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Production and characterization of somatic hybrids between *Solanum melongena* L. and *S. sisymbriifolium* Lam.*

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Summary. Protoplasts of 6-azauracil (AU) resistant cell lines of *Solanum melongena* L. were fused with protoplasts of *S. sisymbriifolium* Lam. to create somatic hybrids between these sexually-incompatible species. Following fusion, colonies were selected which were capable of growth in medium containing 1 mM AU. These colonies were placed on medium containing zeatin which had been shown to stimulate anthocyanin production during shoot organogenesis in tissue explants of *S. sisymbriifolium* but not in *S. melongena.* A total of 37 anthocyanin-producing colonies were identified from which 26 hybrid plants were regenerated. The morphological traits intermediate to those of the parents included: flower colour, leaf shape, and trichome density. Cytogenetic analysis revealed that all hybrids were aneuploids but their chromosome numbers were close to the expected number of 48. Isozyme analysis revealed that nuclear genes of both parents were expressed in the hybrids. In addition, isoelectric focussing of the large subunit of ribulose 1,5-bisphosphate carboxylase (Rubisco) provided evidence that each hybrid expressed only the *S. sisymbriifolium* chloroplast genome. All hybrids regenerated thus far have been sterile.

Key words: *Solanum melongena* – *S. sisymbriifolium* – Somatic hybridization – Chloroplasts – Isoenzymes – Rubisco

Introduction

There have been several reports of protoplast fusion and hybrid plant regeneration among *Solanum* species. Melchers et al. (1978) obtained somatic hybrids between a dihaploid *S. tuberosum* and *Lycopersicon esculentum;* subsequently the chilling resistance of these hybrids was tested (Smillie et al. 1979). Fusion of these sexually-incompatible species has also been described by Shepard et al. (1983). In both cases the resulting hybrids were sterile and backcrosses to either parent proved unsuccessful. Butenko et al. (1982) reported the regeneration of a single somatic hybrid between *S. tuberosum* + *S. chacoense.* This plant displayed resistance to potato virus Y even though both parents were susceptible. Somatic hybrids between haploid *S. tuberosum* and an atrazine-resistant biotype of *S. nigrum* have also been regenerated and the atrazine tolerance trait, which is chloroplast encoded, was inherited in some lines (Binding et al. 1982). Somatic hybrids between *S. tuberosum* and *S. brevidens* have been regenerated as an attempt to transfer desirable traits from the wild species into cultivated potato (Barsby et al. 1984).

The non-tuberous *Solanum* species have not previously been utilized in somatic hybridization although they constitute numerically the vast majority of the genus including the economically-important member, eggplant (*S. melongena*). Several desirable agronomic traits have been identified in the wild South American species *S. sisymbriifolium* Lam., including resistance to root-knot nematodes (Fassuliotis and Bhatt 1982), and carmine spider mites (Schalk et al. 1975). Resistance to these pests is unknown in the eggplant gene pool and several unsuccessful attempts to cross these species sexually have been made (Fassuliotis 1975; Seck 1983). Protoplast fusion offers the possibility of bypassing complex sexual barriers between species and may therefore serve as a method of transferring desirable traits into eggplant. Prerequisite methodology for protoplast culture in both *S. melongena* and *S. sisym-*

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briifolium and for plant regeneration in *S. melongena* has been developed (Gleddie et al. 1985 b, c). This study was therefore undertaken to achieve somatic hybridization between these species with the long term objective of transferring desirable traits into eggplant.

Materials and methods

Seed of *S. sisymbriifolium* Lam. (Garden No. S. 136) was obtained from Dr. S. Marsh, University Botanical Gardens, Birmingham, U.K.; *S. melongena* L. cv. 'Imperial Black Beauty' seed was obtained from Stokes Seeds Ltd., St. Catharines, Ont.

A cell suspension culture of *S. sisymbriifolium* established from stem callus was maintained in liquid MS (Murashige and Skoog 1962) medium supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), and used for protoplast isolation (Gleddie et al. 1985 c). Two cell suspensions (AU^R8 and AU^R10) of eggplant (isolated by D. F. Brown, Bishop's University, Lennoxville, Quebec) which were capable of growth in the presence of 1 mM 6-azauracil (AU) were also maintained in the same medium. Methods for establishing eggplant suspension cultures have been previously described (Gleddie et al. 1983 a).

The growth rate of each suspension culture was measured in the presence and absence of AU by filtering out large cell aggregates on a 1,000 μm nylon filter. The small cell aggregates were then suspended in liquid medium at a density of 0.2 g fr. wt./ml. One-half ml of this suspension was pipetted onto sterile filter paper (Whatman No. 1, 5.5 cm diameter) placed on agar-solidified Kao (1977) medium containing 2 mg/l 2,4-D and various concentrations of filter-sterilized AU and maintained at 25 °C under continuous fluorescent light (30 $\mu E\ m^{-2}\ s^{-1}$). The fresh weight of cells on filter paper was determined every second day for three weeks.

