Theor Appl Genet (1994) 89: 775–782
© Springer-Verlag 1994

R. G. Novy · J. P. Helgeson

Somatic hybrids between *Solanum etuberosum* and diploid, tuber bearing *Solanum* clones

Received: 1 March 1994 / Accepted: 18 April 1994

Abstract Electrofusion was used to obtain somatic hybrids between *Solanum etuberosum* (2n=2x=24) and two diploid potato lines. These hybridizations were conducted to determine if haploid×wild species hybrids are better fusion partners than conventional S. tuberosumGp. Tuberosum haploids. Restriction fragment length polymerase (RFLP) analyses of the putative somatic hybrids confirmed that each parental genome was present. The somatic hybrids between *S. etuberosum* and a haploid *S. tuberosum* clone, US-W730, were stunted and had curled, purple leaves. In contrast, somatic hybrids between *S. etuberosum* and a haploid×wild species hybrid (US-W 730 haploid×*S. berthaultii*, were vigorous and generally tuberized under field conditions. These hybrids were designated as E+BT somatic hybrids. Analyses of 23 E+BT somatic hybrids revealed a statistically significant bias towards the retention of *S. etuberosum* chloroplasts. Stylar incompatibilities were observed when the E+BT somatic hybrids were used as pollen donors in crosses with *S. tuberosum* cultivars. Reciprocal crosses did not show this incompatibility. The progeny were vigorous and had improved tuber traits when compared to the maternal E+BT parent. RFLP analyses of three sexual progeny lines confirmed the presence of all 12 *S. etuberosum* chromosomes. In two of these lines, RFLPs that marked each of the 24 chromosome arms of S. etuberosum were present. However, RFLP markers specific for regions on chromosomes 2, 7, and 11 were missing from the third clone. Because other markers for these chromosomes were present in the progeny line, these results indicated the likelihood of pairing and recombination between *S. etuberosum* and *S. tuberosum* chromosomes.

Key words Somatic hybrid · *Solanum etuberosum* *S. tuberosum* · *S. berthaultii* · RFLP

Introduction

Solanum etuberosum, a diploid (2n=2x=24) species, has virus and frost resistances that could be useful to breeders of the cultivated potato, *S. tuberosum* Gp. Tuberosum (2n=4x=48). Classified as 1EBN according to the endosperm balance number hypothesis (Johnston and Hanneman 1982), this species cannot be readily crossed with tetraploid (4EBN) or diploid (2EBN) forms of *S. tuberosum*.

The use of bridge species has been one means of circumventing crossability barriers (Hermsen and Taylor 1979). Bridging crosses, however, require a careful monitoring of the desired trait at each step to ensure that it is not lost prior to final sexual hybridization with *S. tuberosum*. Somatic hybridization, a procedure that directly combines the genome of the wild species with *S. tuberosum*, is an alternative to the use of bridge species (Helgeson 1989). In many cases, somatic hybrids can be crossed directly to *S. tuberosum* (Ehlenfeldt and Helgeson 1987).

Tubers of tetraploid somatic hybrids between non-tuber bearing *Solanum* species and Gp. Tuberosum species are often very small and misshapen (Austin et al. 1985). Poor tubers and reduced vigor are also often seen in haploids of Gp. Tuberosum (n=2x=24). However, haploid×wild species hybrids often show improved vigor and tuberization in comparison with their respective haploid parents (Hermundstad and Peloquin 1986). The somatic hybridizations reported here were conducted to determine if haploid×wild species hybrids are also better fusion partners than conventional Gp. Tuberosum haploids.

Using electrofusion, we hybridized a clone of *S. etuberosum* to two diploid potato lines. The first potato line was a *S. tuberosum* Gp. Tuberosum haploid, US-W 730 (2n=2x=24); the second line was a sexual hybrid between US-W 730 and *S. berthaultii*. Thus, the two diploid lines differed only by the addition of 12 chromosomes of *S. ber-*

Communicated by G. Wenzel

R. G. Novy (✉)[1] · J. P. Helgeson
Agricultural Research Service, U.S. Department of Agriculture, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

Present address:
[1] Rutgers University-Cook College, Blueberry and Cranberry Research Center, Penn State Forest Rd., Chatsworth, NJ 08019, USA

thaultii. Following somatic hybridizations, restriction fragment length polymorphism (RFLP) analyses were conducted to verify the hybridity of the regenerated clones and to determine if any losses of chromosomes or sets of chromosomes had occurred. Somatic hybrids and their sexual progeny from crosses with tetraploid *S. tuberosum* lines were also evaluated under field conditions.

Materials and methods

Plant material

Solanum etuberosum ('PI 245939') was obtained from the Inter-Regional Potato Introduction Project (IR-1) at Sturgeon Bay, Wis. Seeds were germinated *in vitro* on propagation medium (Haberlach et al. 1985), and one clone (designated 16-1) was selected for use in somatic hybridizations because it readily regenerated plants from protoplasts.

