifested as foliar damage, cracks on the roots (especially on the crown) crown rot and root

rot (Tucker, 1974; Tucker and Cox, 1978; Palta and Simon, 1993). In addition, freeze-thaw injured foliage left in the field is subjected to secondary biotic stresses resulting in rot which often enters the root. Carrots are preferably harvested by machines, which pulls on the foliage. A damage to foliage can prevent mechanical harvest. The cracks are rot in the crown and root tissue decreases the yield and quality of carrots.

From a systematic study of 13 breeding lines of carrots we found that freeze-thaw injury observed on the foliage in the field was highly correlated to freezing tolerance of leaf tissue (measured as ion leakage) determined by controlled freeze-thaw test in our laboratory (Table I). Crown rot was significantly correlated to freezing tolerance of the foliage. Considerable variation existed for the freezing tolerance among the lines we tested.

Table I: Spearman's Rank Correlation Coefficient Between Freeze-Thaw Injury in the Field and Various Parameters Important in Freezing Tolerance and Freezing Avoidance of Carrot Plants. Source: Palta and Simon (1993).

Freeze-thaw injury in the field	Spearman's rank correlation coefficients		
	Freezing tolerance parameter % ion leakage		Freezing avoidance parameter
	Leaf tissue	Root tissue	Crown position in relation to soil surface
On foliage	0.88**	0.53*	0.59*
Crown cracks	0.50	0.11	0.68*
Crown rot	0.68**	0.52	0.61*

* Significant at $\alpha = 0.05$.

** Significant at $\alpha = 0.01$.

In this study we also found that prevention of cracks in the root tissue was related to freezing avoidance of the root. Since carrot root is relatively rigid and dense tissue cracks develop as soon as ice forms in this tissue. This meant that avoidance of ice formation in the root and crown tissue was essential for the prevention of cracks in these tissues. The carrot breeding lines we tested varied considerably for the crown position in relation to soil surface. In some lines, the crown was up to 3.5 cm above the soil surface, whereas in other lines, crown position was about 1 cm below soil surface. Clearly the carrot crowns and

roots below the soil surface will be better at avoiding ice in the tissue, thus avoiding cracks. A significant correlation between crown cracks (observed in the field) and crown position in relation to soil surface was found (Table I).

Thus a breeding strategy for obtaining a desired carrot plant (alleviate frost related injuries) should include, separate selection for freezing tolerance of foliage and freezing avoidance of crown and root, and then recombination of these traits to get the desired plant (Figure 4). By combining the characteristics that avoid ice in the crown tissue (crown position below soil surface) with the characteristics that reduce foliage and root injury by ice (freezing tolerance of foliage) we may be able to obtain the desired plant.

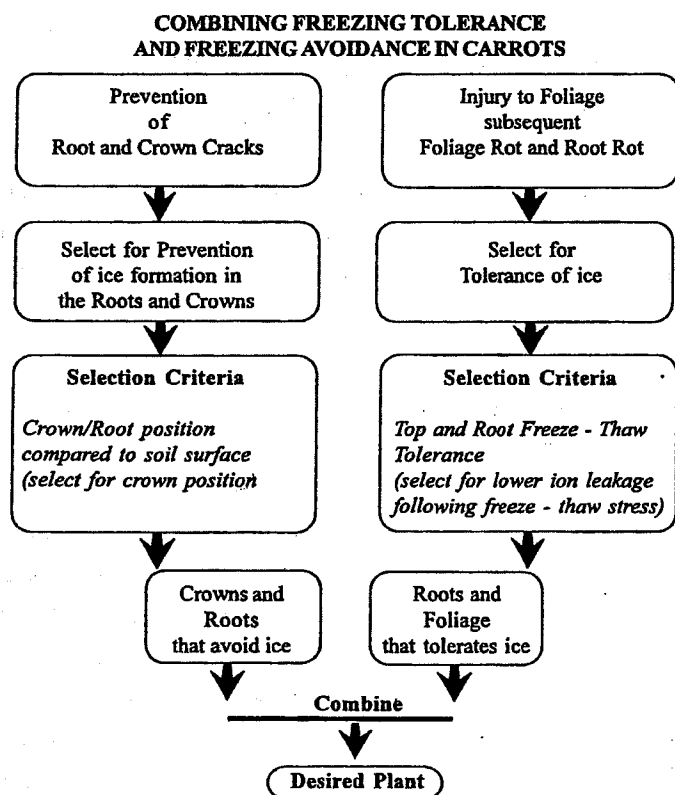


Figure 4. A flow diagram illustrating possible strategy for improving frost survival of carrot plants. Source: Palta and Simon (1993).

