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Production of somatic hybrids between frost-tolerant *Solanum commersonii* and *S. tuberosum*: characterization of hybrid plants

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Abstract. Somatic fusion of mesophyll protoplasts was used to produce hybrids between the frost-tolerant species *Solanum commersonii* ($2n = 2x = 24$) and dihaploid *S. tuberosum* ($2n = 2x = 24$). This is a sexually incompatible combination due to the difference in EBN (Endosperm Balance Number, Johnston et al. 1980). Species with different EBNs as a rule are sexually incompatible. Fifty-seven hybrids were analysed for variation in chromosome number, morphological traits, fertility and frost tolerance. About 70% of the hybrids were tetraploid, and 30% hexaploid. Chloroplast counts in stomatal guard cells revealed a low frequency of cytochimeras. The frequency of aneuploids was relatively higher at the hexaploid level (hypohexaploids) than at the tetraploid level (hypotetraploids). The somatic hybrids were much more vigorous than the parents, and showed an intermediate phenotype for several morphological traits and moderate to profuse flowering. Hexaploid hybrid clones were less vigorous and had a lower degree of flowering than the tetraploid hybrid clones. All of the hybrids were female fertile but male sterile except for one, which was fully fertile and self-compatible. Many seeds were produced on the latter clone by selfing and on the male-sterile clones by crossing. The somatic hybrid plants showed an introgression of genes for frost tolerance and an adaptability to cold from *S. commersonii*. Therefore, the use of these somatic hybrids in breeding for and in genetic research on frost tolerance and cold-hardening is suggested.

Key words: Frost tolerance – Male sterility – Potato – *Solanum commersonii* – Somatic hybridization

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Introduction

Solanum commersonii Dun. is a tuber-bearing wild relative of cultivated potato (*S. tuberosum* L.). Its frost tolerance and ability to acclimatize to cold is well known. In contrast to *S. tuberosum*, for which a killing temperature of around −3 °C has been reported, *S. commersonii* has shown a killing temperature of −11.5 °C or −4.5 °C with or without an appropriate hardening treatment, respectively (Chen and Li 1980). The two species also show differences in several morphological and physiological characters related to frost tolerance (Palta and Li 1979; Li 1985). In addition, resistances to biotic stress and other agronomic traits of interest have been detected in various accessions of *S. commersonii* (Hanneman and Bamberg 1986; Johnston et al. 1986; Chavez et al. 1988; Hawkes 1990). However, its use in potato breeding and genetic studies has been hampered so far by a difficulty to cross diploid *S. commersonii* (2n = 2x = 24, EBN = 1) either with dihaploid (diploid) (2n = 2x = 24, EBN = 2) or tetraploid (2n = 4x = 48, EBN = 4) *S. tuberosum* (Chavez et al. 1988; Novy and Hanneman 1991).

In potato, somatic hybridization has been used between *S. tuberosum* and various wild species, such as *S. chacoense*, *S. phureja*, *S. brevidens*, *S. berthaultii* and *S. circaeifolium*, and the resulting hybrids were tested generally for resistance to biotic stress (Butenko and Kuchko 1980; Austin et al. 1985; Puite et al. 1986; Pehu et al. 1990; Serraf et al. 1991; Mattheij et al. 1992). In one study, somatic hybrids (*S. tuberosum* (+) *S. brevidens*) were tested for resistance to abiotic stress (Preiszner et al. 1991), but the response to hardening was not investigated. Further, the potential value of somatic hybrids for potato breeding may depend on various factors. The combination of parental genotypes, the ploidy level and the genome dosage of hybrids can influence plant morphology and vigour, tuberization, length of stolons, virus resistance, the activity of enzymes related to insect resistance, meiotic behaviour, pollen viability and crossability, as has been shown in somatic hybrids of *S. tuberosum* (+) *S. brevidens* or *S. tuberosum* (+) *S. berthaultii* (Austin et al. 1986; Austin and Helgeson 1987; Ehlenfeldt and Helgeson 1987; Fish et al. 1988a; Pehu et al. 1989, 1990; Serraf et al. 1991).

Recently, we have developed a protocol for regenerating plants from mesophyll protoplasts of various genotypes of *S. commersonii* (Cardi et al. 1990). One of the well-responding genotypes of *S. commersonii* was used for protoplast fusion with *S. tuberosum* to produce somatic hybrid plants. In this paper, data on morphological and cytological features as well as on fertility and frost tolerance of several somatic hybrid plants are presented. The transfer of frost tolerance and adaptability to cold from *S. commersonii* to the hybrid plants is demonstrated.

