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Tolerance to low temperatures and tuber soft rot in hybrids between *Solanum commersonii* and *Solanum tuberosum* obtained through manipulation of ploidy and endosperm balance number (EBN)

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With 2 tables

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Abstract

The objectives of this study were to evaluate the tolerance to low temperatures and tuber soft rot in hybrids between *Solanum commersonii* and *Solanum tuberosum*. The experimental materials consisted of F_1 triploid, BC_1 pentaploid–near pentaploid and BC_2 tetraploid–near tetraploid hybrids. The F_1 triploids had a freezing tolerance and acclimatization capacity closest to *S. commersonii*. This indicated that the endosperm barriers which prevent the introgression of 1EBN *S. commersonii* into 4EBN *S. tuberosum* had been overcome. Indeed, the triploids produced $2n$ eggs, thus giving a compatible maternal to paternal EBN ratio in the hybrid endosperm generated by the $3x$(2EBN) $\times$ $4x$(4EBN) crosses. The tolerance to low temperatures of BC_1 and BC_2 hybrids was lower than that of the F_1. However, a number of genotypes were identified which were able to withstand temperatures down to -5°C. Some BC_2 hybrids were also tested for their tolerance to tuber soft rot, and some resistant hybrids were detected. A number of them combined the capacity for cold acclimatization with tolerance to tuber soft rot. These hybrids have an EBN of 4; they are fertile and have been used in backcrosses with 4EBN *S. tuberosum*.

Key words: *Solanum commersonii* — *Solanum tuberosum* — *Erwinia carotovora* — $2n$ gametes — acclimatization capacity — endosperm balance number (EBN) — germplasm introgression

It is well known that while the cultivated potato *Solanum tuberosum* Group Tuberosum ($2n = 4x = 48$) (tbr) has narrow genetic bases, its wild relatives provide a rich, unique and diverse source of genetic variation (Hanneman 1989). Genes for resistance to biotic and abiotic stresses, for characteristics such as high solid content, good chipping quality and tuber colour can easily be found in these species. Wild *Solanum* species vary in ploidy from diploid ($2n = 2x = 24$) to hexaploid ($2n = 6x = 72$). Over 70% are diploid and thus can easily be crossed with haploids ($2n = 2x = 24$) extracted from tbr varieties.

However, a number of diploid species are sexually isolated from tbr haploids and from other diploid species because of endosperm abortion in the hybrid seed. To explain these crossing barriers Johnston et al. (1980) postulated that each *Solanum* species has a hypothetical endosperm balance number (EBN), ranging from 1 to 4, and that a 2 : 1 maternal to paternal EBN ratio in the hybrid endosperm is a necessary condition for its normal development. Thus, only cross combinations with the same EBN result in normal endosperm development. Sexually isolated diploid species have an EBN of 1 (Johnston and Hanneman 1982), making crosses with tbr haploids and most of the wild species (2EBN) very difficult because the EBN ratio requirement is not satisfied.

Among 1EBN wild species, *S. commersonii* (cmm) has several useful traits, including resistances to Verticillium wilt, potato virus X and tuber soft rot (Hanneman and Bamberg 1986, Sirianni 1997). This species is also very resistant to low temperatures and, among wild *Solanum* species, has the best capacity for cold acclimatization after exposure to low temperatures (Palta and Simon 1993). These traits are very important given that frost is often a major factor in reducing tuber yield, and that the cultivated potato is frost-sensitive and unable to cold acclimatize (Chen and Li 1980).

One simple and efficient way to overcome the EBN barriers of *Solanum* species is to consider the EBN as their effective ploidy level, which can be manipulated by raising and lowering chromosome sets. With this in mind, the chromosome number and EBN of cmm were doubled (Cardi et al. 1993) and $4x$(2EBN) cmm was crossed with $2x$(2EBN) hybrids. Through the function of $2n$ eggs, the F_1 triploids obtained were used in $3x \times 4x$ crosses with tbr to produce fertile BC_1 hybrids, which were further backcrossed to tbr to generate the BC_2 progeny. The ploidy and the cytological and molecular characterization of F_1, BC_1 and BC_2 hybrids have been reported previously (Carputo et al. 1995, 1997a, Barone et al. 1999). In this paper we summarize the evaluation of resistance traits found in these hybrids obtained through the manipulation of effective ploidy levels.