Genetic Variation Exists for Speeds of Acclimation and Deacclimation

Speeds of acclimation and deacclimation appear to be two of the several important factors responsible for frost and winter survival of plants. Ability of gain freezing stress resistance rapidly in response to decreasing air temperatures will certainly be advantageous for survival from unexpected very cold spell (freezing temperatures) in fall. On the other hand not able to deacclimate rapidly in response to mid-winter thaw will be of advantageous against late winter cold spells and spring freezes. Thus in addition to acclimation ability and nonacclimated tolerance it will be important to be able to select for rapid acclimation and slow deacclimation abilities. Our recent studies suggest that genetic variation for these traits exist (Sutinen et al., 1992).

We investigated seasonal changes in the freezing stress resistance of needles of red pine (*Pinus resinosa* Ait.) and Austrian pine (*Pinus resinosa* Arnold) trees (Sutinen et al., 1992). Red pine needles attained much higher freezing stress resistance during the mid-winter than Austrian pine (Figure 5). Thus red pine needles have much higher capacity to acclimate than Austrian pine even though they both have similar nonacclimated freezing stress resistance. Red pine needles also acclimated and deacclimated faster than Austrian pine needles (Figures 5 and 6). During fall there was a sharp increase in the freezing stress resistance of red pine needles whereas the freezing stress resistance of Austrian pine needles increased gradually (Figure 5). During the spring red pine needles deacclimated rapidly and its needles were less hardy than Austrian pine needles in late April (Figure 6). These results help explain why red pine is better adapted to very cold winters and shorter growing season in the northern latitudes.

Plasma Membrane Lipids Associated with Genetic Variability in Freezing Tolerance and Cold Acclimation Ability in *Solanum* Species

Plasma membrane is regarded as a key site of injury by freeze-thaw stress (Palta and Li, 1980; Stephonkus, 1984; Arora and Palta, 1988; Iswari and Palta, 1989; Palta, 1989). Since cold acclimation results in increased tolerance to freeze-thaw stress, it follows that this membrane must undergo changes to withstand a higher intensity of stresses imposed by freeze-thaw stress. Over the last 20 years changes in membrane lipids that coincide with increase in freezing tolerance during cold acclimation have been investigated. The results of early studies are highly controversial. These studies were based on the notion that an increase in the fatty acid unsaturation would increase membrane "fluidity" which could help stabilize membrane structure and function at low temperatures. This idea was extended from the purposed association of chilling injury with the lipid phase transition temperatures (Raison et al., 1971). In early studies on membrane lipid changes during cold acclimation some researchers reported an increase in the