Material and methods

Plant material

Seeds of diploid *Solanum commersonii* PI 243503 (coded Cmm1) were sown, and shoot cultures were maintained as previously described (Cardi et al. 1990). Shoot cultures of a dihaploid *S. tuberosum* clone (coded SVP11) were bleached by adding the herbicide SAN 9789 to the culture medium (Puite et al. 1986). Somatic hybrids were obtained by the electrofusion of mesophyll protoplasts of *S. commersonii* and *S. tuberosum*. Procedures for protoplast isolation and fusion, and plant regeneration will be reported elsewhere.

After regeneration, hybrid shoots were maintained on Murashige and Skoog medium (1962) supplemented with 3% sucrose and 0.8% agar (pH 5.8).

Cytology

To estimate the ploidy level of plants, the chloroplast number in stomatal guard cells was scored in lower epidermal strips of leaves from *in vitro*-grown plantlets. At least 10–20 guard cell pairs of stomatal cells were analysed in fully expanded leaves.

Chromosome number was determined in root tips of *in vitro*-grown plantlets of a random sample of 35 hybrids. At least one shoot per callus was analysed, but when more than one shoot per callus was available, generally two or three were used for chromosome counts. Root tips were pre-treated with 2 m*M* 8-hydroxyquinoline for 4 h at room temperature, fixed in absolute alcohol:acetic acid (3:1) for at least 24 h and then stored in 70% alcohol at 4 °C. Prior to staining with Feulgen, the root tips were hydrolysed in 1 *N* HCl for 8 min at 60 °C. Chromosome counts were generally based on five well-spread cells.

Morphology

Rooted shoots of the two parents and 57 hybrid clones orginating from 21 independent calli were transferred to sterilized soil in styrofoam trays and gradually adapted to greenhouse conditions at about 20 °C under high humidity. In general, 5 plants per clone were transplanted into plastic pots 18 cm in diameter in an air-conditioned greenhouse (about 15–25 °C) in March; these were analysed throughout the culture season. Tubers were harvested in October.

Morphological traits were scored according to Huaman et al. (1977). The following characters were recorded: number of primary stems, plant height, anthocyanin on stems, leaf and leaflet axils, petioles and rachis, length of the sixth leaf from the apex, leaf dissection, number of primary and secondary leaflets, shape of the terminal leaflet base, width/length of the terminal and subterminal leaflet, pubescence of the upper surface of the terminal leaflet, degree of flowering, shape and colour (upper and lower surface) of the corolla, presence and shape of tubers and length of stolons.

An analysis of variance was carried out for some traits by comparing the variability between shoots from different calli with that between clones from the same callus. The effects of ploidy level, aneuploidy and callus within ploidy groups were tested. A callus for which the chromosome number of the regenerated plants was not available was omitted from the statistical analysis.

Fertility

Pollen production was scored in all of the hybrid clones and in *S. commersonii* by shaking mature flowers with a battery-operated vibrator or by opening and scraping the anthers with a pair of forceps. The pollen was stained with 1% acetocarmine to determine pollen stainability. In some clones, pollen germination was tested *in vitro* using the protocol of Pallais (1985).

One of the somatic hybrid plants was male fertile and self-compatible, and therefore the pollen was used for crossing with the other hybrid clones. The latter were considered to be female fertile if they produced berries and seeds.

Frost tolerance

Frost tolerance of hybrid and parental clones was tested using the electrolyte leakage test described by Sukumaran and Weiser (1972) with minor modifications. Rooted shoots were transferred to 14 cm-pots and adapted to *in vivo* conditions in a growth chamber held at 25 °/20 °C day/night (14 h light and about 8 klux). Five to six weeks after transplanting, terminal leaflets from fully expanded leaves were used for the test.

The response of the genotypes to hardening treatments was tested by keeping the potted plants at 5 °C (14 h light, 2 klux) for 2 weeks prior to collecting the leaflets for the test.