Protoplasts of both species were isolated and cultured according to methods described (Gleddie et al. 1985 b, c). Protoplasts were cultured with or without 1 mM AU, in liquid Kao medium supplemented with 1 mg/l 2,4-D, 3 mg/l naphthaleneacetic acid (NAA), and 1 mg/l benzylaminopurine (BAP).

For fusion, 10^6 protoplasts of each species were mixed in a 16×125 mm glass tube and centrifuged at $100 \times g$ for 5 min. Fusion was achieved according to the method described by Douglas et al. (1981 a) and a total of three experiments were performed.

For post-fusion culture, the protoplasts were resuspended in Kao medium containing 1 mg/l 2,4-D, 3 mg/l NAA and 1 mg/l BAP with or without 1 mM AU at a density of 10^5/ml and dispensed as one ml thin layers in 15×60 mm Falcon plastic petri dishes. The dishes were sealed with parafilm and incubated in a water-saturated environment inside plastic containers under low light (7 $\mu E\ m^{-2}\ s^{-1}$) at 25 °C. Efficiency of protoplast division was determined by scoring 100 protoplasts per dish, in five separate dishes per treatment, after 7 days of culture. After 14 days of culture, the dishes were transferred to a higher light intensity (30 $\mu E\ m^{-2}\ s^{-1}$) and 7 days later each dish was supplied 0.5 ml of Kao medium supplemented with 1 mM AU and 0.1 M glucose. At this time the dishes were removed from the plastic boxes and maintained thereafter in the same conditions at 70% R.H.

Six weeks post-fusion, macroscopic cell colonies were counted, isolated with forceps, and placed onto Kao medium lacking glucose and AU but supplemented with 0.8% Difco agar, 2% (w/v) sucrose, 0.1 mg/l 2,4-D, and 1 mg/l BAP. The colonies grew rapidly on this medium and after 14–28 days they were transferred to MS or Kao medium lacking 2,4-D and BAP but supplemented with 1 mg/l zeatin in order to screen for anthocyanin production and induce shoot organogenesis. Cytokinin-supplemented medium was previously shown to promote purple shoot primordia on leaf explant cultures of *S. sisymbriifolium* (Gleddie et al. 1985 c). Due to the distinctly green appearance of eggplant colonies on this medium, it was theorized that cell colonies growing in the presence of 1 mM AU and also producing anthocyanins would be somatic hybrids. Selected colonies which turned purple were usually transferred to fresh MS medium supplemented with 1 mg/l zeatin. Often several transfers were necessary to achieve shoot regeneration. Once these shoots had developed one or two normal leaves, they were transferred to agar-solidified hormone-free MS medium. Rooted shoots were assigned line numbers, transferred to 7 cm diameter plastic pots containing sterilized vermiculite, watered with a commercial fertilizer solution (20 : 20 : 20, N : P : K) weekly, and maintained in a growth chamber with 70% R.H. and 25 : 20 °C (day : night) temperature under a 16-h daylight (30 $\mu E\ m^{-2}\ s^{-1}$) supplied by fluorescent lights. When the plants had developed to the 5–6 leaf stage with adequate roots, they were transplanted to 13 cm diameter pots containing a soil : peat : sand mixture (1 : 2 : 1). Plants of the parental species *S. sisymbriifolium* and *S. melongena* regenerated directly from leaf explant cultures were maintained under the same conditions in the greenhouse.

Morphological data from all plants was collected after two months of greenhouse growth. During this period the plants were also examined cytologically. Excised root tips were pretreated in water for 24 h at 0 °C fixed for 24 h in acetic : ethanol (1 : 3) and stained in Snow's solution (Snow 1963).

Analysis of isoenzymes was performed on three or four healthy young leaves of regenerated plants after 1–3 months of greenhouse growth. Protein determination was by a modified Lowry technique (Wetter 1977). Sample preparation, polyacrylamide gel electrophoresis, and staining for alanyl aminopeptidase (AAP), 6-phosphogluconate dehydrogenase (6 PGDH), leucine aminopeptidase (LAP), and peroxidase were as described by Wetter and Dyck (1983).

Crystallization of ribulose 1,5-disphosphate carboxylase/oxygenase (Rubisco) from eggplant leaves by the method described by Lowe (1977) was unsuccessful and, as a substitute, the protein was prepared from *Nicotiana tabacum* leaves. Polyclonal antisera against the crystalline Rubisco was prepared in rabbits by D. R. Bundle, National Research Council, Ottawa. Rubisco from eight hybrid lines and the two parental species was immunoprecipitated, carboxymethylated, and isoelectric focussed on slab gels according to Wetter and Dyck (1983).

