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Genetic Analysis of Frost Hardiness Traits in Tuber-Bearing *Solanum* Species

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The inheritance of frost hardiness and cold acclimation potential traits was studied in three segregating populations derived from a cross between *Solanum commersonii Dun.* PI 243503 (*cmm*) and *Solanum cardiophyllum* Lindl., PI 184762 (*cph*), two parental genotypes with contrasting frost hardiness and cold acclimation potential. The levels of frost hardiness and cold acclimation potential were expressed as the LT_{50}, the temperature at which 50% of the cells in leaf discs were killed, as measured by the ion leakage method, following a controlled freeze test. There was considerable variation in both frost hardiness and cold acclimation potential in all three segregating populations (F_1, F_1 x *cmm*, and F_1 x *cph*). Frost hardiness and cold acclimation potential were not correlated, suggesting that these two traits are under independent genetic control. The analysis of generation means indicated that the variation for both traits could be best explained by an additive-dominance model, with additive gene effects the most important. Broad-sense heritability was 0.73 and 0.74 in the F_1 population, for frost hardiness and cold acclimation potential, respectively, and was 0.85 for either trait in the F_1 x *cmm* population, indicating that these two traits are highly inheritable. Our results suggest that it should be possible to incorporate the frost hardiness and cold acclimation traits from *S. commersonii* into cultivated potato species.

Key words: Frost injury, generation mean analysis, heritability, potato

Frosts are often the major factor limiting potato production in the temperate zone and in the highlands of the Andean Tropics of South America. In the Andean countries alone, it is estimated that over 400,000 ha of potatoes are threatened by frost injury every year (Estrada et al., 1993; Barrientos et al., 1994; Van Eck, 1995; Vega and Bamberg, 1995). The commonly cultivated potato, *Solanum tuberosum* L, possesses little frost hardiness, whereas many of the tuber-bearing *Solanum* species are much hardier (Chen and Li, 1980). Superior horticultural potential of *S. tuberosum* should be combined with the frost hardiness traits from the hardy relatives.

As the wild *Solanum* species are potential sources of frost hardiness genes, screening of many genotypes for frost hardiness has been conducted both in controlled environmental conditions (Li, 1977; Palta and Li, 1979; Chen and Li, 1980), and in field conditions (Vega and Bamberg, 1995). In these screening efforts, many *Solanum* species were identified that exhibited frost hardiness far superior to that of the cultivated species. Furthermore, potato plants have greater sensitivity to cold injury when grown under warm temperatures (>10°C), compared to when they are growing under cool temperatures (between about 0 and 10°C) (Chen and Li, 1980). In this paper we define frost hardiness as the freezing tolerance of a plant growing under warm conditions, and cold acclimation potential as the freezing tolerance of a plant after a period of cold hardening.

Among the frost hardy *Solanum* species that have been identified, *Solanum commersonii* (*cmm*) has been considered a good source of frost hardiness genes. Therefore, many attempts have been made to incorporate frost hardiness into the cultivated potato (Cardi et al., 1993a, b; Nyman and Waara, 1997). These studies have been done with only limited data on the inheritance of frost hardiness in *Solanum* species. One study investigated the inheritance of frost hardiness and cold acclimation potential in F_1 and backcross populations of *S. commersonii* (*cmm*) and *Solanum cardiophyllum* (*cph*) (Stone et al., 1993). They found that frost hardiness and cold acclimation potential traits are under independent genetic control. Furthermore, the analysis of generation means indicated that all of the variance for frost tolerance and cold acclimation capacity could be best explained by an additive-dominance model with both traits being partially recessive. From a relatively small population, they were able to recover the parental phenotypes and thus suggested that both

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traits are controlled by relatively few genes. Because the study was conducted on a very small number of genotypes (7 F_1s, 21 F_1 × *cmm* and 19 F_1 × *cph*), these results need verification using larger populations.

With the goal of constructing a molecular marker linkage map and to locate quantitative trait loci (QTL) affecting frost hardiness and cold acclimation traits in *Solanum* species, we followed the procedures of Stone et al. (1993) to produce the F_1 and backcross populations from *S. commersonii* × *S. cardiophyllum*. In this communication, we present the phenotypic variation for frost hardiness and cold acclimation potential, and we also re-examine the mode of inheritance of those two traits in these populations.

