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Expression of nuclear-cytoplasmic genomic incompatibility in interspecific *Petunia* somatic hybrid plants*

L. S. Schnabelrauch [1], F. Kloc-Bauchan [2] and K. C. Sink [3]

[1] Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, USA
[2] U.S.D.A., Field Crops, Cell Culture and Nitrogen Fixation Laboratories, Beltsville, MD 20705, USA
[3] Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA

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Summary. Somatic hybrid plants were regenerated following calcium-high pH fusion of the unidirectional, sexually incompatible cross of *Petunia parodii* wild-type leaf mesophyll protoplasts with protoplasts from a cytoplasmic determined chlorophyll-deficient mutant of *P. inflata*. Genic complementation to chlorophyll synthesis and sustained growth in the selective medium was used to visually identify hybrid calluses. Hybrid calluses were subsequently regenerated to shoots, rooted, and confirmed as somatic hybrids by their intermediate floral and leaf morphology based on comparison to the $2n=4x=28$ sexual counterpart, dominant anthocyanin expression in the corolla, chromosome number, and peroxidase and maleic dehydrogenase isozyme patterns. Certain cytologically stable somatic hybrids displayed aberrant reproductive and floral morphologies including subtle to moderate corolla and leaf pigment variegation, floral dimension changes and reduced pollen viability. In contrast, cytologically unstable somatic hybrids showed various degrees of aneuploidy coupled with corolla splitting, and irregularities in reproductive organs such as double stigmas and styles in addition to reduced pollen viability. Postulated mechanisms to account for these phenotypic changes in stable and unstable somatic hybrids include nuclear-cytoplasmic genomic incompatibility, chromosome loss in a biparental cytoplasm, or a phenomenon similar to hybrid dysgenesis occurring as a result of somatic fusion.

Key words: Protoplast fusion – Somaclonal variation – *Petunia parodii* – *P. inflata*

Introduction

Somatic hybridization offers the possibility of bypassing interspecific or intergeneric sexual incongruity to allow more extensive use of wide hybridizations in crop improvement.

Present breeding constraints such as seasonal flowering differences, maturation periods and ecological restrictions could theoretically become secondary as protoplast fusion technologies are perfected and exploited (Thomas et al. 1979; Shepherd et al. 1980; Larkin and Scowcroft 1981). However, it has become increasingly evident from published research that such vast application to breeding so far is, at most, conjectural. In reality, genetic problems associated with cell culture derived plants are not trivial and direct benefits to plant improvement have yet to be realized in any major crop species that could have been more quickly and economically obtained using conventional mutagenesis and breeding techniques.

In attempting to extend the range of hybridization possible by using protoplast fusion, the breeder is faced with difficulties in recovering the highest efficiency of quantitative traits important in determining yield and quality components. In many species, regenerated plants have subtle changes in flowering time, plant height, heading dates, and chlorophyll content suggesting major changes in photosynthetic efficiency which, when coupled with reproductive irregularies such as lower pollen viability and seed set, lead to reductions in the major quantitative components of crop productivity. Years of ideotype breeding of a crop species apparently can be successfully undone by one regeneration cycle in in vitro culture. On the other hand, contributions of tissue culture to the area of disease resistance (Krishnamurthi 1974; Matern et al. 1978; Shepherd et al. 1980) is undeniable. However, in order for tissue culture to be a viable breeding tool in the next decade, identification and quantification of factors leading to phenotypic changes in culture derived plants must be made and channeled toward desired breeding goals.

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Toward this end, we have quantified phenotypic changes, particularly floral and reproductive organ differences, in hybrid plants regenerated after calcium-high pH fusion treatment. The regenerated plants have been proven to be hybrid based on isozyme patterns, chromosome numbers, morphological characteristics, subsequent breeding behavior (Schnabelrauch and Sink 1984), and organellar DNA restriction fragment analysis (Clark et al. 1984).

