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Freezing tolerance and tuber production in selfed and backcross progenies derived from somatic hybrids between *Solanum tuberosum* L. and *S. commersonii* Dun.

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Abstract Selfed and backcross progenies developed from tetraploid somatic hybrids between *Solanum tuberosum* (tbr) and *S. commersonii* (cmm) were characterized for nonacclimated freezing tolerance (NA) and acclimation capacity (ACC) (two independent genetic components of freezing tolerance) under controlled environments. The segregation covered 28% and 71% of the parental range for NA and ACC, respectively, with the distribution skewed toward the tbr parent. Therefore, ACC appeared to be relatively easier to recover in the segregating generation. Some first backcross progeny had greater freezing tolerance than the cultivated parent primarily through the increase in ACC. When grown in the field, the improved freezing tolerance observed in the selfed progeny under controlled conditions was confirmed. Among NA, ACC, and freezing tolerance after acclimation (AA, which is the cumulative performance of NA and ACC), AA exhibited the highest correlation coefficient with field frost tolerance. In addition to freezing tolerance, vine maturity and tuber traits including tuber yield, tuber number per plant, mean tuber weight, and specific gravity were also segregating. No significant correlation between undesirable tuber traits and freezing tolerance was detected. Vine maturity and freezing tolerance were significantly correlated, so more careful selection for earliness was necessary in incorporating freezing tolerance. Yield comparable or superior to the backcross parent Wis AG 231 and an early Canadian cultivar, 'Sable', was found in many backcross progeny and some selfed progeny. The observed high yield can be attributed to the increase in mean tuber weight as well as tuber number. Moreover, a high portion of progeny had a specific gravity higher than 1.085, and some greater than 1.1. The implications derived from this study in breeding for freezing tolerance and further use of these materials are discussed.

Key words Potato · *Solanum tuberosum* · Freezing tolerance · Cold acclimation · Tuber traits

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Introduction

Many valuable characters have been identified in *Solanum commersonii* (cmm), a diploid tuber-bearing wild species endemic to Argentina, Paraguay, Uruguay, and Brazil (Correll 1962). These potentially useful characters include high specific gravity (Ehlenfeldt and Hanneman 1988), resistances to diseases and pests (Hanneman and Bamberg 1986) such as bacterial wilt (Kim et al. 1993), potato virus X (Tozzini et al. 1991), and potato tuber moth (Chavez et al. 1988), heat tolerance (Davis 1941; Palta et al. 1981) and, particularly, freezing tolerance (Chen and Li 1980; Palta et al. 1981; Vega and Bamberg 1995). *S. commersonii* is the most cold-hardy potato species when conditioned for a period of time with cool nonfreezing temperatures (i.e., *acclimated*) (Chen and Li 1980).

In addition to the triploid sexual hybrids obtained by crossing tetraploid cmm with haploid *S. tuberosum* (tbr) (Novy and Hanneman 1991; Carputo et al. 1995, 1997), tetraploid somatic hybrids between cmm and haploid tbr have been produced to capture the desirable traits of cmm in the cultivated potato (Cardi et al. 1993; Kim et al. 1993; Nyman and Waara 1997). These somatic hybrids can be either selfed or directly crossed with cultivated potatoes (Cardi et al. 1993; Chen et al. 1996; Palta et al. 1997; Nyman and Waara 1997). The use of these materials in breeding programs is therefore encouraging.

A recent study from our group has demonstrated that freezing tolerance is actually comprised of two independent genetic components, namely nonacclimated freezing tolerance (NA) and acclimation capacity (ACC) (Stone et al. 1993). Most studies to date aimed at improving freezing tolerance have not distinguished these two components. By characterizing NA and ACC separately, we found that NA in the tbr (+) cmm somatic hybrids was as low as the sensitive fusion parent tbr, whereas ACC did increase significantly and approximated to the parental mean (Chen et al. 1996; Palta et al. 1997). The expression of freezing tolerance in some of the somatic hybrids was already comparable to that of the hardy pure species mainly through the improvement in their ACC over tbr (Chen et al. 1996). In selfed progeny, a segregation for freezing tolerance has also been noted (Palta et al. 1997; Seppanen et al. 1998). Thus, in either selfed or backcross progeny a combination of freezing tolerance and agronomic traits may be recoverable.

