Somatic hybridization in the genus *Solanum*: *S. tuberosum* and *S. brevidens*

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**ABSTRACT**

Somatic hybrid plants were regenerated from fused mesophyll protoplasts of an albino potato (*Solanum tuberosum* spp. *tuberosum*) variant and *Solanum brevidens*, a non-tuber bearing species which is sexually incompatible with *S. tuberosum*. These somatic hybrid plants represent the first example of direct hybridization between potato and members of the taxonomic group *Etuberosa*, and offer the potential for introgressing valuable germplasm from *Solanum* species outside the sexually compatible range into a worldwide crop species.

**INTRODUCTION**

*Solanum brevidens* Phil. is an attractive potential donor of disease and physiologic resistances for the cultivated potato (*S. tuberosum* spp. *tuberosum*) (Jones, 1979; Ross and Rowe, 1965). There is a rigid crossability barrier between the tuberous species and non-tuberous diploids of the *Etuberosa* series (Pandey, 1962; Kamanna and Hermansen, 1981). Recent attempts to effect genetic exchange have centered on techniques utilizing 'bridging' species and doubling the chromosome number of the resultant normally sterile hybrids prior to crossing with *S. tuberosum* spp. *tuberosum* (Hermansen and Taylor, 1979; Hermansen et al., 1981; Ehlenfeldt and Hanneman, 1983). Somatic hybridization presents an alternative and potentially more expedient approach to overcoming barriers imposed by sexual incompatibility by directly combining the genotypes of potato cultivars with those of wild species possessing such features as resistance to biological or environmental stress. The synthesis of somatic hybrids between *S. brevidens* and the cultivated potato facilitates a realistic evaluation of the potential applications of somatic hybridization in potato breeding.

**MATERIALS AND METHODS**

Source of Protoplasts

In the present study, leaf protoplasts of a stable albino protoclone (protoplast-derived clone) of the potato cultivar 'Russet Burbank' (designated as clone 116) were fused with wild-type mesophyll protoplasts of *S. brevidens*. That the 116 protoclone is a stable albino is evidenced by the fact that of the 5 x 10^7 protoplast-derived calli (p-calli) observed thus far, none exhibited reversion to a green color. P-calli of protoclone 116 did not undergo shoot morphogenesis according to the standard regeneration protocol suitable for its parent but was nonetheless capable of doing so when exposed to a substantially different medium sequence involving higher sucrose concentrations and lower osmotic pressures (unpublished). The visual albinism marker and different requirements for induction of shoot morphogenesis figured prominently in the selection scheme devised for recovering somatic hybrids. P-calli of *S. brevidens* have been induced to undergo shoot regeneration but will neither do so nor survive on the shoot inductive medium (Medium D) for potato (Barsby and Shepard, 1983). Nelson et al. (1983) reported 3% shoot formation after 10 weeks on Medium D; however we recorded no shoot formation under these conditions for this particular *S. brevidens* accession. Protoplasts were isolated according to the methods of Shepard and Totten (1977).

Fusion of protoplasts and selection of hybrids

Protoplasts were fused using a combination of polyethylene glycol and calcium ions at high pH. Fusion-treated protoplast populations were plated under conditions suitable for the survival of both species (Shepard et al., 1980; Barsby and Shepard, 1983). P-calli arising from fusion treated preparations were transferred to Medium C as previously described (Shepard et al., 1980) and individuals which displayed green pigmentation after 3-4 weeks were moved to Medium D. After 3-6 weeks on Medium D, 10% of the rapidly growing green p-calli had undergone shoot morphogenesis. Non-green members of the population were either white in color (presumed 116 p-calli which were not hybrids) or dark brown (presumed senerescent *S. brevidens* p-calli). P-calli with shoots were transferred to Medium T for shoot elongation and rooting (Shepard 1982). Plantlets were transferred to sterile vermiculite and maintained in an environmentally controlled growth cabinet. Figure 1 shows a typical somatic hybrid plant (right) alongside *S. brevidens* (left) and the albino *S. tuberosum* shoot culture.

Chloroplast DNA Analysis

Chloroplast DNA (cpDNA) was extracted from 5 to 15g of leaves from the albino protoclone of *S. tuberosum*, *S. brevidens* and somatic hybrids essentially according to the methods of Palmer (1982). A major modification which greatly increased the rapidity of this method was that the two cesium chloride (CsCl) gradient and dialysis steps were not employed. Instead, cpDNA was purified from the lysis mixture by three phenol-chloroform extractions. Subjecting this cpDNA to CsCl gradients caused a decrease in concentration but not a decrease in the amount of contaminating nuclear and mtDNA. Electrophoresis was according to the methods of Kemble et al. (1980).
Fraction I Protein Analysis
Ribulose-1,5-bisphosphate carboxylase (RubPcase) small subunits were analysed in polyacrylamide gels according to the methods of Cammaerts and Jacobs (1980) with the following modifications. The sample buffer contained 50mM Tris-HCl, 5mM EDTA, 10mM sodium metabisulfite, 1% polyvinylpyrrolidone, pH 7.6. Isoelectric focusing gels contained 2% ampholines comprising 1 part pH 5-8 and 2 parts pH 3-7. The gels were electrophoresed for 16 hours at 5W constant power and a final voltage of 1000V.

Fig. 1. Somatic hybrid (right) produced by fusion of protoplasts of S. tuberosum (albino variant, center) and S. brevidens (left).

RESULTS AND DISCUSSION
To date 32 putative somatic hybrid plants have been isolated. All hybrid plants grew vigorously under standard plant growth conditions (Shepard, 1980) and displayed morphological features intermediate between the two parents. Several exhibited extensive axillary shoot development and thickening of the highly pigmented stem which are notable characters of S. brevidens. Analysis of six of the somatic hybrids has thus far been completed and is based on the number of somatic chromosomes present in root tip cells, polypeptide patterns in isoelectric focusing gels of the small (nuclear-encoded) subunit of RubPcase, and origin of cpDNA. All somatic hybrids displayed the RubPcase small subunit polypeptides of both parental species (Fig. 2). Agarose gel electrophoresis of restriction enzyme fragmented cpDNA revealed the presence of only the S. brevidens chloroplast genome (Fig. 3). These restriction enzyme analyses are accurate to the 0.1% level (Schiller et al. 1983), and provide evidence that the plants are true hybrids and not merely chimeras.

Mitotic chromosome counts revealed that four of the six hybrid plants analyzed approached the octoploid condition, having between 92 and 96 chromosomes. Two plants possessed 72 chromosomes and are apparent hexaploids. Possible explanations for the differing ploidy are that: a) the octoploid hybrids resulted from fusions between two S. brevidens (2n = 2X = 24) protoplasts and one S. tuberosum protoplast (2n = 4X = 48); b) fusion of one of each protoplast type was followed by the restitution division of the S. brevidens nucleus prior to synchronous division and subsequent fusion of the two dissimilar nuclei; or c) cells in the leaf tissue of S. brevidens were in the

Fig. 2 Isoelectric focusing in polyacrylamide gel of RubPcase subunits from lane 1, S. brevidens; 2, S. tuberosum cultivar 'Russet Burbank'; 3 and 4, two somatic hybrids. LS and SS refer to the positions of the large subunit polypeptides and small subunit polypeptides respectively.

Fig. 3 Electrophoresis on 1% agarose gel of Bam HI fragmented cpDNAs from lane 1, parental albino protoclone of S. tuberosum; 2, parental S. brevidens; 3-8, six different somatic hybrids. Lane m contains size marker fragments produced by independent digestions of lambda DNA with Eco RI and Hae III.