The *S. tuberosum*, Gp. Tuberosum haploid, US-W 730, was established *in vitro* on propagation medium from the surface-sterilized eyes of tubers supplied by R. E. Hanneman Jr., USDA/ARS-Madison, Wis. Clone 463-4, a haploid×wild species hybrid derived from the cross of US-W 730 with *S. berthaultii* ('PI 265857') was provided by S. J. Peloquin and Lisa Darmo, University of Wisconsin, Madison

When combined in somatic hybrids, the *S. etuberosum* genome component was designated as "E," the contribution of the *S. tuberosum*, US-W 730 clone was designated as "T", and the portion contributed by the *S. tuberosum*×*S. berthaultii* hybrid (clone 463-4) was designated as "BT." Thus, somatic hybrids between *S. etuberosum* and US-W 730 were designated as E+T hybrids. Somatic hybrids between *S. etuberosum* and clone 463-4 (US-W 730×*S. berthaultii*) were designated E+BT hybrids. Following the same notation, the sexual progeny from crosses of E+BT hybrids with *S. tuberosum* were designated EBT×T.

Protoplast isolation, electrofusion and regeneration

Plant cultural conditions and protoplast isolation procedures were as described by Haberlach et al. (1985) except where indicated. For protoplast isolation, plants were grown *in vitro* at 20°C on 15 ml of propagation medium in 25×150-mm test tubes for 3–4 weeks (because of its slower growth *in vitro*, it was necessary to use 5- to 6-week-old *S. etuberosum* plants). The flotation step was not used; leaves excised from plants were immediately placed in conditioning medium. After 24 h at 4°C, the leaves were filtered from the conditioning medium, chopped finely with a straight-edged blade, and placed in enzyme digest medium for 16 h at 28°C. The digest medium of Haberlach et al. (1985) was modified by adding fatty acid-free bovine serum albumin (0.2% BSA; Sigma A-7030), deleting PVP, and utilizing sucrose at 120 g/l. The rinse medium contained only sucrose (125 g/l) and BSA (0.2%) at pH 6.5. This was necessary because the concentration of salt in the standard rinse solution was too high for electrofusion. Following two rinses, the protoplast density was diluted to approximately 10^6/ml by the addition of fusion medium containing sucrose (20 g/l), mannitol (71.6 g/l) and $CaCl_2$ (73.5 mg/l). A dilution of protoplasts to a final concentration of 5×10^5/ml was done with the same medium after the actual protoplast density was determined with a hemacytometer.

Electrofusion of protoplasts was conducted with the use of a Cell-Electrofusion Processor #001002 (DEP Systems) with 2×35 mm stainless steel electrodes resting in a petri dish. The electrodes were sterilized in a 100×25 mm petri plate by soaking in 70% ethanol for 5 min. After air-drying, they were placed in a 100×15-mm sterile petri dish. A drop (440 µl) containing protoplasts to be fused was then added to the gap between the electrodes. Protoplasts were aligned into short chains between the electrodes by the application of a 90 V/cm, 500-KHz alternating current for approximately 30–45 s. Fusion of the protoplasts then proceeded following a direct current pulse (100 µs, 1250 V/cm). The alternating current was then slowly decreased to zero over 20–30 s.

The protoplast density was adjusted to 1.65×10^5 protoplasts/ml by the addition of liquid SKM-A medium, a modified version of Hunt and Helgeson's (1989) SKM medium. The modification consisted of adding an additional 5.0 g/l of glucose and 10 g/l of D-mannitol, bringing the osmolarity to approximately 490 mOsm/kg. Petri dishes containing the possible hybrid cells were wrapped with parafilm and incubated for 16–17 h in darkness at 21°C.

After 16–17 h incubation, protoplasts were plated onto agar-solidified SKM-A according to the bi-layer procedure of Hunt and Helgeson (1989). Newly plated protoplasts were kept in darkness for 16–17 h. Then, a low light regime (20 $\mu E\ m^{-2}\ s^{-1}$) was created by covering plates with cheesecloth and illuminating them with cool-white fluorescent lights . Incubator temperatures were 24°C during the day (16 h) and 21°C at night (8 h). The cheesecloth covering was removed 7–10 days after plating to give a light level of 50–60 $\mu E\ m^{-2}\ s^{-1}$. By that time, viable protoplasts had formed cell walls and some had undergone cell division.

When the microcalli were visible to the naked eye, the medium in the bi-layer plates was cut into six equal pie-shaped wedges. Two wedges each were layered onto 20 ml of agar-solidified culture medium (the culture medium of Haberlach et al. 1985 to which 2 mg/l zeatin was added) in a 100×15 mm plastic petri dish. Light illumination was then increased to 90 $\mu E\ m^{-2}\ s^{-1}$. Under these conditions calli turned green. When they had grown to about 1 mm in diameter, calli were transferred to fresh culture medium at a density of 50 calli/plate for further growth. Green calli which grew to 2–3 mm were then transferred (50 calli/plate) to differentiation medium (Haberlach et al. 1985) to promote shoot morphogenesis. Calli with shoot initials were placed upon proliferation medium to promote shoot elongation. The proliferation medium (PM-M#2) was modified from the version of Austin and Cassells (1983) by decreasing sucrose by 5.0 g/l, adding 10.0 g/l additional mannitol, including 1.0 g/l of casein hydrolysate, and providing 2.0 mg/l (rather than 10 mg/l) GA_3. Shoots were removed and allowed to root in test tubes containing propagation medium.

Genomic and chloroplast analyses

Total cellular DNA was isolated from frozen young leaves by the method of Saghai-Maroof et al. (1984). Subsequent digest, electrophoresis, and blotting of DNA are as described by Williams et al. (1990). For blots, each lane contained approximately 6 µg of DNA.

