Theor Appl Genet (1999) 98: 995–1004

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Expression of freezing tolerance in the interspecific F_1 and somatic hybrids of potatoes

Received: 21 July 1998 / Accepted: 29 September 1998

Abstract The expression of freezing tolerance was examined in interspecific F_1 and somatic hybrids of potatoes using 20 species and 34 different combinations between hardy and sensitive species. In the field, the frost tolerance of hybrids resembled either that of the hardy parent, the sensitive parent, or the parental mean, depending on the species combination and the genomic ratio (ratio of the number of sets of chromosomes contributed from each parent). Similar phenomena were observed when the non-acclimated freezing tolerance (NA) and the acclimation capacity (ACC) (two independent genetic components of freezing tolerance) were evaluated separately under controlled environments. In general, the expression level of freezing tolerance was higher in hybrids with more genomes contributed from the hardy parent than from the sensitive parent. In addition, the effectiveness or combining ability of genes conferring freezing tolerance from the hardy species also showed some influence on the expression of freezing tolerance. All three parameters, namely NA, ACC and acclimated freezing tolerance (AA) (NA plus ACC), were significantly correlated to the frost tolerance exhibited in the field. This indicates that the controlled freezing test used in this study could provide a good estimate of field performance. The implications of these results in breeding for freezing tolerance in potatoes are discussed.

Communicated by B. S. Gill

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Key words Freezing tolerance · Potato · Interspecific F_1 · Somatic hybrids · Genomic ratio

Introduction

Freezing stress is one of the major factors limiting the yield and geographical range of potatoes production. In spite of its importance, breeding for improved freezing tolerance has progressed very slowly over the years in potatoes (Hermsen 1987; Palta et al. 1997; Valverde et al. 1997) as well as in other crops (Grafius 1981; Marshall 1982; Limin and Fowler 1991). In addition to the lack of effective selection criteria and breeding schemes, is slow progress could be blamed on a limited understanding of the underlying genetics. Although most studies have concluded that freezing tolerance is a multigenic trait (Mastenbroek 1956; Richardson and Weiser 1972; Palta and Simon 1993; Stone et al. 1993), there is no consensus on the mode of gene action governing the expression of this trait in potatoes. Contradictory reports regarding whether this trait is dominant can be found. For example, Mastenbroek (1956) indicated that freezing tolerance depends on a number of dominant genes with a quantitative or cumulative effect. Vavilova (1978) also described frost tolerance as a dominant trait in interspecific F_1 hybrids. Stone et al. (1993), on the other hand, concluded that freezing tolerances with or without acclimation are both partially recessive. The recessive nature of freezing tolerance was further supported by the results of Tucci et al. (1996).

In potatoes, more than 30 wild species with a distinct freezing tolerance have been recognized and could be a potential source of genetic variability for the improvement of this trait (Li and Palta 1978; Palta and Li 1979; Vega and Bamberg 1995). The transfer of freezing tolerance from wild germplasm into cultivated species has been attempted by interspecific hybridization

through sexual means and somatic fusion (Okuno 1951; Ross and Rowe1965, 1969; Richardson and Estrada 1971; Austin et al. 1986; Estrada 1987; Preiszner et al. 1991; Cardi et al. 1993; Kim et al. 1993; Bamberg et al. 1994; Carputo et al. 1997; Nyman and Waara 1997). Unfortunately, the polyploid nature of the species and the crossing barriers involved have made genetic analysis and data interpretation both complex and difficult.

Freezing tolerance is actually composed of two independent genetic components, non-acclimated freezing tolerance (NA) and acclimation capacity (ACC) (Stone et al. 1993). Non-acclimated freezing tolerance is the ability to survive freezing temperatures without acclimation, while ACC is the ability to increase the freezing tolerance in response to chilling temperature exposure. It is impossible to separate these two components in field evaluations. To-date most studies aimed at understanding the genetics of freezing tolerance have not distinguished NA and ACC as separate genetic components.

The present study was undertaken to characterize the expression of NA and ACC under controlled environments in addition to field evaluations for frost damage. Our goals were to investigate the genetic control of freezing tolerance by using interspecific F_1 and somatic hybrids containing genomes from both hardy and sensitive species. Because ion leakage from excised leaflets subjected to a controlled ice nucleation and a simulated freeze-thaw stress (Steffen et al. 1989) provides a precise test of freezing tolerance, this method was employed in NA and ACC analyses to discern the small but significant differences among genotypes (Stone et al. 1993). *Solanum* species are known to vary greatly in both NA and ACC (Li et al. 1979; Chen and Li 1980), and therefore represent an ideal material to study the subject addressed above. The information gained from this study should be valuable for understanding the genetics of this important trait as well as for freezing tolerance breeding and germplasm enhancement.

In this report, *frost* tolerance refers to results in field conditions, while *freezing* tolerance refers to results from controlled laboratory tests or more general situations.