MATERIALS AND METHODS

Plant Materials

Two diploid, tuber-bearing *Solanum* species were used for this study. These were *S. commersonii* Dun (*cmm*), a frost tolerant species that possesses the ability to cold acclimate and *S. cardiophyllum* Lindl. (*cph*), a frost sensitive species which lacks the ability for cold acclimation (Chen and Li, 1980). Seeds of *S. commersonii* (PI 243503) and *S. cardiophyllum* (PI 184762) were obtained from The Inter-Regional Potato Introduction Station in Sturgeon Bay, Wisconsin. They were germinated and grown in a soil mix of #8 pumice:peat moss:clay loam soil (v/v/v 2:1:1), and the resulting seedling clones were grown to maturity in the greenhouse. Controlled pollination under greenhouse conditions was conducted to generate 73 F_1s (*cmm* x *cph*). Individual F_1 seedlings were clonally propagated by cuttings. One of the F_1 seedling clones was used to produce two backcross populations: 28 individuals of F_1 × *cmm*, and 85 individuals of F_1 x *cph*. The F_1 seeds were germinated in sand under mist conditions. Backcross seeds were sterilized, germinated, and propagated on Murashige and Skoog (1962) agar medium with no growth regulators. Plantlets propagated in vitro for each genotype, were transferred to 4″ pots with a soil mix of #8 pumice:peat moss:clay loam soil (v/v/v 2:1:1). Plants were kept on the mist bench in the greenhouse for two weeks, and were then transferred to 6″ pots with the same substrate. Plants were grown in a 20° ± 2°C greenhouse with a 14-h photoperiod and light intensity of about 180 µS^{-1}m^{-2}. All plants were fertilized every two months with Nutricote type 100 (Plant Products LTD, Brampton, Ontario, Canada), a controlled-release fertilizer.

Evaluation of Frost Tolerance

Frost hardiness and cold acclimation potential were measured for 6 *cmm* genotypes, 6 *cph* genotypes, and all individuals of the F_1 and backcross populations.

Ten-week-old plants of individual clones were divided into two groups: one group was used to measure frost tolerance without cold acclimation (for frost hardiness). The other group of plants was moved to a cold room (2°C, 21.8 µS^{-1}m^{-2}, 12-h photoperiod) for two weeks (for cold acclimation potential). Frost hardiness and cold acclimation potential were estimated by exposing leaf discs to a controlled freeze test (Sukumaran and Weiser, 1972). Briefly, fully expanded second leaves were excised and leaf discs were punched out, placed in test tubes (25 × 150 mm), and incubated in a low temperature bath (model LT-50DD, NESLAB Instruments, Newington, NH). When the bath temperature reached −1°C, ice nucleation was initiated by adding a small piece of ice to each tube. The samples were held at −1.5°C for an additional 1 h., and then the temperature was lowered at a rate of 1°C/h for non-acclimated samples, and at 2°C/h for cold acclimated samples. Tubes were removed from the cooling bath at a specific temperatures, and were thawed in a refrigerator overnight.

Freezing injury was estimated by the ion leakage method (Sukumaran and Weiser, 1972). Freezing tolerance was expressed as LT$_{50}$, defined as the temperature at which 50% of the cells were killed. Thawed leaf discs were transferred to individual vials containing 10 mL of deionized water at room temperature. Samples were vacuum infiltrated for 10 min at 25 in. Hg. Afterwards, samples were shaken for 1 h and conductivity was measured with a YSI model 35-conductance meter (Yellow Spring, OH, USA). Once the conductivity was measured, all samples were frozen in a −20°C freezer for 24 h, thawed at room temperature, shaken for 1 h, and then the total conductivity for each sample measured. Percent ion leakage was calculated from triplicate samples, the results were plotted as a function of freezing temperature. The experiment was repeated three times for all genotypes.