Materials and Methods

Plant materials: F_1 triploid, BC_1 pentaploid–near pentaploid and BC_2 tetraploid–near tetraploid cmm–phu–tbr sexual hybrids were used. The triploid hybrids were obtained from crosses between a tetraploid (2EBN) clone of PI 243503 of cmm obtained by *in vitro* tissue culture (Cardi et al. 1993) and 2EBN diploid hybrids between *S. tuberosum* Group Phureja (phu) and *S. tuberosum* Group Tuberosum haploids (Carputo et al. 1995). BC_1 hybrids were produced through $3x \times 4x$ crosses between triploid hybrids and tbr varieties. BC_2 hybrids were obtained by backcrossing two pentaploids with the varieties 'Blondy', 'Carmine' and 'Tollocan' and the breeding line Wis 482 (Carputo et al. 1997a).

Screening for freezing tolerance: Clonally propagated F_1 (five genotypes), BC_1 (eight genotypes) and BC_2 hybrids (19 genotypes) were evaluated for freezing tolerance. Also used in this screening were the parental genotypes (cmm and the phu–tbr hybrid). The tests were carried out using the electrolyte leakage procedure (Sukumaran and Weiser 1972, Stone et al. 1993) before and after cold acclimatization. Such a procedure has previously been employed for potato germplasm evaluation and is very reproducible (Stone et al. 1993). It also allows the detection of small differences among genotypes. Four plants from each genotype were grown in a growth chamber under cool white fluorescent lamps (350–400 μmol/m^2 s^{-1}) at 18–20°C (dark/light) for non-acclimatization studies. For cold acclimatization studies, two plants per genotype were transferred to a cold room (4–2°C, light/dark) at 100 μmol/m^2 s^{-1} for 2 weeks. This procedure is known to produce fully acclimatized plants (Steffen and Palta 1986). Mature expanded leaves were put into culture tubes and submerged in a glycol bath at 0°C. Three replicates per genotype were used in each temperature treatment. Temperature was lowered 0.5°C every 30 min. The control treatment consisted of three replicates per genotype kept on ice at 0°C. After 30 min at the desired freezing temperature, the tubes were placed on ice to thaw overnight. Thawed leaves were then cut, vacuum-infiltrated in 25 ml distilled deionized water and shaken for 1 h at room temperature. Freezing damage was assessed by evaluating ion leakage from thawed leaf samples with a conductivity meter. For each genotype, a freezing curve was constructed by plotting the per cent ion leakage (mean $\pm$ SD from three separate measurements) vs. freezing temperatures. The maximum conductivity, representing total ion leakage for each sample, was determined after autoclave heat killing. The minimum ion leakage was determined by measuring the ion leakage from the controls held at 0°C. This method is well established for precise determination of freezing tolerance (Steffen and Palta 1986, Stone et al. 1993, Teutonico et al. 1993). The freezing tolerance (non-acclimatized, NAFT; acclimatized, ACFT) for each genotype was determined by calculating the temperature at 50% of freezing injury (LT_{50}), according to the logistic model described by Janácek and Prásil (1991). Statistical differences between LT_{50}s of genotypes tested were calculated with the program LV50 version 2.1 (Janácek and Prásil 1991).

Screening for tuber soft rot resistance: Tubers from 15 BC_2 hybrids and four tbr varieties were used for the test. The tbr varieties included 'Majestic', 'Dèsirèe', 'Draga' and 'Spunta' as controls. Strain 009 of *Erwinia carotovora* ssp. *carotovora* (Ecc) was provided by the International Potato Centre, Lima, Peru. Tubers were inoculated according to the procedure described by Carputo et al. (1997b). Three to five holes (2 cm deep) were made in each tuber using a sterilized drill. One hole was filled with distilled water as a control and the others were inoculated with 20 μl of bacterial suspension. After 72 h incubation at 24°C in a dew chamber, the diameter of the rotted area was measured. Following the scale given by Carputo et al. (1997b), genotypes with an average diameter of rotted area smaller than 4 mm were classified as resistant, those with a diameter of decay between 4 and 6 mm as intermediate, and those with a diameter of rotted area larger than 6 mm as susceptible.