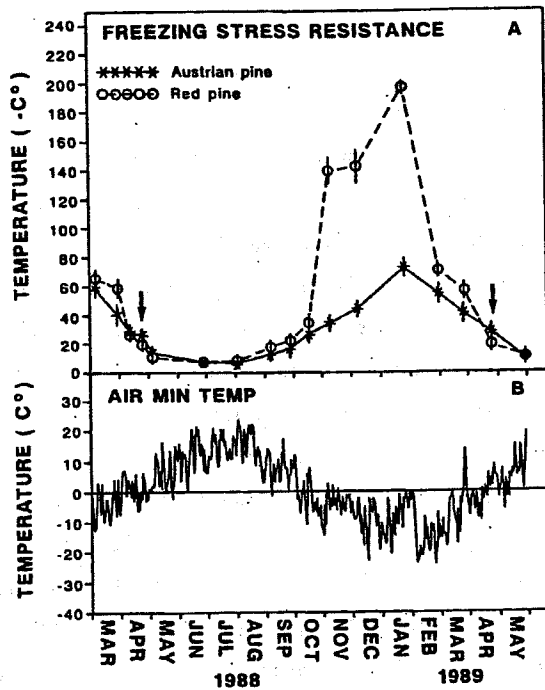


Figure 5 (A) Seasonal changes in freezing stress resistance of Austrian and red pine needles. The sampling was done between March 7, 1988 and May 31, 1989. Current-year needles were used except in June. In July, the newest needles were used when they were fully expanded. Freezing stress resistance was estimated by the electrolyte leakage method except during winter (November-February), when it was estimated by visual observation. The arrows show the period when red pine needles are deacclimating faster than Austrian pine needles. (B) The daily minimum air temperature (2 m above ground level) at the experimental site.
Source: Sutinen et al. (1992).

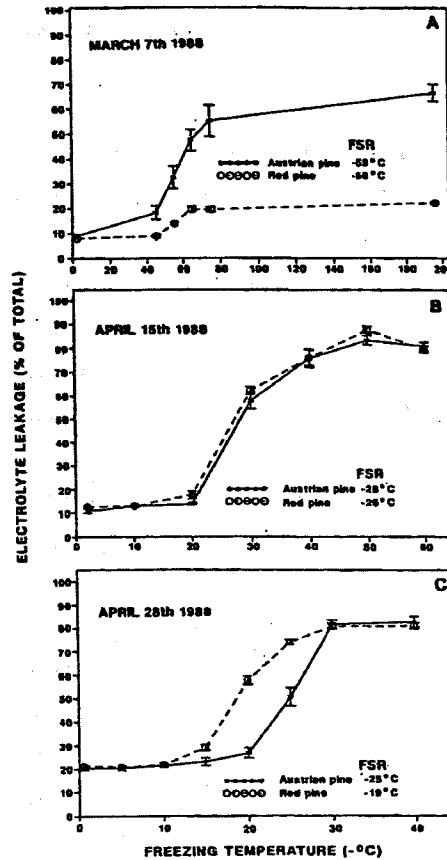


Figure 6. Changes in the relationship between percent electrolyte leakage and freezing temperatures (laboratory assay for estimating freezing stress resistance, FSR) during deacclimation in spring in Austrian and red pine needles.
Source: Sutinen et al. (1992).

proportion of unsaturated fatty acids (de la Roche et al., 1972; Grenier et al., 1972; Willemot, 1975 and 1977) while others failed to detect such changes (de la Roche et al., 1975; de la Roche, 1979; Vigh et al., 1985). These contradictory results can be explained, in part by the fact that these researchers analyzed the lipid composition of whole tissues, organelle preparation or crude membranes rather than purified membrane of interest.

Only in limited studies changes in the lipid composition during cold acclimation of purified plasma membrane has been studied (Lynch and Steponkus, 1987; Uemura and Yoshida, 1984; Yoshida and Uemura, 1984). However, in these studies it is impossible to distinguish lipid changes related to cold acclimation *per se* (i.e. increase in freezing tolerance), from lipid changes that can result from low temperature adjustment of plant metabolism. We have avoided this problem in our recent study (Palta et al., 1993a). We compared plasma membrane lipid changes during cold acclimation in genetically related potato species. In this study the cultivated potato species *Solanum tuberosum* (freezing sensitive and unable to cold acclimate) was compared with the wild potato species *Solanum commersonii* (freezing tolerant and able to cold acclimate). Comparisons were made before and after cold acclimation.

Under normal growing conditions (nonacclimated) the wild *S. commersonii* has a higher degree of freezing tolerance (-4.5°C) compared with the cultivated *S. tuberosum* (-2.5°C). Under these conditions there were some lipid compositional differences between the two species (Table II-IV). These included, a lower sterol/phospholipid ratio (Table II), lower total membrane lipid per mg membrane protein (Table II) and a higher unsaturated/saturated fatty acid ratio (Table IV) in *S. commersonii* as compared with *S. tuberosum*. In addition *S. commersonii* had a higher proportion of acylated sterol glycosides (mol % of total sterol lipid) and a lower proportion of free sterols (mol % of total sterol lipid).