There are many reports on the association of various desired traits in potatoes, especially the tuber traits (Maris 1969, 1988; Gopal et al. 1994; Serquen and Peloquin 1996). However, little information is available regarding the association between freezing tolerance and tuber traits. Although no correlation between reaction to frost and traits such as yield has been indicated (Ross and Rowe 1965; Estrada 1978), no systematic or quantitative analyses on this subject have been accomplished.

The objectives of the study presented here were: (1) to examine the segregation of NA and ACC in selfed and backcross progenies; (2) to test the association between freezing tolerance and agronomic traits; (3) to identify promising material for use in breeding programs.

Materials and methods

Plant materials

The parental somatic hybrids, designated HA26-5 and HA05-1, were generated by protoplast fusion between cmm (PI 320266, $2n = 2x = 24$) and a haploid clone of tbr cv 'Superior' (US-W 13122, $2n = 2x = 24$) (Kim et al. 1993). A chromosome number of 48 in both HA26-5 and HA05-1 has been verified by root-tip counts of chromosomes (Kim et al. 1993). These materials were kindly provided by Dr. John P. Helgeson, USDA/ARS, Department of Plant Pathology, University of Wisconsin, Madison. Two types of progenies were used for this study: (1) selfed progeny, produced by selfing HA26-5; (2) backcross progeny, derived by crossing either HA26-5 or HA05-1 as the female parent with the elite tbr breeding line Wis AG 231. Wis AG 231 is a selected breeding line from the University of Wisconsin Potato Breeding Program. This breeding line was used instead of cv 'Superior' because of the availability of pollen and the consideration of allelic diversity to avoid inbreeding.

Controlled freezing test

Plants transplanted from culture were grown in a controlled environment room at the Biotron facility (Madison, Wis.) for 6–7 weeks at 20°/18°C light/dark. A 14-h photoperiod at 400 μmol photon $m^{-2}s^{-1}$ was used. For cold acclimation, the temperature was lowered to 4°/2°C light/dark (100 μmol photon $m^{-2}s^{-1}$) for 2 weeks. These conditions have been shown to result in full acclimation in tuber-bearing *Solanum* species (Steffen and Palta 1986).

Excised leaflets were subjected to a simulated freeze-thaw stress for the determination of freezing tolerance before and after acclimation. A modification of the protocol by Steffen et al. (1989) was used. 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 for those samples for measuring ion leakage at 0°C, which were directly put on ice without being subjected to a freeze-thaw treatment. After 30 min, the temperature in the glycol bath was lowered to −0.5°C and held there for 30 min. Then the temperature was lowered to −1°C and held there 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 there for 1 h. 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 with a YSI conductance meter (Yellow Springs, Ohio). Thawed leaflets were sliced into 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 total ion content for each sample was determined after autoclaving for 15 min at 121°C. Percentage ion leakage at each temperature was obtained as $(R_1/R_2) \times 100\%$. The freezing curve was constructed by plotting mean percentage ion leakage of three subsamples versus 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). The difference between NA and freezing tolerance after acclimation (AA) was defined as ACC.

Field experiments

The field trials were conducted at the University of Wisconsin Peninsular Agricultural Research Station, Sturgeon Bay, Wisconsin in both 1996 and 1997. In 1996, plants were grown from tubers in the greenhouse and transplanted to the field in mid-July. Fifty genotypes of selfed progeny with six plants per genotype were used and assigned at random to plots. A randomized complete block design with three replications and nine plants per entry was used to assess 6 genotypes of backcross progeny, two fusion parents, two somatic hybrids (HA26-5 and HA05-1), and the backcross parent, Wis AG 231. The plant spacing was 60 cm within rows and 120 cm between rows for both progeny and parental lines. Species populations previously identified as frost sensitivity standards (Vega and Bamberg 1995) were also planted. After frosts occurred in early October of 1996, which caused severe injury to the sensitive species used as standards, plots were rated for frost damage on 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.

Since the haploid tbr fusion parent started senescence before frosts occurred, the score obtained from *S. polytrichon* (plt) planted at adjacent plots was used as a reference. Based on controlled freezing tests, the hardiness level of plt was similar to haploid tbr before and after acclimation.