Results

Freezing tolerance

The freezing tolerances of nonacclimatized and acclimatized F_1, BC_1, BC_2 hybrids, and their diploid parents *S. commersonii* and UP88-P5 are reported in Table 1. *S. commersonii* confirmed its high NAFT and ACFT values, which were significantly different from those of all the other genotypes (–4.9°C and –9.5°C, respectively). The phu–tbr parent UP88-P5 was sensitive in non-acclimatized conditions (NAFT value of –2.6°C), whereas it showed some acclimatization capacity (ACFT value of –4.4°C). The F_1 triploid hybrids showed killing temperatures distributed between the parental values, both with and without acclimatization. On average, killing temperatures were –3.5°C and –6.2°C in non-acclimatized and acclimatized conditions, respectively. The best genotype was B10, with NAFT and ACFT values significantly higher than those of the other hybrids (–4.1°C and –7.5°C, respectively).

BC_1 and BC_2 hybrids showed a similar behaviour. The NAFT values of BC_1 genotypes ranged from –3.2°C (hybrid P5) to –2.5°C (hybrids P7 and T1), that of BC_2 genotypes from −3.0°C (hybrids PTH-A1, PTH-F3 and PTH-F5) to –1.9°C (hybrid PTH-B10). These values are similar to those reported for tbr varieties (Chen and Li 1980, Palta et al. 1993). Conversely, the ACFT values of BC_1 and BC_2 hybrids were often higher than tbr, which is not able to cold acclimatize. The most interesting genotype was the BC_1 clone P3, whose ACFT value (–5.5°C) was significantly higher than that of the phu–tbr parent and of all the other BC_1 and BC_2 hybrids. P3 was also the backcross genotype with the largest difference between acclimatized and non-acclimatized killing temperatures (around 3°C). Interestingly, this genotype came from $3x \times 4x$ crosses involving the triploid B10, which was the most resistant triploid genotype. Also interesting were BC_2 hybrids PTH-E10 and PTH-F4b, with an ACFT value of –4.8°C and a difference between acclimatized and non-acclimatized killing temperatures over 2°C. Although their ACFT value was not significantly different from that of the phu-tbr parent, the percentage of ions leaked by acclimatized PTH-E10 and PTH-F4b between –2.5°C and –4°C was always low ($< 30\%$), thus giving an S-shaped freezing curve that was more similar to that of cmm than to that of phu–tbr (not shown).

Tuber soft rot resistance

Results from the screening tests are presented in Table 2. A wide range in susceptibility was displayed by the genotypes tested, with a diameter of rotted area ranging from 12.6 mm (cv. 'Majestic') to 2.5 mm (clone PTH-E3). 'Draga' was the best control, with intermediate resistance (diameter of rotted area 5.3 mm). All the other tbr controls were very susceptible, with diameters of rotted areas larger than 9 mm. Out of 15 PTH hybrids tested, three (PTH-F7, PTH-E3 and PTH-E10) were classified as resistant according to the scale adopted. From the viewpoint of breeding, it is interesting to note that among these resistant genotypes one (PTH-F7) has a euploid chromosome number. Of the other PTH hybrids, seven were classified as intermediate (diameter of rotted area of 4–6 mm) and five as susceptible (diameter of rotted area of larger than 6 mm).

Discussion

The breeding scheme used to introgress tolerance genes from the sexually isolated species *S. commersonii* into the *S. tuberosum* gene pool is based on the production of F_1 triploids, BC_1 pentaploids and BC_2 iper-tetraploid hybrids (Carputo et al. 1997a). These hybrids are characterized by different genomic ratios of the parents. The triploids have two haploid genomes of the frost resistant, cold acclimatizing cmm and one of phu–tbr, whereas BC_1 pentaploids have two haploid genomes of cmm and three of phu–tbr. BC_2 hybrids should have a further reduction of the cmm genome. Clearly, having more genomes from cmm increased the expression of tolerance. In fact, within the hybrids analysed, F_1 triploids were those that displayed the highest NAFT and ACFT values.

Killing temperatures of triploid B10 so close to cmm strongly suggested that freezing tolerance *per se* and acclimatization capacity were transmitted from the 1EBN form of cmm to the 2EBN form of B10. These results demonstrate that the

Table 1: Freezing tolerance (°C) of non-acclimatized (NAFT) and acclimatized (ACFT) F_1, BC_1 and BC_2 *Solanum commersonii–S. tuberosum* hybrids and their diploid parents (cmm and phu–tbr). The difference between acclimatized and non acclimatized killing temperature (Δ(ACFT-NAFT)) is also given