Materials and methods

Plant materials

The parental species used for interspecific F_1 and somatic hybrid production are listed in Table 1 and the species combinations are summarized in Table 2. Because of the availability of hybrid seed, different accessions might be used as parental species in a combination (Table 2). The wild species accessions were supplied by the Inter-Regional Potato Introduction Station at Sturgeon Bay, Wis. All crosses were performed either on intact plants or on cut stems (Peloquin and Hougas 1959) in the greenhouse and the Biotron facility (UW-Madison, Wis). The hybrids and parental species were grown in the greenhouse and then transplanted to the field for frost tolerance ratings. For controlled freezing assays, the materials were maintained either in the greenhouse or at the Biotron. All the hybrid seedlings for field study in 1995 were propagated clonally for replication. But since the variation in scores for frost tolerance among

Table 1 Description of parental species used for interspecific F_1 and somatic hybrid production

Species	Abbreviation	Class	EBN[a]	Ploidy
S. acaule	acl	Hardy	2	4*x*
S. acaule	8*x*-acl	Hardy	4	8*x*[b]
S. arnezii	arz	Sensitive	2	2*x*
S. berthaultii	ber	Sensitive	2	2*x*
S. brachistotrichum	bst	Sensitive	1	2*x*
S. brevidens	brd	Hardy	1	2*x*
S. bukasovii	buk	Hardy	2	2*x*
S. chacoense	chc	Sensitive	2	2*x*
S. commersonii	cmm	Hardy	1	2*x*
S. commersonii	4*x*-cmm	Hardy	2	4*x*[c]
S. cardiophyllum	cph	Sensitive	1	2*x*
S. demissum	dms	Hardy	4	6*x*
S. leptophyes	lph	Sensitive	2	2*x*
S. microdontum	mcd	Sensitive	2	2*x*
S. megistacrolobum	mga	Hardy	2	2*x*
S. multidissectum	mlt	Hardy	2	2*x*
S. oplocense	opl	Sensitive	4	4*x*
S. polytrichon	plt	Sensitive	2	4*x*
S. sucrense	scr	Sensitive	4	4*x*
S. sanctae-rosae	sct	Hardy	2	2*x*
S. tuberosum (haploid)	hap	Sensitive	2	2*x*
S. tuberosum	tbr	Sensitive	4	4*x*
S. toralapanum	tor	Hardy	2	2*x*

[a] EBN = Endosperm Balance Number
[b] Artificial chromosome-doubled species of tetraploid *S. acaule*
[c] Population derived from a chromosome-doubled individual of diploid *S. commersonii*

Table 2 Species combinations and respective accessions employed in this study

Combination (Plant introduction number)
acl (210029) × arz (545958)
acl (210029) × ber (498102)
acl (210029) × chc (230862)
acl (210029) × hap (H.D.F.20.1*)
acl (210029) × lph (545991)
acl (210029) × mcd (498121)
acl (472715) × plt (186545)
8*x*-acl (230530) × opl (473042)
8*x*-acl (230530) × scr (498301)
brd (218228) (+) tbr (203900)
buk (473494) × ber (498102)
buk (498220[a], 473494[b], 473492[c]) × chc (230862)
buk (473494) × hap (H.D.F.20.1)
buk (473492[a], 473494[b]) × mcd (208866[a], 473176[b])
chc (197758) × acl (210029)
cmm (243503) × bst (545817[a], 545820[a,b])
cmm (243503) × cph (186549[a,b], 251729[a], 283063[a])
cmm (320266) (+) hap (US-W13122, a haploid clone of cv Superior)
4*x*-cmm (243503) × arz (545958)
4*x*-cmm (243503) × ber (310981[a,b], 498099[a], 498141[a])
4*x*-cmm (243503) × buk (473494)
4*x*-cmm (243503) × chc (133618, 230862)
4*x*-cmm (243503) × hap (H.D.F.20.1)
4*x*-cmm (243503) × mcd (473176[a,b], 498124[a])
dms (186552) × tbr (Wis AG 231, an elite breeding line)
hap (H.D.F. 20.1) × 4*x*-cmm (243503)
mcd (208866) × buk (473492)
mcd (208866) × tor (458396)
mga (210029) × mcd (208866)
mlt (498304) × hap (H.D.F.20.1)
plt (186545) × buk (473494)
sct (230464) × hap (H.D.F.20.1)
sct (230464) × mga (500031)
tor (458396) × mcd (208866)

[a,b,c] Accessions used in 1995, 1996 field trials and controlled freezing assays, respectively. *H.D.F.20.1 was obtained from H. DeJong, Agriculture Canada, Fredericton, New Brunswick

seedlings within the same family was small, according to 1995 field trials, different seedlings were used for replications in 1996. The somatic hybrids were kindly provided by Dr. Helgeson, USDA/ARS, Department of Plant Pathology, University of Wisconsin, Madison. The clonal plantlets of somatic hybrids, fusion parents, some F_1 hybrids and parental species from sterilized culture were maintained in the Biotron.