Hybridization probes for nuclear analyses were a subset of the single-chromosome specific probes used in mapping the tomato and potato genomes (Bonierbale et al. 1988; Tanksley et al. 1992). Twenty-five probes were selected which provided at least 2 probes/chromosome and which showed DNA polymorphisms between *S. etuberosum* and US-W 730 (Fig. 1).

Chloroplast analyses were done with a *Petunia* probe (P3) by the method of Spooner et al. 1991. Radiolabeling of the probe DNA and subsequent hybridization and washing were as described by Williams et al. (1990).

Field assessment of somatic hybrids

Three somatic hybrid clones from each fusion, the fusion parents, and cultivars 'Kennebec' and 'Atlantic' were evaluated at the University of Wisconsin Agricultural Research Stations at Hancock and Sturgeon Bay, Wis. The E+BT clones were obtained from three separate calli; the E+T clones were taken from two different calli. The experimental design was a randomized complete block with three replicates. Each experimental unit consisted of five transplants spaced at 30 cm with 92 cm between rows. Evaluated traits included tuber number, yield, tuber weight, and survival. Survival was assessed 1.5 months after transplanting to the field.

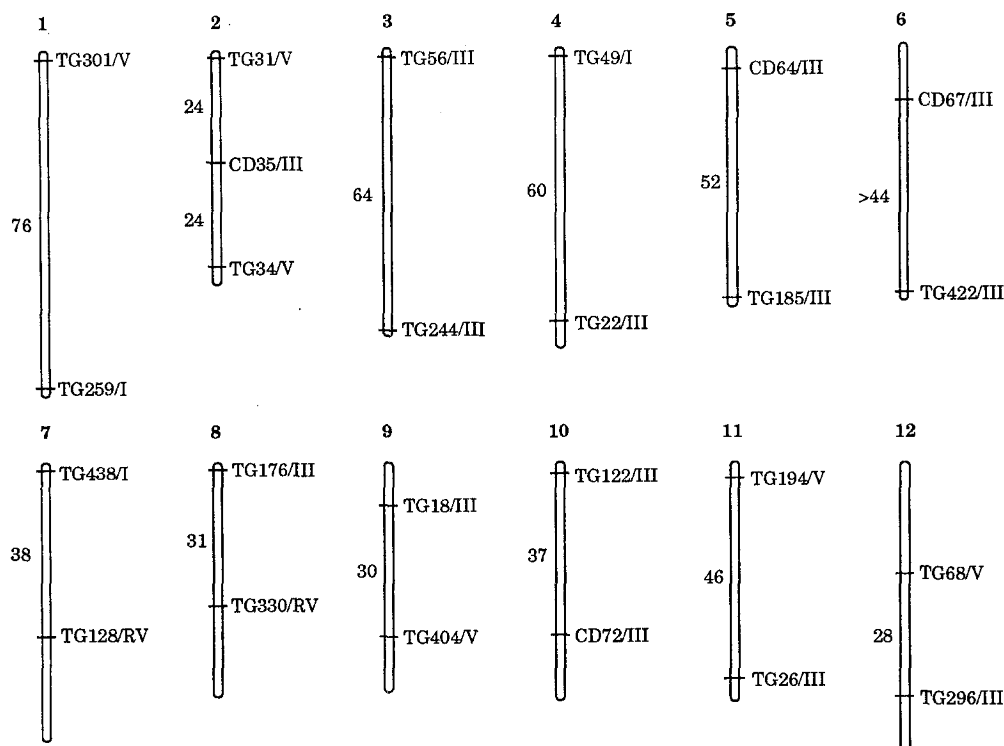

Fig. 1 Schematic representation of *Solanum* chromosomes with locations of markers used in the genomic analysis of somatic hybrids and sexual progeny. Each probe/enzyme combination detected polymorphisms between *S. etuberosum* and the fusion parents 463-4 and US-W 730, *III Hin*dIII, I *Eco*RI, V *Eco*RV. The estimated distance between markers is given in centiMorgans (cM), and was based on the summation of two point distances between the intervening markers presented in the Cornell University potato molecular map (Tanksley et al. 1992)

Cytology and stylar analyses

Ploidy levels of somatic hybrids and their sexual progeny was determined by root tip or flower bud chromosome counts as described by Novy and Hanneman (1991).

Stylar analysis was done using aniline blue (0.05% aniline blue in 0.1 N K_2PO_4) as described by Novy and Hanneman (1991) except that pistils or styles were collected 2 or 3 days after pollination. Pollinated styles treated with aniline blue showed fluorescent pollen tubes when exposed to UV light (356 nm).

Crosses

Crosses were performed in a greenhouse or growth room. Greenhouse light was supplemented by high pressure sodium vapor lights; growth rooms were illuminated with cool-white fluorescent lights. An attempt was made to maintain photoperiods of 15–16 h and temperatures of 18°C under both regimes, however the temperature fluctuated ±4°C in the greenhouse. Plants to be used as females were emasculated 1 day prior to anthesis. Either fresh or refrigerated pollen (no more than 1 week old) was used in crosses. Fruit were allowed to mature on the plant for 1–1.5 months following pollination. Harvested fruit were then allowed to ripen an additional month prior to the extraction of seed. Seed were then aseptically extracted from the mature fruit and soaked in a sterile aqueous solution of 1500 to 2000 mg/l of GA_3 for 24 h. Seeds were germinated aspectically in test tubes containing propagation medium (Haberlach et al. 1985).