Estimation of Genetic Parameters and Heritability

The means and variances of frost hardiness and cold acclimation potential were calculated for a group of seedlings of parental PIs, F_1, and 2 backcross generations. By using the LT_{50} of individual F_1, $F_1 \times$ *cmm*, and $F_1 \times$ *cph* populations, phenotypic correlation coefficients for both frost tolerance and cold acclimation potential were calculated, and their significance was evaluated by a t-test. The result from a Bartletts test for variance homogeneity (Sokal and Rohlf, 1981) indicated that the population variances were homogeneous for the cold hardiness trait but not for cold acclimation potential. The Joint Scaling test of Mather and Jinks (1982) was used to estimate the genetic parameters: **m** which is the midpoint between parental means, [**d**] the additive gene effects, and [**h**] the dominance gene effects. These genetic components were calculated through the Joint Scaling test, with weighted least squares (WLS), a multiple linear regression, using the reciprocals of the squared standard errors of each mean as weights (Mather and Jinks, 1982). The S-PLUS program for Windows was then used to resolve the information matrix produced. The squared root of the diagonal products of the inverted matrix was employed to calculate the standard error of the genetic parameters. With these genetic estimates, an expected mean was calculated for each generation. The same results were obtained by calculating those genetic parameters following the Beaver and Mojisdis (1988) least squared regression method. The goodness of fit for the additive-dominance model for frost hardiness was calculated as $X^2_{k\text{-}p} = \Sigma_{k\text{-}p}(O\text{-}E)^2 \times W$ (Mather and Jinks, 1982). Since the variance homogeneity test yielded different results for cold acclimation potential, the formula $X^2_{k\text{-}p} = (Y\text{-}CM)'\, SN^{-1}\, (Y\text{-}CM)$ was used for estimating the goodness of fit of the model, following the Beaver and Mojisdis (1988) notation and method. The degree of dominance of both traits for the three segregating generations was calculated as the h/d ratio, where −1 or +1 corresponds to complete dominance towards the least or most hardy parent, respectively, and 0 represents additive rather than dominance effects. In addition, the broad sense heritability (h^2) for the F_1, which is one of the sources of phenotypic variation in a pseudo-testcross, and backcross populations was calculated for both frost hardiness and cold acclimation potential according to Poehlman and Sleper (1996) as:

$$h^2 = \frac{\sigma^2(\text{segregating population}) - \sigma^2(\text{parental clones})}{\sigma^2(\text{segregating population})}$$

RESULTS

Frost Hardiness and Cold Acclimation Potential

As shown in Table 1, the means for frost hardiness of the parental genotypes were −5.7 ± 0.2°C for *cmm* and −2.8 ± 0.1°C for *cph*, while cold acclimation potential was −10.2 ± 0.1°C and −3.5 ± 0.2°C for *cmm* and *cph*, respectively. In the F_1 population, the mean frost hardiness was −4.3 ± 0.1°C, which was exactly the mean of the two parental genotypes. Cold acclimation potential for the F_1 population was −6.4 ± 0.1°C , which was very close to the mean of the two parental genotypes. When the backcross of F_1 x *cph* was evaluated, means of the whole population were −3.4 ± 0.1°C and −5.0 ± 0.1°C for frost hardiness and cold acclimation potential, respectively. Means of the backcross $F_1 \times$ *cmm* for the same traits were −4.8 ± 0.2°C and −7.9 ± 0.3°C, respectively.

Table 1. Frost hardiness and cold acclimation potential mean calculated for *Solanum commersonii* and *S. cardiophyllum*, the F_1 between these two species and backcrosses to the parents.

Generation	N	Frost Hardiness (LT_{50}, °C)		Cold Acclimation Potential (LT_{50}, °C)	
		Observed Mean	Expected[1] Mean	Observed Mean	Expected[1] Mean
cmm	6	−5.7±0.2	−5.6	−10.2 ± 0.1	−10.1
$F_1 \times$ *cmm*	28	−4.8±0.2	−4.9	−7.9±0.3	−8.7
F_1	73	−4.3±0.1	−4.2	−6.4±0.1	−7.3
$F_1 \times$ *cph*	85	−3.4±0.1	−3.4	−5.0±0.1	−5.5
cph	6	−2.8±0.1	−2.6	−3.5±0.2	−3.6
Goodness of fit		P > 0.05		P > 0.05	
R^2		0.999		0.991	

[1] Expected means were calculated by the weighted least squared regression (WLS).
Values are presented with ±EM.