Genotype	Pedigree	Killing temperature (°C)[1] NAFT	ACFT	Δ(ACFT–NAFT) (|°C|)
F_1				
A1 ($2n = 36$)	cmm × phu–tbr	–3.40 c	–5.68 de	2.28
B1 ($2n = 36$)	cmm × phu–tbr	–3.27 c	–6.40 c	3.13
B3 ($2n = 36$)	cmm × phu–tbr	–3.42 c	–6.42 cd	3.0
B10 ($2n = 36$)	cmm × phu–tbr	–4.10 b	–7.48 b	3.38
C1 ($2n = 36$)	cmm × phu–tbr	–3.13 cd	–5.09 e–g	1.96
BC_1				
P3 ($2n = 60$)	B10 × tbr	–2.71 e–g	–5.49 e	2.78
P5 ($2n = 60$)	B10 × tbr	–3.23 c	–3.95 l–o	0.72
P6 ($2n = 67$)	C1 × tbr	—	–3.75 l–p	—
P7 ($2n = 60$)	C1 × tbr	–2.50 lk	–4.17 i–l	1.67
P11 ($2n = 58$)	B3 × tbr	–2.67 e–h	–4.08 j–m	–1.41
P12 (—[2])	B3 × tbr	–2.59 f–j	–4.33 f–o	–1.74
P15($2n = 58$)	A1 × tbr	–2.67 e–h	–4.30 g–l	1.63
T1($2n = 60$)	C1 × tbr	–2.48 i–k	–3.26 pq	0.78
BC_2				
PTH-A1 ($2n = 49$)	P5 × tbr	–2.99 d	–4.65 fg	1.66
PTH-A2 ($2n = 48$)	P5 × tbr	–2.70 e–g	–4.02 k–n	1.32
PTH-B7 ($2n = 51$)	tbr × P5	–2.63 f–i	–4.12 i–l	1.49
PTH-B10 ($2n = 53$)	tbr × P5	–1.86 m	–3.06 q	1.20
PTH-C4 ($2n = 51$)	P3 × tbr	–2.25 lm	–3.40 p	1.15
PTH-C5 ($2n = 54$)	P3 × tbr	–2.70 e–h	–4.58 f–h	1.88
PTH-D12 ($2n = 54$)	P5 × tbr	–2.51 lk	–3.59 o–p	1.08
PTH-D13 ($2n = 51$)	P5 × tbr	–2.61 f–j	–4.53 f–h	1.92
PTH-D14 ($2n = 54$)	P5 × tbr	–2.52 h–k	–4.50 f–i	1.98
PTH-E8 ($2n = 52$)	tbr × P3	–2.39 kl	–4.31 g–l	1.92
PTH-E10 ($2n = 56$)	tbr × P3	–2.66 e–h	–4.80 f	2.14
PTH-F3 ($2n = 52$)	P5 × tbr	–2.97 d	–3.80 m–o	0.83
PTH-F4 ($2n = 52$)	P5 × tbr	–2.73 ef	–4.14 i–l	1.41
PTH-F4b ($2n = 51$)	P5 × tbr	–2.73 ef	–4.86 f	2.13
PTH-F5 ($2n = 54$)	P5 × tbr	–3.03 d	–4.59 fg	1.56
PTH-F6 ($2n = 51$)	P5 × tbr	–2.41 k	–3.68 n–p	1.27
PTH-F7 ($2n = 48$)	P5 × tbr	–2.78 e	–4.23 i–l	1.45
PTH-F9 ($2n = 54$)	P5 × tbr	–2.70 e–g	–4.49 f–k	1.79
PTH-F10 ($2n = 53$)	P5 × tbr	–2.71 e–g	–3.99 l–n	1.28
cmm (PI 243503; $2n = 24$)		–4.88 a	–9.45 a	4.57
phu-tbr (UP88-P5; $2n = 24$)		–2.59 g–j	–4.44 f–k	1.85

[1] Within each column, values followed by the same letter are not significantly different at $P = 0.05$
[2] Data not available.

endosperm barriers which prevent the introgression of 1EBN cmm genes into the 4EBN tbr gene pool had been overcome. In fact, the 2EBN triploids produced $2n$ eggs (Carputo et al. 1997a), thus allowing a compatible maternal to paternal EBN ratio following $3x$(2EBN) × $4x$(4EBN) crosses. This is important considering that, usually, the bottleneck of breeding schemes based on triploid bridges is the simultaneous selection for $2n$ gamete production and for the target traits in the triploids (Watanabe et al. 1992).