Field studies

The plant materials were planted at the Peninsular Agricultural Research Station, Sturgeon Bay, Wis, in June, 1995. The experiment was conducted with two replicates in a split-plot design with families of different *crosses* randomly allocated to main plots and with *generations* composed of F_1 hybrids and two parents randomly assigned to subplots. Except when fewer seedlings were obtained, generations were represented as 18 F_1 hybrids and nine plants for each parental species. The F_1 hybrids and parents were planted in nine-hill rows. The spacing was 45 cm between rows and 60 cm between hills. In cases where desirable seedling numbers were not obtained, a tbr cultivar was used to fill out the rows. The entire block was bordered by tbr cultivar plants.

In 1996, a similar experiment was conducted again at Sturgeon Bay. The field layout and experimental design were the same as in 1995 except that three replicates were used.

The evaluation for frost tolerance was done after frosts severe enough to differentiate the hardiness of standard species had occurred (Vega and Bamberg 1995). The extent of frost damage was scored visually by the following scale:

0 = no damage,
1 = slightly bronzing on the upper leaves,
2 = some top leaflets killed,
3 = all top leaves killed,
4 = all leaves and petioles killed,
5 = leaves and stems (whole plant) killed.

In 1995 the differentiating frosts occurred on September 22 and 23 and the plants were rated 2 days later. The thermograph record in the field showed that the temperature was lower than $-1°C$ for 3 h and $-4°C$ for 1 h. In 1996 the plants were inspected for frost injury on October 15 and 16 after frosts on October 10 and 11. Although the temperature and the duration were not monitored, the severity of the frosts was comparable to that of 1995 according to the level of damage on the standard species. Because haploid tbr started senescence before the frost occurred, the scores obtained from plt were used instead. Based on a controlled freezing test, the hardiness level of plt resembled that of haploid tbr under both non-acclimated and acclimated conditions.

Controlled freezing tests

The plantlets obtained from sterilized culture were transferred to 8-l pots with Jiffy Mix (Jiffy products of America, Inc., W. Chicago, Ill.) and grown in a controlled environment room at the Biotron. After 6–7 weeks at 20/18 °C light/dark with a 14-h photoperiod and about 400 μmol photon $m^{-2}s^{-1}$, terminal leaflets from fully expanded leaves were excised for non-acclimated freezing tolerance (NA) assay. To achieve cold acclimation, the temperature was lowered to $4/2°C$ light/dark with 14 h light at about 100 μmol photon $m^{-2}s^{-1}$ for an additional 2 weeks prior to collecting leaflets. These conditions were previously shown to result in full acclimation in tuber-bearing *Solanum* species (Steffen and Palta 1986). Seedlings grown in the greenhouse were transplanted once before being planted in 6-inch pots. Leaflets were harvested for the NA test when plants were 6–8 weeks old. The plants were then moved to a cold room at the Biotron for acclimation using the same conditions mentioned above following 1–2 weeks of recovery and re-growth from first sampling. For sexual F_1 hybrids, at least ten seedlings were used for each cross with the exception of acl × lph and 4*x*-cmm × hap, for which only seven and eight seedlings were available, respectively.

Freezing tolerance of the plant materials, including NA, acclimated freezing tolerance (AA, i.e., freezing tolerance after acclimation), and acclimation capacity (ACC), was determined using the protocols 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 in the case of samples for measuring ion leakage at 0°C which were put directly 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 h. 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 h. Further cooling below $-1.5°C$ was at the 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 pre-determined 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 with a YSI conductance meter (Yellow Springs, Ohio). 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 h before conductivity readings (R_1) were taken. The maximum conductivity (R_2) representing the total ion content for each sample was determined after autoclaving for 15 min at 121°C. The percent of ion leakage at each temperature was obtained as $(R_1/R_2) \times 100\%$. The freezing curve was constructed by plotting the mean percent ion leakage of three subsamples vs the freezing temperature. 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). ACC was defined as the difference between NA and AA.

Statistical analysis

The dominance and recessive estimates were calculated as the deviation of each hybrid family from the corresponding parental mean by a two-tailed *t* test. For the field studies, data were analyzed by single degree-of-freedom contrast-comparisons for the differences between the F_1 and the parental mean using the GLM procedure of SAS (SAS Institute, Cary, N.C.). The Spearman's rank correlation coefficients between field performance and the different components of freezing tolerance as determined by controlled freezing tests were obtained by using the CORR SPEARMAN procedure of SAS.

