

Expression of nonacclimated freezing tolerance and cold acclimation capacity in somatic hybrids between hardy wild *Solanum* species and cultivated potatoes

Yu-Kuang H. Chen¹, Jiwan P. Palta^{1,*}, John B. Bamberg², Heiyoung Kim³, Geraldine T. Haberlach⁴ & John P. Helgeson⁴

¹Department of Horticulture, University of Wisconsin, Madison, WI 53706, U.S.A.; ²USDA/Agricultural Research Service, US Potato Genebank, 4312 Hwy 42, Sturgeon Bay, WI 54235, U.S.A.; ³Department of Agrobiolgy, Dongguk University, Seoul, Korea; ⁴Department of Plant Pathology and USDA/Agricultural Research Service, University of Wisconsin, Madison, WI 53706, U.S.A.; (*author for correspondence)

Received 15 June 1998; accepted 18 November 1998

Key words: freezing tolerance, potato, *Solanum brevidens*, *Solanum commersonii*, *Solanum tuberosum*, somatic hybrids

Summary

The expression of freezing tolerance was characterized in interspecific somatic hybrids between *S. tuberosum* (tbr) and two cold-hardy wild species, *S. brevidens* (brd) and *S. commersonii* (cmm). The nonacclimated freezing tolerance (NA) and acclimation capacity (ACC, increase in freezing tolerance in response to low nonfreezing temperature), two main genetic components of freezing tolerance, were evaluated separately. In contrast to cmm, which exhibited excellent NA and ACC, the freezing tolerance of brd was mainly due to ACC. However, the ACC of brd was only moderately expressed in the somatic hybrids. The NA of cmm was also suppressed in combination with tbr genomes. However, with acclimation, some of the tbr (+) cmm somatic hybrids achieved freezing tolerance comparable to pure hardy species such as brd used in this study. Analysis of chloroplast DNA type by RFLP markers revealed no significant difference in ACC between somatic hybrids carrying chloroplasts from either tbr or cmm. The reasons for the reduced expression of freezing tolerance from either the brd or cmm parent and the utilization of these somatic hybrids in breeding programs are discussed.

Abbreviations AA – acclimated freezing tolerance; ACC – acclimation capacity; NA – nonacclimated freezing tolerance

Introduction

Somatic hybrids between *Solanum tuberosum* (tbr) and diploid wild species *S. brevidens* (brd) and *S. commersonii* (cmm) have been produced to incorporate valuable traits from these wild species into cultivated potatoes (Austin et al., 1985, 1986; Kim et al., 1993). In addition to disease resistances (Austin et al., 1985, 1988; Helgeson et al., 1986; Kim et al., 1993), the wild species, brd and cmm, exhibit remarkable freezing tolerance (Ross & Rowe, 1965; Li & Palta, 1978; Palta & Li, 1979; Vega & Bamberg, 1995). Since somatic

hybrids with potato are now available, it is possible to assess the expression of freezing tolerance in interspecific hybrids with hardy-to-sensitive genomic ratios which are not easily synthesized by sexual crosses. To realize the breeding potential of these somatic hybrids, the expression of freezing tolerance also needs to be characterized.

While most studies in the inheritance of freezing tolerance have concluded that freezing tolerance is a complex trait of multigenic nature (Richardson & Weiser, 1972; Palta & Simon, 1993; Palta, 1994), there is no consensus on the mode of gene action

controlling the expression of this trait. Both dominant (Mastenbroek, 1956; Vavilova, 1978; Tiwari & Garg, 1982) and recessive (Stone et al., 1993; Tucci et al., 1996) effects have been reported in potatoes. In this regard, somatic hybrids offer a means to examine the dominance-recessive relationship for the trait of interest (Craig et al., 1994). Since chromosomal changes such as deletions (Williams et al., 1993) and aneuploids can occur during the production of somatic hybrids, an extensive examination of somatic hybrids with additional information such as chromosome number is helpful to alleviate the disadvantage of this approach.

Many differential changes occur within the cytoplasm of hardy and less hardy species in response to low temperature, including ultrastructure (Kimball & Salisbury, 1973; Li & Palta, 1978) and membrane lipid composition (Palta et al., 1993; Uemura & Steponkus, 1994). Thus, cytoplasmic effect may be involved in the expression of freezing tolerance. Although some studies have indicated that cytoplasm has no significant effect on freezing tolerance in wheat (Gullord et al., 1975; Limin & Fowler, 1984; Sutka, 1994), its importance in potatoes is still not clear. Owing to the early segregation of chloroplasts into the two parental types during regeneration (Lössl et al., 1994), typically only one of parental chloroplast types is present in mature regenerants. Therefore, somatic hybrids are one way that cytoplasmic effects based on the chloroplast genome may be revealed.