Results

Cell suspensions of wild-type eggplant, two azauracil-resistant eggplant lines and *S. sisymbriifolium* were tested for growth on agar-solidified medium containing various concentrations of AU. The Au-resistant eggplant cell cultures, AU^R8 and AU^R10, were able to grow equally well on either 0.5 or 1.0 mM AU at about one-half the rate they grew on AU-free medium (Fig. 1 a). Wild-type eggplant cells were severely inhibited in the

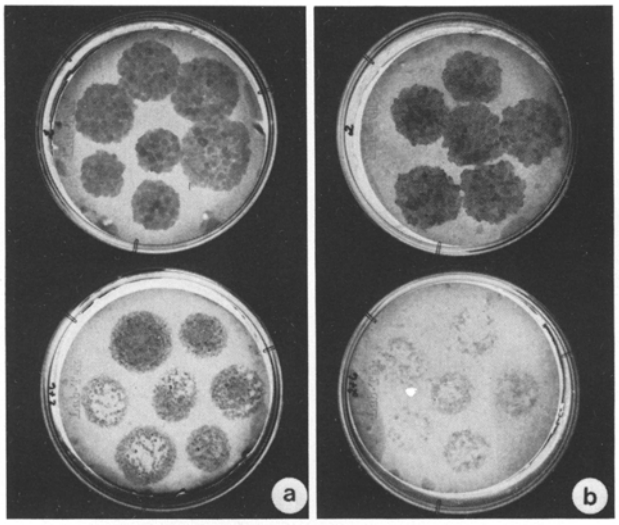

Fig. 1. **a** Cells of AU^R10 after 25 days of growth on medium containing 1 mM AU (*lower dish*) and lacking AU (*upper dish*). **b** Cells of *S. sisymbriifolium* after 25 days of growth on medium containing 1 mM AU (*lower dish*) and lacking AU (*upper dish*)

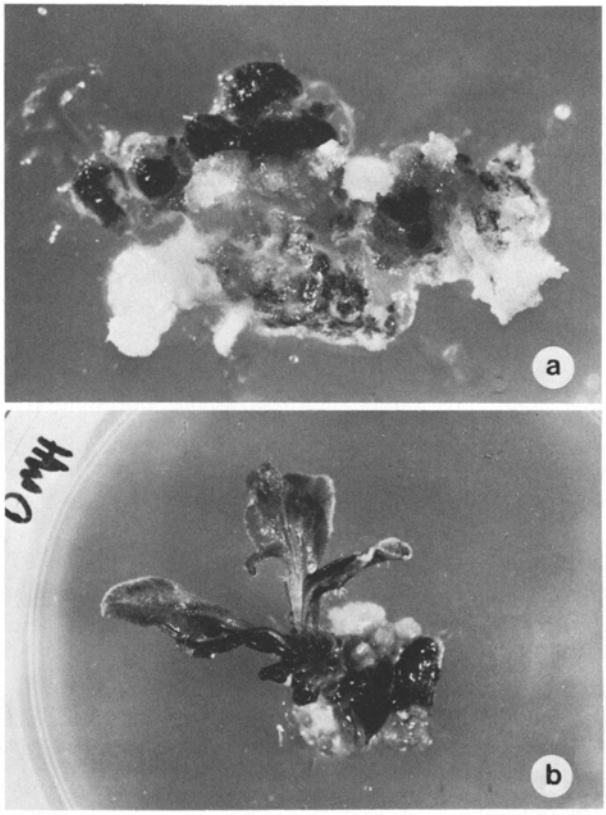

Fig. 2. **a** Hybrid colonies of *S. melongena* + *S. sisymbriifolium* after selection on AU and kinetin medium. Note the dark appearance of the anthocyanin-producing colonies. **b** Shoot regeneration from a hybrid purple colony

presence of 1 mM AU. Cells of *S. sisymbriifolium* were not severely affected by 0.5 mM AU while 1 mM was strongly inhibitory (Fig. 1 b). These observations indicated it was possible to distinguish between AU^R8 or AU^R10 and *S. sisymbriifolium* cells growing in AU after 20 days.

Protoplasts of AU^R8 and AU^R10, obtained at yields of 6×10^5 and 8×10^5 per g fr. wt., respectively, were cultured in the presence and absence of 1 mM AU. They were compared with *S. sisymbriifolium* protoplasts cultured in similar conditions. All three populations were capable of cell-wall reformation within three days of culture. The first division of AU^R8 and AU^R10 protoplasts occurred after seven days of culture in the presence or absence of AU and the frequency of division and plating efficiency were not adversely affected by 1 mM AU (Table 1). After 42 days in liquid medium, it was possible to visually identify the colonies of AU^R8 and AU^R10 and transfer them to agar medium. The first division of *S. sisymbriifolium* protoplasts was noted after four days in medium lacking AU. The division efficiency after 7 days was 5% and after 30 days a plating efficiency of 0.045% was recorded. In 0.5 mM AU the division efficiency was also 5%, while the plating efficiency was 0.063%. In 1 mM AU, however, division did not occur.