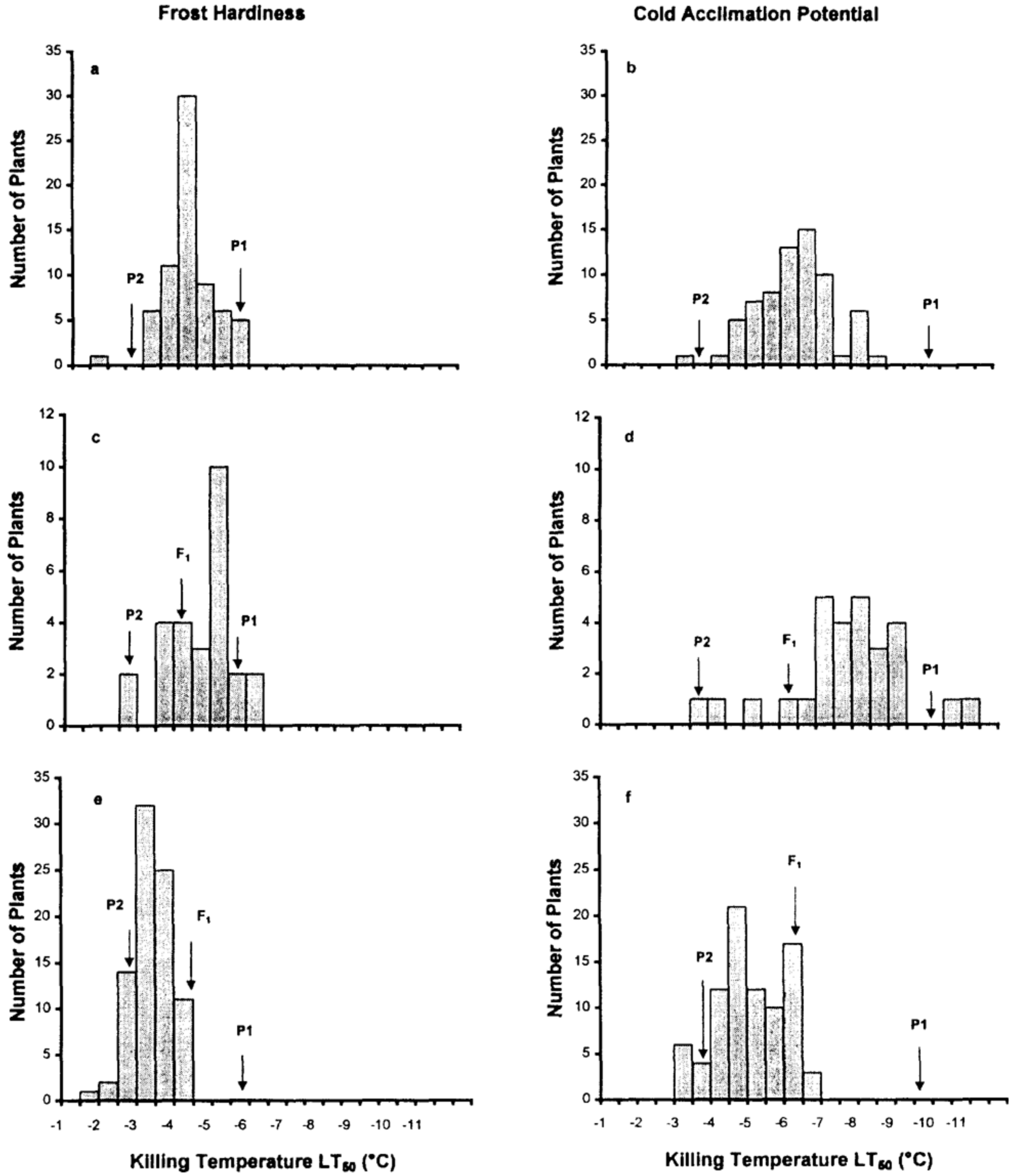

Figure 1. Distribution of progeny derived from a cross between *S. commersonii* (*cmm*, P1) and *S. cardiophyllum* (*cph*, P2) for frost hardiness (a, c, and d) and cold acclimation potential (b, d, and f). a & b : F_1 (*cmm* × *cph*); c & d : F_1 × *cmm*; and e & f. F_1 × *cph*.

Table 2. Correlation analysis between frost hardiness and cold acclimation potential in segregating populations of a cross between *S. commersonii* and *S. cardiophyllum*, and backcrosses of the F_1 to the parents.