Freezing tolerance is composed of at least two independent genetic components, nonacclimated freezing tolerance (NA) and acclimation capacity (ACC) (Stone et al., 1993). While NA is the ability to survive freezing temperature when grown under normal conditions, ACC is the ability to increase the freezing tolerance after a period of low nonfreezing temperature exposure. Most studies to date on characterizing the genetic expression of freezing tolerance have not distinguished between these two components.

Freezing tolerance has been characterized on both tbr (+) brd and tbr (+) cmm somatic hybrids generated in different laboratories (Preisner et al., 1991; Cardi et al., 1993; Nyman & Waara, 1997). In these somatic hybrids, some fertile and hardy material was identified. However, it is very difficult to correlate directly the published results obtained by different freezing protocols to our series of studies aimed at understanding the genetic control of freezing tolerance. For example, no distinction was made between NA and ACC in the case of tbr (+) brd somatic hybrids.

In addition, potted plants were frozen and visually scored for injury and recovery (Preisner et al., 1991). This method is qualitative and does not allow one to distinguish small but significant differences among genotypes. Here we report the results of evaluation of NA and ACC on both tbr (+) brd and tbr (+) cmm somatic hybrids produced by Austin et al. (1985, 1986) and Kim et al. (1993), respectively. Information is also presented on the direction of dominance of genes controlling freezing tolerance and the influence of chloroplast DNA type on freezing tolerance.

Materials and methods

The somatic hybrids used in this study were: (1) Two hexaploid tbr (+) brd somatic hybrids: A206 and A937 were obtained from a fusion between brd (PI 218228, $2n = 2x = 24$) and tbr (PI 203900, $2n = 4x = 48$) (Austin et al., 1986). (2) Four tetraploid tbr (+) brd somatic hybrids: A4068 and A4324 were obtained from a fusion between brd (PI 218228, $2n = 2x = 24$) and *S. tuberosum* 77-19 ($2n = 2x = 24$); A704 and A1200 were generated by fusing brd (PI 245763, $2n = 2x = 24$) with *S. tuberosum* 77-1 ($2n = 2x = 24$) (Austin et al., 1985). Both *S. tuberosum* 77-19 and 77-1 were diploid lines derived from a *S. tuberosum* Gp. Phureja-Stenotomum population by H. De Jong, Agriculture Canada, Fredericton, New Brunswick. (3) Six tetraploid tbr (+) cmm somatic hybrids: all clones were obtained from a fusion between cmm (PI 320266, $2n = 2x = 24$) and a haploid clone of tbr cv Superior (US-W 13122, $2n = 2x = 24$) provided by S. J. Peloquin at the University of Wisconsin, Madison (Kim et al., 1993). Chromosome numbers of both hexaploid tbr (+) brd somatic hybrids and tetraploid tbr (+) cmm somatic hybrids have been verified by root-tip chromosome counts (Helgeson et al., 1993; Kim et al., 1993). Plantlets obtained from *in vitro* culture were transferred to 8 liter pots containing Jiffy Mix (Jiffy products of America, Inc., W. Chicago, IL) and grown in a controlled environment room at the University of Wisconsin Biotron facility (Madison, WI). After 6 to 7 weeks at 20/18 °C light/dark with a 14-hr photoperiod and about 400 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, terminal leaflets from fully expanded leaves were excised for NA assay. To achieve cold acclimation, temperature was lowered to 4/2 °C light/dark with 14 hr light at about 100 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ for an additional two weeks prior to collecting the leaflets. These conditions were previously shown to result in full acclimation

in tuber-bearing *Solanum* species within two weeks (Steffen & Palta, 1986).

Freezing tolerance of the plant materials was determined by the protocol of Steffen et al. (1989) with modification. Leaflets were placed in covered test tubes (25 × 200 mm) and submerged in a glycol bath (Forma Scientific, Model 2323, Marietta, Ohio) at 0 °C except samples for measuring ion leakage at 0 °C which were directly put on ice without subjecting them to a freeze-thaw treatment. After 30 min, the temperature in the glycol bath was lowered to -0.5 °C and held for 30 min. Then the temperature was lowered to -1 °C and held for 1 hr. A small piece of ice was added to each tube for initiating ice nucleation after 30 min at -1 °C. Thereafter, the temperature was lowered to -1.5 °C and also held for 1 hr. Further cooling below -1.5 °C was at a rate of 0.5 °C every 30 min until -7 °C, and 1 °C every 30 min below -7 °C. Tubes were removed from the freezing bath at predetermined temperatures and thawed on ice overnight prior to evaluation of injury. At each temperature three replications were evaluated.

