Theor Appl Genet (1987) 73:395–402

Fertility of somatic hybrids from protoplast fusions of *Solanum brevidens* and *S. tuberosum*

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Received July 8, 1986; Accepted August 19, 1986
Communicated by I. Potrykus

Summary. Two sets of somatic hybrids between *Solanum brevidens* (2x) and *S. tuberosum* (2x and 4x) were evaluated for male fertility, meiotic regularity and female fertility. The somatic hybrids were tetraploids from 2x+2x fusions and hexaploids from 2x+4x fusions. Pollen stainability ranged from 0 to 83% in tetraploids and from 0 to 23% in hexaploids. The tetraploids had more regular meiosis, lower levels of micropollen and fewer unassociated chromosomes than hexaploids. However, except for a low level of selfing, the pollen of both sets of hybrids was ineffective in pollinations. The tetraploids, as females, crossed poorly with 2x and 4x tester species and selfed only at low levels. The hexaploid fusion hybrids also crossed poorly with the 2x tester species and selfed only to a limited degree; however, they crossed well with 4x testers. Seed set in crosses with *S. tuberosum* Group Andigena, and *S. tuberosum* Group Tuberosum cultivars 'Kathadin' and 'Norland' averaged 16.7, 15.6 and 28.6 seeds per fruit, respectively. Progeny from these crosses had 5x or nearly 5x ploidy levels. The results indicate that reasonable levels of female fertility can be obtained in somatic fusion hybrids of *S. brevidens* and *S. tuberosum*.

Key words: Protoplast – Somatic fusion – *Solanum brevidens* – *Solanum tuberosum* – Endosperm balance number

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Introduction

Wild species have traditionally been good sources of genes for crop improvement. The potential for subsequent utilization of the germplasm after initial combination with domestic materials however can be a serious problem.

In some cases, vigorous F1 hybrids produced from interspecific crosses have proven to be sterile or cross incompatible (Karpechenko 1927; Rick 1951; Thomas and Pratt 1981). In certain other cases advanced crosses succeeded only with difficulty (de Wet et al. 1973; CIMMYT 1978; Hermsen et al. 1981).

Fertility and crossability are also important for materials produced by somatic fusions (Melchers et al. 1978; Binding et al. 1982; Gleddie et al. 1985). One of the major goals of fusions is to unite partners which are sexually incompatible. In this way genes from wild species (e.g. disease resistances) might be incorporated into breeding lines.

We have produced tetraploid and hexaploid hybrid plants from protoplast fusions between *Solanum brevidens*, a 2x non-tuber-bearing species and 2x and 4x *S. tuberosum* materials (Austin et al. 1985b, 1986). One aim of the experiments was to transfer the potato leaf roll virus (PLRV) resistance of *S. brevidens* into Group Tuberosum materials. Many of the hybrids between *S. brevidens* PI 218228 and 4x *S. tuberosum* PI 203900 exhibited both the resistance to PLRV of the *S. brevidens* parent and the hypersensitivity to Race 0 of *Phytophthora infestans* of the *S. tuberosum* parent (Helgeson et al. 1986).

The next step in the practical incorporation of PLRV resistance from *S. brevidens* into *S. tuberosum* is the sexual transfer of PLRV resistance from somatic hybrids to conventional breeding lines. Since *S. brevidens* normally cannot be crossed directly to *S. tuberosum*, and since the intermediate hybrids usually have lowered fertility and abnormal chromosome pairing

(Ramanna and Hermsen 1979b; Ehlenfeldt and Hanneman 1984), the fertility of these fusion hybrids is an important concern. We report on the fertility, pollen viability, meiotic configurations and crossability of 4x and 6x somatic hybrids of *S. brevidens* and *S. tuberosum*.

Materials and methods

The tetraploid somatic hybrids were obtained by Austin et al. (1985b) from a fusion between *S. brevidens* PI 235763 (2n = 2x = 24) and *S. tuberosum* 77-1 (2n = 2x = 24) (a *S. tuberosum* Gp. Phureja-Stenotomum selection obtained from H. De Jong, Agriculture Canada). The hexaploid hybrids were obtained by Austin et al. (1986) from a fusion of *S. brevidens* PI 218228 (2n = 2x = 24) and *S. tuberosum* PI 203900 (2n = 4x = 48). Clones of the original fusion plants have been maintained in vitro as described by Haberlach et al. (1985). *S. brevidens* PI 218228, PI 245763, *S. tuberosum* PI 203900, *S. chacoense* bulk population 83-4211, *S. tuberosum* Gp. Andigena bulk population 83-6002 and the cultivars 'Katahdin' and 'Norland' were obtained from the Inter-Regional Potato Introduction Project (IR-1) at Sturgeon Bay, WI.