Following three fusion experiments between AU^R10 and *S. sisymbriifolium* protoplasts, numerous cell colonies were regenerated. The average frequency of division among PEG-treated protoplasts was 14% after 14 days and an average of 26 colonies per dish were recovered after 42 days of continuous culture in 1 mM AU. This represented a plating efficiency of 0.026%. Using unfused protoplasts, no *S. sisymbriifolium* cell colonies were recovered under these conditions while the plating efficiency was 0.046% with AU^R10 protoplasts. From a mixture of both species without PEG added the plating efficiency was 0.039%. At 42 days of culture the suspensions containing colonies were layered on agar-solidified medium containing 0.1 mg/l 2,4-D and 1 mg/l BAP. Of the total estimated 740 colonies present after PEG treatment from three experiments, 250 produced rapidly-growing, white callus in two weeks but no purple regions or shoot primordia. These colonies were then placed onto agar-solidified medium containing 1 mg/l zeatin as the sole growth regulator in an attempt to induce shoot regeneration. Within three weeks 37 colonies showed distinct localized purple areas (Fig. 2 a). Shoots were regenerated from each of the 37 purple colonies (Fig. 2 b) and to date 26 separate lines have been rooted and established in soil. Cell colonies which resulted from a mixture of two species without PEG fusion treatment often turned green on this medium (a trait specific for eggplant) but gave no purple primordia.

Table 1. Effect of 6-azauracil on protoplast cultures of AU^R8, AU^R10 and *S. sisymbriifolium*

AU Concentration	0			1.0 mM		
Cell line	AU^R8	AU^R10	*Solanum sisymbrii-folium*	AU^R8	AU^R10	*Solanum sisymbrii-folium*
1st division (days)	7	7	4	7	9	no division
Division efficiency (%)	18	21	5	15	20	0
Plating efficiency (%)	0.35	0.51	0.045	0.23	0.47	0

Note: Protoplasts were isolated from the cell suspensions and cultured according to "Materials and methods"

Table 2. Morphological and chromosome analysis of *S. melongena, S. sisymbriifolium* and their somatic hybrids

Plant	Mature height (cm)	Leaf[a] spines	Flower colour	Mean petal no./flower	Mean no. of flowers/ cyme	Mean no. of flowers/ plant	Mean corolla width (cm)	Chromosome no.
Eggplant cv. 'Imperial Black Beauty'	35	–	purple	7 (fused)	1	6	4.5	24
S. sisymbriifolium	60	+ + + + +	white	5 (fused)	6	16	4.5	24
Somatic hybrid								
line 5	18	–	light blue	5.6 (fused)	9	37	2.3	38–43
line 6	13	+	blue	5.5 (fused)	6	48	2.6	43–45
line 7	17	+	light blue	6.5 (separate)	8	112	2.4	45
line 9	15	–	blue	5.5 (separate)	8	64	2.1	44 (45)
line 14	20	–	white	5.4 (separate)	4	22	1.2	43–45
line 15	14	–	light blue	5.8 (separate)	6	42	3.2	45
line 16	16	+	white	7.0 (separate)	7	88	3.4	44 (45)

Note: Plants were analyzed after being established in soil in the greenhouse for 2–6 months
[a] Leaves were scored on a scale from – = no spines to + + + + + = extremely spiny

Morphological characteristics of the plants regenerated from purple colonies were compared with those of plants of eggplant and *S. sisymbriifolium* regenerated directly from leaf explant cultures (Table 2). Due to difficulties in rooting some lines and the death of others after transplantation, morphological data on most plants was gathered at various times ranging from 1–2 years after the initial fusion experiments. The potential hybrid regenerants were less vigorous and more fragile than the parental species. The height of each 'hybrid' was considerably less than either parental species (Table 2, Fig. 3 a).

Morphological characteristics intermediate between those of eggplant and *S. sisymbriifolium* provided preliminary evidence supporting the theory that the selected regenerants were hybrids. The most striking difference between the parental species was in their leaves (Fig. 3 c). Eggplant has oval leaves which are pubescent but not spiny. *Solanum sisymbriifolium* leaves, on the other hand, have pinnately-lobed leaves, and many 0.4 and 1.0 cm long spines along their adaxial and abaxial surfaces. A range of morphological variation was observed amongst the leaves of each regenerant as well as between the regenerated lines. Although the hybrids occasionally produced spines on their leaves or petioles, in most respects, including shape, the leaf morphology more closely resembled that of eggplant. Many hybrid leaves showed signs of variegation, retarded development of one lateral half of the leaf created by the midvein, and extreme variation in blade and petiole size (Fig. 3 c). The leaves of eggplant and *S. sisymbriifolium* plants regenerated from tissue culture were, by contrast, very uniform.