The phenotypic spectrum of reproductive and morphological changes in plants derived from the somatic combination of *P. parodii* leaf mesophyll protoplasts and *P. inflata* chlorophyll deficient cell line have been quantified and suggest, in a broad sense, that nuclear-cytoplasmic genome incompatibility may be responsible for the phenotypic irregularities observed.

Materials and methods

Seeds of *P. parodii* were germinated biweekly on V.S.P. planting medium (Bay Houston Towing Co.) and maintained at 25 ± 2 °C under a photon fluence flux of 80 μmol $Em^{-2}s^{-1}$ (400–700 nm) (GE96T12CW) on a 16 h photoperiod. After transplanting to 6-cell plastic packs, vegetative growth continued under greenhouse conditions of 25 ± 2 °C minimum night temperature and fluctuating day temperatures supplemented with 680 μmol $Em^{-2}s^{-1}$ (400–700 nm) (GE96T12CW) 10 h daily. Plants were fertilized at each watering with 150 ppm 20N-8.6P-16.6K aqueous solution adjusted to pH 6.5 with phosphoric acid. Leaf harvesting, sterilization using a 10% solution of commercial 5.25% NaClO, and abaxial epidermis removal were followed as described (Hayward and Power 1975).

Protoplast isolation

Extensive trials indicated that optimum yield and viability of leaf protoplasts of *P. parodii* were obtained using 1.5% Meicelase (Meiji Seika Kaisha, Ltd), 0.1% Macerase (Calbiochem) and 13% mannitol in CPW salts (Frearson et al. 1973) at pH 5.8. The protoplast isolation procedure for *P. inflata* (Power et al. 1979) was modified to produce more efficient and reliable yields in our laboratory (Schnabelrauch et al. 1981). The isolation enzyme was 2% Cellulysin (Calbiochem), 1% Macerase, 1% Driselase (Kyowa Hakko, Kogyo Co. Ltd) and 8% Mannitol in CPW salts at pH 5.8. Protoplasts were collected, counted, and diluted in culture medium consisting of Murashige and Skoog (1962) inorganic salts and vitamins, 2.0 mg/l α-naphthaleneacetic acid, 0.5 mg/l 6-benzylamino purine and 9% mannitol at pH 5.8.

Protoplast fusion

Calcium-high pH fusion (Keller and Melchers 1973) of *P. parodii* with *P. inflata* protoplasts was conducted with both species at a starting density of 2.0×10^5 pplts/ml. After fusion, the protoplasts were plated in liquid medium at a final density of 5×10^4 pplts/ml in 5 cm plastic petri dishes and cultured at 25 ± 2 °C under a continuous photon fluence flux of 18–22 μmol $Em^{-2}s^{-1}$ (400–700 nm) (GE96T12CW). Aliquots of culture medium containing 6% and 3% mannitol were added after 44 and 65 days, respectively. Calluses were transferred to solidified (0.8% agar) MS medium with 2 mg/l trans-zeatin to regenerate shoots. Such shoots were removed and rooted in 25 days on MS-0, established in soil and transferred to a greenhouse for subsequent growth, flowering and evaluation.

Chromosome determination

Chromosome counts of somatic hybrid plants were made using pollen mother cells. Immature flower buds, approximately 0.2 cm in length, were fixed in Carnoy's solution, followed by the removal of the anthers and subsequent staining with aceto-propiocarmine.

Pollen viability

Pollen viability was determined by counting the dark, fully round grains mounted in an aqueous solution of iodine-potassium-iodide. Flowers were collected one day before anthesis and placed in vials of water until anther dehisence. Counts of

Table 1. Phenotypic variability in regenerated plants from the fusion of *P. parodii* + *P. inflata*