Pollen was collected from field-grown plants in mid-August, 1984. Pollen stainability determinations were made using a 1% acetocarmine stain (1% carmine in 45% aqueous acetic acid). A minimum of 200 grains were counted for each determination.

Crosses were made on cut-stems in air-conditioned greenhouses in 1984 and in screened cold frames in 1985. Stems were placed in milk bottles filled with water to which a small amount of fungicide (Arasan, Du Pont) had been added. All flowers were emasculated in the bud stage. After pollination, fruits were allowed to mature on the cut stems for one month, then removed and allowed to ripen an additional month prior to seed extraction. Seeds were treated with 1,500 ppm gibberellic acid (GA_3, Sigma) to promote uniform germination.

Meiotic analyses were made on buds fixed for 48 h in a 6 : 3 : 2 solution of methanol, chloroform and 45% propionic acid (saturated with ferric acetate), and stored in 90% ethanol. Anther squashes were stained with a 1% acetocarmine solution.

Somatic chromosome counts were performed on root tips pre-treated with an aqueous 8-hydroxy-quinoline (0.29 g/l) for 4 h, and then fixed in an ethanol-acetic acid (3 : 1) solution for at least 24 h. Root tips were prepared by hydrolyzing in 1N HCl at 60 °C for 10 min and then rinsing with tap water for a minimum of 5 min. Root tips were squashed in 1% lacto-propionic orcein (1% orcein in equal volumes of lactic and propionic acid).

Styles for stylar compatibility analysis, were fixed in FAA (5% formalin – 5% acetic acid – 90% ethanol) approximately 48 h after pollination. After fixing, styles were softened for 8–24 h in 8N NaOH, then rinsed in tap water for 1 h. Styles were stained in a solution of 0.1% aqueous aniline blue of 0.1 N K_2HPO_4 4 h then observed for fluorescence at 356 nm.

Results

Pollen viability and male fertility

S. brevidens PI 245763 and PI 218228 had 81% and 82% pollen stainability, respectively. The *S. tuberosum* materials 77-1, and PI 203900, had stainabilities of 43% and 11%, respectively. None of the *S. brevidens* or *S. tuberosum* materials produced appreciable micro-pollen.

Male fertility in the fusion hybrids varied widely, ranging from the very good (83% stainable) to the very poor (0% stainable or no pollen shed) (Fig. 1), and micro-pollen was observed in all fusion clones examined (Fig. 2a). The tetraploids and hexaploids differed considerably in their overall male fertility. Among the tetraploids, a total of 108 clones from 23 different fusion calli were evaluated. Stainabilities ranged from 0 to 83%. Almost half (52 of 108) had pollen stainabilities of less than 1%, 20% had stainabilities of 1% to 10% and 36% had stainabilities ranging from 11% to 83%. Among the hexaploids, pollen stainability was determined for 105 plants originating from 20 different fusion calli. Values for pollen stainability ranged from 0 to 23%. Overall, 30% of the clones had less than 1% stainable pollen, 43% had between 1 and 10% stainable pollen, and 27% had stainability levels between 10% and 25%.

Interspecific testcrosses using pollen from 4x and 6x fusion hybrids were unsuccessful regardless of ploidy or genotype of the female involved. For these tests, *S. chacoense* (chc), *S. tuberosum* Gp. Andigena (adg), and the cultivar 'Red Pontiac' were used as females. Examination of styles in interspecific crosses revealed an arrest of pollen tube growth in the upper sections of the styles. This finding is in agreement with those of Hermsen et al. (1981) regarding unilateral stylar incompatibility in sexual hybrids with Series Etuberosa species. Limited success was achieved in using fusion hybrid pollen in selfing. Upon selfing, pollen tube growth was retarded in hexaploids, but tube growth in tetraploids appeared normal.