Results

Callus culture and regeneration

S. etuberosum protoplasts and those from the potato parents, US-W730 and 463–4 were plated in the experiments along with the fusion products. *S. etuberosum* protoplasts and some cells that had undergone the electrofusion procedure gave visible calli 2 weeks after the electrofusion and plating. On the other hand, neither US-W 730 nor 463-4 protoplasts showed cell division when plated by themselves.

Shoots were first removed from calli 3 months after electrofusion, and the majority were obtained over an additional 6-week period. Since regeneration from US-W 730 and 463-4 cells was not expected, regenerated shoots were expected to be either: (1) *S. etuberosum* diploids derived from non-fused cell lines, (2) *S. etuberosum* tetraploids, or higher-ploidy types from auto-fusions or spontaneous doublings in culture, or 3) tetraploid and higher-ploidy somatic hybrids.

Initially, all shoots that formed were removed from calli and placed on medium for rooting. During this process it was observed that regenerating shoots on calli could be categorized on the basis of trichome density and pigmentation of stems and leaves. Most shoots were green and had few or no trichomes, traits characteristic of regenerated shoots of *S. etuberosum*. However, some shoots had purple pigmentation and abundant trichomes. Once rooted, these purple-pigmented shoots with trichomes developed into *in vitro* plants with morphologies differing from *S. etuberosum*. These were selected as possible fusion hybrids.

RFLP analyses of somatic hybrids

Three chromosome-specific RFLP probes, TG68 (chromosome *12*), TG259 (ch. *1*), and TG422,(ch. *6*) were used to analyze 23 possible somatic hybrids (Fig. 2). In general,

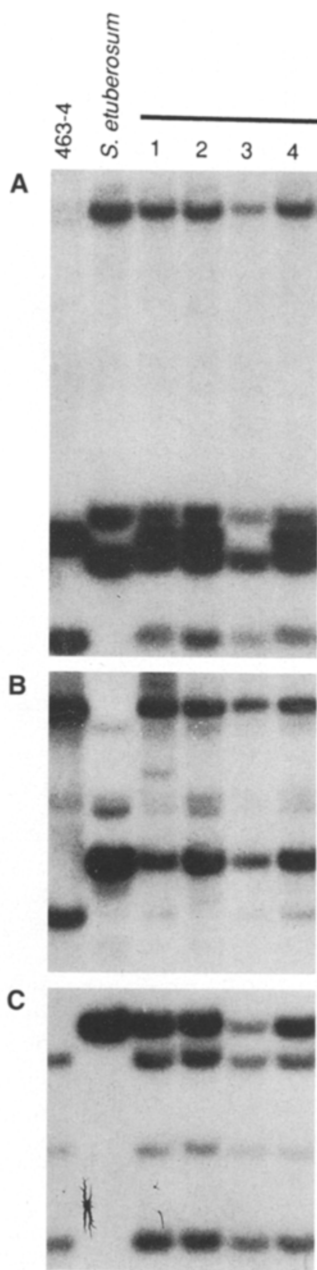

Fig. 2 Autoradiograms from Southern blots of genomic DNA from fusion parents (463-4, 16-1) and four of their E+BT somatic hybrids hybridized with **A** probe TG68 from chromosome 12. Genomic DNA had been digested with *Eco*RV. Somatic hybrid 3 is missing the upper diagnostic band of 463-4. **B** Probe TG259 from chromosome 1; genomic DNA had been digested with *Eco*RI. **C** Probe TG422 from chromosome 6; genomic DNA had been digested with *Hin*dIII

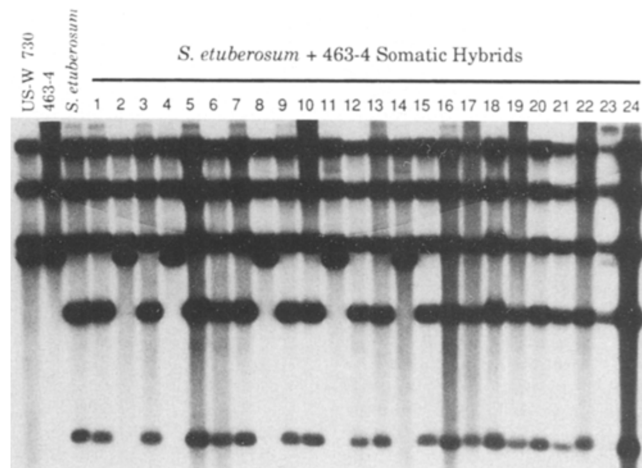

Fig. 3 Autoradiogram from the hybridization of a Southern blot of total DNA (*Hin*dIII digests) with chloroplast probe P3. E+BT somatic hybrids 2, 4, 8, 11, and 14 display only the bands of the 463-4 parent, while the remaining hybrids show the bands of the *S. etuberosum* parent (16-1). DNA of hybrid 23 had incomplete digestion with *Hin*dIII that did not allow for a conclusive chloroplast categorization

the putative somatic hybrids, which had been selected on the basis of morphology, had all of the diagnostic bands of both fusion parents. The exceptions were 3 somatic hybrids that lacked 463-4 (BT) marker bands when probed with TG68 (ch. *12*) and 1 somatic hybrid that lacked the TG422 (ch. 6) marker bands of the 463-4 parent.