Results

Cytology

In potato the ploidy level of the plants can be reliably determined by counting the number of chloroplasts in stomatal guard cell pairs. This procedure enabled the ploidies of 60 hybrid plants to be tentatively characterized, based on a comparison with the stomatal chloroplast number per guard cell pair in the diploid parental genotypes Cmm1 and SVP11 (14.0 ± 1.9 and 14.1 ± 1.3, respectively). For 35 hybrid plants, the somatic chromosome numbers in root-tip cells were counted. From the data in Fig. 1 a, it can be seen that 51 hybrid plants showed 19–27 chloroplasts per guard cell pair, 7 plants 28–37 chloroplasts and 2 plants 43–46 chloroplasts. Chromosome counts revealed the occurrence of hypotetraploids ($2n = 43-47$), tetraploids ($2n = 48$), hypohexaploids ($2n = 63-70$) and hexaploids ($2n = 72$) at frequencies 37.1%, 37.1%, 17.2% and 8.6%, respectively (Fig. 1 b). A comparison of the mean chloroplast number per guard cell pair with the chromosome number of the hybrid plants showed a highly significant correlation ($r = 0.80$, $P < 0.01$) between the two parameters (Fig. 1 c). Two hybrid clones showed octoploidy on the basis of chloroplast number (i.e. 43.5 ± 4.4 and 45.7 ± 2.6), but 1

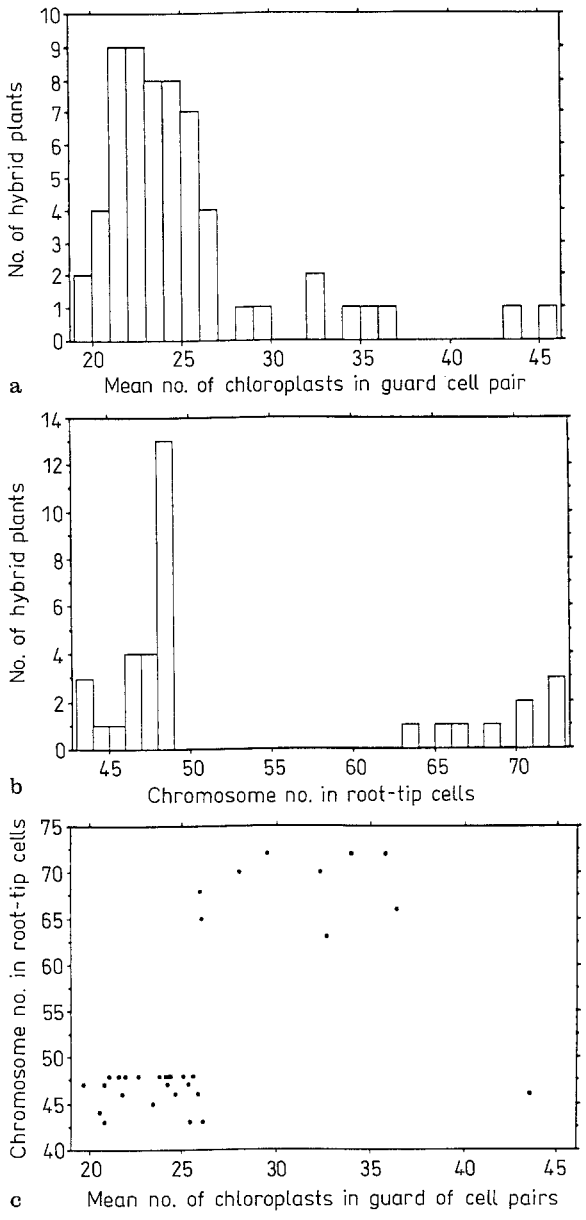


Fig. 1 a–c. Mean chloroplast number in guard cell pairs of leaf epidermis (**a**), chromosome number in root-tip cells (**b**) and correlation between mean chloroplast number per guard cell pair of leaf epidermis and chromosome number in root-tip cells (**c**) of somatic hybrid plants of *S. commersonii* (+) *S. tuberosum*

had 46 chromosomes only. The chromosome number of the other clone was not determined.

Morphology

After transfer to soil, the hybrid plants grew much more vigorously than the parental genotypes. Phenotype varied among hybrids, some clones being more similar to Cmm1 or intermediate between parents, and others, generally hexaploids, resembling SVP11. At flowering, several morphological traits were analysed in both the hybrid and parental clones, and the results are summarized in Table 1. In general, the hybrids showed heterotic vigour for some characteristics and were intermediate between parents for others. They appeared to be much taller than the parents (Fig. 2a) and had one to four primary stems. Anthocyanin was distributed mostly on stems, leaf and leaflet axils, but occasionally also on petiole and rachis. Leaves were in general weakly dissected, and the terminal leaflet was round at the base, showing an intermediate degree of pubescence (Fig. 2b).