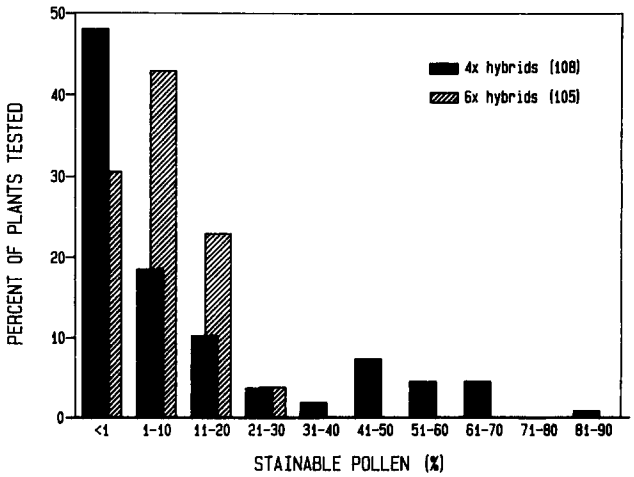

Fig. 1. Pollen stainability distributions for plants from 4x and 6x *S. brevidens* + *S. tuberosum* somatic cell fusions. Numbers in parentheses indicate number of plants examined

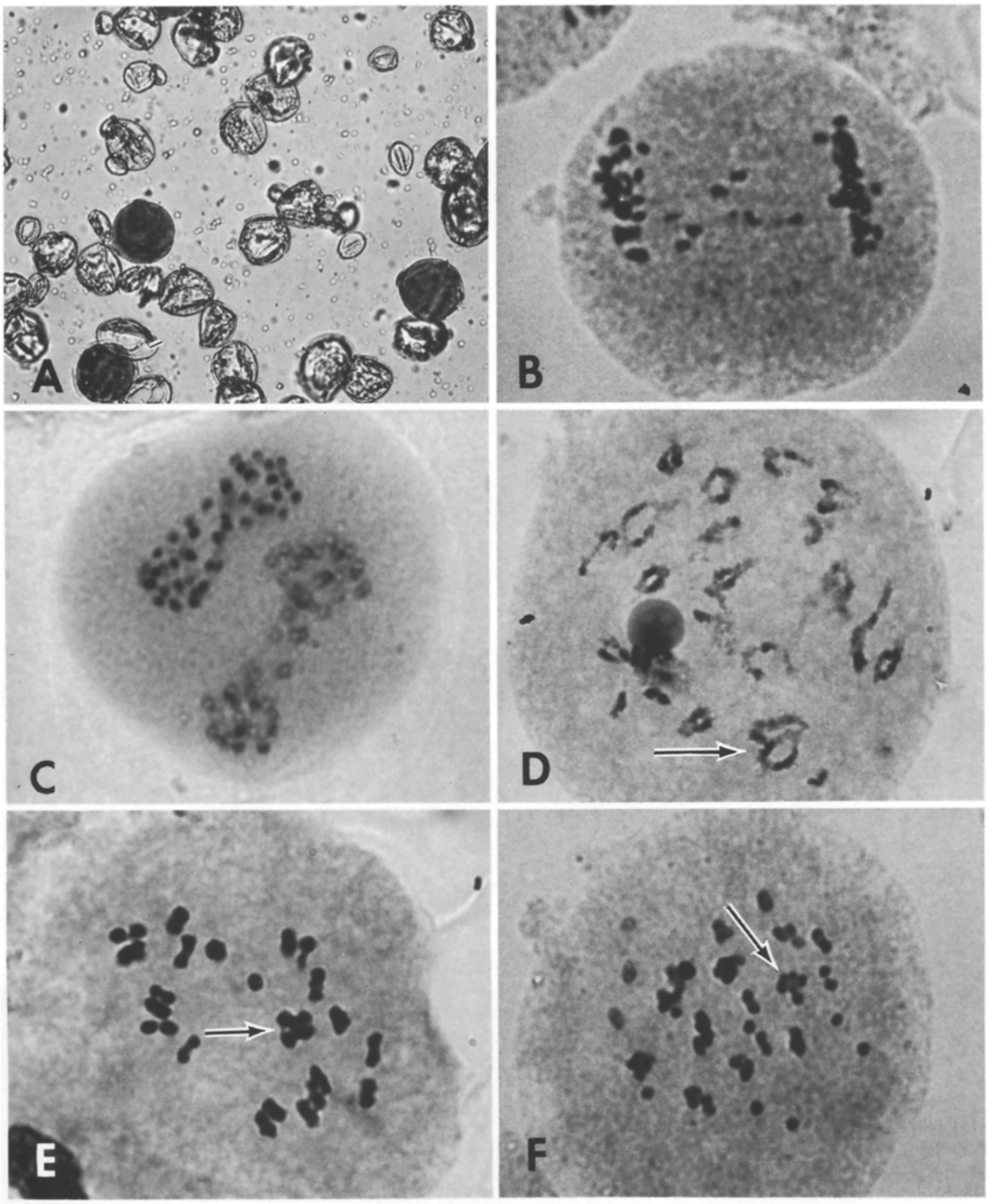

Fig. 2. **A** Pollen from 6x *S. brevidens* + *S. tuberosum* fusion hybrid showing micro-pollen typical of 4x and 6x fusions (hybrid 1691). **B** Lagging chromosomes at anaphase-telophase I in 6x *S. brevidens* + *S. tuberosum* fusion hybrid (hybrid 249). **C** Dumbell-shaped configuration at anaphase-telophase II in 4x *S. brevidens* + *S. tuberosum* fusion hybrid (hybrid 722). **D** Diplotene in 4x *S. brevidens* + *S. tuberosum* fusion hybrid. Quadrivalent at lower right (hybrid 1628). **E** Metaphase I in 4x *S. brevidens* + *S. tuberosum* fusion hybrid. Quadrivalent figure in center (hybrid 1628). **F** Metaphase I in 6x *S. brevidens* + *S. tuberosum* fusion hybrid. Note multivalent formation (hybrid 462)

Cytology

For ploidy determinations, buds of over 114 tetraploid and 94 hexaploid hybrids were examined. Meiosis overall was similar in both 4x and 6x somatic hybrids. In all plants, a certain number of unassociated chromosomes were observed which had failed to orient on the metaphase I plate. In some cells, these chromosomes appeared to be paired and undergoing independent division, but often they appeared to be unpaired. In many clones, chromosomes which had oriented on the metaphase plate, appeared to lag behind in division at anaphase I, and to arrive late at the telophase I nuclei, giving the impression of non-synchronous division (Fig. 2 B). Most of the chromosomes which did not align on the metaphase I plate appeared to remain unassociated with any metaphase plate through the second meiotic division, a few however appeared associated with telophase II nuclei at the end of the second meiotic