A more detailed genomic characterization of 14 E+BT and 4 E+T somatic hybrids was conducted with 25 probes covering all 12 chromosomes. All somatic hybrids carried all of the bands unique to *S. etuberosum*. The 4 E+T hybrids also had all of the US-W730 diagnostic bands. At least one 463-4-specific RFLP was present for each chromosome in each E+BT hybrid. However, the loss of 463-4 marker bands was observed in 5 of the 14 E+BT somatic hybrids. Four of these clones lacked one 463-4 diagnostic band; 2 lacked TG 68 (ch. *12*) and 2 lacked TG56 (ch. *3*) and TG 194 (ch. *11*) , respectively. The remaining clone lacked bands for both TG 68 (ch. *12*) and TG 438 (ch. *7*).

Chloroplast analyses of somatic hybrids

Chloroplast analyses of 24 E+BT somatic hybrids with probe P3 identified 18 hybrids with *S. etuberosum* chloroplasts and 5 hybrids with *S. tuberosum* chloroplasts (Fig. 3). (DNA of 1 somatic hybrid was incompletely digested and thus a definitive categorization of its chloroplast type was not possible.) The observed deviation from an expected 1:1 ratio was statistically significant at the 2.5% level (χ^2 value of 6.26 with 1 *df*).

Description of somatic hybrid plants

Test tube plants of E+BT somatic hybrids were vigorous with an upright growth habit similar to that of the fusion parent 463-4. *S. etuberosum* plantlets tended to have a bushier growth habit. The growth of these somatic hybrids in the field and greenhouse was typically vigorous with a sprawling, indeterminate growth pattern. Leaves were darker green and larger than those of the diploid parents. Flowering was profuse, with 5–8 dark purple, pentagonal flowers per inflorescence.

Chromosome counts of 13 E+BT hybrids indicated that all but 1 were at or near the expected tetraploid level (45–48 chromosomes). The exception, although difficult to assess cytologically, had many more than 48 chromosomes, as well as improved tuberization with respect to the tetraploids. Thus, it was probably a hexaploid (2n=6x=72) de-

Table 1 Location means for yield (g/hill), tuber number, tuber weight, and % survival of three parental clones, their respective somatic hybrids, and two commercial cultivars

Clone	Description	Grams/hill[a]		Tubers/hill[a]		Tuber weight[a] (g)		Survival[a] (%)	
		Hancock	St. Bay	Hancock	St. Bay	Hancock	St. Bay	Hancock	St. Bay
463-4	US-W 730 × *ber*	1048a[c]	294a	18.2a	6.5bc	58c	45b	100a	93a
US-W 730	Tuberosum haploid	227c	60b[d]	6.6c	4.5bc[d]	35d	14c[d]	93a	40cd
16-1	*S. etuberosum* (etb)	0.0	0.0	0.0	0.0	0.0	0.00	93a	60bc
Atlantic	Cultivar	1140a	261a	7.5c	3.7c	152a	72a	100a	100a
Kennebec	Cultivar	1126a	368a	12.7b	6.6bc	89b	58ab	100a	87ab
2-7-4A	E+BT fusion hybrid	391b	110b	18.6a	18.6a	21e	7d	93a	87ab
2-9-1A	E+BT fusion hybrid	226c	79b	22.0a	13.7ab	10f	6d	100a	93a
2-1-3A	E+BT fusion hybrid	0.0	0.0	0.0	0.0	0.0	0.0	60b	40cd
1-1-1A	E+T fusion hybrid	0.0	0.0	0.0	0.0	0.0	0.0	93a	27de
1-1-6A	E+T fusion hybrid	0.0	0.0	0.0	0.0	0.0	0.0	100a	7e
1-1-1C	E+T fusion hybrid	0.0	0.0	0.0	0.0	0.0	0.0	0	0

[a] Entries with 0.00 values were not included in data analyses due to their biased effect (reduction) on the error variance
[b] *S. berthaultii*
[c] Trait means followed by the same letters are not significantly different at P=0.05 (Fisher's LSD)
[d] Missing one entry. Values in the LSD comparison were adjusted using harmonic means

rived from the fusion of two 463-4 protoplasts with one *S. etuberosum* protoplast.

Of 34 E+BT somatic hybrids tested, 7 (21%) shed pollen. Pollen staining of these 7 hybrids ranged from 45% to 75%. Male sterility was not associated with chloroplast type or the presence or absence of a particular RFLP band.

As with the E+ BT hybrids,growth of the E+T somatic hybrids in test tubes was generally good, with hybrids displaying the erect growth of the haploid parent US-W 730. When these somatic hybrids were transplanted to the greenhouse or field, however, their leaves curled and developed purple coloring on the underside of the leaves. Shoot and root growth was severely retarded. Of ten lines counted, seven had 45–48 chromosomes, at or near the expected tetraploid level. Three clones were hexaploids, or nearly so (65–72 chromosomes).

Field analyses of somatic hybrids

A field study with 3 tetraploid somatic hybrids of each type was designed to evaluate survival, tuber weight, tuber number, and yield (Table 1). Survival of the transplanted clones was 93–100% (with 2 exceptions) at Hancock, Wis., where fields were regularly irrigated every 2–3 days. The exceptions were 2-1-3-A (60% survival) and clone 1-1-1C (0% survival). Survival rates at Sturgeon Bay, where water was only supplied after transplanting and during periods of prolonged dryness, were generally lower. Under these conditions, E+T somatic hybrids 1-1-1A and 1-1-6A showed a dramatic decrease in survival when compared to their counterparts at Hancock. As at Hancock, clone 1-1-1C also did not survive under field conditions at Sturgeon Bay. The E+BT somatic hybrids did not show this marked difference in survival between locations, although 2 clones showed a 6–7% decrease in survival at Sturgeon Bay and 1 clone showed a 20% decrease.