The dihaploid clone SVP11 did not flower under the environmental conditions of this study, but in other environments (greenhouse at Wageningen, the Netherlands, or screenhouse at Camigliatello, Italy) the development of small white flowers was occasionally observed. In contrast, Cmm1 flowered profusely and continuously. The flowers were typically stellate shaped, pinkish on the upper part of corolla and deep purple on the lower part. Most hybrid clones also showed normal flowering, but in hexaploids, it was generally delayed and not persistent. Flower colour and size varied among the hybrid clones. The colour of the flowers resembled that of *S. commersonii* but was generally lighter. The flower was larger than that of the parent. It was pentagonal in shape and in some cases semi-stellate (Fig. 2c).

All hybrids produced tubers under long-day conditions (Fig. 2d), like the *S. tuberosum* parent, but formed long stolons, which is typical of the wild parent *S. commersonii*.

The hybrid plants regenerated from independent calli varied, in general, for several characters, whereas those derived from the same callus were similar. The effect of callus was significant ($P<0.05$ or $P<0.01$) for all of the traits analysed (Tables 2 and 3). When compared to tetraploid hybrids ($4x$), the hexaploid hybrids ($6x$) were significantly shorter in height and had a lower number of primary stems and leaflets, larger subterminal leaflets and a reduced degree of flowering. In addition, the $6x$ hybrids had a smaller leaf, a reduced number of secondary leaflets, and a larger terminal leaflet (data not shown). In some cases, hexaploids could be recognized by their darker and thicker leaflets with irregular shape. The effect of aneuploidy was significant at both the $4x$ and $6x$ levels for plant height and only at the $4x$ level for the number of primary leaflets and the degree of flowering. In fact, both values were lower in hypotetraploids than in tetraploids. Residual variation within calli belonging to the same ploidy group was significant for the number of primary stems (ploidy groups A and B) and leaflets (groups B and C), plant height (group C) and subterminal leaflet width/length (group B). The number of primary stems was equally variable in tetraploid and hypotetraploid hybrids, ranging in both cases from 1 to 3, whereas leaf traits analysed in hybrid clones were significantly different among hypotetraploid calli (group

Table 1. Morphological characters and fertility in parental clones Cmm1 (*S. commersonii*) and SVP11 (*S. tuberosum*) and somatic hybrids *S. commersonii* (+) *S. tuberosum*

Characters	Cmm1	SVP11	Somatic hybrids
Branching habit	Branched	Single	Single, Branched
Plant height	Medium	Medium	Tall
Anthocyanin distribution[a]	S, LA, LfA	Absent	S, LA, LfA, (P, R)
Leaf dissection	Scarce	Scarce	Weak
Shape terminal leaflet (base)	Cuneate	Heart shaped	Rounded
Hairiness upper leaflet (surface)	Glabrous	Strongly pubescent	Glabrescent, Pubescent
Flowering	Present	Absent	Present[e]
Shape of corolla	Stellate	–	Pentagonal, Semistellate
Colour of corolla (upper)[b]	Pink	–[d]	Whitish/Pink striped
Colour of corolla (lower)[b]	Purple	–	Whitish/Purple striped
Pollen production	Abundant	–	None[f]
Tuberization (long day)	Absent	Present	Present
Stolon length	Long[c]	Short	Long
Shape of tubers	Round	Oblong	Oblong

[a] S, Stem; LA, leaf axil; LfA, leaflet axil; P, petiole; R, rachis. ( ) Present only in some clones
[b] Intensity and distribution of colour varied widely among hybrid clones
[c] Under short-day conditions
[d] White, when flowers were obtained
[e] In some hexaploid hybrids, flowering delayed or absent
[f] Abundant in one clone

B), but not among tetraploid ones (group A). Further, significant variation was observed for plant height and number of leaflets among hexaploid calli (group C).