Freezing injury was assessed by measuring ion leakage (Steffen et al., 1989) with a YSI conductance meter (Yellow Springs, OH). Thawed leaflets were sliced into 5 mm strips, suspended in 25 ml of distilled water, infiltrated for 6 min by using a vacuum pump, and then shaken for 1 hr before conductivity readings (R_1) were taken. The maximum conductivity (R_2) representing total ion content for each sample was determined after autoclaving for 15 min at 121 °C. Percent ion leakage at each temperature was obtained as $(R_1/R_2) \times 100\%$. The freezing curve was constructed by plotting mean percent ion leakage of three subsamples vs freezing temperature (see Figure 1). The freezing tolerance for each test clone was calculated from its respective freezing curve by determining the temperature at which the midpoint of the maximum and minimum ion leakage values occurred (Sutinen et al., 1992; Stone et al., 1993). The difference between NA and freezing tolerance after acclimation (AA) was defined as ACC. Since ACC is the gain in freezing tolerance after cold acclimation, it is always expressed as a positive value.

For chloroplast type analysis, total cellular DNA was isolated from frozen young leaves as described by Gill et al. (1991). The DNA was digested with *EcoRV* restriction enzyme, separated on 0.9% agarose gel, and then blotted to a Zeta-probe GT membrane (Bio-Rad). The S6 chloroplast DNA probe from *Petunia* (Sytsma & Gottlieb, 1986) was used to distin-

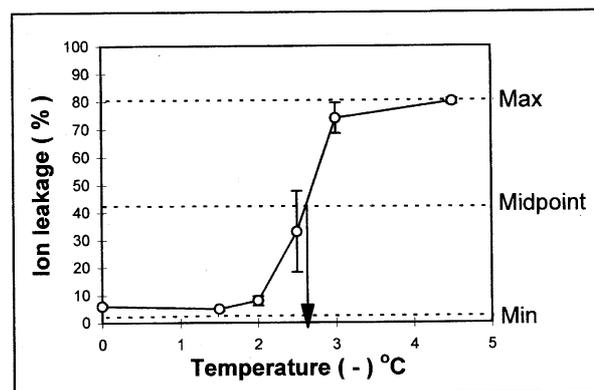


Figure 1. Determination of freezing tolerance based on ion leakage measurements after freezing excised leaves to a series of temperatures: An example from a *S. brevidens* clone (PI 218228) before acclimation. Vertical bars represent SD for three subsamples at each temperature. The freezing tolerance of this genotype (arrow) was deduced from the midpoint of the maximum and minimum ion leakage values.

guish the chloroplast contribution of *S. tuberosum* and *S. commersonii* based on the results of Spooner and Castillo-T. (1997).

Results

Somatic hybrids between tbr and brd

Before acclimation, the freezing tolerance of brd PI 218228 was -2.4 °C and PI 245763 was -2.8 °C (Table 1), which is within the general range of sensitive species (Palta and Li, 1979; Chen and Li, 1980). However, the freezing tolerance of both brd clones increased more than 2 °C with acclimation (Table 1), indicating the freezing tolerance of brd was derived mainly from its ACC. Regardless of the ability of brd to acclimate, none of the somatic hybrids displayed ACC appreciably different from tbr (Table 1). Therefore, little of the ACC from brd was expressed in these somatic hybrids.

Somatic hybrids between tbr and cmm

Even though the differences in the NA between cmm and tbr parents used for somatic hybridization were about 2 °C (Table 2), NA of the somatic hybrids was not significantly different from that of tbr (Table 2). In contrast to tbr (+) brd somatic hybrids, however, the tbr (+) cmm somatic hybrids expressed ACC significantly higher than the sensitive tbr parent but clearly

Table 1. Nonacclimated freezing tolerance (NA) and acclimation capacity (ACC) of *S. tuberosum* (tbr) (+) *S. brevidens* (brd) somatic hybrids and fusion parents

| Clone | Ploidy | (°C) | |
|---|--------|-------------------------|------------------|
| | | NA | ACC ^a |
| I. Hexaploid somatic hybrids | | | |
| Parents: | | | |
| tbr (PI 203900) | 4x | -2.2 ± 0.1 ^b | 1.2 ± 0.1 |
| brd (PI 218228) | 2x | -2.4 ± 0.3 | 2.7 ± 0.2 |
| Somatic hybrids | | | |
| A206 | 6x | -2.0 ± 0.3 | 1.3 ± 0.4 |
| A937 | 6x | -2.5 ± 0.3 | 1.2 ± 0.1 |
| II. Tetraploid somatic hybrids ^c | | | |
| (1) Parents: | | | |
| tbr (De Jong 77-19) | 2x | -2.2 | 0.2 |
| brd (PI 218228) | 2x | -2.4 | 2.5 |
| Somatic hybrids: | | | |
| A4068 | 4x | -2.5 | 0.7 |
| A4324 | 4x | -2.7 | 0.0 |
| (2) Parents: | | | |
| tbr (De Jong 77-1) | 2x | -2.4 | 0.5 |
| brd (PI 245763) | 2x | -2.8 | 2.1 |
| Somatic hybrids: | | | |
| A704 | 4x | -2.6 | 0.8 |
| A1200 | 4x | -2.6 | 0.8 |

^aACC is the increase in freezing tolerance added by acclimation and is calculated as: |AA - NA|.