Tuber Traits

All 3 E+BT somatic hybrids were late maturing; green vines and flowers were present 3.5 months after planting. Tuberization occurred in 2 of the 3 (Table 1 and Fig. 4A). Tubers from these 2 E+BT clones were produced in large numbers, and no significant differences for tubers/hill were found between clones. However, clone 2-7-4A with larger tubers, gave a significantly higher yield at Hancock (g/hill, Table 1).

The E+T somatic hybrids did not tuberize nor did they even form stolons. At harvest, surviving plants showed only swelling at the base of the plant. Due to the poor vigor and complete lack of tuberization in the E+T hybrids, comparisons with E+BT hybrids were not repeated a second year.

Crossing of somatic hybrids

Sexual hybridization between E+BT somatic hybrids and 4x Gp. tuberosum cultivars was attempted. Five hundred and three pollinations utilizing 22 4x somatic hybrid clones as females produced 99 berries and 24 seeds. Reciprocal crosses using Gp. Tuberosum cultivars as the female parent yielded few berries and no seeds.

It was hypothesized that poor seed set might reflect stylar incompatibilities. To test this possibility, stylar analyses were conducted to assess pollen-tube growth in each of the two crosses. When somatic hybrids were used as females, no obstruction of Gp. tuberosum pollen tube growth was seen. In contrast, blockage of pollen tube growth was seen in the reciprocal crosses. Generally, pollen tubes were stopped in the upper one-third of the Gp. tuberosum styles; a few tubes sometimes managed to reach three-quarters of the length of the style. None traversed the entire length. Similar findings were seen in self-pollinations of somatic

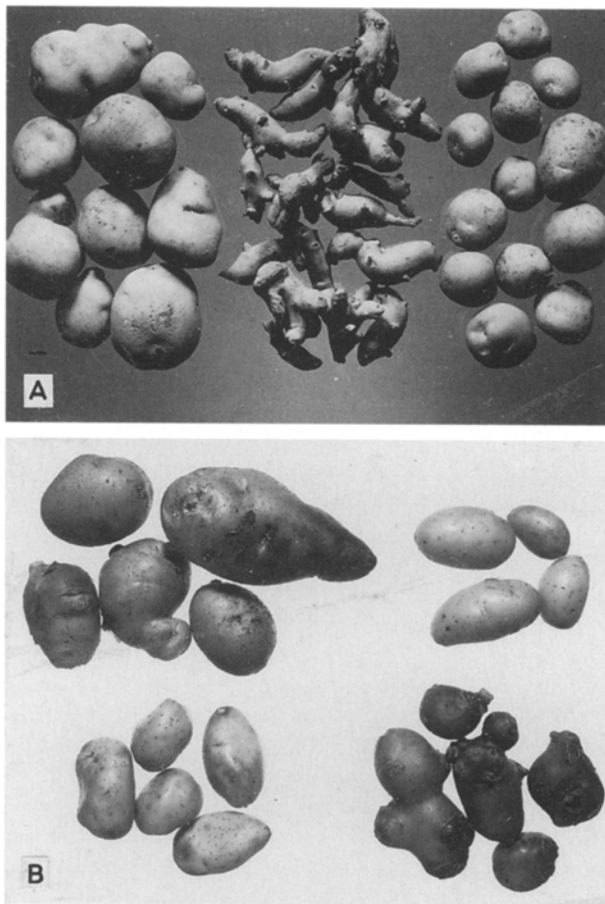

Fig. 4 **A** Field tubers of 463-4 (*left*), E+BT hybrid 2-7-4A (*middle*), and US-W 730 (*right*). **B** Greenhouse tubers of four EBT×T clones. E+BT hybrid 2-7-4A was the maternal parent of the EBT×T clone that produced the tubers in the *top right*

hybrids, where cessation of tube growth occurred in the upper one-half of the style.

Sexual progeny of E+BT somatic hybrids

Only 5 of the 24 seeds obtained from crosses of E+BT clones with Gp. Tuberosum cultivars germinated and produced plants. Chromosome counts of 4 of these EBT×T progeny indicated they were at or near the tetraploid level (47–48 chromosomes). Under greenhouse conditions, 4 of the 5 displayed normal growth patterns. The fifth had normal early development. Later, its apical meristem produced twisted, distorted leaves, and meristematic growth eventually ceased.

Tuberization occurred in all EBT×T clones under greenhouse conditions. A noticeable improvement in tuber size and appearance was observed (Fig. 4B) when comparisons were made to tubers of the somatic hybrid parents. All progeny showed intense purple pigmentation of stolons and less intense pigmentation of tubers. This pigmentation trait was apparently derived wholly or in part from the *S. etuberosum* parent, which also influenced the expression of purple pigmentation in the stems and leaves of the somatic hybrids and the sexual progeny.

In the greenhouse, only 2 EBT×T clones flowered: 1 was male-sterile, and the other, EBT×T-3, had 38% stainable pollen. These 2 clones were used as females in crosses with Gp. tuberosum cultivars. The male-sterile plant was also female sterile. However, EBT×T-3 produced an average of 6 seeds/berry (from 29 pollinations and 21 berries).