Fertility

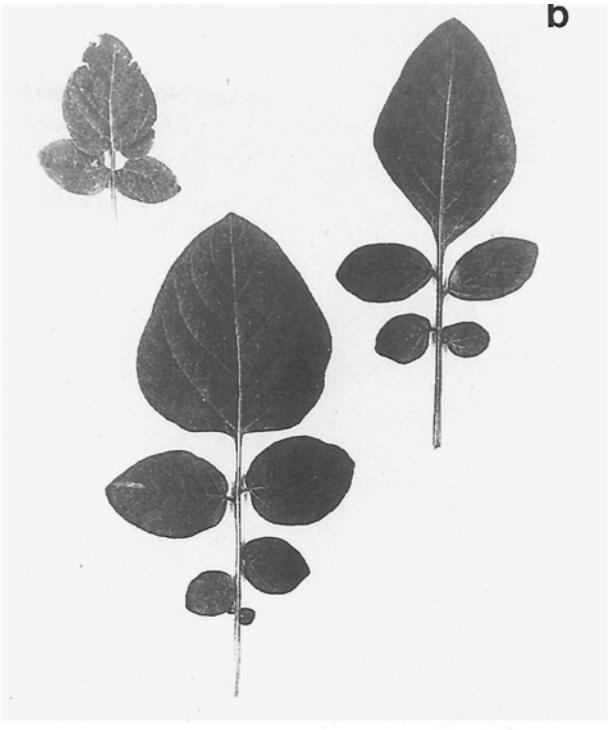
The wild parent *S. commersonii* flowered profusely and had an abundant production of pollen that showed approximately 100% stainability and approximately 50% germinability *in vitro*. Most somatic hybrids showed profuse flowering, but all were male sterile except for 1 clone. They generally had divergent, light-yellow and incompletely developed anthers (Fig. 2e), although normal-looking anthers were also present in some sterile hybrids, and did not produce any normal pollen grains. Anther dehiscence was almost absent. Only after the anthers were opened could some irregularly shaped and degenerated structures be observed (Fig. 2f). These structures were faintly stainable with acetocarmine, but failed to germinate *in vitro* and even to develop into normal microspores. In contrast, the male-fertile hybrid clone SH9A had normal yellow anthers and produced abundant pollen with 80–95% stainability and approximately 20% germinability *in vitro*. In addition, this clone showed normal berry and seed set after selfing, indicating that it was also female fertile and self-compatible. When the pollen of the clone SH9A was used in crosses with several male-sterile somatic hybrid clones, berries and viable seeds were obtained in most cases, suggesting that the hybrids were female fertile. Parthenocarpic berries were observed in some male-sterile hybrids, but the berries were more elongated than the seeded ones.

Frost tolerance

The results of freezing tests on the tetraploid somatic hybrid SH9A and the parental genotypes are reported in Fig. 3. The hybrid clone showed a higher frost tolerance than the *S. tuberosum* parent SVP11 either with or without a hardening pretreatment. In the absence of the hardening pretreatment, SH9A showed less damage than SVP11 up to −1.5 °C. However, after the hardening pretreatment, the percentage of injury of the hybrid clone in the range from 0 °C to −6 °C was always lower than that of SVP11. The estimated LT_{50} of SVP11, Cmm1 and SH9A was 0 °C, −3.8 °C and −0.7 °C without any pretreatment and −0.8 °C, −9.8 °C and −3.3 °C after 2 weeks at 5 °C, respectively. Thus, in the hybrid clone adaptation to low temperature increased frost tolerance by 2.6 °C. This was intermediate to the values of 6 °C and 0.8 °C shown by the wild parent *S. commersonii* and the cultivated parent *S. tuberosum*, respectively.

Fig. 2a–f. Illustrations on morphological and fertility features of somatic hybrids: **a** Plants at flowering stage of *S. commersonii* (*left*), a hybrid clone (*centre*) and *S. tuberosum* (*right*); **b** leaves of *S. commersonii* (*upper row, right*), *S. tuberosum* (*upper row, left*) and a hybrid clone (*lower row*); **c** flowers of *S. commersonii* (*right*) and of a hybrid clone (*left*); **d** tubers of *S. tuberosum* (*upper row*) and of two randomly chosen hybrid clones (*lower row*). *Bar*: 5 cm; **e** anthers of a male-sterile clone; **f** degenerated structures found in the anthers of male-sterile clones