The combination of freezing tolerance and acclimatization capacity in triploid B10 is felt to be very important from the standpoint of breeding, in that unlinked genes control these two traits in the potato (Stone et al. 1993). In the genus *Solanum*, species with a degree of tolerance to low temperature rarely combine these two traits. They are generally either cold tolerant but unable to acclimatize (e.g. $2x$(2EBN), *S. sanctae-rosae*) or able to acclimatize but cold sensitive (e.g. $4x$(4EBN), *S. oplocense*) (Chen and Li 1980).

Although the tolerance of BC_1 and BC_2 hybrids was, in general, significantly lower than that of the F_1, we were able to identify a number of genotypes able to acclimatize down to around –5°C. We believe that genotypes with a higher tolerance will be found when the sample size is enlarged. The availability of genotypes with the capacity to cold acclimatize is very important in breeding for an early potato. In temperate Mediterranean areas, the potato is planted from late autumn to early spring; in those conditions, frost is normally preceded by a cool period which allows plants to cold acclimatize. The capacity to cold acclimatize, even just 1–2°C, is also very advantageous in the tropical highlands of South America, where potato is a major staple food; it would increase the areas where potato can be grown.

The accession of *S. commersonii* used in this study is highly resistant to tuber soft rot by *E. carotovora* ssp. *carotovora* (Sirianni 1997). A good level of resistance was also found in the phu–tbr parent. Thus, a sample of BC_2 hybrids was also tested for their resistance to tuber soft rot, and resistant genotypes were detected. Finding genotypes with resistance to soft rot is promising in the search for resistant varieties. As reported by Zimnoch-Guzowska and Lojkowska (1993) and Wolters and Collins (1995), the use of resistant cultivars is the best way to control tuber soft rot caused by *Erwinia* spp. Some of the hybrids with the highest level of resistance to tuber soft rot (e.g. PTH-E10, PTH-F5 and PTH-F7) also showed the capacity to

Table 2: Diameter of rotted area of tuber after artificial inoculation with *Erwinia carotovora* ssp. *carotovora* of BC_2 *Solanum commersonii–S. tuberosum* hybrids and four control varieties

Genotype	Diameter of rotted area (mm[1])
'Majestic' ($2n = 48$)	12.6 a
'Dèsirèe' ($2n = 48$)	11.1 ab
'Spunta' ($2n = 48$)	9.2 bc
PTH-B10 ($2n = 53$)	9.1 bc
PTH-D10 ($2n = 51$)	8.4 b-d
PTH-A7 ($2n = 50$)	8.4 b-d
PTH-A5 ($2n = 52$)	7.1 c-e
PTH-D16 ($2n = 54$)	7.1 c-e
'Draga' ($2n = 48$)	5.3 d-f
PTH-D13 ($2n = 51$)	4.9 ef
PTH-B8 ($2n = 53$)	4.8 ef
PTH-F9 ($2n = 54$)	4.7 ef
PTH-A1 ($2n = 49$)	4.6 ef
PTH-E8 ($2n = 52$)	4.6 ef
PTH-B7 ($2n = 51$)	4.4 ef
PTH-F5 ($2n = 54$)	4.3 ef
PTH-F7 ($2n = 48$)	3.4 f
PTH-E10 ($2n = 56$)	3.3 f
PTH-E3 ($2n = 57$)	2.5 f

[1] Values followed by the same letter are not significantly different at $P = 0.05$ on the basis of Duncan's multiple range test.

cold acclimatize. These genotypes can be used as parental lines in backcrosses to transfer multiple resistances to the tbr gene pool.

In conclusion, manipulations of ploidy and EBN resulted in new genetic material potentially valuable for potato breeding. It is remarkable that most of BC_1 and BC_2 genotypes of this study, despite their aneuploidy, did not show the morphological anomalies normally found in aneuploids of other species (Khush 1973). We were able to transfer tolerance genes from the sexually isolated *S. commersonii* into a usable form at the 2EBN level of F_1 triploid hybrids. In addition, even in a small sample size of BC_2 hybrids, we could identify genotypes that combined the capacity to cold acclimatize with resistance to *Erwinia* spp. (e.g. PTH-E10). Preliminary studies also indicated that these BC_2 hybrids have good yield potentials under long-day conditions (data not shown). This is important given that *S. commersonii* normally produces tubers only under short-day conditions. The BC_2 hybrids have an EBN of 4; they are fertile and have already been used in crosses with 4EBN *S. tuberosum* Group Tuberosum varieties to produce the BC_3 generation.

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References

Barone, A., A. Sebastiano, and D. Carputo, 1999: Chromosome pairing in *Solanum commersonii–S. tuberosum* sexual hybrids detected by *commersonii*-specific RAPDs and cytological analysis. Genome **42,** 218—224.