^bValues are the means of two determinations ± SD.

^cOnly one determination was done for tetraploid tbr(+)brd somatic hybrids.

lower than the cmm parent (Table 2). It was also noticed that half of the tbr (+) cmm somatic hybrids exhibited AA (the additive performance of NA and ACC) beyond -5 °C (Table 2), which was the hardiness level comparable to some pure hardy species such as brd used in this study. As an example to demonstrate the relative change in freezing tolerance before and after acclimation, the freezing curves of the self-compatible clone, HA26-5, and the fusion parents were presented in Figure 2. While the nonacclimated freezing curve of HA26-5 was closer to that of tbr, the acclimated freezing curve of this somatic hybrid was separated more from that of tbr and was more centrally located between the parental curves. The ion leakage data of HA26-5 after acclimation were always lower than that of tbr parent up to -5 °C (Figure 2).

Analysis of chloroplast DNA showed that four of the somatic hybrids had the tbr chloroplast genome, while the other two had the chloroplast type of cmm (Figure 3; Table 2). Neither mixtures of banding pat-

tern from both fusion parents nor occurrence of new bands indicating a recombination event was detected (Figure 3). However, no significant difference in ACC was found between somatic hybrids with either cmm or tbr type of chloroplast DNA ($p = 0.97$).

Discussion

Based on visual inspection of the damage and the recovery of plants after subjecting acclimated plants to different freezing temperature, Preiszner et al. (1991) indicated that the two hexaploid tbr (+) brd somatic hybrids produced by their group exhibited an intermediate degree of freezing tolerance as was compared to the parents. With separate evaluation of NA and ACC, we found that the freezing tolerance in brd mainly came from ACC and the difference in ACC between hexaploid somatic hybrids and tbr was not significant. In addition to the materials tested in the

Table 2. Nonacclimated freezing tolerance (NA), acclimation capacity (ACC), and chloroplast DNA type of *S. tuberosum* (tbr) (+) *S. commersonii* (cmm) somatic hybrids and fusion parents

| Clone | Ploidy | (°C) | | Chloroplast DNA type |
|------------------|--------|-------------------------|------------------|----------------------|
| | | NA | ACC ^a | |
| Parents: | | | | |
| tbr (US-W13122) | 2x | -2.4 ± 0.2 ^b | 0.6 ± 0.0 | tbr |
| cmm (PI 320266) | 2x | -4.2 ± 0.1 | 3.8 ± 0.0 | cmm |
| Somatic hybrids: | | | | |
| HA 04-7 | 4x | -2.5 ± 0.1 | 1.4 ± 0.5 | tbr |
| HA 05-1 | 4x | -2.8 ± 0.3 | 2.6 ± 0.3 | tbr |
| HA 09-3 | 4x | -2.7 ± 0.0 | 2.4 ± 0.2 | cmm |
| HA 21-3 | 4x | -2.3 ± 0.1 | 2.0 ± 0.1 | cmm |
| HA 21-5 | 4x | -2.2 ± 0.1 | 1.8 ± 0.3 | tbr |
| HA 26-5 | 4x | -2.6 ± 0.0 | 2.9 ± 0.3 | tbr |

^aACC is the increase in freezing tolerance added by acclimation and is calculated as: |AA - NA|.

^bValues are the means of two determinations ± SD.