Results and discussion

Dominance-recessive relationship in the expression of freezing tolerance

Genetic variablilty for frost tolerance among species and their hybrids was visually discernible after the differential frosts had occurred in both 1995 and 1996. Rating scores of hybrids minus the parental mean revealed that frost tolerance can either appear to be dominant or recessive, depending on the species used in the crosses. This was obvious because both positive and negative values were obtained (Tables 3 and 4). The ratio of hybrid hardiness level to the parental difference ranged from 26% to 100% in 1995 and from 10% to 94% in 1996 (Table 3 and Table 4, respectively). In other words, the expression in the hybrids varied from the level of the sensitive parent to that of the hardy parent in both years. When the parental contributions of genomes (genomic ratio; ratio of the number of sets of chromosomes contributed from each parent) were taken into consideration, it was noticed that the hybrids having more genomes from the hardy parent than from the sensitive parent tended to express greater hardiness than those having an equal contribution from both parents. The crosses for which the hardy parents had a higher ploidy exhibited hardiness either higher than the parental mean or approximate to that of the hardy parent. The scores of hybrids produced by parents with equal ploidy were either less than or close to the parental means. Exceptions were cmm × cph and cmm × bst in 1995 and cmm × cph and cmm (+) hap in 1996, whose hybrids had a genomic ratio of 1:1 but exhibited hardiness levels higher than the parental mean. The hardiest F_1 hybrids were those derived from crosses between hardy species, such as 4*x*-cmm × buk and sct × mga.

Some of the hybrids and parent combinations were also studied under controlled environments by evaluating NA and ACC separately. In addition, combinations encompassing a broader range of genomic ratios were included to further explore the influence of genomic ratio. Except for 4*x*-cmm × hap, all hybrids which were produced using a higher ploidy from the hardy parent had a greater NA than their parental mean, while hybrids produced using parents with the same genome number or more genomes from the sensitive parent tended to have less NA and had a freezing tolerance either lower than or close to the parental mean (Table 5). Similar results were found in the tests for ACC with the exception of mlt × hap and acl × plt (Table 6). Thus, the results from NA and ACC analyses were generally in agreement with what had been observed in the field.

The results presented here may partly explain the contradictory observations regarding whether freezing tolerance is a dominant trait. For instance, Mastenbroek (1956) used acl as the donor for freezing tolerance in crosses with tbr. Since this was a cross with a 2:1 hardy to sensitive genomic ratio, it is no surprise that he indicated that tolerance seems to be governed by dominant genes. Other than acl, cmm is another hardy species that has been widely used for freezing tolerance studies. According to the Endosperm Balance Number (EBN) theory (Johnston et al. 1980; Johnston and Hanneman 1982), cmm, a diploid species, has been assigned as a 1EBN species. It crosses with haploid tbr (2*x*) or other 2EBN species only when its chromosomes have been doubled or when 2n gametes are produced. Therefore, it is likely that the hardy to sensitive genomic ratio was 2:1 in many cases where cmm was involved. This may be the reason why Vavilova (1978) concluded that frost resistance is dominant in the F_1 hybrids. However, when a diploid, 1EBN species cph is used to cross with cmm, recessiveness can be expected as was shown in Stone et al. (1993). The average freezing tolerance of the hybrid families between tbr haploid and 2*x* sct was closer to the susceptible parent according to Tucci et al. (1996). Recessiveness was also expected from their results since a genomic ratio of 1:1 was present in the hybrids.

Correlation between field evaluation and controlled freezing assays

Correlation coefficients were calculated for the associations of field rating scores with freezing tolerance determined before and after acclimation and ACC under a controlled environment from those materials used in both field and controlled-environment studies. NA,

Table 3 Frost tolerance rating scores of interspecific F_1 hybrids and parental species (P_1 and P_2) from the 1995 field study. A negative sign in the column of "Hybrids-PM" indicates that hybrids are more hardy than PM (parental mean)

Cross	P_1	P_2	Hybrids	Hybrids – PM	Ratio[a]	Genomic ratio[b]
acl × arz	0.2	4.2	1.4	−0.8*	70%[c]	2:1
acl × ber	0.0	3.3	0.9	−0.8*	73%	2:1
acl × chc	0.0	3.4	0.7	−1.0	79%	2:1
chc × acl[d]	3.1	0.0	0.6	−0.9*	81%	2:1
acl × hap	0.0	3.3	0.4	−1.3	88%	2:1
acl × mcd	0.0	4.0	1.0	−1.0**	75%	2:1
8*x*-acl × opl	0.1	1.1	0.1	−0.5*	100%	4:2
8*x*-acl × scr	0.0	3.0	0.4	−1.2*	87%	4:2
4*x*-cmm × arz	0.0	4.1	0.2	−1.9**	95%	2:1
4*x*-cmm × ber	0.0	3.6	0.3	−1.5*	92%	2:1
4*x*-cmm × chc	0.0	2.8	0.4	−1.0	86%	2:1
4*x*-cmm × mcd	0.1	3.3	0.2	−1.5**	97%	2:1
hap × 4*x*-cmm	3.0	0.0	0.3	−1.2	90%	2:1
cmm × bst	0.0	3.5	0.1	−1.6*	97%	1:1
cmm × cph	0.0	3.0	1.1	−0.5	63%	1:1
buk × ber	0.0	3.3	2.1	0.5*	36%	1:1
buk × chc	0.1	3.1	2.3	0.7	27%	1:1
buk × mcd	0.0	3.9	2.1	0.2	46%	1:1
mcd × buk[d]	3.1	0.0	2.3	0.8*	26%	1:1
mcd × tor	4.0	0.3	2.9	0.7	30%	1:1
tor × mcd[d]	1.0	4.3	3.0	0.4	39%	1:1
mga × mcd	0.0	3.5	2.2	0.5	37%	1:1
4*x*-cmm × buk[e]	0.0	0.0	0.0	0.0	–	2:1