Leaf morphology	Percent hybrid plants	Parental phenotype	
		P. inflata	*P. parodii*
Shape			
Ovate	100	+	+
Apex			
Rounded	3.8	–	–
Obtuse	84.6	+	+
Acute	11.6	–	–
Base			
Rounded	26.9	–	–
Acute	38.5	+	–
Attenuate	34.6	–	+
Margins			
Entire	73.1	–	+
Undulate	15.4	+	–
Revolate	11.5	–	–
Pigmentation			
Variegated	26.9	–	–
Green	73.1	+	+
Trichomes			
Hirsululous	42.3	–	–
Hirtellous	53.9	+	+
Subglabrous	3.8	–	–
Corolla morphology			
Limb			
Symmetrical	64.3	–	+
Assymmetrical	35.7	+	–
Lobe			
Pointed	57.1	–	+
Round	42.8	+	–
Throat area Pigmentation			
Dark	64.3	+	–
Light	21.4	–	+
Halo	14.3	–	–
Limb variegation			
Acyanic flecks	28.6	–	–
Acyanic sectors	28.6	–	–
Pale/dark magenta cyanic sectros	50	–	–
Uniform color	14.3	+	+

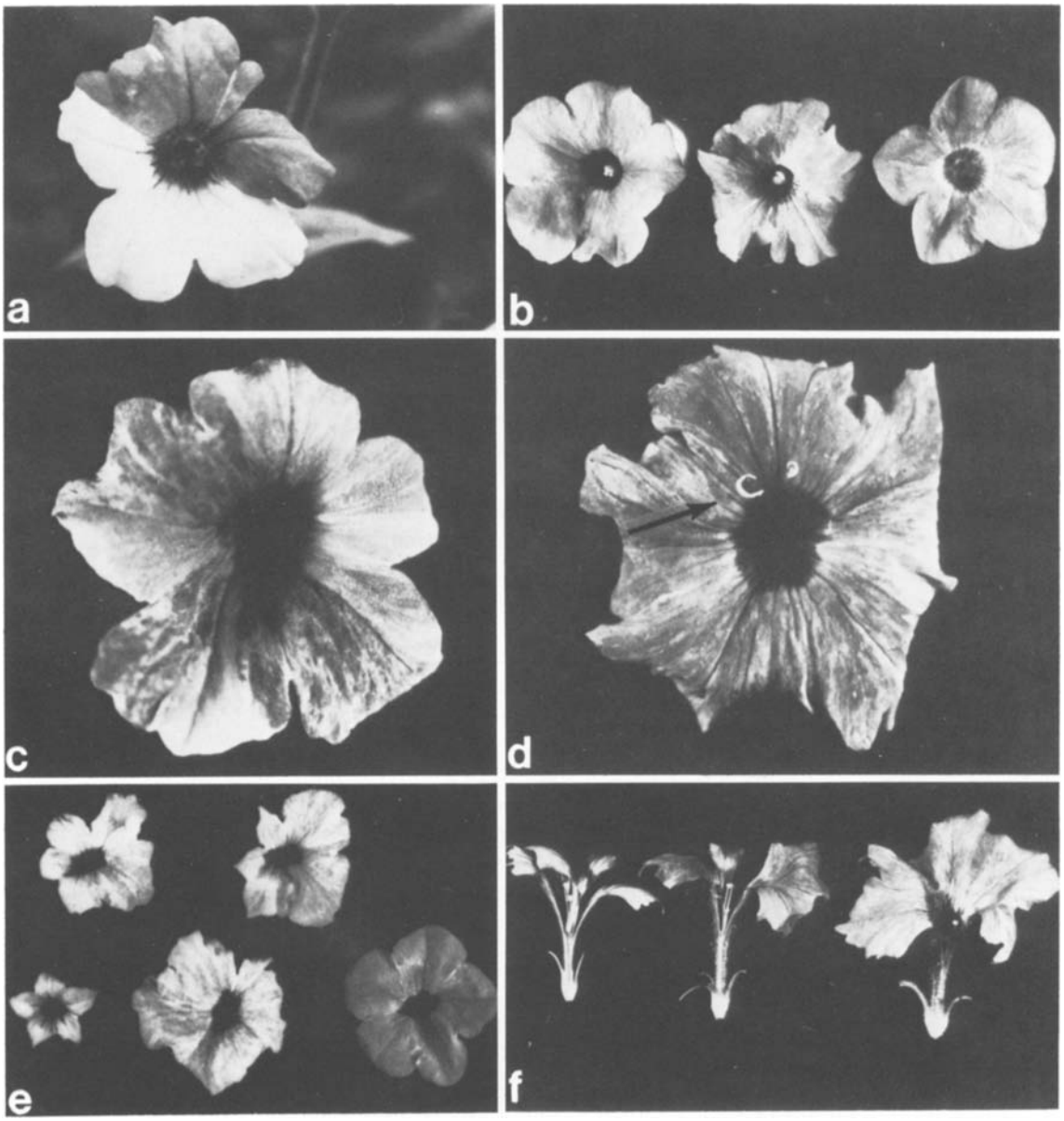

Fig. 1. **a** Sectored corolla of somatic hybrid plant 11-1; **b** Anthocyanin variegation patterns (*left to right*): stable, acyanic sectored, minute acyanic flecks; **c** Anthocyanin variegation patterns consisting of pale and dark mottled areas; **d** Acyanic petaloid structure (*arrow*); **e** Tobacco-like flower arrays from somatic hybrid plant 15-2; **f** Split blossom classes (*left to right*): narrow split blossom, wide split blossom, deeply lobed blossom

5–10 random microscopic fields (100×) were made on a minimum 500 pollen grains.

Morphological traits

Total floral length, corolla tube length, limb diameter, style length and anther filament attachment length were measured using the procedures applied to the sexual hybrid (Sink et al. 1978). In addition, variant phenotypes were noted including morphological changes in reproductive organs and in the distribution of anthocyanin in corolla limbs. Quantitative measures of floral organs were made for the variant morphological phenotypes. Leaf characters were evaluated as to shape, chlorophyll variegation patterns, presence of trichomes, ruffled or undulating margins, and developmental anomalies.

Isozymes

Based on differences between the parental species, peroxidase and maleic dehydrogenase isozyme patterns were selected as biochemical markers to confirm putative somatic hybrid plants. Plant material preparation, starch gel electrophoresis and the staining procedures of Scandalios (1970) were followed.

Results

Leaf characters

Regenerated plants displayed a range of leaf apex and base shapes, pigmentation and epidermal trichomes