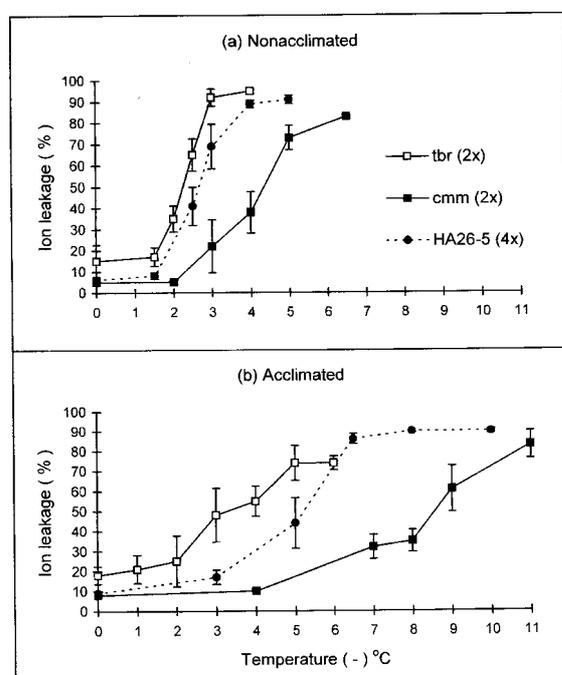


Figure 2. Nonacclimated (a) and acclimated (b) freezing curves of one *S. tuberosum* (tbr) (+) *S. commersonii* (cmm) somatic hybrid, HA26-5, and its fusion parents. Vertical bars represent SD for three subsamples at each temperature.

present study that are different from theirs, the difference found could be due to the freezing protocols used. Cardi et al. (1993) examined the freezing tol-

erance of one tetraploid tbr (+) cmm somatic hybrid and reported that the freezing tolerance determined by electrolyte leakage for tbr, cmm, and the hybrid clone was 0 °C, -3.8 °C, and -0.7 °C before acclimation and -0.8 °C, -9.8 °C, and -3.3 °C after acclimation, respectively. Although they suggest that their hybrid clone showed a higher freezing tolerance than the sensitive parent with or without acclimation, the freezing tolerance of the hybrid even with acclimation was still relatively low compared to the hardy parent. The improvement in this hybrid over the potato cultivars indeed was quite limited. However, more promising tbr (+) cmm somatic hybrids with AA comparable to pure hardy species were identified in our study. According to our results, the hardiness for tbr and cmm were -2.4 and -4.2 °C before acclimation and -3.0 and -8.0 °C after acclimation, respectively (Table 2). The hardiness level of somatic hybrids ranged from -2.2 to -2.8 °C before acclimation and -3.9 to -5.5 °C after acclimation (Table 2). Similar to our work, some of the tbr (+) cmm somatic hybrids evaluated by Nyman & Waara (1997) are also quite hardy.

By using the somatic hybrids of sensitive *Hibiscus rosa-sinensis* and hardy *Lavatera thuringiaca*, it has been shown that freezing tolerance is recessive to sensitivity (Vazquez-Thello et al., 1996). Analogously, the cold hardiness (which is similar to AA mentioned in the present study) from rye or crested wheatgrass (*Agropyron cristatum*) was also poorly expressed in

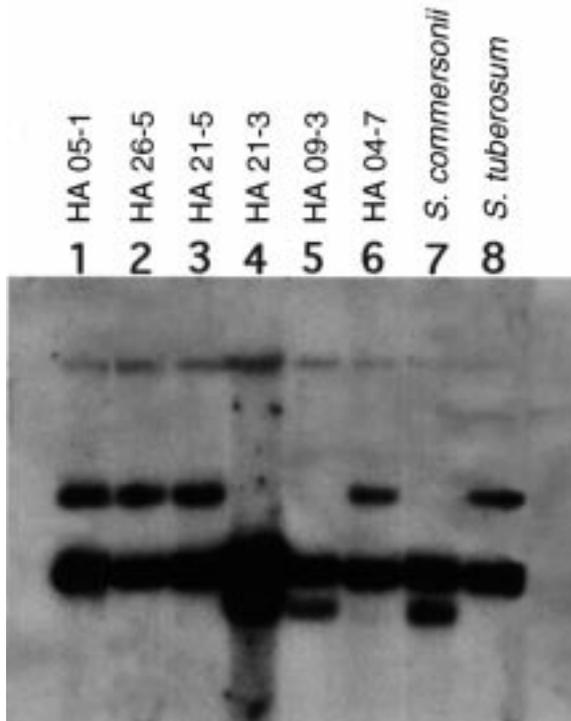


Figure 3. Autoradiograph of a Southern blot from the hybridization of *EcoRV*-digested total DNA with chloroplast probe, S6. Lanes 1–6, somatic hybrids; lane 7, *S. commersonii*; lane 8, *S. tuberosum*.

the amphiploids when combined with wheat genomes (Dvorak & Fowler, 1978; Limin & Fowler, 1988; Limin et al., 1985, 1995). Therefore, our results are generally consistent with the conclusions from previous studies in other crops except for the expression of ACC in tbr (+) cmm somatic hybrids. Although the interaction between species used in the fusion can not be ruled out as the cause for the observed variation or reduction in the expression of NA and ACC, other factors such as the recessiveness of the genes conferring NA and ACC could be also operating in this scenario. In addition, evidence from a parallel study in interspecific F_1 hybrids of potatoes (Chen et al., 1996, 1999; Palta et al., 1997) and studies in wheat (Limin & Fowler, 1988, 1989) have demonstrated that the ratio of parental genomes in the hybrids can affect the expression of freezing tolerance. In other words, the expression of both NA and ACC can be improved by increasing the contribution of hardy genome in the hybrids. Therefore, the sensitive-to-hardy genomic ratio could be another important determinant in the expression of NA and ACC.