*, **Significant at the 0.05 and 0.01 probability level, respectively.
[a] Calculated by the following formula: $|(\text{Hybrids} - \text{Sensitive parent})/(P_1 - P_2)| \times 100\%$
[b] Ratio of hardy: sensitive genome number in the hybrids
[c] $> 50\%$ indicates that frost tolerance is somewhat dominant; $< 50\%$ indicates that frost tolerance is somewhat recessive
[d] Indented entries indicate reciprocal crosses
[e] Cross between hardy species

Table 4 Frost tolerance rating scores of interspecific F_1 or somatic hybrids and parental species (P_1 and P_2) from the 1996 field study. A negative sign in the column of "Hybrids – PM" indicates that hybrids are more hardy than PM (parental mean)

Cross	P_1	P_2	Hybrids	Hybrids – PM	Ratio[a]	Genomic ratio[b]
acl × ber	0.0	4.0	1.3	−0.7	68%[c]	2:1
acl × chc	0.0	4.4	0.8	−1.4**	82%	2:1
acl × lph	0.0	3.4	0.5	−1.2**	85%	2:1
acl × mcd	0.0	4.3	1.5	−0.7**	65%	2:1
8*x*-acl × opl	0.0	1.7	0.1	−0.7**	94%	4:2
8*x*-acl × scr	0.0	3.5	0.3	−1.4**	91%	4:2
4*x*-cmm × ber	0.0	4.3	0.3	−1.9**	93%	2:1
4*x*-cmm × mcd	0.0	4.2	0.6	−1.5**	86%	2:1
dms × tbr	0.1	3.8	1.6	−0.3	59%	3:2
cmm × cph	0.0	3.1	1.3	−0.2	58%	1:1
cmm (+) hap	0.0	4.1	0.4	−1.7	90%	2:2
acl × plt	0.0	3.9	2.0	0.1	49%	2:2
buk × ber	0.2	3.9	2.6	0.5*	35%	1:1
buk × chc	0.0	4.1	2.5	0.4	39%	1:1
buk × hap	0.0	3.9	2.1	0.2	46%	1:1
buk × mcd	0.0	4.1	3.0	0.9**	27%	1:1
mcd × tor	4.3	0.1	3.9	1.7**	10%	1:1
tor × mcd[d]	0.3	4.5	3.8	1.3**	17%	1:1
mga × mcd	0.2	4.2	3.2	1.0**	25%	1:1
mlt × hap	0.6	3.9	2.3	0.1	49%	1:1
sct × hap	0.0	3.8	2.3	0.4	40%	1:1
4*x*-cmm × buk[e]	0.0	0.0	0.0	0.0	–	2:1
sct × mga	0.0	0.0	0.0	0.0	–	1:1
sct × mlt	0.0	0.3	0.0	−0.2	–	1:1

*, **Significant at the 0.05 and 0.01 probability level, respectively
[a] Calculated by the following formula: $|(\text{Hybrids} - \text{Sensitive parent})/(P_1 - P_2)| \times 100\%$
[b] Ratio of hardy: sensitive genome number in the hybrids
[c] $> 50\%$ indicates that frost tolerance is somewhat dominant; $< 50\%$ indicates that frost tolerance is somewhat recessive
[d] Indented entry indicates reciprocal crosses
[e] Crosses between hardy species

Table 5 Non-acclimated freezing tolerance (NA[a], ºC) of interspecific F_1 or somatic hybrids and parental species (P_1 and P_2) from controlled freezing test. A negative sign in the column of "Hybrids-PM" indicates that hybrids are more hardy than PM (parental mean)