Comparative studies of cold acclimating and nonacclimating species allowed us to differentiate, lipid changes associated with increased freezing tolerance following cold acclimation, from lipid changes that can result from metabolic adjustment to low temperature. Lipid changes detected only in acclimating species (*S. commersonii*) included an increase in phosphatidylethanolamine (Table III), a decrease in sterol/phospholipid ratio (Table II), an increase in linoleic acid (Table IV) and a decrease in linolenic acid (Table IV). These changes were either opposite or absent in the nonacclimating species *S. tuberosum*, suggesting an association of these plasma membrane lipid changes with cold acclimation.

Table II. Lipid composition of the plasma membrane isolated from nonacclimated (NA) and cold acclimated (CA) *Solanum tuberosum* and *Solanum commersonii* leaf tissue.
Source: Palta et al. (1993a)

Values \pm SE of three separate samplings expressed as μmol per mg membrane protein and as mole percent of total lipid.

<i>Solanum tuberosum</i>	NA	CA	NA	CA
	$\mu\text{mol} \cdot \text{mg}^{-1} \text{ protein}$		mol % of total lipid	
Phospholipid	0.53 \pm 0.05	0.55 \pm 0.02	46.4 \pm 1.5	46.8 \pm 1.2
Total Sterols	0.52 \pm 0.03	0.55 \pm 0.05	45.0 \pm 1.1	46.2 \pm 1.7
Cerebroside	0.07 \pm 0.01	0.06 \pm 0.01	6.5 \pm 1.3	5.0 \pm 0.6
MGDG	0.01 \pm 0.00	0.01 \pm 0.00	0.9 \pm 0.2	0.9 \pm 0.3
DGDG	0.01 \pm 0.00	0.01 \pm 0.00	1.3 \pm 0.3	1.1 \pm 0.1
Total	1.15 \pm 0.08	1.19 \pm 0.06		
Sterol/ Phospholipid	0.96	0.99	0.97	0.99

<i>Solanum commersonii</i>	NA	CA	NA	CA
	$\mu\text{mol} \cdot \text{mg}^{-1} \text{ protein}$		mol % of total lipid	
Phospholipid	0.41 \pm 0.03	0.50 \pm 0.03	48.3 \pm 2.0	51.1 \pm 1.4
Total Sterols	0.36 \pm 0.07	0.42 \pm 0.06	41.6 \pm 2.8	40.7 \pm 2.0
Cerebroside	0.05 \pm 0.00	0.05 \pm 0.00	6.1 \pm 0.5	4.9 \pm 0.8
MGDG	0.02 \pm 0.00	0.02 \pm 0.00	2.2 \pm 0.2	1.9 \pm 0.1
DGDG	0.02 \pm 0.00	0.01 \pm 0.00	1.8 \pm 0.2	1.4 \pm 0.2
Total	0.86 \pm 0.11	1.01 \pm 0.10		
Sterol/ Phospholipid	0.88	0.81	0.86	0.80

Table III. Phospholipid composition of the plasma membrane isolated from nonacclimated (NA) and cold acclimated (CA) *Solanum tuberosum* and *Solanum commersonii* leaf tissue.
Source: Palta et al. (1993a).

Values \pm SE of three separate samplings expressed as μmol per mg membrane protein as mole percent of total lipid, and as mole percent of total.

<i>Solanum tuberosum</i>	NA	CA	NA	CA	NA	CA
	$\mu\text{mol} \cdot \text{mg}^{-1} \text{ protein}$		mol %		mol % Total Lipid	
PC	0.20 \pm 0.01	0.20 \pm 0.00	38.4 \pm 1.4	36.6 \pm 0.7	17.7 \pm 0.1	17.2 \pm 0.7
PE	0.20 \pm 0.02	0.21 \pm 0.01	35.7 \pm 0.6	37.6 \pm 0.1	16.6 \pm 0.3	17.6 \pm 0.4
PA	0.06 \pm 0.01	0.06 \pm 0.00	10.2 \pm 1.5	10.0 \pm 0.4	4.7 \pm 0.8	4.7 \pm 0.3
LPC	0.02 \pm 0.00	0.02 \pm 0.00	4.3 \pm 0.5	4.4 \pm 0.4	2.0 \pm 0.3	2.1 \pm 0.2
LPE + PI	0.04 \pm 0.01	0.04 \pm 0.00	7.5 \pm 0.6	7.7 \pm 0.1	3.5 \pm 0.4	3.6 \pm 0.1
PG	0.02 \pm 0.00	0.02 \pm 0.00	3.9 \pm 0.2	3.6 \pm 0.5	1.8 \pm 0.1	1.7 \pm 0.2
Total	0.53 \pm 0.05	0.55 \pm 0.02	46.4 \pm 1.5	46.8 \pm 1.2		
PC/PE	0.93	1.03				