Tubers of backcross progeny were harvested on October 17 (93 days after planting). Because selfed progeny were late in vine maturity, their tuber harvest was postponed until October 30, which was 106 days after planting.

Tubers of 6 genotypes from selfed progeny selected on the basis of freezing tolerance and tuber production in 1996, all of the backcross progeny from 1996, and the parental lines were sprouted and planted in the field again in mid-June 1997. In addition, 5 more genotypes of backcross progeny and an early Canadian cultivar, 'Sable', were included in the evaluation. Since cmm did not tuberize under field conditions at Sturgeon Bay due to its need of short daylengths for tuberization, it was not planted for comparison in 1997. The experimental design was a completely random design with two replications and 8 plants for each genotype per replication was used. The spacing within and between rows was 30 cm and 60 cm, respectively. All clones were harvested on October 23, which was 126 days after planting.

Evaluation of tuber traits and vine maturity

The traits evaluated included tuber weight per plant, tuber number per plant, mean tuber weight, tuber number > 55 mm (marketable), tuber number > 88 mm (large), and specific gravity. Mean tuber weight was obtained by dividing the total tuber weight by the number of tubers. The specific gravity was calculated using the weight in air/weight in water method based on the following formula: specific gravity = tuber weight in air/(tuber weight in air − tuber weight in water). This value is used to estimate the dry matter content of the tubers (Dean 1994). Vine maturity was measured only in 1997 by the degree of vine senescence, which was recorded on October 16, 119 days after planting. A scale of 0 = very early (vine already dead and dried up) to 5 = very late (still flowering) was used.

Statistical analysis

The comparison of genotype means and the analysis of correlation coefficients among traits were done by using the PROC GLM and PROC CORR (SPEARMAN) procedures of the SAS statistical package (SAS Institute 1995), respectively.

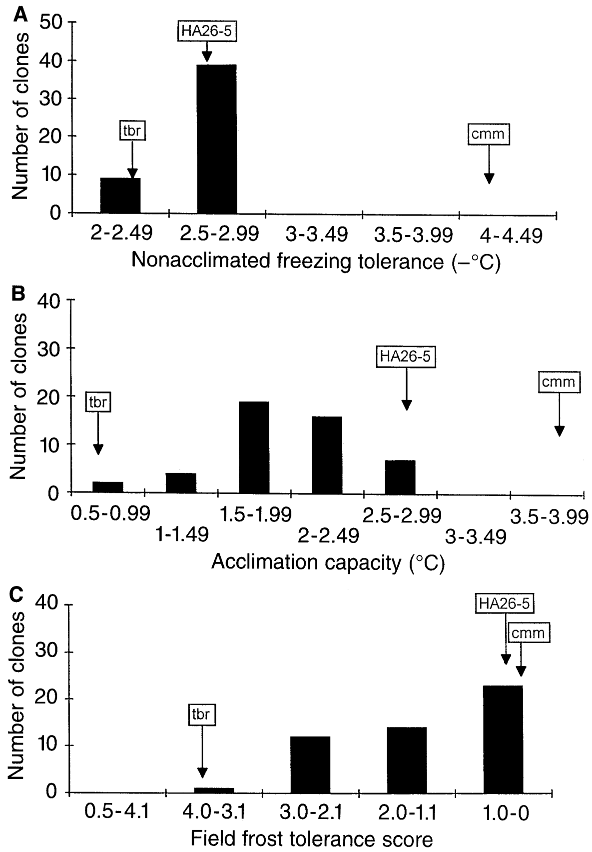

Fig. 1A–C Frequency distribution of nonacclimated freezing tolerance (**A**), acclimation capacity (**B**), and field frost tolerance score (**C**) in selfed progeny derived from a *S. tuberosum* (*tbr*) (+) *S. commersonii* (*cmm*) somatic hybrid, HA26-5. *Boxes* with *arrows* indicate the level of the parents

Results

Segregation of NA, ACC, and field frost tolerance in the selfed and backcross progeny

The NA derived from cmm was poorly recovered in the selfed progeny. Segregation of NA was 28% of the parental range with a skew toward the tbr parent (Fig. 1A). Segregation of ACC ranged from the level of the sensitive fusion parent, tbr, to that of the somatic hybrid HA26-5 and covered 71% of the parental difference (Fig. 1B). In other words, the improved freezing tolerance in selfed progeny was mainly due to an increase of ACC. Under field conditions, while some of the selfed progeny were as sensitive as the tbr parent, several clones of selfed progeny survived the frosts with little or no damage (Fig. 1C). However, the frosts were severe enough to discriminate among the selfed progeny but not between some of the progeny and the hardy parent cmm.