Combination	P_1	P_2	Hybrids	Hybrids – PM	Ratio[b]	Genomic ratio [c]
acl × chc	−4.3	−2.1	−3.6	−0.4**	68%[d]	2:1
acl × lph	−5.5	−2.6	−4.8	−0.8*	75%	2:1
4*x*-cmm × hap	−4.8	−2.8	−3.5	0.3*	35%	2:1
dms × tbr	−4.8	−2.7	−4.1	−0.3**	67%	3:2
buk × chc	−3.8	−2.1	−2.1	0.9**	0%	1:1
buk × hap	−4.0	−2.8	−3.4	0.0	50%	1:1
cmm × cph	−4.5	−1.6	−2.6	0.4**	35%	1:1
cmm (+) hap	−4.2	−2.4	−2.5	0.8**	6%	2:2
mcd × tor	−2.0	−3.3	−2.5	0.1**	39%	1:1
tor × mcd[e]	−3.3	−2.0	−2.5	0.2**	39%	1:1
mlt × hap	−4.2	−2.8	−3.1	0.4**	21%	1:1
acl × plt	−5.6	−2.9	−3.5	0.8**	22%	2:2
plt × buk	−2.9	−4.0	−2.9	0.6**	0%	1:2
4*x*-cmm × buk[f]	−4.4	−4.1	−4.4	−0.1	–	2:1

*,**Significant at the 0.05 and 0.01 probability level, respectively
[a] NA is the freezing tolerance when plants are grown under normal condition (20/18°C day/night), i.e. without acclimation
[b] Calculated by the following formula: $|(\text{Hybrids} - \text{Sensitive parent})/(P_1 - P_2)| \times 100\%$
[c] Ratio of hardy : sensitive genome number in the hybrids
[d] > 50% indicates that NA is somewhat dominant; < 50% indicates that NA is somewhat recessive
[e] Indented entry indicates reciprocal crosses
[f] Cross between hardy species

Table 6 Acclimation capacity (ACC[a], °C) of interspecific F_1 or somatic hybrids and parental species (P_1 and P_2) from controlled freezing test.
A negative sign in the column of "Hybrids-PM" indicates that hybrids are less hardy than PM (parental mean)

Combination	P_1	P_2	Hybrids	Hybrids – PM	Ratio[b]	Genomic ratio[c]
acl × chc	1.5	0.5	2.1	1.1**	160%[d]	2:1
acl × lph	2.4	1.6	2.6	0.6	125%	2:1
4*x*-cmm × hap	3.6	0.5	3.1	1.0*	84%	2:1
dms × tbr	1.8	0.5	1.7	0.5**	92%	3:2
buk × chc	2.6	1.1	1.3	−1.6**	13%	1:1
buk × hap	2.4	0.5	1.4	−0.1	47%	1:1
cmm × cph	5.1	0.6	2.2	−0.7**	36%	1:1
cmm (+) hap	3.8	0.6	2.2	0.0	50%	2:2
mcd × tor	0.7	2.5	1.3	−0.3**	33%	1:1
tor × mcd[e]	2.5	0.7	1.3	−0.3*	39%	1:1
mlt × hap	1.2	0.5	1.3	0.5**	114%	1:1
acl × plt	1.9	0.4	1.7	0.5**	87%	2:2
brd (+) tbr	2.7	1.2	1.3	−0.6*	7%	2:4
plt × buk	0.4	2.4	1.3	−0.1	45%	1:2
4*x*-cmm × buk[f]	4.1	2.3	3.2	0.0	50%	2:1

*,**Significant at the 0.05 and 0.01 probability level, respectively
[a] ACC is the increase in freezing tolerance after exposure to 4/2°C day/night for 14 days, i.e. with acclimation
[b] Calculated by the following formula: $|(\text{Hybrids} - \text{Sensitive Parent})/(P_1 - P_2)| \times 100\%$
[c] Ratio of hardy : sensitive genome number in the hybrids
[d] > 50% indicates that ACC is somewhat dominant; < 50% indicates that ACC is somewhat recessive
[e] Indented entry indicates reciprocal crosses
[f] Cross between hardy species

AA, and ACC were all significantly correlated to field rating scores (Fig. 1). Therefore, freezing tolerance determined by controlled freezing tests could provide a good predictor of field frost tolerance. Studies from rapeseed also showed that controlled freezing tests predicted the winter survival observed in the field (Teutonico et al. 1993), although NA was not correlated significantly with winter survival in this case. In our study, AA had the highest correlation with field performance ($r_s = 0.92$) among NA, AA, and ACC. The explanation for this may depend on the fact that the frosts in 1995 and 1996 took place after a period of gradually