<i>Solanum commersonii</i>	NA	CA	NA	CA	NA	CA
	$\mu\text{mol} \cdot \text{mg}^{-1} \text{ protein}$		mol % total lipid		mol % phospholipid	
PC	0.18 \pm 0.01	0.19 \pm 0.01	42.6 \pm 0.2	36.3 \pm 1.5	20.6 \pm 0.9	18.5 \pm 1.0
PE	0.15 \pm 0.01	0.21 \pm 0.01	36.2 \pm 0.6	41.2 \pm 0.8	17.5 \pm 0.9	21.1 \pm 0.9
PA	0.04 \pm 0.00	0.05 \pm 0.01	9.5 \pm 1.7	9.6 \pm 2.0	4.6 \pm 1.0	4.9 \pm 1.1
LPC	0.01 \pm 0.00	0.02 \pm 0.01	2.6 \pm 0.6	3.4 \pm 0.8	1.2 \pm 0.2	1.7 \pm 0.4
LPE + PI	0.02 \pm 0.00	0.03 \pm 0.01	5.5 \pm 0.5	3.4 \pm 0.8	2.6 \pm 0.1	3.0 \pm 0.3
PG	0.02 \pm 0.00	0.02 \pm 0.01	3.7 \pm 0.9	3.7 \pm 0.8	1.8 \pm 0.4	1.9 \pm 0.4
Total	0.41 \pm 0.03	0.51 \pm 0.04			48.3 \pm 2.0	51.1 \pm 1.4
PC/PE	1.17	0.87				

Table IV. Fatty acid composition of phosphatidylcholine and phosphatidylethanolamine from plasma membrane isolated from nonacclimated (NA) and cold acclimated (CA) *Solanum tuberosum* and *Solanum commersonii* leaf tissue.

Values expressed as weight percent of total PC or PE fatty acids

Fatty Acid	<i>Solanum tuberosum</i>				<i>Solanum commersonii</i>			
	Phosphatidylcholine		Phosphatidyl-ethanolamine		Phosphatylcholine		Phosphatidyl-ethanolamine	
	NA	CA	NA	CA	NA	CA	NA	CA
16:0	36.5	32	39.9	36.8	28.2	24.1	31.7	29.5
16:1	0.6	0.6	0.5	0.4	0.7	0.7	0.5	0.9
18:0	4.3	6.1	3.6	3.8	4.2	3.8	3.2	2.8
18:1	4.7	6.5	2.2	2.7	12.3	12.1	5.2	5
18:2	34.4	33.3	35.9	38.1	33.3	39.8	40.5	43.5
18:3	18.4	19.9	15.1	15.9	19	17.1	17	16.7
20:0	0.7	1.1	2.4	1.9	1.7	1.8	1.5	1.3
18:2/18:3	1.88	1.67	2.38	2.4	1.75	2.33	2.38	2.6
18:2/16:0	0.94	1.04	0.9	1.04	1.18	1.65	1.28	1.47
Unsaturated/ Saturated	1.38	1.54	1.17	1.34	1.92	2.35	1.74	1.97

Clearly, plasma membrane lipids that were associated with the development of freezing tolerance during cold acclimation were different from lipids associated with a higher degree of freezing tolerance in the nonacclimated state. For example, although the hardy species *S. commersonii* had higher proportion of unsaturated fatty acids in the nonacclimated state, yet this ratio increased during cold acclimation in both the acclimating and nonacclimating species (Table IV). Furthermore 18:2 increased and 18:3 decreased with cold acclimation in *S. commersonii* although there were no significant differences in the contents of 18:2 or 18:3 among the two species in the non acclimated state (Table IV). These results suggest that the differences in freezing tolerance in the nonacclimated conditions and the ability to increase freezing tolerance during cold acclimation cannot be explained by the same biochemical and/or genetic mechanisms. These results are in agreement with our recent results (Stone et al., 1993) on separate genetic control for nonacclimated tolerance and capacity to acclimate (Figures 1-3).