a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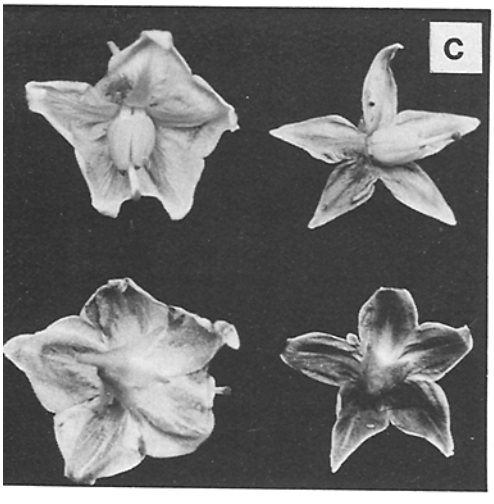
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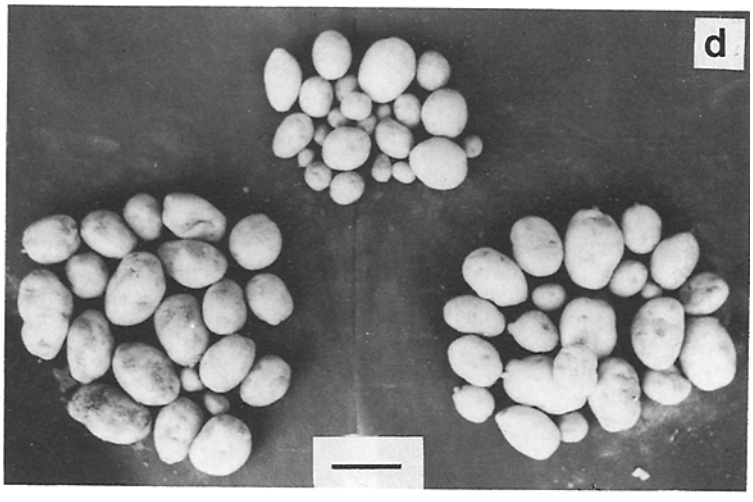
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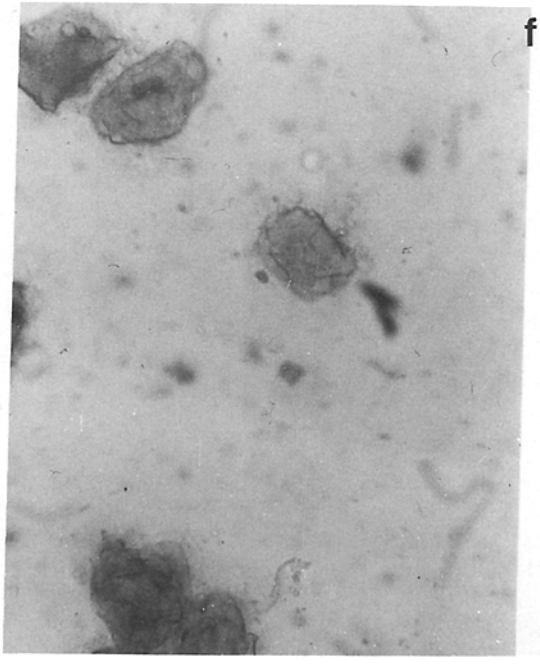
f

Table 2. Mean values (minimum and maximum in parentheses) of various morphological traits in somatic hybrids and parental clones. Hybrid clones regenerated from the same or different calli were grouped according to chromosome number

Ploidy group (chromosome no.)	Number of hybrid calli/ regenerated clones	Primary stems (no.)	Plant height (cm)	Primary leaflets (no.)	Width/length of subterminal leaflets	Degree of flowering[b]
Somatic hybrids[a]						
A (48)	7/16	1.7 (1.0–3.1)	96.5 (86.9–105.5)	3.1 (2.5–3.5)	0.59 (0.52–0.63)	5.2 (5.0–6.0)
B (43–48)	7/30	1.9 (1.0–3.0)	103.6 (93.7–117.5)	2.9 (2.2–3.1)	0.60 (0.55–0.70)	4.3 (3.5–5.2)
C (72)	2/3	2.0 (1.5–2.5)	92.5 (80.0–105.5)	2.4 (2.0–2.7)	0.71 (0.65–0.76)	4.0 (3.0–5.0)
D (63–70)	4/7	1.25 (1.0–1.5)	83.6 (77.5–90.0)	2.3 (2.0–2.5)	0.66 (0.58–0.70)	3.3 (3.0–3.7)
Parents[a]						
Cmm1 (24)	–	6.5	52.5	4.5	0.45	7.0
SVP11 (24)	–	1.0	52.5	2.0	0.55	1.0