division and may have been incorporated later. The 4x and 6x fusion hybrids differed sharply with regard to numbers of unassociated chromosomes. In the 4x somatic hybrids, 45% of the dyads had no unassociated chromosomes. One or two unassociated chromosomes were seen in another 30% of the dyads (Fig. 3 A). In contrast, only 14% of the dyads in 6x plants had no unassociated chromosomes. Two unassociated chromosomes were observed most commonly (22%) in the dyads examined (Fig. 3 A). In the hexaploid fusion plants, the abnormal classes with 1, 2, 3 or 4 unassociated chromosomes were each larger than the normal (0) class. This trend continued into pollen production. In the 4x fusion, 41% of the pollen tetrads were normal (0 micro-pollen grains), and 37% had only one micro-pollen grain (Fig. 3 B). In the 6x fusions, only 4% of all tetrads were normal, and three micro-pollen grains per tetrad was the most common (25%).

Although unincorporated chromosomes were often present, meiosis otherwise appeared normal. Occasional chromosomal bridges were observed at anaphase I, and some dumbell-shaped nuclei were observed at what was probably telophase II (Fig. 2 C), but neither was very common.

Early stages of meiosis were examined in a few clones of each fusion. In tetraploids, diplotene pairing was primarily bivalent, although trivalent and quadrivalent configurations were seen in most cells. Multivalent pairing, seldom exceeded two or three multivalents per cell (Fig. 2 D). Similar pairing configurations were seen at metaphase I (Fig. 2 E). In hexaploids, observations of pairing configurations were much more difficult. Multivalents and chains of chromosomes were often seen, but numbers of chromosomes involved were difficult to determine. Occasional hexavalents were observed on metaphase I plates, but the complete resolution of multivalent associations was not possible (Fig. 2 F).