Molecular analyses of the sexual progeny clones

Three clones, including the fertile clone mentioned above, were analyzed with 25 RFLP probes to assess the sexual transmission of *S. etuberosum* chromosomes from the E+BT parent. The somatic hybrid parents of these 3 clones had all 25 *etuberosum* marker bands, indicating that at least one copy of each *S. etuberosum* chromosome arm had probably been present.

No loss of *S. etuberosum* diagnostic bands were observed for 2 of the clones. The remaining clone, EBT×T-3, showed the loss of regions on chromosomes 2, 7, and 11 (identified by the molecular probes TG31, TG438 and TG194, respectively). Markers mapping to other regions of each of these chromosomes were present, indicating that only partial losses of chromosomes 2, 7, and 11 had occurred.

Discussion

Selection of somatic hybrids directly from calli was facilitated by shoot pigmentation and the presence of trichomes on newly regenerated shoots. Also, the inability of the protoplasts of parental US-W 730 and 463-4 lines to divide on the selected medium greatly reduced the number of nonhybrid calli regenerated. As in studies with *S. brevidens* (Austin and Helgeson 1987), the ability of one partner (S. etuberosum) to regenerate from protoplasts allowed production of somatic hybrids with the non-regenerating parental lines.

The preferential loss of chromosomes or sets of chromosomes has been reported in some somatic hybrids (Pental et al. 1986; Pijnacker et al. 1987). RFLP analyses showed that this was not the case with the somatic hybrids examined in this study. Only 5 of the E+BT hybrids showed loss of parental diagnostic bands. Of these, 4 had lost one marker; the remaining line lost two. In no case had a somatic hybrid lost all of the markers for a given chromosome.

Chloroplasts in somatic hybrids generally are of one parental-type, and not both types (Pelletier 1986; Kumar and Cocking 1987). This was also observed in the E+BT somatic hybrids, where either the *S. etuberosum* or the *S. tuberosum* Gp. Tuberosum chloroplast, but not a mix of the two, predominated. However, in contrast to other studies (Austin and Helgeson 1987; Pehu et al. 1989), a statistically significant preferential retention of *S. etuberosum* pa-

rental chloroplasts was observed. *S. etuberosum* chloroplasts were even retained in a hexaploid E+BT clone, the probable product of two potato cells fusing with one *S. etuberosum* cell. With somatic hybridizations of *Nicotiana*, Bonnett and Glimelius (1983) found that species with only slight chloroplast genome divergence exhibited a random chloroplast segregation pattern in their somatic hybrids. Conversely, those *Nicotiana* species with a greater disparity between chloroplast genomes were more likely to show a selective advantage for one type of chloroplast in the somatic hybrid cells. Considering the large dissimilarity (>21 mutational differences) between *S. tuberosum* and *S. etuberosum* chloroplast genomes (Hosaka et al. 1984), the observations of Bonnett and Glimelius may help to explain the biased retention of *S. etuberosum* chloroplasts.

Pronounced differences in vigor and yield were seen between the E+T and E+BT somatic hybrids. The poor vigor of the E+T hybrids may be an expression of hybrid lethality or semi-lethality similar to what has been previously reported in *Solanum* (Emme 1937; Propach 1940). The improved performance of the E+BT somatic hybrids appears to be the result of the substitution of 12 *S. tuberosum* chromosomes with *S. berthaultii* chromosomes. This chromosome substitution may have removed gene(s) of *S. tuberosum* responsible for the poor vigor of the E+T hybrids. Conversely, the allelic diversity and heterozygosity contributed by the 12 *S. berthaultii* chromosomes may have masked the expression of the *etuberosum-tuberosum* incompatibility in E+BT somatic hybrids.

Crosses of the E+BT somatic hybrids with *S. tuberosum* Gp. Tuberosum cultivars were possible, but limited by stylar incompatibilities and seed lethality. Stylar incompatibilities have been reported for the self-compatible Etuberosa species and their hybrids when used as males in crosses (Hermsen et al. 1981; Ehlenfeldt and Helgeson 1987; Novy and Hanneman 1991). Ehlenfeldt and Helgeson (1987) reported no inhibition of pollen tube growth upon selfing of the *S. brevidens+S. tuberosum* Gp. Phureja-Stenotomum tetraploid somatic hybrids. This was not the situation when E+BT somatic hybrids were selfed, as pollen tube growth was arrested in the upper one-half of the style.

Four of five sexual progeny were vigorous plants with improved tuber traits as compared to their somatic hybrid parents. The marked improvement of tuber characteristics following one cross to Gp. tuberosum cultivars is a trend generally observed with somatic hybrids involving the series Etuberosa species (Austin and Helgeson 1987; Helgeson et al. 1993). All five progeny appeared to be at, or near, the expected tetraploid level. Molecular analyses of two of the progeny lines indicated that each had a complete set of 12 *S.* e*tuberosum* chromosomes. This was probably due to preferential intra-genomic pairing of S. etuberosum chromosomes during meiosis of the E+BT parent. A third progeny line was missing some diagnostic bands for chromosomes 2, 7, and 11. This suggests the possibility that intergenomic pairing and recombination may have occurred, a prerequisite for effective introgression of traits from *S. etuberosum* into potato breeding lines. Further crosses of clone EBT×T-3 with a Gp. Tuberosum cultivar were successful. Thus, it appears that the somatic hybrid-derived materials will have the fertility necessary for their effective use in breeding programs.