[a] The parental and hybrid clones were propagated in vitro and transferred to the greenhouse
[b] The degree of flowering was rated from 1 (=absent) to 7 (=profuse) (Huaman et al. 1977)

Table 3. Mean squares from the analysis of variance for various morphological traits in hybrid clones. Ploidy groups (A–D) as in Table 2

Source of variation	*df*	Number of primary stems	Plant height	Number of primary leaflets	Width/length of subterminal leaflets	Degree of flowering
Calli	19	1.43**	211.95*	0.30**	0.0082*	1.45*
Ploidy level	1	1.37*	986.09**	2.75**	0.0503**	8.50**
Tetraploids vs hypotetraploids	1	0.01 NS	522.01*	0.33*	0.0015 NS	4.08*
Hexaploids vs hypohexaploids	1	0.63 NS	380.03*	0.04 NS	0.0003 NS	1.72 NS
Calli within ploidy group	16	1.57**	133.68 NS	0.17*	0.0065 NS	0.82 NS
Calli within ploidy group A	6	2.26**	83.32 NS	0.15 NS	0.0020 NS	0.16 NS
Calli within ploidy group B	6	1.81**	184.70 NS	0.19*	0.0120*	1.50 NS
Calli within ploidy group C	1	0.67 NS	416.67*	0.37*	0.0081 NS	2.67 NS
Calli within ploidy group D	3	0.05 NS	38.05 NS	0.06 NS	0.0037 NS	0.18 NS
Clones within calli	36	0.24	91.26	0.08	0.0040	0.68

* Significant at $P<0.05$; ** significant at $P<0.01$; NS, not significant

Discussion

The use of chloroplast number in stomatal guard cells as an index of the ploidy level in potato was first reviewed by Frandsen (1968). In anther-derived potato plants a consistently higher mean chloroplast number was observed in plants multiplied *in vitro* than in those grown *in vivo*. However, a highly positive correlation between the two scores could be expected and was indeed found by Singsit and Veilleux (1991). In the present study, chloroplast counts in stomatal guard cells of *in vitro*-grown plants allowed a quick and reliable estimate of the ploidy levels of somatic hybrid plants regenerated from fusion products between the cultivated species *S. tuberosum* and the wild species *S. commersonii*. Chromosome counts facilitated the classification of a few ambiguous cases as well as the analysis of aneuploids and cytochimeras. On the basis of chromosome analysis, 74% of the somatic hybrid plants (70% of the calli) were shown to be tetraploid and 26% (30% of the calli), hexaploid. Of the 2 clones with chloroplast counts indicative of $8x$ ploidy levels, the one with 46 chromosomes and a normal phenotype was a periclinal chimera that probably originated by the somatic doubling of cells from the L_1 layer. Ploidy levels higher than the expected $4x$ (i.e., $2x$ (+) $2x$) may derive either from multiple fusions or from single fusions involving diploid G_2 or tetraploid G_1 mesophyll cells, and also from the somatic doubling of fusion products (Puite et al. 1986; Ramulu et al. 1989; Wijbrandi et al. 1990). Chromosome elimination occurred equally at