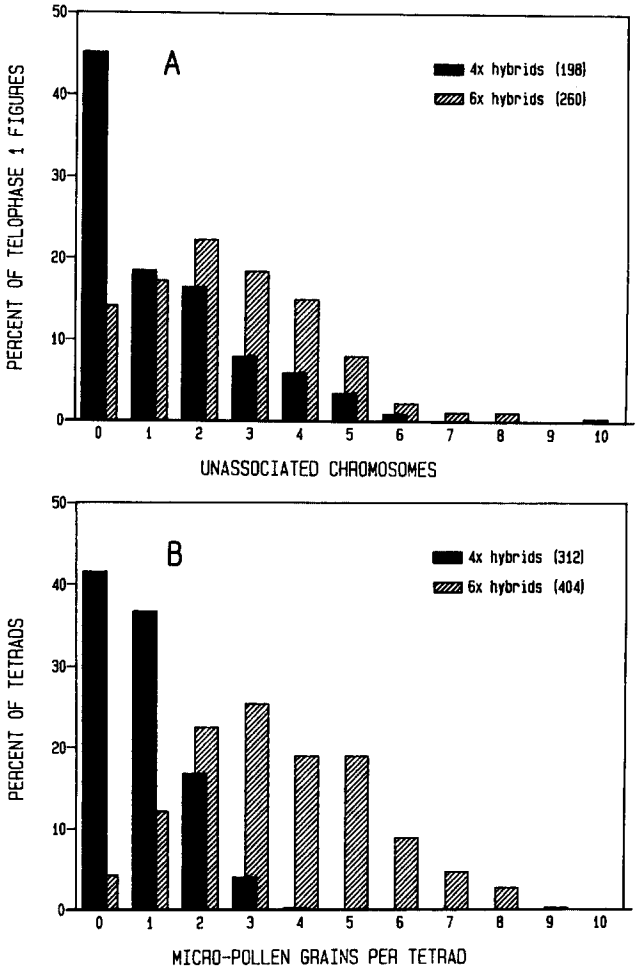

Fig. 3. A Unassociated chromosomes at telophase I and **B** micro-pollen grains per tetrad in meiosis of 4x and 6x *S. brevidens* + *S. tuberosum* fusion hybrids. Values are an average for three 4x hybrids, 704, 778 and 1,009 and four 6x hybrids, 209, 219, 242 and 464; equal numbers of cells were examined in each clone at each ploidy level; numbers in parentheses indicate number of microspore mother cells or tetrads examined

Female fertility

Female fertility was evaluated by seed set. To provide an adequate measure of fertility, both self pollinations (which are expected to be compatible in polyploids) and cross pollinations with tester species were done.

Among the 4x somatic hybrids only a small amount of selfing was possible (3.7 s/f average) despite the apparent lack of stylar inhibition. The 6x somatic hybrids also set a very low number of selfed seed, averaging 1.8 s/f. Stylar examination of the hexaploids, however, had revealed limited pollen tube growth.

A notable feature of self seed of 4x fusion plants was the large size of the seeds produced. Although the numbers of seeds set per fruit were relatively low, the seeds were substantially larger than those set in other successful crosses with these clones (Fig. 4). Seeds from selfed 6x plants was not consistently larger than seeds from other crosses with these lines.

In interspecific crosses, the 4x fusion plants had only modest levels of female fertility. In test crosses with 2x chc only 3 seeds were obtained from 84 fruit (0.04 s/f). In crosses with 4x adg only 25 seeds were obtained from 59 fruit (0.4 s/f) (Table 1).

The crosses of the 6x somatic hybrids with testers were more successful. Crosses of the 6x plants with 2x *S. chacoense* averaged 1.7 s/f (Table 2), and crosses with 4x adg averaged 15.7 s/f (range 1.3 to 51.6 s/f). By comparison, control crosses of adg × adg yielded 93 s/f. The 6x fusion hybrids were also used in additional crosses with the cultivars 'Katahdin' and 'Norland'. With 'Katahdin', seed set averaged 15.6 s/f (range 1.9 to 37.7 s/f), and with 'Norland', 28.7 s/f (range 7.6 to 53.8 s/f). Seed sizes in 6x fusion hybrid × adg crosses did not differ appreciably from those seen in adg × adg crosses. Seeds from 6x fusion hybrid × cultivar crosses were similar in size or slightly smaller than adg × adg crosses.

Progeny

In most cases, the observed ploidy of seedling progeny matched the levels expected from the union of 1n gametes from the respective parents, with an occasional observance of progeny arising from 2n or aneuploid gametes (Table 3). About 1% of the plants grown were dwarfs for which the chromosomes were not counted due to weak growth.

Crosses of 6x fusion hybrids with 4x adg consistently produced 5x progeny. Of 63 progeny evaluated only one plant deviated from the 5x level. The plant, from clone 216 × Andigena, had 55 rather than 60 chromosomes.