(Table 1). The predominant leaf shape was ovate, but leaf apex varied being round, obtuse or acute. Leaf bases ranged from round, acute to attenuate. Regenerated plants also showed revolate leaf margins, a trait frequently seen in plants regenerated from in vitro culture (Barbier and Duleiu 1980) but not normally expressed by either parent. One obvious character change in some hybrids was a variegated leaf pattern consisting of a light green mid-rib area outlined by a band of ivory colored tissue, juxtaposed to a dark green leaf margin. Regenerates also displayed varying degrees of surface hairness (Table 1).

Corolla morphology

The corolla of *P. inflata* is symmetrical, magenta, with round lobes and a short floral tube. In contrast, the corolla of *Petunia parodii* is slightly asymmetric, white, has pointed lobes, and the tube is long. *Petunia parodii* also has a faint purple veination in the outer throat area (Sink et al. 1978).

Corollas of all somatic hybrids displayed the dominant magenta coloration and varied from slight to pronounced asymmetry. The hybrids also segregated for round or pointed corolla lobes (Table 1). In addition, the majority or regenerants produced phenotypes with anthocyanin variegation in the corolla limbs (Figs. 1a–c) and are discussed in detail elsewhere (Schnabelrauch and Sink 1984).

Floristic data taken on magenta somatic hybrids, white-flowered presumably *P. parodii* parental types, the tetraploid sexual hybrid (MSU 77-17) and tetraploid *P. parodii* (MSU 77-22-4) indicated that white flowered regenerants (43-1, 43-7 and 48-2) differed from the magenta-flowered sexual hybrid 77-17 $2n=4x$ in three categories: the ratio of total floral length/tube length, style length/tube length, and filament attachment as a percentage of floral tube length (Table 2). These plants had floral dimensions that were not significantly different from tetraploid *P. parodii*, except for slightly smaller limb diameter in the regenerates. However, it is clear from mitochondrial DNA restriction fragment analysis that these plants have some cytoplasmic contributions from both parents (Clark et al. 1984). Stable $2n=4x=28$ somatic hybrids (5-4, 15-1, 15-4, 15-5, 15-6 and 21-5) were not significantly different from 77-17 in the ratio of style length/tube length. Generally, total corolla length/tube length and filament attachment tended to be greater in stable somatic hybrids compared to the tetraploid sexual hybrid.