Although NA of backcross progeny was similar to that of tbr, about half of the progeny exhibited on ACC higher than that of the tbr parent by more than 1°C (Table 1). Backcross progeny generally were earlier in vine maturity and some were senescent before frosts occurred, so field frost tolerance was not scored for backcross progeny. However, those backcross progeny relatively late in vine maturity appeared slightly hardier than the sensitive species in the 1996 field frost test.

The correlation coefficients between field rating scores and freezing tolerance determined under controlled environments were 0.19, −0.46, and 0.55 for NA, ACC, and AA, respectively. Therefore, AA showed the highest correlation coefficient with frost tolerance ($P < 0.001$).

Field evaluation of tuber traits in the selfed and backcross progenies in 1996

The parental lines used in the fusion differed in vine maturity and tuberization in the field. At harvest the

Table 1 Nonacclimated freezing tolerance (NA), acclimated freezing tolerance (AA), and acclimation capacity (ACC) of the fusion parents, somatic hybrids, backcross parent, and derived progeny

Clone	(°C)		
	NA	AA	ACC
Parental lines			
(1) Fusion parents			
tbr (US-W 13122)	-2.4 ± 0.2[b]	-3.0 ± 0.2	0.6 ± 0.0
cmm (PI 320266)	-4.2 ± 0.1	-8.0 ± 0.1	3.8 ± 0.0
(2) Somatic hybrids			
HA26-5	-2.6 ± 0.0	-5.4 ± 0.3	2.9 ± 0.3
HA05-1	-2.8 ± 0.3	-5.4 ± 0.0	2.6 ± 0.3
(3) Backcross parent			
tbr (Wis AG 231)	-2.3 ± 0.0	-3.3 ± 0.4	1.0 ± 0.4
Backcross progeny[a]			
BC (1)	−2.1	−3.8	1.7
BC (2)	−2.6	−3.8	1.2
BC (5)	−2.3	−4.3	2.0
BC (6)	−2.2	−4.0	1.8
BC (7)	−2.2	−3.4	1.1
BC (8)	−2.4	−4.3	1.8
Clones used only in 1997:			
BC (9)	−3.0	−3.7	0.7
BC (10)	−2.8	−4.6	1.8
BC (11)	−3.1	−4.5	1.4
BC (13)	−3.0	−4.9	1.9
BC (14)	−2.4	−4.9	2.5
Selected selfed progeny derived from HA26-5			
(x) 4	−2.8	−4.4	1.6
(x) 9	−2.8	−5.2	2.4
(x) 11	−2.5	−5.5	3.0
(x) 34	−2.8	−5.3	2.5
(x) 96	−2.6	−4.4	1.8
(x) 125	−2.8	−4.6	1.8

[a] All of the backcross progeny were derived from HA05-1 × Wis AG 231, except BC (1), which was from HA26-5 × Wis AG 231
[b] Values are the means of two determinations ± SD. At each determination three replicated samples were subjected to a simulated frost

vines of haploid tbr parent were already dried up, while the cmm parent was still flowering and had no tubers. The somatic hybrids produced immature tubers and were late in vine maturity, so they were intermediate.

Tuber production was generally low in 1996, which may have partly resulted from late planting. The planting was intentionally delayed in order to obtain plants which were not senescing when field frosts occurred. Nevertheless, segregation for tuber traits was revealed by the considerable variation in total tuber weight, tuber number, mean tuber weight and specific gravity in both selfed and backcross progenies (Fig. 2A–D; Table 2). The vine maturity also appeared to be segregating, although scores for individual genotypes were not recorded. Based on the data of freezing tolerance and tuber yield, 6 genotypes of selfed progeny were selected for use in the 1997 field trials. The information regarding their freezing tolerance and tuber yield is included in Table 1 and Table 2, respectively.