A second morphological distinction between eggplant and *S. sisymbriifolium* was in flower colour, shape and structure (Fig. 3 b). Eggplant had dark purple

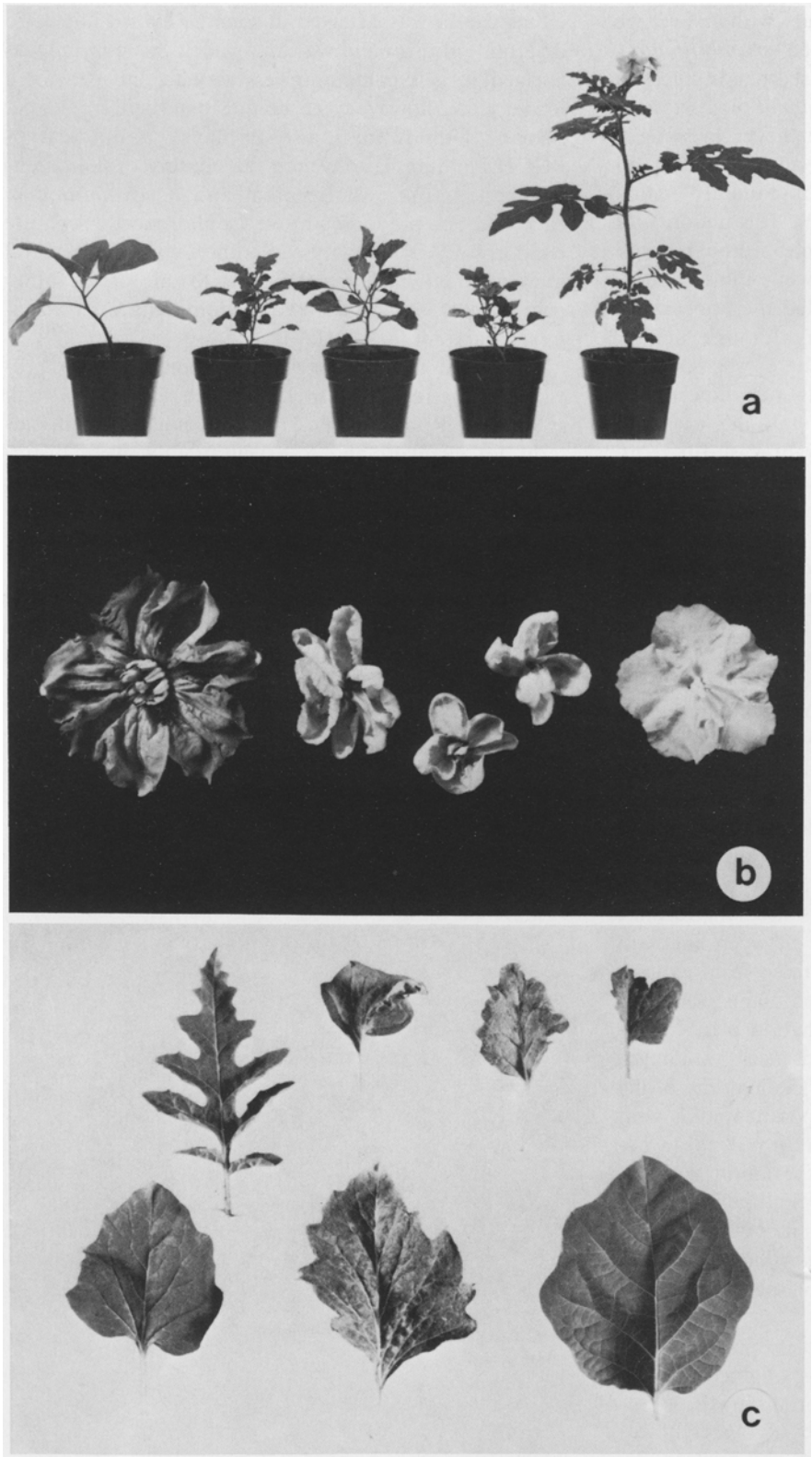

Fig. 3. **a** Regenerated plants of *S. melongena* (*left*), somatic hybrid plants 3, 5, 7 (*center*), and *S. sisymbriifolium* (*right*). **b** Flowers of *S. melongena* (*left*), somatic hybrids 3, 5, 7 (*center*), and *S. sisymbriifolium* (*right*). **c** Leaves of *S. melongena* (*lower right*), *S. sisymbriifolium* (*upper left*), and five somatic hybrid lines

flowers composed of seven fused petals, with an average of seven anthers per plant. Flowers of *S. sisymbriifolium*, on the other hand, had five white fused petals, and five anthers. The flowers of *S. sisymbriifolium* plants turned light purple with increasing age but never became as dark as eggplant flowers. The somatic hybrids all flowered very prematurely and consequently many, small flowers were formed (Table 2). The majority of the hybrid flowers were light purple although some plants had predominantly white flowers. Floral abnormalities amongst the hybrids included the appearance of separate petals in every flower although neither parental species had this trait. There was extreme variation in the number of petals per flower, the observed range being four to ten and the variation amongst flowers on any one regenerant was also great. Although most hybrid flowers produced a normal style and stigma, very few produced anthers and among all of the flowers observed on hybrid regenerants, none had anthers containing pollen. In addition to exhibiting severe sterility the anthers which developed often showed other abnormalities such as fusion to petals; in one case, the anthers all developed into new flower buds.