Unstable (less than 28 chromosomes) magenta-flowered types differed from 77-17 primarily in total corolla length/tube length and filament attachment. Total length/tube length was generally greater for these hybrids than tetraploid *P. parodii* or $2n=4x$ sexual hybrids, indicating the short floral tube contribution from *P. inflata*. Filament attachment percentage varied from values approaching 57.6% for the $2n=4x=28$ *P. parodii* to less than 29%.

Chromosome numbers of regenerated plants varied between 20 and 36 (Table 3). Stable $2n=4x=28$ hybrids remained so even after prolonged greenhouse culture.

Table 2. Variation in corolla characteristics in somatic hybrid plants

Plant no.	Total length (cm) / Tube length	Limb diameter (cm)	Style length (mm) / Tube length	% Filament attachment
5-4	1.85*	5.2*	0.732	33.2
11-1	1.44*	4.5	0.766	39.2
14-3	1.59	4.9	0.883	51.7*
15-1	1.69*	4.1*	0.774	39.0
15-2	1.73*	4.5	0.780	43.8*
15-3	1.74*	4.4	0.744	43.5*
15-4	1.62	5.0	0.741	37.7
15-5	1.73*	4.2*	0.769	41.5*
15-6	1.70*	5.0	0.775	28.5*
15-7	1.65	4.0*	0.752	38.5
21-1	1.71*	4.0*	0.805*	34.9
21-5	1.82*	4.8	0.754	38.3
28-1	1.66	4.5	0.592*	35.0
38-5	1.51	3.6*	0.731	45.7*
43-1	1.34*	5.2*	0.912*	59.2*
43-7	1.37*	4.9	0.868*	55.3*
48-2	1.33*	3.9*	0.825*	53.1*
77-22-4	1.31*	5.1	0.892*	57.6*
77-17	1.58	4.7	0.734	35.6

* Means are significantly different ($P=0.05$) compared to the $2n=4n=28$ sexual hybrid (77-17) using Dunnett's procedure

Table 3. Chromosome number and morphological characteristics of somatic hybrid plants

Plant no.	Chromosome no.	Phenotype		
		Pollen color	Compatibility[a]	Pollen viability (%)
77-1	14	Blue	SI	92.0
77-a	14	Yellow	SC	99.0
77-17	28	Purple	SC	90.6
5-4	28	Yellow	SI	27.8
14-3	36	Ivory	SC	71.7
15-1	28	Blue	SC	64.6
15-2	26–28	Blue	SC	44.4
15-3	27	Ivory	SI	sterile
15-4	28	Ivory	SC	65.3
15-5	28	Ivory	SC	66.3
15-6	28	Blue	SC	83.6
15-7	26–28	Ivory	SC	45.2
20-1	–	Blue	SI	–
21-1	26	Ivory	SC	42.4
21-5	28	Ivory	SC	44.4
28-1	20	Blue	SI	31.8
38-5	26	Ivory	SI	45.4

[a] SI = Self incompatible; SC = Self compatible

Pollen color, viability, and self-compatibility alleles also differed in these plants, giving the same range of variation shown by aneuploid types.