Generation	r
F_1	0.06^{ns}
F_1 x *cph*	0.05^{ns}
F_1 x *cmm*	0.03^{ns}

^{ns} = Not significant at P = 0.05

Table 4. Broad sense heritability of frost hardiness and cold acclimation potential for a cross between *S. commersonii* and *S. cardiophyllum*, and backcrosses of the F_1 to the parents.

Generation	Broad Sense Heritability	
	Frost Hardiness	Cold Acclimation Potential
F_1	0.73	0.74
F_1 x *cmm*	0.85	0.85
F_1 x *cph*	0.45	0.45

The distribution of the frost hardiness (a) and cold acclimation potential (b) of the F_1 population is shown in Figure 1. The majority of the F_1 individuals fell between the two parental genotypes for both traits. In the backcross population of F_1 × *cmm* (Fig. 1, c and d), the distribution of both traits shifted to more negative values (i.e. hardier), whereas the backcross population of the F_1 × *cph* (Fig. 1, e and f) tended to shift to less negative values (i.e. more tender). Similar results were reported from backcross populations in blueberry (Arora et al., 1997). In addition, some transgressive segregants were observed in all segregating populations, with a higher number of them in the backcross populations (Fig. 1, c–f).

By using the individual LT_{50}s from all segregating populations, a correlation analysis of frost hardiness vs. cold acclimation potentials gave rise to very low and non-significant r-values (Table 2).

Genetic Analysis of Frost Hardiness and Cold Acclimation Potential Traits

According to Stone et al. (1993), the additive-dominance model proposed by Cavalli (1952), could be used to explain the inheritance of frost hardiness and cold acclimation potential in *Solanum* species. The model includes an estimation of the mean, additive, and dominance (m, [d], and [h], respectively) genetic parameters, calculated through the Joint Scaling test with weighted least squares (WLS) (Mather and Jinks, 1982). Based on these parameters (Table 3), the expected means for all populations in both traits were calculated (Table 1). The results indicated that both observed and expected means were not significantly different from each other. The significance of these genetic parameters for each trait was analyzed by a t-test. We found non-significance for [h], which is the dominance component of the model. Since [h] was not significant for either trait, a verification of the usefulness of the additive-dominance model was performed using the data presented in Table 1. The goodness of fit for each trait, following the Beaver and Mosjidis (1988) and Mather and Jinks (1982) methodologies, indicated that the model was appropriate. Moreover, the very high values of R^2 calculated for both traits corroborated the appropriateness of the model to explain the variance in each trait. Also, as a way to verify the results in Table 3, the degree of dominance was calculated following Mather and Jinks (1982). The h/d ratios for each generation in each trait were very low (0.05-0.36) indicating that additive gene effects explain a large portion of the genetic variation.

Heritability

We calculated broad sense heritability using the variances of the parental clones, as an estimate of the environmental variance, and the three individual segregating populations (F_1, F_1 × *cmm*, and F_1 × *cph*), as the source of variation. As shown in Table 4, the

Table 3. Estimates of genetic parameters for frost hardiness and cold acclimation potential (LT_{50}, °C) from segregating populations from the cross of *S. commersonii* by *S. cardiophyllum*

Genetic Parameter	Components of stress tolerance	
	Frost Hardiness	Cold Acclimation Potential
m (mean value)	$4.1 \pm 0.1^{**}$	$6.8 \pm 0.1^{**}$
[d] (additive gene effects)	$1.5 \pm 0.1^{**}$	$3.2 \pm 0.1^{**}$
[h] (dominance gene effects)	0.1 ± 0.1^{ns}	-0.5 ± 0.2^{ns}

**= $P < 0.01$, ^{ns} = Not significant at P = 0.05.

broad sense heritability values for frost hardiness were 0.73, 0.85, and 0.45 for F_1, F_1 × *cmm*, and F_1 × *cph* population, respectively; and was 0.74, 0.85, and 0.45 for cold acclimation potential for F_1, F_1 × *cmm*, and F_1 × *cph* population, respectively.