Table V. Correlation coefficients of plasma membrane fatty acid contents purified from nonacclimated and cold acclimated leaves (generated from *S. commersonii* and *S. cardiophyllum*, their F₁ hybrid, and backcrosses to each parent) with relative freezing tolerance (RFT) in nonacclimated and acclimated states and of changes in the fatty acid contents with change in freezing tolerance following cold acclimation (cold acclimation capacity = acclimated RFT - nonacclimated RFT).

Population	Trait	Correlation coefficients		
		Fatty acid		
		16:0	18:2	18:3
Total	Nonacclimated RFT	0.65**	0.29 ^{NS}	-0.65**
	Acclimated RFT	0.20 ^{NS}	0.54**	-0.68**
	Cold acclimation capacity	-0.54**	0.45*	-0.01 ^{NS}
<i>commersonii</i>	Nonacclimated RFT	0.07 ^{NS}	0.58I	-0.79II
	Acclimated RFT	-0.20 ^{NS}	0.83**	-0.86**
	Cold acclimation capacity	-0.24 ^{NS}	0.65**	-0.06 ^{NS}
<i>cardiophyllum</i>	Nonacclimated RFT	0.70*	-0.21 ^{NS}	-0.03 ^{NS}
	Acclimated RFT	-0.36 ^{NS}	0.24 ^{NS}	-0.31 ^{NS}
	Cold acclimation capacity	-0.64*	0.33 ^{NS}	-0.17 ^{NS}

Contents of Specific Plasma Membrane Fatty Acids Co-segregate with Nonacclimated Freezing Tolerance Which are Distinct from the Fatty Acids Contents that Cosegregate with Capacity to Acclimate

As discussed above, we have recently demonstrated that the two major components of freezing tolerance (i.e. nonacclimated tolerance and capacity to acclimate) are under independent genetic control (Stone et al., 1993). We have now analyzed the plasma membrane lipid composition of these segregating *Solanum* populations. We found that relative contents of specific fatty acids correlated with specific traits of freezing stress resistance (Harbage et al., 1992; Table V). The correlation between fatty acid contents and different traits were only highly significant when backcross populations of the two parents were analyzed separately (Table V). For example 18:2 contents were correlated best in the *S. commersonii* backcross population. These results

further provide evidence that distinct biochemical and genetic mechanisms are responsible for nonacclimated freezing tolerance and acclimation capacity. Furthermore these results demonstrate that separate examination of closely related populations is necessary to establish relationships between fatty acids and components of freezing tolerance.

Plasma Membrane Linoleic Acid (18:2) Appears to Have an Important Role in Freezing Tolerance

The results of our studies suggest an interesting potential role of 18:2 in freezing tolerance. An increase in 18:2 in both the phosphatidylcholine and phosphatidylethanolamine was found only cold acclimating species *S. commersonii* (Palta et al. 1993a; Table IV). Furthermore, 18:2 contents best correlated with acclimated freezing tolerance and cold acclimation capacity (Table V). An increase in 18:2 in plasma membrane enriched fraction following cold acclimation in potato species was reported by us about five years ago (Palta and Meade, 1989). We have also found similar increases in 18:2 with increase in freezing tolerance during cold acclimation in cranberry leaves (Abdallah and Palta, 1989) and pine needles (Sutinen et al., 1989) in fall and winter. It is possible that 18:2 fatty acid has a specific interaction with key membrane enzymes such as ATPases (Palmgren et al., 1988) which are known to be sensitive to freeze-thaw stress (Palta, 1989; Iswari and Palta, 1989). The reason for this could be that 18:2 has larger molecular areas as compared to 18:0 and 18:1 and a smaller length (Cook, 1985). Thus an increase in 18:2 following cold acclimation may be responsible for imparting cryostability to plasma membrane.

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