Biochemical analyses

Peroxidase (Fig. 2) and maleic dehydrogenase (Fig. 3) isozyme profiles were analyzed from leaves of in vitro shoot cultures of somatic hybrids and parental *P. inflata* and *P. parodii* plants. For both enzymes, hybrid extracts shared specific bands of both *P. parodii* and *P. inflata.*

The hybrid nature of calluses satisfying the selection criteria was also substantiated by peroxidase and maleic dehydrogenase isozyme profiles of regenerated plants. When stained for peroxidase, *P. parodii* ($2n = 2x = 14$, wild type) leaf material from shoot culture resolved 4 bands. *Petunia inflata* cytoplasmic albino resolved 3 of the slower migrating bands of *P. parodii,* but, in addition, had 2 faster migrating bands that were not observed for *P. parodii.* Somatic hybrids possessed the 4 *P. parodii* peroxidase bands and frequently had the 2 fast migrating bands of *P. inflata.* For the mitochondrial marker isozyme maleic dehydrogenase, the banding pattern between parental lines was very distinctive. Hybrids shared the slow and fast migrating band differences seen in the parental lines and, in addition, had faster bands indicating perhaps smaller fragments in hybrids which remained as larger fragments in the parental lines.

Phenotypic developmental abnormalities

Pigment intensity changes. In addition to flecking in the corollas, several somatic hybrids also showed pigment intensity changes. Some plants initially flowered with traces of very pale pink, initially classified as white flowered type, but progressively darkened to the standard magenta color of the sexual hybrid after prolonged greenhouse growth (5-4). Such a situation mimics the rabbit ear rogue described by Burr and Burr (1981). The reverse situation was also found; dark

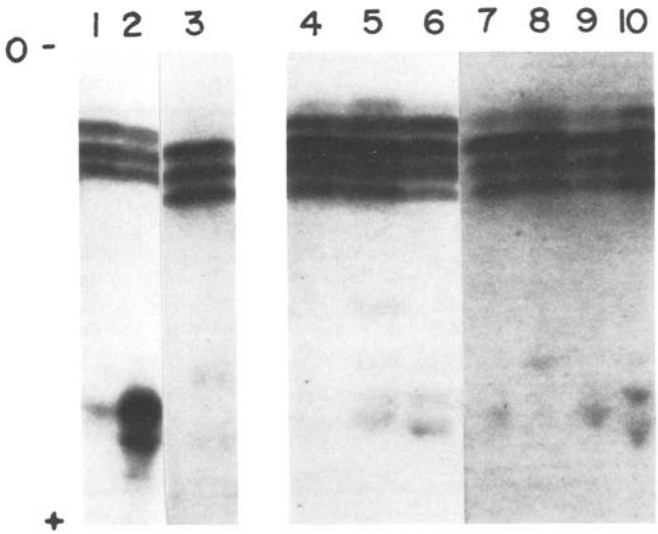

Fig. 2. Peroxidase isozyme patterns of leaf extracts from parental and somatic hybrid lines following starch gel electrophoresis. *Lanes 1–3: P. inflata* (wild type), *P. inflata* (cytoplasmic albino), *P. parodii* (wild type). *Lanes 4–10:* somatic hybrids 11-1, 14-3, 15-1, 43-1, 48-2 and 48-18

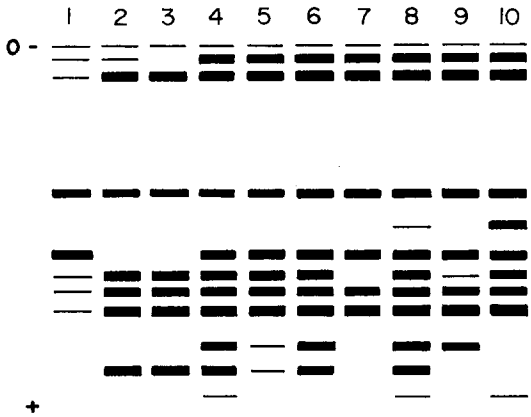

Fig. 3. Maleic dehydrogenase isozyme patterns from leaf extracts of parental and somatic hybrid lines following starch gel electrophoresis. *Lanes 1–3:* parents *P. parodii* (wild type), *P. inflata* (cytoplasmic albino), and *P. inflata* (wild type). *Lanes 4–10:* somatic hybrid plants 21-5, 15-2, 15-3, 48-2, 15-6, 48-18 and 48-8