Crosses of 6x fusion hybrids with 2x chc produced a number of ploidy combinations (Table 3). Some progeny had somewhat less than 4x complements, others

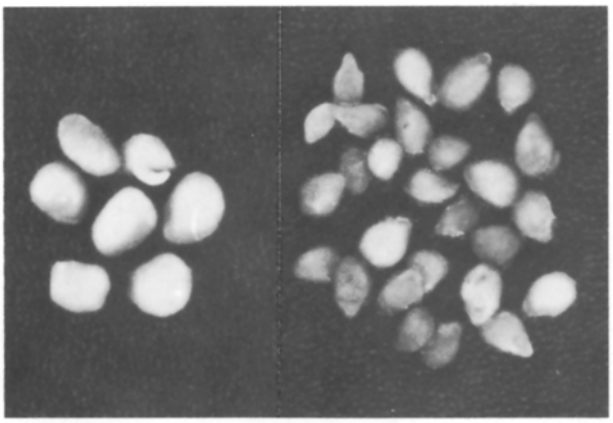

Fig. 4. Self seed (left) of 4x *S. brevidens* + *S. tuberosum* fusion hybrid (hybrid 704) and sib-seed of *S. tuberosum* Group Andigena

Table 1. Seed set in selfings and crosses of 4x fusion hybrids between *S. brevidens* (PI 245763) and *S. tuberosum*) (77-1) as females with 2x(2EBN) *S. chacoense* (chc) or 4x(4EBN) *S. tuberosum* Group Andigena (adg) as males

Clone	x 2x chc		x 4x adg		Selfed	
	p/f/s[b]	s/f[c]	p/f/s	s/f	p/f/s	s/f
704a[a]	11/6/1	0.2	12/7/8	1.1	6/3/6	2.0
759b	10/9/1	0.1	9/7/1	0.1		
764b	1/0/0	0	1/0/0	0		
766b	6/6/0	0	6/3/3	1.0		
770b	3/3/0	0	6/3/3	1.0		
772b	8/6/0	0	5/5/1	0.2	7/7/37	5.3
774b	12/10/0	0	13/9/4	0.4		
780b	9/2/0	0	8/1/0	0		
782b	12/11/0	0	9/0/0	0		
783b	24/20/0	0	16/14/0	0		
788b	11/10/0	0	7/4/2	0.5		
843c	2/2/1	0.5	1/1/0	0		
1,009d	6/5/0	0	6/5/3	0.6	4/3/5	1.7
overall	115/84/3	0.04	99/59/25	0.4	17/13/48	3.7

[a] Identical letters following clone number indicate plants originating from same callus
[b] p/f/s = pollinations/fruit/seed
[c] s/f = seed per fruit

Table 2. Seed set in selfings and crosses of 6x fusion hybrids between *S. brevidens* (PI 218228) and *S. tuberosum* (PI 203900) as females with 2x(2EBN) *S. chacoense* and 4x(4EBN) tester lines as males[a]

Clone	x 2x chc		x 4x adg		x 4x 'Katahdin'		x 4x 'Norland'		Selfed	
	p/f/s[c]	s/f[d]	p/f/s	s/f	p/f/s	s/f	p/f/s	s/f	p/f/s	s/f
56e[b]	7/7/0	–0–	8/7/9	1.3						
57e	9/8/4*	0.5			37/11/116*	10.5	34/18/297*	16.5	7/3/1*	0.3
205e	12/5/1*	0.2			38/14/69*	4.9	31/1184*	7.6		
209e	14/9/11	1.2	13/4/192	48.0	50/230403*	17.5	34/15/499*	33.3		
211e	10/7/6*	0.9			39/10/195*	19.5	49/22/635*	28.9		
216e	8/8/4*	0.5	17/14/85	6.1	47/19/37*	1.9	19/8/78*	9.8	6/5/1*	0.2
219e	8/8/7	1.1	3/3/32	10.7	43/22/268*	12.2	24/7/252*	36.0		
240f	9/8/8*	1.0			21+/10/272*	27.2	47/15/486*	32.4	9/7/21*	3.0
242f	20/8/8	1.0	21/17/63	3.7	11/9/73*	8.1				
249f	10/10/5	2.0	9/5/258	51.6	41/10/315*	31.5	35/18/874*	48.6	8/6/11*	1.8
462g	15/12/8	0.7	8/8/161	20.1					10/6/0	0
464g	8/6/7	1.2	10/9/119	13.2						
903h					30/17/124*	7.3	19/4/215*	53.8		
919i					37/15/227*	15.1	26/11/274*	24.9		
920i					16/9/191*	21.2	11/10/280*	28.0		
937g					42/25/548*	21.9	74/11/272*	24.7		
1,690h					23/7/264*	37.7	17/6/306*	51.0		
1,691h	12/10/6*	0.6	7/4/199	49.8	36/9/153*	17.0	16/2/67*	33.5	7/5/23*	4.6
1,692h	11/9/5	0.6			35/13/217*	16.7	18/9/217*	24.1		
1,861g	13/9/13*	1.4			18/2/45*	22.5	25/5/93*	18.6		
overall	166/124/93	0.8	96/71/1,118	15.7	564/225/3,517	15.6	479/172/4,929	28.6	47/32/57	1.8