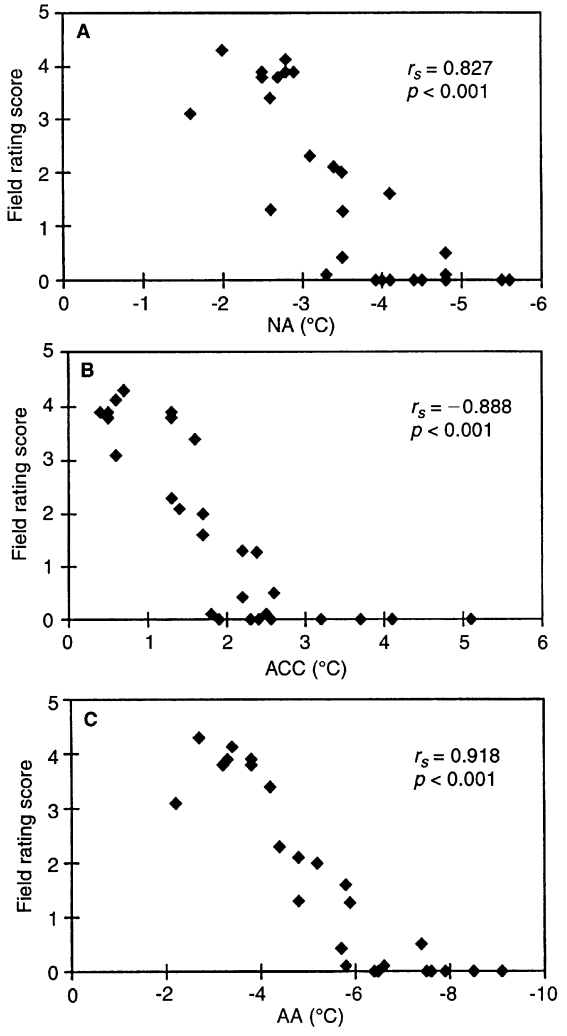

Fig. 1 Correlation between field rating scores and non-acclimated freezing tolerance (*NA*) **(A)**, acclimation capacity (*ACC*) **(B)**, and acclimated freezing tolerance (*AA*) **(C)**, in the interspecific F_1 hybrids, somatic hybrids, and the parental species used in studies conducted both in the field and under controlled environments; *r*s, Spearman's rank correlation coefficient

cooling weather in late fall, thus allowing the expression of AA prior to the frost episode. These results indicate that survival from stresses following a fall frost episode in nature in north temperate regions like Sturgeon Bay tend to correspond to AA as measured under laboratory conditions.

Due to the inherent limitations associated with field evaluation, such as the unpredictability of frost and the variability in the field, controlled freezing tests have advantages in selection programs for freezing tolerance. Nevertheless, the field performance is still the final standard for validating a controlled freezing assay. The good correlation between field observations and the laboratory results confirms that our protocol for controlled freezing assays is reliable and is practical for estimating freezing tolerance.

Field frost severity and the determination of freezing tolerance expression

When the plants possessed a AA of more than −5°C, they could survive the frost exposure of the field trials in 1995 and 1996 without visible damage. This might explain why F_1 families from cmm × cph and cmm (+) hap somatic hybrids showed a higher tolerance than the parental mean despite their genomic ratio of 1 : 1. For example, the mean freezing tolerance after acclimation of cmm × cph F_1 was −4.8°C (−2.6 plus −2.2 from Tables 5 and 6, respectively). It is no surprise that field frost damage scores of cmm × cph F_1 ranged around 0 and 1, which is less than the parental mean of 1.5. This resulted in an appearance of dominance in terms of the expression of frost resistance. However, when tested under a controlled environment, the NA and ACC of cmm × cph F_1 were both recessive and less than the parental means (Table 5 and Table 6). As in the example given above, the severity of freezing stress must be taken into consideration in interpreting data from genetic studies that have adopted approaches such as field survival trials and controlled freezing tests using a single low temperature. Depending upon the temperature applied, freezing tolerance may appear as a dominant character under low-stress or a recessive character under high-stress environments (Sutka and Veisz 1988; Limin and Fowler 1991; Sutka 1994). Therefore, it is more informative to use a series of test temperatures in freezing tolerance evaluation.

Genomic ratio and the expression of freezing tolerance

The wide range of freezing tolerance and ploidy levels found within *Solanum* has made it possible to determine the effect of the genomic ratio on the expression of freezing tolerance. However, since many species were involved in producing various hybrids with different genomic ratios, the effect of genomic ratio was confounded by the species effect. Ideally, combinations of different genomic ratios should be derived from only two species with various ploidies in order to eliminate the species effect. But due to the pre- or post-zygotic crossing barriers, such as EBN, realistically the successful crosses between different species are allowed to take place only at certain ploidy levels. In spite of the difficulties encountered, the influence of genomic ratio on freezing tolerance was further demonstrated by the comparison of cmm (+) hap somatic hybrids (ratio 1 : 1) with sexual F_1 hybrids of 4*x*-cmm and hap (ratio 2 : 1). The 1 : 1 fusion hybrids had an NA only 5% of the difference between two parents, while the 2 : 1 sexual hybrids had an NA of 35% (Table 5). In ACC, the ratios of cmm (+) hap somatic hybrids and sexual F_1 hybrids were 50% and 84%, respectively (Table 6). Therefore, having more genomes from cmm increased

the expression of both NA and ACC. Crosses of the hardy species acl and buk with the sensitive species plt and chc may provide additional evidence. The ratios of the F_1 hardiness level to the parental difference in acl were higher than buk in terms of ACC in derived F_1 hybrids even though the same sensitive parents were used and the ACC of buk was higher than that of acl.