DISCUSSION

There was a clear difference in LT_{50} between non-acclimated and acclimated plants of a particular genotype, and also between individual genotypes. The test was repeated three times, and the variation among runs was quite small (<13%). This small variation was also reflected in the very small individual standard errors. This is probably due to the sampling procedure we used and the controlled environment where our plant material was grown. As a result there was an increase in the precision of the genetic estimates ($R^2 = 0.99$) and the inferences made from the results obtained, suggesting that the methodology we used for assessing frost tolerance was reliable and reproducible. Moreover, our LT_{50} values from controlled freezing of leaf discs was also consistent with the frost hardiness data obtained from field grown plants where *cmm* is one of the five most hardy *Solanum* species, and *cph* is one of the most frost susceptible species (Vega and Bamberg, 1995).

The inheritance of non-acclimated freezing tolerance (equal to frost hardiness) and acclimation capacity (equal to cold acclimation potential minus frost hardiness) was previously reported (Stone et al., 1993). However, the populations used were relatively small (7 F_1s, 21 F_1 × *cmm* and 19 F_1 × *cph*). Our results support the conclusions that frost hardiness and cold acclimation potential are under independent genetic control, and relatively few genes control these traits. Nonetheless, there are also some differences between our results and those reported by Stone et al. (1993). First, there was a significant variation in the F_1 generation for both traits in our study. This is not surprising since both parental clones used in this study are highly heterozygous. Second, we found that the generation mean for the F_1 was almost at the midpoint of the two parents, whereas Stone et al. (1993) found that the F_1 mean was closer to the *cph* parent. Third, in the analysis of genetic parameters, our results indicated that additive gene effects could explain most of the phenotypic variation, whereas Stone et al. (1993) concluded that both traits were partially recessive.

One simple explanation for the difference could be the much larger population sizes used in this study. Furthermore, in the case of cold acclimation potential, we used the LT_{50}s of cold acclimated plants. It may not be equivalent to the cold acclimation capacity, which is the net increase in frost hardiness after a fixed duration of cold conditioning. The difference in the terms used to express the relative level of frost tolerance after cold acclimation may also explain why our conclusions are different.

According to our results, there was a very low correlation between frost tolerance and cold acclimation potentials (Table 2). This suggests that different genes control the expression of these two traits. Similar results were obtained by Stone et al. (1993) in *Solanum* species, as well as by Teutonico et al. (1995) in oilseed *Brassica*.

An additive-dominance model adequately explains the gene action of frost hardiness and cold acclimation. Additive gene effects rather than dominance effects explain the majority of the phenotypic variation. Tibbits et al. (1991) also observed similar results in inter-specific hybrids of pine, based on h/d ratios. Theoretically, an h/d = 0 indicates that the variance is totally due to additive gene effects, and an h/d = 1 indicates that the variance is totally due to dominant gene effects. In our research, all h/d ratios for both traits were <0.37, indicating that frost hardiness and cold acclimation capacity traits are inherited in a predominantly additive manner. Looking at the high broad-sense heritability values, it is possible to infer that the narrow-sense heritability values should also be high, further suggesting the additive inheritance of both traits. This conclusion is also supported by the results of Tibbits et al. (1991). Moreover, these results also imply that both of these two traits are controlled by more than one gene.

The polygenic inheritance of frost hardiness has been reported in plant species including Pine (Norell et al., 1986); and Brassica (Teutonico et al., 1995). Based on the observation that parental genotypes could be recovered from relatively small populations, Stone et al. (1993) concluded that only a few genes are controlling these two traits. Our results agree with theirs.

In the past decade, *cmm* has been the major source of frost hardiness genes for improving the frost tolerance of the cultivated potato (Nyman and Waara, 1997). Since the heritability values for the F_1 population are 0.73 and 0.74 for frost hardiness and cold acclimation potential, respectively, and 0.85 for either trait in the backcross of F_1 × *cmm*, reasonable advance in the introgression of both traits to the cultivated potatoes should be possible. Thus, information on the inheritance of frost hardiness traits will be useful for breeding efforts to transfer those traits to the cultivated potato. It will also serve as the foundation for further work on QTL analysis of frost hardiness-related traits. Based on our segregating population, a molecular marker linkage map has been constructed. The phenotypic data and the marker linkage map are being used for QTL analysis. Such information will be essential for advancing our understanding of the molecular mechanism of cold acclimation and for developing a better breeding strategy for improving the frost tolerance of cultivated potato.

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