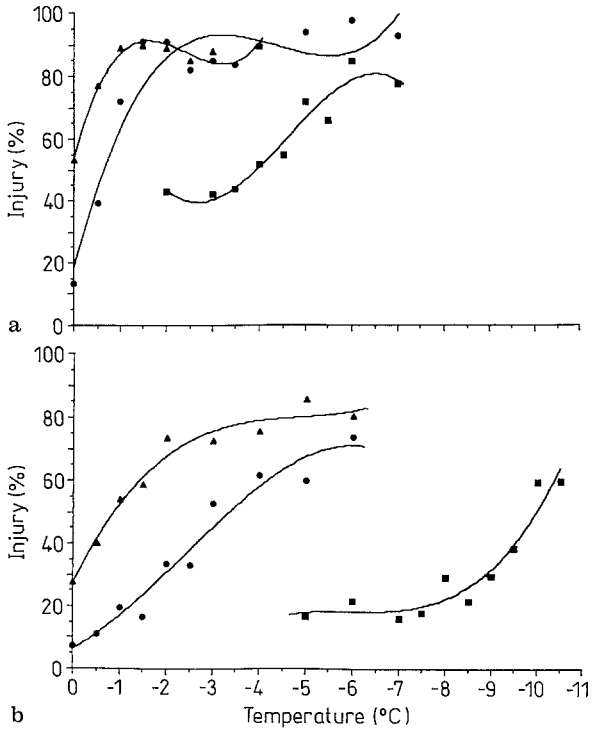

Fig. 3a, b. Percentage of injury determined on the basis of the electrolyte leakage test after freezing stress at different temperatures of non-hardened (**a**) and hardened (**b**) *S. commersonii* Cmm1 (*squares*), *S. tuberosum* SVP11 (*triangles*) and somatic hybrid SH9A (*circles*)

both the tetraploid and hexaploid levels, resulting in hypotetraploid and hypohexaploid hybrid plants. Chromosome loss is a well-known phenomenon in somatic interspecific hybrids of potato and other species (Puite et al. 1986; Fish et al. 1988b; Ramulu et al. 1989; Wijbrandi et al. 1990; Preiszner et al. 1991; Mattheij et al. 1992).

The increased vigour of the hybrids both *in vivo* and *in vitro* is mainly due to the expression of heterosis, since a high heterozygosity was observed in the hybrids; in this respect somatic hybridization is comparable to sexual crosses between desynaptic FDR clones (Jongedijk et al. 1991). Increased vigour after intra- or interspecific fusions in potato has also been reported by other authors (Austin et al. 1985; Puite et al. 1986; Debnath and Wenzel 1987, Fish et al. 1988b). Similar to the variation observed in the hybrids of *S. tuberosum* (+) *S. brevidens* (Fish et al. 1988a) and *L. esculentum* (+) *L. peruvianum* (Wijbrandi et al. 1990), that found among the hybrid clones is mainly due to the ploidy level of regenerated plants. However, some of the variation within ploidy level can be attributed to either aneuploidy or somaclonal variation that is independent of differences in chromosome number. Finally, the differences in growth habit and flowering observed among hexaploid hybrid clones might depend on the relative genome dosage of the two parents; however further analyses on the genome composition of the hybrids are necessary for testing this hypothesis.

No significant variation in tuberization was found among the somatic hybrid clones. Previously, several authors (Austin et al. 1986; Fish et al. 1988a; Pehu et al. 1989; Preiszner et al. 1991) reported that the relative parental genome dosage and ploidy level influence the tuberization of hybrid plants when a non-tuberizing species is used as a fusion partner.

All of the somatic hybrid plants, except for one, were male sterile. The analysis of microsporogenesis in male-sterile hybrids revealed spindle abnormalities during meiosis II and a precocious degeneration of tapetum (Conicella et al. 1992). The observed male sterility results from an interaction between the parental idiotypes, since both parental clones produce viable pollen (Mattheij et al. 1992; this study). Previous investigations (Matsubayashi 1983; Hosaka et al. 1984; Perl et al. 1991) pointed out that *S. commersonii* diverged from *S. tuberosum* and other South American species with respect to both the cytoplasmic and nuclear genome. It is improbable that structural chromosome changes are involved in male sterility in these somatic hybrids because they combine intact diploid parental genomes. On the other hand, nuclear-organellar (mitochondrial) interactions seem to play an important role, as suggested by the data on potato hybrids and cybrids involving *S. commersonii* (Novy and Hanneman 1991; Perl et al. 1991).

The female fertility of the somatic hybrids (*S. tuberosum* (+) *S. commersonii*) can be exploited by using them as female parents in crosses with *S. tuberosum* for obtaining valuable recombinants. On the other hand, the male-fertile somatic hybrid clone can be used either for reciprocal backcrosses or for selfing.

Freezing tests clearly showed that the tetraploid hybrid clone SH9A expressed not only a higher frost tolerance but also a better adaptability to cold than *S. tuberosum*. This is interesting since, besides their use in breeding programmes, tetraploid somatic hybrids and their progenies obtained by selfing or crossing can be used for studying morphological and physiological mechanisms involved in the development of hardening response and frost tolerance in *S. commersonii* (Palta and Li 1979; Li 1985).

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