The phenotypic variability observed in floral and leaf characteristics was paralleled by cytological variation in the somatic hybrids. Cytological analysis of the *S. sisymbriifolium*, AU^R10, and AU^R8 cell cultures, indicated that these cells were predominantly diploid ($2\underline{n}=24$), although many aneuploid and polyploid cells were observed. Since it has not been possible to regenerate plants from the cell lines used for fusions, cytological comparisons were made between eggplant and *S. sisymbriifolium* plants regenerated from explant cultures. In both cases, all regenerated plants were normal diploids ($2n=24$). The somatic hybrid plants, however, were often mixoploids composed of cells with different chromosome numbers (Table 2). The majority of the regenerants were predominantly hypo-aneuploids with chromosome numbers ranging from $2\underline{n}=38$ to 48 between different lines. The greatest variation was observed in one plant of line 5 which had cells ranging from $2\underline{n}=38$ to 45 in the same root tip. Similar morphology of eggplant and *S. sisymbriifolium* chromosomes precluded the identification of specific chromosomes in the hybrids (Fig. 4).

Electrophoretic separation of leucine amino-peptidase, alanyl aminopeptidase, 6-phosphogluconate dehydrogenase and aspartate aminotransferase isoenzymes confirmed the hybrid nature of the eight regenerants analyzed. In each hybrid the nuclear genomes of both parental species were expressed since the isoenzyme banding patterns resembled a mixture of the parental enzymes (Fig. 5 a). In the case of leucine aminopeptidase a novel band which was not present in either parent was detected in somatic hybrid line 7. To determine if this band was an artifact, this enzyme was assayed in gels containing less protein and run for a longer time, however, the unique band still appeared. Somatic hybrid line 7 also displayed a different 6-PGDH banding pattern than the mixture of the parental extracts (Fig. 5 a). Eggplant and *S. sisymbriifolium* each had one predominant electrophoretically-separate band of 6-PGDH activity while line 7 contained several minor bands which were specific to one or the other parent yet it was missing the predominant *S. sisymbriifolium* band for 6-PGDH.

Evidence for nuclear hybridization was also provided by the results of isoelectric focussing of the small subunit of Rubisco (Fig. 5 b). The small subunit patterns for eggplant and *S. sisymbriifolium* were distinguishable and both patterns appeared in the somatic hybrids analyzed (Fig. 5 b), indicating that Rubisco nuclear genes of both parents were expressed in the somatic hybrids.

Separation of the large subunits by isoelectric focussing revealed that only the *S. sisymbriifolium* chloroplast genome was expressed in all hybrids