The variability among symmetric somatic hybrids from a given fusion combination has been described in potatoes (Austin et al., 1986; Fish et al., 1988; Preiszner et al., 1991; Cardi et al., 1993). The variability can be attributed to several reasons such as somaclonal variation, genetic changes stimulated by fusion, novel cytoplasmic composition, and nucleus-organellar interaction. Fortunately, the self-fertile clone, HA26-5, and the most cross-fertile clone, HA05-1, were the two hardiest clones among the six tbr (+) cmm somatic hybrids.

Evidence has shown that the large subunit of Rubisco (D-ribulose 1,5-bisphosphate carboxylase/oxygenase) from cmm is structurally more stable to freeze-thaw cycles than that from tbr (Huner et al., 1981). In the present study, however, no difference in ACC can be detected between tbr (+) cmm somatic hybrids with chloroplasts either from cmm or tbr (Table 2). Similarly, the ACC of tbr (+) brd somatic hybrid A206 and A937 was nearly the same (Table 1) despite the difference in their chloroplast type (Kitzinger and Helgeson, unpublished data, as cited in Helgeson et al., 1993). Therefore, the chloroplast DNA type is likely to have little influence on the expression of ACC. In addition, no significant difference was detected between the reciprocal crosses of hardy and sensitive *Solanum* species. Taken together, these results indicate that the expression of ACC and the cytoplasmic responses occurring under low temperature conditions seem to be controlled mainly by nuclear genes.

Among the somatic hybrids tested, tbr (+) cmm somatic hybrids with AA more than -5 °C, such as HA05-1 and HA26-5, are particularly noteworthy in terms of breeding for freezing tolerance. As mentioned above, the freezing tolerance in these somatic hybrids already reaches the hardiness level to be recognized as a hardy species. Therefore, the fertility and crossability found in these hardy clones is encouraging. Freezing tolerance and tuber traits of the selfed and backcross progeny derived from our tbr (+) cmm somatic hybrids are being evaluated.

Acknowledgments

We thank Dr Kenneth J. Sytsma for providing the *Petunia* chloroplast DNA probe. This work was supported by a USDA/NRI grant 93-37100-8924 to J. P. P. and J. B. B. and by the College of Agriculture and Life

Sciences (Hatch grant 142C737 to J. P. P.), University of Wisconsin, Madison, WI 53706.