Evidence from the Triticeae also indicates that freezing tolerance is favored by having a higher dosage from the hardy parental genomes than from the sensitive parent (Limin and Fowler 1988, 1989). In the Triticeae, the freezing tolerance of interspecific hybrids and induced amphiploids between hardy species and common wheat was significantly hardier than the parental mean only when the hardy species-to-wheat genomic ratio was 5 : 3. When the genomic ratios of parents were 1 : 3, 2 : 3, or 1 : 1, on the other hand, the expression of freezing tolerance in their progeny ranged from that of the wheat parent to the level of the parental mean. Because common wheat has a high ploidy level (6*x*), the hardy species are generally either at the same ploidy level or are less than hexaploid. For example, the two most cold hardy species, rye (*Secale cereale*) and crested wheatgrass (*Agropyron cristatum*), are both diploid. As a result, only one combination with tall wheatgrass (*Thinopyrum ponticum*, 10*x*), which has mild freezing tolerance, produced hybrids showing hardiness higher than the parental mean and this was used as an indication of the importance of chromosome dosage. In addition, the dosage effect once again was confounded by a species effect. In contrast, our results explored the role of genomic ratio by including multiple combinations, in some cases without the confounding effect of species (by using cmm-tbr hybrids).

Molecular mechanisms, such as the competition among the different regulatory elements, the strength of promoters and the efficiency of transcription factors of both parental genomes, have been proposed to explain the phenomenon observed (Limin et al. 1995). However, it is not yet known how the genomic ratio or dosage effect operates to manipulate the expression of freezing tolerance.

Combining ability and species choice in breeding for freezing tolerance

While the genomic ratio may have a significant influence on determining the expression of freezing tolerance, the combining ability of species or the ability of genes from different species to confer freezing tolerance would be another important consideration in breeding for freezing tolerance in terms of species choice. For instance, hybrids of 4*x*-cmm with arz, ber, chc, and mcd always showed less damage and had a higher ratio of F_1 hardiness level to the parental difference than hybrids of these sensitive species with acl (Table 3). In 1996 field trials, F_1 hybrids derived from 4*x*-cmm when crossed with either ber or mcd also exhibited more frost tolerance than hybrids with acl (Table 4).

Implications for breeding

Based on our results and many previous reports (Ross and Rowe 1965, 1969; Richardson and Estrada 1971; Vavilova 1978; Estrada 1987), it is clear that interspecific F_1 hybrids with an improved freezing tolerance over the non-hardy parent does not appear to be difficult to obtain if at least one parent is cold hardy (Tables 3, 4, 5, and 6). Some hybrids produced from crossing or fusing tbr with hardy species are particularly promising for use as the initial materials in breeding for freezing tolerance. However, the dosage effect determining the expression of freezing tolerance should be taken into consideration in subsequent crosses with tbr for the improvement of the economically important traits of hybrids. From this point of view, recurrent selection is needed to maximize the number of alleles conferring freezing tolerance in a genetic background suitable for cultivation. Fortunately, damage by frost in most cases is caused by only a few degrees centigrade in exceeding the limit of tolerance of cultivated potatoes (Estrada 1987). In other words, 2–3°C increase in the frost tolerance of cultivated potatoes would ensure a successful crop in many frost prone areas (Li 1985). Our data also demonstrated that genotypes with an AA of around or over −5°C, which is about 2°C more cold-hardy than tbr, were able to survive the frosts in both 1995 and 1996. Therefore, once the dosage of genes conferring freezing tolerance can be built up in the initial materials, or when species with extreme freezing tolerance such as cmm are chosen to start with, some losses in freezing tolerance during advanced breeding procedures are affordable.

Conclusion

From the present study, there appears to be two determinants for the expression of freezing tolerance in potatoes: the effectiveness or combining ability of genes existing in the hardy species, and the effect of the hardy to sensitive genomic ratio of the parents. In general, when equal genomes from both parents or more genomes from sensitive parents were combined in the hybrids, either sexually or somatically, freezing tolerance less than or closer to the parental mean can be predicted. However, the suppressing effect of sensitive genomes could be partially overcome by increasing the number of hardy genomes. A freezing tolerance higher than or similar to the parental mean is expected for combinations with higher genome numbers from the hardy parent. Although similar findings have been reported in the Triticeae, this has not been investigated in

Solanum species to our knowledge. The information provided here should be valuable in utilizing wild germplasm to improve the freezing tolerance of cultivated potatoes and in understanding the genetic and physiological mechanisms of freezing tolerance in potato species.

Acknowledgments 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, Wis 53706.

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