Since AA evaluated under the controlled environment exhibited the highest correlation coefficient with field rating scores, only the association between AA and other traits was determined in addition to the field frost tolerance score. No significant correlation between freezing tolerance (represented by field scores or AA) and tuber yield, tuber number, mean tuber weight, and specific gravity was detected in this population (Table 3).

Field evaluation of vine maturity and tuber traits in 1997

The yield in all of the clones was higher in 1997 than that in 1996 with the exception of the somatic hybrid HA26-5 (Table 4). This increase in yield varied from one-to ninefold higher than the previous year when the clones used in both years were compared. Despite the difference in tuber yield between 1996 and 1997, a significant correlation was detected in tuber number, mean tuber weight, and marketable tuber number between the 2 years (Table 5). Thus, the genotypes which produced either greater tuber weight or a higher tuber number in 1996 also had the similar tendency in 1997.

When comparisons were made among different genotypes, a promising finding was that the performance of many progeny clones was comparable or superior to the backcross parent, Wis AG 231, or the cultivar 'Sable' in terms of tuber production (Table 4). In addition, 11 out of 17 progeny clones had a specific gravity significantly higher than that of the haploid tbr parent and Wis AG 231 (Table 4).

The freezing tolerance of the new backcross progeny incorporated in the 1997 field evaluation is given in Table 1. With the exception of vine maturity, AA was not significantly correlated with specific gravity or other tuber traits (Table 3) as was also the case for selfed progeny in 1996.

Discussion

Recovery of freezing tolerance in the progeny

Both field trials and controlled freezing tests demonstrated that improved freezing tolerance can be identified in either set of progeny derived from tbr (+) cmm somatic hybrids. From controlled freezing tests some selfed progeny exhibited an AA higher than that of tbr by 2°C, which is about the hardiness level of some hardy pure species (Li 1977; Palta and Li 1979; Chen at al. 1998). It is not surprising that little or no damage could be detected on some of the selfed progeny after the frosts and that their scores were in the range of 0 to 1. In contrast, sensitive species used as standards such as *S. chacoense* and *S. microdontum* were severely

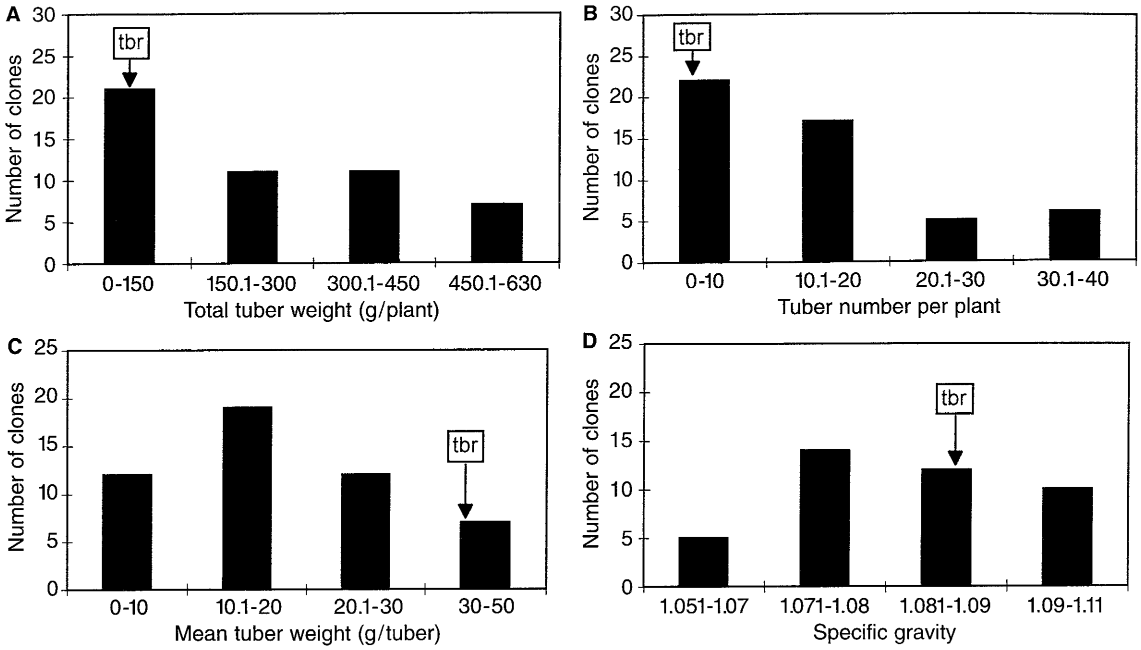

Fig. 2A–D Frequency distribution of total tuber weight (**A**), tuber number per plant (**B**), mean tuber weight (**C**), and specific gravity (**D**) in selfed progeny derived from a *S. tuberosum* (*tbr*) (+) *S. commersonii* somatic hybrid, HA26-5. *Box* with *arrow* indicates the level of *tbr* fusion parents