[a] Data with *, from 1985, all others from 1984
[b] Identical letters following clone number indicate plants originating from same callus
[c] p/f/s = pollinations/fruit/seed
[d] s/f = seed per fruit

Table 3. Chromosome counts of progeny from selfs of somatic hybrids of *S. brevidens* + *S. tuberosum*, and test crosses with *S. chacoense* (chc) and *S. tubersosum* Group Andigena (adg)

Ploidy of fusion parent	Clone no.	Selfed ploidy	x 2x chc ploidy	x 4x adg ploidy
4x	704	4x (5)[a]	4x (1)	4x (4), 52 (1)
	759	–	–	4x (1)
	766	–	–	4x (1)
	770	–	–	4x (1)
	772	4x (6)	–	–
	774	–	–	4x (2)
	1,009	4x (5)	–	–
6x	56	–	–	5x (5)
	209	–	4x (4), 7x (1)	5x (6)
	216	–	–	5x (5), 55 (1)
	219	–	4x (1), 42 (1)	5x (6)
	240	6x (7)	–	–
	242	–	< 4x (1)	5x (5)
	249	–	46 (1)	5x (5)
	462	–	4x (1), 46 (1)	5x (7)
	464	–	4x (2), 5x (3)	5x (5)
	1,691	–	–	5x (5)
	1,692	6x (6)	–	–

[a] Numbers in parentheses represent the number of plants having preceding chromosome number on ploidy value

were 4x, 5x and even 7x. It is probable that the 5x plants came from normal megagametophytes (1n = 3x = 36) from 6x fusion hybrids combined with 2n (= 2x = 24) gametes from chc. The single 7x progeny arose most likely from a 2n (= 6x = 72) gamete from the fusion hybrid combined with a normal gamete from chc.

Discussion

The results of this study demonstrate that it is possible to obtain useful amounts of female fertility in fusion hybrids between *S. brevidens* and cultivated *S. tuberosum*.

Further, our results indicate that actual pollination rather than stainability will be critical for evaluations of the fertility of fusion hybrids. Despite high levels of stainability, the 4x somatic hybrids performed poorly in crosses. These hybrids were self-fertile at only low levels and cross compatible as females at very low levels. The 6x hybrids generally had more abnormalities in meiosis and less stainable pollen; however, they performed better in crosses than did the 4x lines. Like

the 4x hybrids, they were self-fertile only at low levels, but unlike the tetraploid hybrids, they had good cross compatibility with 4x tester species. It is noteworthy that cross pollination proved the most useful test of fertility, despite the fact that self pollination might be expected to be the most compatible. The high male fertility of the tester lines may be the reason for this superiority.

It is unknown at this point what effect cytoplasm has on the fertility of these hybrids. Further studies should reveal if the cytoplasm found in the fusion plants has any significant effect on their fertility or their progeny's.

In terms of fertility in fusion hybrids, the selection of parents is critical. Careful selection and evaluation of parents should be made for both ploidy level and fertility. Clearly, in these interspecific fusions, the 2x+4x combination yielded a hexaploid product that was more desirable in terms of cross-ability. Since proper endosperm development in seeds is directly tied to genome dosage, ploidy level of hybrids is a real concern if further utilization is to occur. On a practical basis, the tuberization of these hexaploid materials is also superior to those of 2x+2x fusions (Austin et al. 1986) and further enhances their desirability.