Table 4. Reproductive characteristics of chloripetalous flowers of somatic hybrid 15-2

Flower phenotype	Reproductive conditions (%)						
	Pistils[a]			Anthers[b]		Pollen viability	Chromosomes no.
	P1	P2	P3	S1	S2		
Narrow split blossom	100	–	–	100	–	57.3	24
Wide split blossom	90	–	10	100	–	70.1	24
Deeply lobed blossom	–	10	90	–	100	84.2	28
Restored ruffled blossom	15	20	65	–	100	15.7	26–28
Normal blossom	–	–	100	10	90	56.7	26–28
Sexual hybrid 77-17 (4x)	–	–	100	–	100	90.6	28

[a] P1 = (2) separate ovaries and styles, styles shorter in length than "normal", with stigmatic somewhat cupped and elliptical in shape
P2 = (1) large ovary with a fasciated double style culminating in a bipartate stigmatic surface
P3 = (1) ovary and one style, usual dimensions
[b] S1 = terminal anther sac degenerate, some proliferation of subterminal tissue
S2 = terminal anther sac normally developed

Table 5. Quantitative measurements of floral traits in modified corolla phenotypes sectoring from somatic hybrid 15-2

Flower phenotype	Mean organ dimensions (cm) (± SE)							
	Chromosome no.	Pollen viability (%)	Total corolla length	Tubular base length	Limb diameter	Total style length	Filament attach length	Filament attach (%)
Narrowly split blossom	24	57.3	4.44 (0.38)	2.74 (0.25)	3.75 (0.65)	1.46 (0.18)	1.36* (0.05)	49.6
Widely split blossom	24	70.1	5.09 (0.23)	2.93 (0.18)	4.40 (0.49)	1.71 (0.29)	1.35* (0.11)	46.1
Deeply lobed blossom	28	84.2	5.24 (0.24)	3.05 (0.13)	4.76 (0.55)	2.12 (0.27)	1.25 (0.09)	41.1
"Normal" blossom	26–28	56.7	5.47 (0.26)	3.16 (0.10)	4.98 (0.38)	2.52 (0.10)	1.32 (0.06)	40.2
77-17 4x sexual hybrid	28	90.6	6.15 (0.33)	3.92 (0.28)	4.72 (0.45)	2.87 (0.12)	1.40 (0.19)	35.7
HSD (0.01)			0.59	0.32	0.79	0.44	0.16	

* Means are not significantly different ($P = 0.01$) when compared by Tukey's method

magenta flowering plants sectored out branches of light pink flowers (15-3, 11-1, 15-2).

Split blossom phenotypes. A further floral modification observed was a split blossom type in some of the hybrids (15-2, 38-2, and 11-1). The degree of splitting was found to follow a linear progression from finely divided to 'restored' or typical floral phenotype in one hybrid plant. Plant 15-2 upon further growth in the greenhouse sectored out branches which displayed flowers with chloripetalous characteristics, morphologically similar to the split blossom described in *Nicotiana* (Clayton 1950).

Flowers with narrowly split blossoms (Fig. 1 f) were significantly ($P=0.05$) correlated with degenerate anthers and double ovaries and styles (Table 4). Ninety percent of widely split blossoms were associated with degenerate anthers, double ovaries and styles. A single instance of a flower with three ovaries and three styles was encountered in this split flower class. Flowers that had ruffled margins with no corolla splitting (i.e. "restored") had viable anthers, but the ovary and style varied between two and the normal condition of one ovary and one style. Flowers of the normal unruffled phenotype on hybrid 15-2 still had 10% anther degeneration.

Quantitative measures of five floral organ dimensions in flower samples from the various chloripetalous corolla types showed significant ($P=0.01$) mean differences, supporting the hypothesis of a successive developmental sequence of the split blossom phenotypes to the normal phenotype (Table 5). Of the floral organs analyzed, total corolla length and total style length showed significant ($P=0.01$) linearity when regressed on the corolla classes ($r=96.12$ and 99.11, respectively). Only the filament attachment length showed low correlation to corolla type.