injured and scored around 4. Therefore, our results from the field evaluation were generally in agreement with those from controlled freezing tests. Among NA, ACC, and AA, AA showed the highest correlation coefficient with frost tolerance. Since the frosts took

Table 2 Tuber yield and specific gravity of the fusion parent, somatic hybrids, backcross parent, and derived progeny in 1996

Clone	Total tuber weight (g/plant)	Tuber number per plant	Mean tuber weight (g/tuber)	Tuber number > 55 mm	Specific gravity
Parental lines					
(1) Fusion parent					
tbr (US-W 13122)	77.8 c[c]	2.4 d	31.2 c, d, e	0.0 e	1.087 c
(2) Somatic hybrids					
HA26-5	156.1 b, c	7.6 b, c, d	21.0 d, e	0.7 d, e	1.062 e
HA05-1	332.8 a, b, c	11.9 a, b	26.4 c, d, e	1.8 b, c, d	1.072 d
(3) Backcross parent					
tbr (Wis AG 231)	274.5 a, b, c	3.6 c, d	71.6 a, b	1.0 d, e	1.071 d
Backcross progeny[a]					
BC (1)	524.1 a	10.8 a, b, c	47.1 b, c, d, e	3.8 a	1.088 c
BC (2)	263.2 a, b, c	5.0 b, c, d	54.3 a, b, c, d	2.0 b, c, d	1.072 d
BC (5)	500.0 a	6.1 b, c, d	82.0 a	3.2 a, b	1.091 b, c
BC (6)	214.4 a, b, c	16.0 a	13.8 e	2.0 b, c, d	1.101 a
BC (7)	222.5 a, b, c	8.7 b, c, d	25.2 c, d, e	1.3 c, d, e	1.062 e
BC (8)	428.4 a, b	7.6 b, c, d	55.9 a, b, c	2.7 a, b, c	1.095 a, b
Selected selfed progeny derived from HA26-5[b]					
(x) 4	508.3	17.7	28.8	3.0	1.095
(x) 9	500.0	14.2	35.3	4.8	1.084
(x) 11	300.0	8.7	34.6	2.3	1.081
(x) 34	630.0	25.0	25.2	3.8	1.078
(x) 96	566.7	30.3	18.7	2.0	1.080
(x) 125	491.7	24.8	19.8	2.8	1.079

[a] All of the backcross progeny were derived from HA05-1 × Wis AG 231, except BC (1), which was from HA268-5 × Wis AG 231
[b] Data are the mean value of six plants
[c] Data are the mean value of three field plots, each with nine plants. Means in a column followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Table 3 Correlation coefficients (r_s)[a] between freezing tolerance (field rating scores or acclimated freezing tolerance, AA) and other traits in 1996 and 1997 (*ND* not determined)

Traits		Vine maturity	Total tuber weight (g/plant)	Ttuber number per plant	Mean tuber weight (g/tuber)	Specific gravity
Field scores	1996	ND	0.12	−0.14	0.26	0.08
AA	1996	ND	0.02	−0.08	0.06	0.11
AA	1997	−0.54*	0.09	−0.10	0.24	−0.28

* Significant at the 0.05 level
[a] Spearman's rank correlation coefficient

Table 4 Vine maturity (VM), tuber yield, and specific gravity of the fusion parent, somatic hybrids, backcross parent, and derived progeny in 1997