References

- Austin, S., M.A. Baer & J.P. Helgeson, 1985. Transfer of resistance to potato leaf roll virus from *Solanum brevidens* into *Solanum tuberosum* by somatic fusion. *Plant Sci* 39: 75–82.
- Austin, S., M.K. Ehlenfeldt, M.A. Baer & J.P. Helgeson, 1986. Somatic hybrids produced by protoplast fusion between *S. tuberosum* and *S. brevidens*: phenotypic variation under field conditions. *Theor Appl Genet* 71: 682–690.
- Austin, S., E. Lojkowska, M.K. Ehlenfeldt, A. Kelman & J.P. Helgeson, 1988. Fertile interspecific somatic hybrids of *Solanum*: a novel source of resistance to *Erwinia* soft rot. *Phytopath* 78: 1216–1220.
- Cardi, T., F.D. Ambrosio, D. Consoli, K.J. Puite & K.S. Ramulu, 1993. Production of somatic hybrids between frost-tolerant *S. commersonii* and *S. tuberosum*: characterization of hybrid plants. *Theor Appl Genet* 87: 193–200.
- Chen, H.H. & P.H. Li, 1980. Characteristics of cold acclimation and deacclimation in tuber-bearing *Solanum* species. *Plant Physiol* 65: 1146–1148.
- Chen, Y.-K., J.B. Bamberg & J.P. Palta, 1996. Expression of freezing tolerance in interspecific F₁ of potatoes. *Am Potato J* 73: 348 (Abstract).
- Chen, Y.-K.H., J.B. Bamberg & J.P. Palta, 1999. Expression of freezing tolerance in the interspecific F₁ and somatic hybrids of potatoes. *Theor Appl Genet* (in press).
- Craig, A.L., I. Morrison, E. Baird, R. Waugh, M. Coleman, P. Davie & W. Powell, 1994. Expression of reducing sugar accumulation in interspecific somatic hybrids of potato. *Plant Cell Rep* 13: 401–405.
- Dvorak, J. & D.B. Fowler, 1978. Cold hardiness potential of Triticale and tetraploid rye. *Crop Sci* 18: 477–478.
- Fish, N., S.H. Steele & M.G.K. Jones, 1988. Field assessment of dihaploid *Solanum tuberosum* and *S. brevidens* somatic hybrids. *Theor Appl Genet* 76: 880–886.
- Gill, K.S., E.L. Lubbers, B.S. Gill, W.J. Raupp & T.S. Cox, 1991. A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). *Genome* 34: 362–374.
- Gullord, M., C.R. Olien & E.H. Everson, 1975. Evaluation of freezing hardiness in winter wheat. *Crop Sci* 15: 153–157.
- Helgeson, J.P., G.T. Haberlach, M.K. Ehlenfeldt, G.J. Hunt, J.D. Pohlman & S. Austin, 1993. Sexual progeny of somatic hybrids between potato and *Solanum brevidens*: potential for use in breeding programs. *Am Potato J* 70: 437–452.
- Helgeson, J.P., G.J. Hunt, G.T. Haberlach & S. Austin, 1986. Somatic hybrids between *Solanum brevidens* and *Solanum tuberosum*: Expression of a late blight resistance gene and potato leaf roll resistance. *Plant Cell rep* 3: 212–214.
- Huner, N.P.A., J.P. Palta, P.H. Li & J.V. Carter, 1981. Comparison of the structure and function of ribulosebisphosphate carboxylase-oxygenase from a cold-hardy and nonhardy potato species. *Can J Biochem* 59: 280–289.
- Kim, H., S.U. Choi, M.S. Chae, S.M. Wielgus & J.P. Helgeson, 1993. Identification of somatic hybrids produced by protoplast fusion between *Solanum commersonii* and *S. tuberosum* haploid. *Korean J Plant Tiss Cult* 20: 337–344.
- Kimball, S.L. & F.B. Salisbury, 1973. Ultrastructural changes of plants exposed to low temperatures. *Am J Bot* 60: 1028–1033.
- Li, P.H. & J.P. Palta, 1978. Frost hardening and freezing stress in tuber-bearing *Solanum* species. In: P.H. Li & A. Sakai (eds), *Plant Cold Hardiness and Freezing Stress*, pp. 49–71. Academic Press, New York.
- Limin, A.E., J. Dvorak & D.B. Fowler, 1985. Cold hardiness in hexaploid triticale. *Can J Plant Sci* 65: 487–490.
- Limin, A.E. & D.B. Fowler, 1984. The effect of cytoplasm on cold hardiness in alloplasmic rye (*Secale cereale* L.) and triticale. *Can J Genet Cytol* 26: 405–408.
- Limin, A.E. & D.B. Fowler, 1988. Cold hardiness expression in interspecific hybrids and amphiploids of the Triticeae. *Genome* 30: 361–365.
- Limin, A.E. & D.B. Fowler, 1989. The influence of cell size and chromosome dosage on cold-hardiness expression in the Triticeae. *Genome* 32: 667–671.
- Limin, A.E., M. Houde, L.P. Chauvin, D.B. Fowler & F. Sarhan, 1995. Expression of the cold-induced wheat gene *Wcs120* and its homologs in related species and interspecific combinations. *Genome* 38: 1023–1031.
- Lössl, A., U. Frei & G. Wenzel, 1994. Interaction between cytoplasmic composition and yield parameters in somatic hybrids of *S. tuberosum* L. *Theor Appl Genet* 89: 873–878.
- Mastenbroek, C., 1956. Some experience in breeding frost-tolerant potatoes. *Euphytica* 5: 289–297.
- Nyman, M. & S. Waara, 1997. Characterization of somatic hybrids between *Solanum tuberosum* and its frost-tolerant relative *Solanum commersonii*. *Theor Appl Genet* 95: 1127–1132.
- Palta, J.P., 1994. Sorting genes controlling freezing stress resistance. In: J.H. Cherry (ed.), *Biochemical and Cellular Mechanisms of Stress Tolerance in Plants*, pp. 569–586. Springer-Verlag, Berlin, Germany.
- Palta, J.P., J.B. Bamberg, Y.-K. Chen, S.E. Vega, L.S. Weiss & B.H. Karlsson, 1997. Understanding genetic control of freezing resistance using potato species as a model system. In: P.H. Li & T.H.H. Chen (eds), *Plant Cold Hardiness: Molecular Biology, Biochemistry, and Physiology*, pp. 67–75. Plenum Press, New York.
- Palta, J.P. & P.H. Li, 1979. Frost-hardiness in relation to leaf anatomy and natural distribution of several *Solanum* species. *Crop Sci* 19: 665–671.
- Palta, J.P. & G. Simon, 1993. Breeding potential for improvement of freezing stress resistance: genetic separation of freezing tolerance, freezing avoidance, and capacity to cold acclimate. In: P.H. Li & L. Christersson (eds), *Advances in Plant Cold Hardiness*, pp. 299–310. CRC Press, Boca Raton, Florida.
- Palta, J.P., B.D. Whitaker & L.S. Weiss, 1993. Plasma membrane lipids associated with genetic variability in freezing tolerance and cold acclimation of *Solanum* species. *Plant Physiol* 103: 793–803.
- Preisner, J., A. Feher, O. Veisz, J. Sutka & D. Dudits, 1991. Characterization of morphological variation and cold resistance in interspecific somatic hybrids between potato (*Solanum tuberosum* L.) and *S. brevidens* Phil. *Euphytica* 57: 37–49.
- Richardson, D.G. & C.J. Weiser, 1972. Foliage frost resistance in tuber-bearing *Solanums*. *HortScience* 7: 19–22.
- Ross, R.W. & P.R. Rowe, 1965. Frost resistance among the *Solanum* species in the IR-1 potato collection. *Am Potato J* 42: 177–185.
- Spooner, D.M. & R. Castillo-T., 1997. Reexamination of series relationships of South American wild potatoes (Solanaceae: *Solanum* sect. *Petota*): Evidence from chloroplast DNA restriction site variation. *Am J Bot* 84: 671–685.
- Steffen, K.L., R. Arora & J.P. Palta, 1989. Relative sensitivity of photosynthesis and respiration to freeze-thaw stress in herbaceous species. *Plant Physiol* 89: 1372–1379.