Cardi, T., V. Iannamico, F. D'Ambrosio, E. Filippone, and P. F. Lurquin, 1993: *In vitro* regeneration and cytological characterization of shoots from leaf explants of three accessions of *Solanum commersonii*. Plant Cell Tiss. Org. Cult. **34,** 107—114.

Carputo, D., T. Cardi, L. Frusciante, and S. J. Peloquin, 1995: Male fertility and cytology of triploid hybrids between tetraploid *Solanum commersonii* ($2n = 4x = 48$, 2EBN) and Phureja–Tuberosum haploid hybrids ($2n = 2x = 24$, 2EBN). Euphytica **83,** 123—129.

Carputo, D., A. Barone, T. Cardi, A. Sebastiano, L. Frusciante, and S. J. Peloquin, 1997a: Endosperm Balance Number manipulation for direct germplasm introgression to potato from a sexually isolated relative (*Solanum commersonii* Dun.). Proc. Natl. Acad. Sci. USA **94,** 12013—12017.

Carputo, D., T. Cardi, M. Speggiorin, A. Zoina, and L. Frusciante, 1997b: Resistance to blackleg and tuber soft rot in sexual and somatic interspecific hybrids with different genetic background. Am. Potato J. **74,** 161—172.

Chen, H. H., and P. H. Li, 1980: Characteristics of cold acclimation and deacclimation in tuber-bearing *Solanum* species. Plant Physiol. **65,** 1146—1148.

Hanneman Jr, R. E., 1989: The potato germplasm resource. Am. Potato J. **66,** 655—667.

Hanneman Jr, R. E., and J. B. Bamberg, 1986: Inventory of tuber-bearing *Solanum* species. Univ. Wisconsin Res. Bull. **533,** .

Janácek, J., and I. Prásil, 1991: Quantification of plant frost injury by nonlinear fitting of an S-shaped function. Cryo-Lett. **12,** 47—52.

Johnston, S. A., and R. E. Hanneman Jr, 1982: Manipulations of Endosperm Balance Number overcome crossing barriers between diploid Solanum species. Science **217,** 446—448.

Johnston, S. A., T. M. den Nijs, S. J. Peloquin, and R. E. Hanneman Jr, 1980: The significance of genic balance to endosperm development in interspecific crosses. Theor. Appl. Genet. **57,** 5—9.

Khush, G. S., 1973: Cytogenetics of Aneuploids. Academic Press, New York, London.

Palta, J. P., and 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, and L. Christersson (eds), Advances in Plant Cold Hardiness, 299—310. CRC Press, Boca Raton.

Palta, J. P., L. S. Weiss, J. F. Harbage, J. B. Bamberg, and J. M. Stone, 1993: Molecular mechanisms of freeze–thaw injury and cold acclimation in herbaceous plants: merging physiological and genetic approaches. In: M. B. Jackson, and C. R. Black (eds), Interacting Stresses on Plants in a Changing Climate. NATO ASI Series, Vol. I 16, 559—680. Springer-Verlag, Berlin-Heidelberg.

Sirianni, P., 1997: Superamento di barriere di incompatibilità interspecifica attraverso la manipolazione della ploidia e dell 'Endosperm Balance Number' per l'introgressione di geni utili in *Solanum tuberosum* L. ($2n = 4x = 48$). MSc Thesis, Univ. of Naples, Naples, Italy.

Steffen, K. L., and 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, and 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.

Sukumaran, N. P., and C. J. Weiser, 1972: An excised leaflet test for evaluating potato frost tolerance. HortSci. **7,** 467—468.

Teutonico, R. A., J. P. Palta, and T. C. Osborn, 1993: *In vitro* freezing tolerance in relation to winter survival of rapeseed cultivars. Crop Sci. **33,** 103—107.

Watanabe, K., C. Arbizu, and P. E. Schmiediche, 1992: Potato germplasm enhancement with disomic tetraploid *Solanum acaule*. 1. Efficiency of introgression. Genome **35,** 53—57.

Wolters, P. J., and W. W. Collins, 1995: Estimation of genetic parameters for resistance to *Erwinia* soft rot, specific gravity, and calcium concentration in diploid potatoes. Crop Sci. **35,** 1346—1352.

Zimnoch-Guzowska, E., and E. Lojkowska, 1993: Resistance to *Erwinia* spp. in diploid potato with a high starch content. Potato Res. **36,** 177—182.