Clone	VM	Total tuber weight (g/plant)	Tuber number per plant	Mean tuber weight (g/tuber)	Tuber number > 55 mm	Tuber number > 88 mm	Specific gravity
Parental lines[a]							
tbr	0[d]	475 e, f[e]	12 e, f	40 g, h, i	3.2 i, j	0.1 f	1.085 h, i
HA26-5	3	103 f	6 f	21 i	0.3 j	0.0 f	1.091 e, f, g, h
HA05-1	3	1382 b, c, d, e	21 d, e, f	69 e, f, g, h, i	12.2 a, b, c, d, e, f, g	2.1 b, c, d, e, f	1.100 b, c, d
Wis AG 231	2	1544 b, c, d	12 e, f	134 a, b, c	7.7 d, e, f, g, h, i	2.0 c, d, e, f	1.084 h, i
Backcross progeny[b]							
BC (1)	1.5	2600 a	36 b, c, d	78 d, e, f, g, h	17.7 a	2.2 b, c, d, e, f	1.091 e, f, g, h
BC (2)	2	2200 a, b	15 e, f	155 a, b	11.1 b, c, d, e, f, g, h	4.9 a, b, c	1.091 e, f, g, h
BC (5)	1.5	1545 b, c, d	21 d, e, f	78 d, e, f, g, h	10.2 c, d, e, f, g, h	1.2 e, f	1.093 d, e, f, g
BC (6)	1	1312 b, c, d, e	57 a	23 i	16.3 a, b	0.3 e, f	1.109 a
BC (7)	2	2201 a, b	22 d, e, f	100 c, d, e	13.3 a, b, c, d	4.3 a, b, c, d	1.083 i
BC (8)	1	1510 b, c, d	19 e, f	79 d, e, f, g	9.8 c, d, e, f, g, h	1.6 d, e, f	1.101 b, c
BC (9)	2	1054 d, e	9 e, f	121 a, b, c, d	6.4 g, h, i	1.6 d, e, f	1.097 b, c, d, e
BC (10)	3	2021 a, b, c	21 d, e, f	101 c, d, e	12.8 a, b, c, d, e, f	6.8 a	1.086 g, h, i
BC (11)	3	1409 b, c, d	8 f	165 a	6.9 f, g, h, i	5.0 a, b	1.088 f, g, h, i
BC (13)	3.5	1342 b, c, d, e	14 e, f	96 c, d, e	8.8 c, d, e, f, g, h, i	3.2 b, c, d, e	1.100 b, c, d
BC (14)	3.5	1072 d, e	16 e, f	78 d, e, f, g, h	7.7 d, e, f, g, h, i	1.7 d, e, f	1.096 b, c, d, e
Selected selfed progeny							
(x) 4	4	1121 c, d, e	35 b, c, d	30 g, h, i	7.8 d, e, f, g, h, i	1.0 e, f	1.097 b, c, d, e
(x) 9	3	1916 a, b, c, d	18 e, f	105 b, c, d, e	14.5 a, b, c	6.9 a	1.099 b, c, d
(x) 11	1.5	1407 b, c, d	25 c, d, e	56 e, f, g, h, i	13.1 a, b, c, d, e	0.6 e, f	1.090 e, f, g, h
(x) 34	2.5	1688 b, c, d	38 b, c	44 f, g, h, i	12.2 a, b, c, d, e, f, g	0.9 e, f	1.094 c, d, e, f
(x) 96	2.5	1156 c, d, e	43 a, b	27 h, i	7.2 e, f, g, h, i	0.4 e, f	1.102 b
(x) 125	3.5	1194 c, d, e	13 e, f	92 c, d, e, f	8.6 c, d, e, f, g, h, i	0.9 e, f	1.101 b, c
Sable[c]	0	988 d, e	8 f	123 a, b, c, d	5.4 h, i, j	2.1 b, c, d, e, f	1.068 j

[a,b] See Table 1
[c] An early maturity Canadian Tuberosum cultivar
[d] Data are the mean score of two field plots. The scores used for maturity evaluation are following: 0 = dead and dried up, 1 = dead, 2 = more than mature, 3 = mature, 4 = less than mature, 5 = flowering
[e] Data are the mean value of two field plots, each with eight plants. Means in a column followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Table 5 Correlation coefficients between 1996 and 1997 for tuber traits

Trait	Total tuber weight (g/plant)	Tuber number per plant	Mean tuber weight (g/tuber)	Tuber number > 55 mm	Specific gravity
r_s[a]	0.26	0.66**	0.56*	0.50*	0.48

*,** Significant at the 0.05 and 0.01 levels, respectively
[a] Spearman's rank correlation coefficient

place after a period of gradually cooling weather in late fall, it allowed the expression of ACC prior to the frost episode. These results indicate that survival from stresses following a fall frost episode in nature in north temperate regions such as Sturgeon Bay tends to correspond to AA as measured under controlled environments.