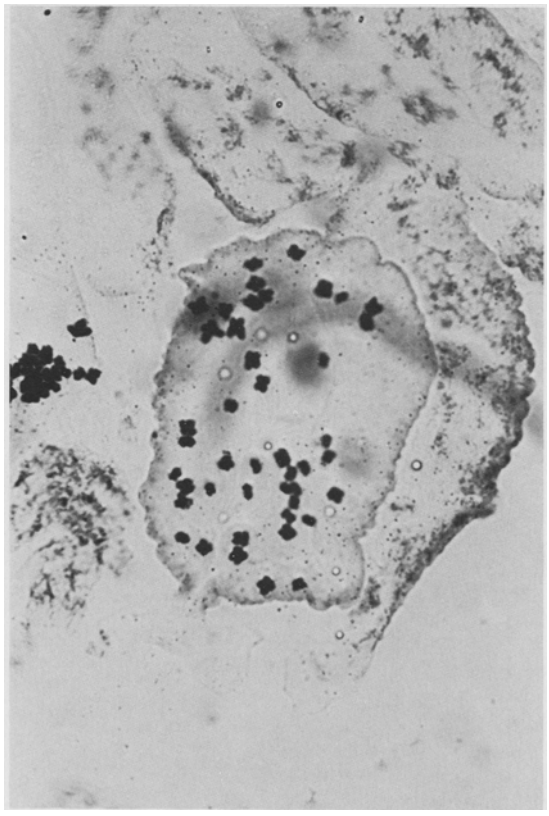

Fig. 4. A root tip cell of somatic hybrid line 7 possessing 43 chromosomes

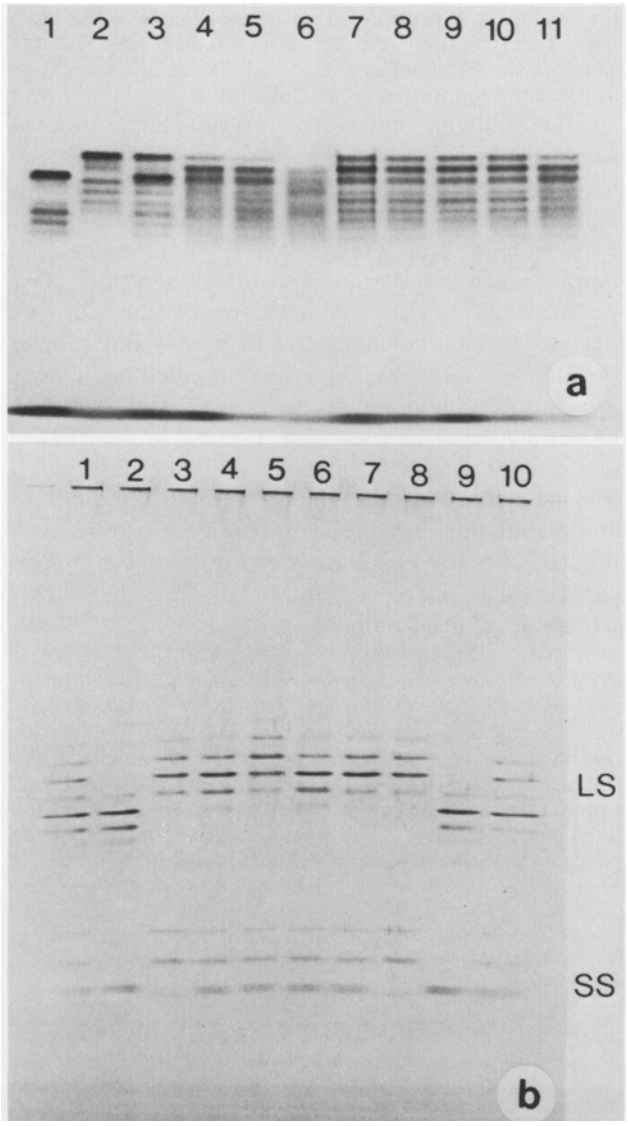

Fig. 5. a Electrophoretic banding patterns of 6-PGDH isozymes. *Lane 1* = eggplant, *2* = *S. sisymbriifolium*, *3* = mixture of the parents, lanes *4* to *11* are hybrid plants 3 b, 5, 7, 8, 9, 10, 11, and 16, respectively. **b** Isoelectric focussing patterns of Rubisco from a mixture of eggplant and *S. sisymbriifolium* (*lanes 1* and *10*), eggplant (*lanes 2* and *9*), *S. sisymbriifolium* (*lanes 3* and *8*), hybrid plants 3 b, 7, 12, 13 are in lanes *4, 5, 6,* and *7,* respectively. *LS* = large subunit, *SS* = small subunit

analyzed. The eggplant large subunits were electrophoretically distinct from those of *S. sisymbriifolium* and could be clearly seen in an artificial mixture of protein extracts of the two species (Fig. 5 b).

Discussion

The development of the screening system for selecting hybrid cells in this study was based on the assumption that resistance to 6-azauracil and capability of synthesizing anthocyanins would be expressed in the hybrids. These assumptions were indeed proven correct indicating that these traits were dominant or semi-dominant. However, our method for hybrid identification was based more on enrichment than on direct selection as large numbers of colonies could develop from unfused or homo-fused eggplant protoplasts. It was necessary to screen 740 colonies on zeatin-supplemented medium in order to identify 37 potential hybrid colonies. A method for selectively restricting the growth of eggplant cells in post-fusion cultures would obviously be desirable. An approach that we considered was the use of metabolic inactivation agents which were originally used to restrict parental cell growth and select somatic hybrids in mammalian cell cultures (Wright 1978). In fact, one such inactivating agent, iodoacetic acid has been successfully used to eliminate one of the parental lines in plant somatic hybridization studies (Sidorov et al. 1981). However, in our study it was not possible to define an iodoacetic acid treatment that consistently inactivated eggplant protoplasts without completely destroying them prior to fusion. More work is needed to define reliable treatments for inactivating plant protoplasts before this approach can be generally applied in plant somatic hybridization studies.

Although the hybrid purple colonies displayed shoot induction, all attempts to regenerate plants from protoplasts isolated from the parental cell suspensions (AU^R8, AU^R10, and *S. sisymbriifolium*) have failed. Ability to undergo organogenesis was, therefore, an integral part of the selection system. Restoration of morphogenic competence has been observed in *N. rustica* + *N. tabacum* (Douglas et al. 1981 a) and *N. rustica* + *N. sylvestris* (Gleddie et al. 1983 b) somatic hybrid colonies after fusion of protoplasts from cell suspensions which had greatly reduced or no morphogenic competence.