Consideration of parental fertility is equally important. Barsby et al. (1984) reported the fusion of *S. brevidens* with the cultivar 'Russet Burbank'. These plants were male sterile, and perhaps female sterile as well. Although selections of *S. brevidens* are generally highly fertile, 'Russet Burbank' is very difficult to use in sexual crosses due to its sterility. This sterility appears to be manifested in the fusion hybrids as well. In our studies, we have also observed problems with sterile fusion parents. A fusion of *S. brevidens* PI 218228 with a male sterile haploid of the cultivar 'Superior' resulted in plants which were both male and female sterile. Fusions between male fertile and male sterile plants can produce fertile hybrids however. A 2x+2x fusion of male sterile and male fertile *S. tuberosum* haploid lines yielded tetraploid hybrids which were highly self-fertile (Austin et al. 1985 a).

Unlike many other fusion hybrids, moderate to high levels of fertility are expressed in *S. brevidens*+*S. tuberosum* fusion hybrids. The retention of fertility is perhaps not surprising in light of the evolutionary relationships among potato species. Most potato species are believed to have evolved in relatively recent times, and because of this have essentially homologous genomes (Ramanna and Hermsen 1979 a). *S. brevidens,* as a non-tuber-bearing species, has been considered to be more distantly related to cultivated potatoes than most other species of Section Petota, yet obviously it still falls within the limits of compatibility required for sexual fertility. Certainly there does appear to be a limit as to how broad of a fusion can be both successful and compatible. Intergeneric fusions of potato and tomato (Melchers et al. 1978) have not proven fertile, and fusions of *S. tuberosum* with *S. nigrum* (Binding et al. 1982) also appear non-utilizable. These however define the farthest limits so far tested for fusions with at least one partner being a tuber-bearing Solanum. Many less distantly related species exist as potential fusion partners.

Solanum brevidens normally cannot be crossed directly with Gp. Tuberosum materials. The block to seed production lies in inadequate endosperm development. According to the Endosperm Balance Number (EBN) hypothesis (Johnson et al. 1980; Johnston and Hanneman 1980) endosperm development in Solanums is dependent upon a 2 : 1 balance of maternal to paternal EBN values in the developing endosperm. Only species with identical EBN numbers appear to cross freely. Thus, *Solanum brevidens,* a 2x(1EBN) species will not cross with either 2x(2EBN) or 4x(4EBN) Gp. Tuberosum materials, but will cross with 2x(2EBN) species if its chromosome complement is doubled making it 4x(2EBN) (Johnston and Hanneman 1982).

According to the EBN hypothesis, the tetraploid somatic hybrids, being fusions of 2x(1EBN) *S. brevidens* and 2x(2EBN) *S. tuberosum,* should be 4x(3EBN). The hexaploids, being fusions of *S. brevidens* and 4x(4EBN) *S. tuberosum* should be 6x(5EBN). Technically, the fusion hybrids should not be crossable with any species. However, the 6x(5EBN) fusion plants cross well with 4x(4EBN) lines. Genetic studies of the EBN system have suggested that a slight excess female dosage may be tolerated. Growth of the endosperm is retarded, but viable seeds are produced (Ehlenfeldt 1984). Limits of this tolerance may explain the success of 6x(5EBN)×4x(4EBN) crosses and likewise the failure of 4(3EBN)×2x(2EBN) crosses. More work is needed in this area and fusion materials, with their unique EBN numbers, may prove very valuable in these studies. Certainly the overall trends suggest that EBN values must be close if successful seed development is to occur, and that consideration of endosperm development may be very important to the further use of these and other fusion hybrids.

The ultimate goal of our work is to use somatic fusions to incorporate genes from wild species such as *S. brevidens* into potato breeding lines. For this reason, the fertility of the fusion hybrids, particularly the hexaploids, is very encouraging. Good yields of seeds have been obtained with three different 4x tester lines. In addition, we have found that the somatic hybrids can retain important characters from both parents. Thus PLRV resistance from *S. brevidens* and late blight resistance from *S. tuberosum* can both be expressed in individual hybrids (Helgeson et al. 1986). Transmission of these genes through meiosis and their expression in sexual progeny is being investigated. Subsequent incorporation of these genes into useful breeding lines will complete the demonstration that protoplast fusion can

be an effective means for broadening the germplasm base of potatoes.

Acknowledgements. The authors would like to thank Dr. Sandra Austin and Dr. R. E. Hanneman Jr. for useful advice and discussions and Steve Vicen for photograph and illustration assistance.

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