Distorted segregation of NA and ACC in selfed progeny

The reasons for the skewed segregation pattern of NA and ACC and why we were unable to fully recover the excellent NA and ACC of the cmm parent in the segregating generation could be due to the dominant effect of freezing-sensitive genes or the small sample size. In view of the tetraploid nature of the materials and polygenic inheritance of freezing tolerance (Richardson and Weiser 1972; Stone et al. 1993), the sample size used in the present study may be too small to recover the cmm parental type. In addition, irregularity during meiosis (Masuelli et al. 1995), gametic or zygotic selection (Giovannini et al. 1993; Kreike and Stiekema 1997), and preferential pairing between homologues from the same species (Williams et al. 1993) might also exist. The expression of freezing tolerance has also been shown to be influenced by the relative dosage of homologues or genomes conferring freezing tolerance and sensitivity (Palta et al. 1997; Chen et al. 1999). This could partly explain the distorted segregation pattern in conjunction with preferential pairing or differential transmission between cmm and tbr chromosomes to the gametes or zygotes.

Correlation between freezing tolerance and other traits

No significant correlation between freezing tolerance (determined by AA or field rating scores) and tuber yield, tuber number, mean tuber weight, and specific gravity was detected in both years of field trials (Table 3), indicating independent genetic controls for freezing tolerance and these tuber traits and the feasibility of combining freezing tolerance and desirable tuber traits. Although a significant association was found between freezing tolerance and late vine maturity, variation for vine maturity was present among freezing-tolerant individuals in the progeny. Therefore, more careful selection appears to be necessary to obtain genotypes with earliness in addition to freezing tolerance.

Tuber traits of the progeny derived from tbr (+) cmm somatic hybrids and implications in further utilization of these materials

Wild *Solanum* species including cmm do not normally tuberize under the long-day conditions of the North Temperate region (Rudorf 1958). Nevertheless, when wild species are crossed to haploids of Tuberosum cultivars, many of the diploid F_1 hybrids can produce greatly improved tuberization over the wild species parent, and in some cases, over the haploid parent as well (Hermundstad and Peloquin 1986). The somatic hybrids HA26-5 and HA05-1 also produced tubers, although they formed long stolons just like cmm parent. HA05-1 produced twice the total tuber weight than its haploid tbr parent (Table 4). On the other hand, HA26-5 may still have some of the short-day requirement for tuberization, so its yield was even lower than that of the haploid tbr parent.

High dry matter content represented by specific gravity is a particularly important trait in potato cultivars used in the potato processing industry because of its association with increased chip yield and lower oil absorption (Dean 1994). Thus, it is encouraging that 16 out of 17 clones showed a specific gravity greater than 1.085 (acceptable processing level) and that 4 of the clones showed a specific gravity greater than 1.1 in 1997 field trials (Table 4).

The consistency between the data from 2 years in terms of clone performance was particularly noteworthy. For instance, tubers of BC(1) consistently outyielded while BC(6) always outnumbered the other backcross progeny. In addition, the specific gravity of tubers in BC(6) was ranked the highest for both years. Clone (x)9 consistently produced a high portion of large tubers.

Our investigation of separate evaluation of NA and ACC reveals that ACC is relatively easier to recover in the segregating generation. ACC may represent a component of freezing tolerance which is genetically simpler as suggested by Stone et al. (1993) and more accessible for breeding manipulation. However, the relative importance of different components of freezing tolerance may vary among seasons and environments in various potato growing regions.

This study demonstrated that it is feasible to combine high levels of freezing tolerance from *S. commersonii* with high tuber yield and specific gravity. A good example is clone (x)9, which had an AA beyond $-5°C$, the highest number of tubers greater than 88 mm, and a specific gravity of 1.099. Since high-yielding clones with improved freezing tolerance and agronomic traits were identified, the usefulness of these materials in breeding programs appears to be promising.

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