Several morphological traits in the *S. melongena* + *S. sisymbriifolium* hybrids were intermediate to those of the parents. These included flower colour, sepal, petal and anther numbers; leaf shape and size; number of spines on leaves and stems; density and type of trichomes. It is common for somatic hybrids of Solanaceous species to show traits intermediate between the parental species. This was the case for hybrids in *Datura* (Schieder 1978), *Nicotiana* (Evans et al. 1980; Douglas et al. 1981 b), *Petunia* (Power et al. 1980), and *Solanum* (Binding et al. 1982). However, some novel morphological traits were observed in our hybrids. Most notable was the occurrence of separate petals on all hybrid flowers (Fig. 3 b) while both parents had only fused petals. Eggplant has seven fused petals and *S. sisymbriifolium* has five whereas hybrid flowers had as many as ten or as few as four petals. The number of flowers per plant was also much greater in all hybrids than in either parent. The hybrids all flowered prematurely, even during in vitro rooting which suggests alterations in endogenous growth regulator metabolism. Novel characteristics relating to flowering have also been observed in tomato + potato somatic hybrids (Melchers et al. 1978).

The variation in chromosome numbers observed in the regenerated hybrids (Table 2) undoubtedly accounted for the tremendous morphological variation amongst the hybrid plants. It could also be the basis for the observed sterility of the hybrids. Since most of the regenerated hybrids were hypoaneuploid, there may have been a preferential loss of chromosomes of one parent in this hybrid combination. Preferential loss of chromosomes from one partner of a fusion combination has been documented for soybean + *N. glauca* (Wetter and Kao 1980), *Daucus carota* + *Petroselinum hortense* (Dudits et al. 1980), and *Datura innoxia* + *Atropa belladonna* (Schieder 1982). A more probable cause for chromosome variation amongst the regenerants may be related to the fact that protoplasts were derived from cell suspensions which had been maintained in vitro for over six months prior to use in hybridization experiments. A cytological analysis of the parental cell suspension revealed that they were predominantly diploid, although considerable variation in chromosome number was observed. In addition, ethyl methanesulfonate (EMS) was used to select the AU resistant cell lines (D. F. Brown, unpublished). This mutagen may have induced other genetic changes, including chromosome loss or rearrangements which also prevented plant regeneration from AU^R cells.

Isozyme analysis revealed that seven hybrids expressed the summation of the parental bands of LAP and 6-PGDH while only one plant (line 7) was unique (Fig. 5 a). This plant was missing a predominant eggplant band of 6-PGDH and expressed a novel LAP band. Line 7 was aneuploid ($2\underline{n} = 45$) and it may be that loss of an eggplant chromosome accounted for the loss of the 6-PGDH band, while a subunit association may have caused the formation of a novel (hybrid) LAP band. Analyses of *Nicotiana* somatic hybrids have not identified any cases where the banding pattern was simply the summation of the parental species (Evans et al. 1980; Douglas et al. 1981 b). In contrast to the *Nicotiana* studies almost all enzymes assayed in this study revealed distinctly different isozyme bands between the parental species. This may be due to the greater taxonomical separation between *S. melongena* and *S. sisymbriifolium*.

Isoelectric focussing of eight lines revealed that all expressed the small Rubisco subunits of both parents thereby providing further evidence for hybridization (Fig. 5 b). Similar analyses have confirmed hybridization between *Nicotiana* (Gleddie et al. 1983 b), and *Solanum* species (Barsby et al. 1984). The large Rubisco subunit of *S. sisymbriifolium* was expressed in all hybrids analyzed (Fig. 5 b). Uniparental expression of the chloroplast genome has been previously reported for some *Nicotiana* somatic hybrids (Evans et al. 1980, 1982; Gleddie et al. 1983 b). However, in many cases the random segregation of parental chloroplasts has been reported (Iwai et al. 1980; Melchers et al. 1978; Scowcroft and Larkin 1981). One reason for the non-random segregation of chloroplasts in this study is that plastid type was closely linked to the selection system. Possibly the ability to produce anthocyanins, which was an essential step in hybrid selection, is specifically associated with *S. sisymbriifolium* plastids.

This study has shown that it is possible to produce somatic hybrids between *S. melongena* and *S. sisymbriifolium* even though it has not been possible to sexually hybridize these species (Seck 1983). Several somatic hybrids have been evaluated for the inheritance of desirable agronomic traits and preliminary tests have revealed that they are completely resistant to root knot nematodes and potentially resistant to spider mites (Gleddie et al. 1985 a). These hybrids could be of value in plant breeding programs providing that the problem of sterility could be overcome. Possible approaches to solving this problem include: 1) 'back fusions' of somatic hybrids with eggplant mesophyll protoplasts and 2) initiation of hybrid cell suspension cultures with the objective of eliminating more *S. sisymbriifolium* chromosomes thereby restoring fertility or partial fertility in the regenerants.

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