References

Austin S, Cassells AC (1983) The regeneration of plantlets from isolated single cells of Irish potato varieties. Proc R Irish Acad 83:53–61

Austin S, Helgeson JP (1987) Interspecific somatic fusions between *Solanum brevidens* and *S. tuberosum*. In: von Wettstein D, Chua NH (eds) Plant molecular biology. Plenum Publ, New York, pp 209–222

Austin S, Baer MA, JP Helgeson (1985) Transfer of resistance to potato leaf roll virus from *Solanum brevidens* into *S. tuberosum* by somatic fusion. Plant Sci Lett 39:75–82

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Bonnett HT, Glimelius K (1983) Somatic hybridization in *Nicotiana*: behavior of organelles after fusion of protoplasts from male fertile and male sterile cultivars. Theor Appl Genet 65:213–21

Ehlenfeldt MK, Helgeson JP (1987) Fertility of somatic hybrids from protoplast fusions of *Solanum brevidens* and *S. tuberosum*. Theor Appl Genet 73:395–402

Emme H (1937) Genetik der Kartoffel II. Bastarde von *Solanum emmeae* Juz. und *S. commersonii* Dun. Arten, welche gegen den Koloradokäfer widerstandshfähig sind. Biol Zh 6:299–310 (Taken from: Bibliogr Genet 16:22

Haberlach GT, Cohen B, Reichert N, Baer M, Towill L, Helgeson JP (1985) Isolation, culture and regeneration of protoplasts for potato and several related *Solanum* species. Plant Sci 39:67–74

Helgeson JP (1989) Somatic hybridization of wild *Solanum* species with potato: a potential source of diversity for breeders. In: Louwes K, Toussant H, Dellart L, (eds) Parental line breeding and selection in potato breeding. Pudoc, Wageningen, pp 87-94

Helgeson JP, Haberlach GT, Ehlenfeldt MK, Hunt G, Pohlman JD, Austin S (1993) Fertile somatic hybrids of potato and wild *Solanum* species: potential for use in breeding programs. Am Potato J 70:437–452

Hermsen JGTh, Taylor LM (1979) Successful hybridization of nontuberous *Solanum etuberosum* Lind. and tuber-bearing *S. pinnatisectum* Dun. Euphytica 28:1–7

Hermsen JGTh, Ramanna MS, Sawor Z (1981) The effect of chromosome doubling on fertility, meiotic behaviour and crossability of *Solanum etuberosum*×*S. pinnatisectum*. Euphytica 30: 33–39

Hermundstad SA, Peloquin SJ (1986) Tuber yield and tuber traits of haploid-wild species F_1 hybrids. Potato Res 289–297

Hosaka K, Ogihara Y, Matsubayashi M, Tsunewaki K (1984) Phylogenetic relationship between the tuberous *Solanum* species as revealed by restriction endonuclease analysis of chloroplast DNA. Jpn J Genet 59:349–369

Hunt GJ, Helgeson JP (1989) A medium and simplified procedure for growing single cells from *Solanum* species. Plant Sci 60: 251–257

Johnston SA, Hanneman RE Jr (1982) Manipulations of Endosperm Balance Number overcomes crossing barriers between diploid *Solanum* species. Science 217:446–448Kumar A, Cocking EC (1987) Protoplast fusion: A novel approach to organelle genetics in higher plants. Am J Bot 74:1289–1303

Novy RG, Hanneman RE (1991) Hybridization between Gp. Tuberosum haploids and 1EBN wild potato species. Am Potato J 151–169

Pehu E, Karp A, Moore K, Steele S, Dunckley R, Jones MGK (1989) Molecular, cytogenetic and morphological characterization of somatic hybrids of dihaploid *Solanum tuberosum* and diploid *S. brevidens*. Theor Appl Genet 78:696–704

Pelletier G (1986) Cybrids as tools in higher plant genetics. In:Mantell SH, Chapman GP, Street PFS (eds) The chondriome: chloroplast and mitochondrial genomes. John Wiley & Sons, New York, pp 213–224

Pental D, Hamill JD, Pirrie A, Cocking EC (1986) Somatic hybridization of *Nicotiana tabacum* and *Petunia hybrida*: recovery of plants with *P. hybrida* nuclear genome and *N. tabacum* chloroplast genome. Mol Gen Genet 202:342–347

Pijnacker LP, Ferwerda MA, Puite KJ, Roest S (1987) Elimination of *Solanum phureja* nucleolar chromosomes in *S. tuberosum+S. phureja* somatic hybrids. Theor Appl Genet 73:878–882

Propach H (1940) Cytogenetische Untersuchungen in der Gattung *Solanum* sect. *Tuberarium*. V. Diploide Artbastarde. Z. indukt. Abstamm. Vererb.-Lehre 78:115–128 (Taken from: Bibliogr Genet 16:19)

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Spooner DM, Sytsma KJ, Conti E (1991) Chloroplast DNA evidence for genome differentiation in wild potatoes (*Solanum* sect. Petota: *Solanaceae*). Am J Bot 78:1354–136

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141-1160

Williams CE, Hunt GJ, Helgeson JP (1990) Fertile somatic hybrids of *Solanum* species: RFLP analysis of a hybrid and its sexual progeny from crosses with potato. Theor Appl Genet 80:545–551