- Steffen, K.L. & J.P. Palta, 1986. Effect of light on photosynthetic capacity during cold acclimation in a cold-sensitive and a cold-tolerant potato species. *Physiol Plant* 66: 353–359.
- Stone, J.M., J.P. Palta, J.B. Bamberg, L.S. Weiss & J.F. Harbage, 1993. Inheritance of freezing resistance in tuber-bearing *Solanum* species: evidence for independent genetic control of nonacclimated freezing tolerance and cold acclimation capacity. *Proc Natl Acad Sci (USA)* 90: 7869–7873.
- Sutinen, M., J.P. Palta & P.B. Reich, 1992. Seasonal differences in freezing stress resistance of needles of *Pinus nigra* and *Pinus resinosa*: evaluation of the electrolyte leakage method. *Tree Physiol* 11: 241–254.
- Sutka, J., 1994. Genetic control of frost tolerance in wheat. *Euphytica* 77: 277–282.
- Sytsma, K.J. & L.D. Gottlieb, 1986. Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* sect. *Peripetasma* (Onagraceae). *Evolution* 40: 1248–1261.
- Tiwari, S.P. & K.C. Garg, 1982. Inheritance of frost resistance in potato. *Curr Sci* 51: 249–251.
- Tucci, M., D. Carputo, G. Bile & L. Frusciante, 1996. Male fertility and freezing tolerance of hybrids involving *Solanum tuberosum* haploids and diploid *Solanum* species. *Potato Res* 39: 345–353.
- Uemura, M. & P.L. Steponkus, 1994. A contrast of the plasma membrane lipid composition of oat and rye leaves in relation to freezing tolerance. *Plant Physiol* 104: 479–496.
- Vavilova, M.A., 1978. Use of wild frost-resistant species of the potato *S. commersonii* Dun. and *S. chomatophilum* Bitt. in interspecific hybridization. In: V.S. Kothekar (ed.), 1985. Systematics, Breeding, and Seed Production of Potatoes, pp. 157–167. Amerind Publishing, New Delhi. Translated from *Bull Appl Bot Genet Breed*, vol 62 (in Russian), by A.K. Dhote.
- Vazquez-Thello, A., L.J. Yang, M. Hidaka & T. Uozumi, 1996. Inherited chilling tolerance in somatic hybrids of transgenic *Hibiscus rosa-sinensis* × transgenic *Lavatera thuringiaca* selected by double-antibiotic resistance. *Plant Cell Rep* 15: 506–511.
- Vega, S.E. & J.B. Bamberg, 1995. Screening the U.S. potato collection for frost hardiness. *Am Potato J* 72: 13–21.
- Williams, C.E., S.M. Wielgus, G.T. Haberlach, C. Guenther, H. Kim-Lee & J.P. Helgeson, 1993. RFLP analysis of chromosomal segregation in progeny from an interspecific hexaploid somatic hybrid between *Solanum brevidens* and *Solanum tuberosum*. *Genetics* 135: 1167–1173.