Reproductive structure changes. Along with corolla variegation and splitting, other morphological abnormalities were apparent upon examination of somatic hybrids. Various abnormalities associated with reproductive structures such as stigmoid anthers were found in 14-3 and in progeny of the sexual sib mating of 15-2×21-5. A single instance of anther sac formation on a stigmatic surface was noted in 15-3. Flowers of 38-5 had anther sac formation at the corolla lobe junctions on the limb surface, with adnate filaments extending up the corolla tube. As mentioned previously, bipartate stigmas (15-2), fasciated styles (15-2 and 38-5) and double stigmas, styles and ovaries (15-2) were observed, and often associated with corolla splitting. The occurrence of six anthers and six corolla lobes together with anther fasciation were found in one flower on plant 15-6. Anthers were also seen with feather-like tissue projections midway up the filament (20-1, 15-3).

Other types of flower modifications were frequently observed in somatic hybrids. Acyanic petaloid structures were found along the midveins of plants 38-5, and 28-1 (Fig. 1 d). Suture-like vein incisions were common to corolla lobes of 38-5 and 28-1. Ruffled margins, and asymmetric corollas were frequently associated with corolla variegation and various combinations of ruffled margins, split corollas, acyanic sectors and petaloid structures were found in some plants (38-5, 20-1). One common corolla limb anomaly was the occurrence of acyanic ear-shaped projections at the junction of corolla lobes (15-2, 21-1, 21-5, 15-3, 15-5, 5-4, 15-6, and TC progeny). Such projections were frequently found at all 5 lobe junctions, but cases of projections at only 1 or 2 lobes were also seen.

Tobacco-like flower form. Hybrid 15-2 sectored branches that produced flowers which phenotypically resembled *Nicotiana* flowers in size and shape (Fig. 1 e). These flowers were produced on glabrous green shoots. Flowers with varying degrees of anthocyanin variegation and *Nicotiana*-like morphology were frequently observed during the course of branch development resulting in flowers which were half sectored and half changed in shape. Albino branches were also observed which produced flowers with extremely cupped corollas and dilute anthocyanin pigmentation extending from the lobe junctions into the center of the corolla.

Discussion

Genetic and epigenetic changes frequently appear in tissue culture-derived plants and it is possible to produce an array of mutants and variants in many species (Meins 1983; Shepherd 1982; Evans et al. 1983; Larkin and Scowcroft 1981). One potential contribution of these plant types to breeding programs and cell genetics lies in developing an understanding of the control mechanisms responsible for these altered phenotypes. Interesting changes in floral characteristics of *Petunia* somatic hybrids have allowed us to quantitatively compare a variety of phenotypes that may have resulted from somaclonal variation, nuclear-cytoplasmic incompatibility or a hybrid dysgenic-type response induced by somatic fusion of a reciprocally incompatible sexual cross.

Wide crosses have been shown to cause morphological abnormalities attributable to chromosomal-cytoplasmic incompatibilities. Disruptions in nucleo-cytoplasmic interaction resulting in split corollas were observed by Clayton (1950) in the first backcross generation of the amphiploid *N. debneyi-tabacum* to *N. tabacum* as the male parent. Such disruptions took the form of male sterility and corolla splitting with

associated anther abnormalities. Further backcrosses substantiated that *N. debneyi* cytoplasm interacting with the *N. tabacum* genome resulted in morphological aberrants (Sand and Christoff 1973), as if the regulatory mechanism for developmental and structural features of the corolla had been upset by some type of incompatible reaction between cytoplasmic factors and nuclear chromosomal elements leading to altered differentiation.

One frequently observed incompatibility reaction in interspecific plastome/genome hybrid plants is chloroplast variegation. In the genus *Oenothera* which has biparental transmission of plastids, phenotypically distinct species can be crossed easily and the resulting interspecific genome/plastome hybrids show various forms of developmental disturbances but maintain stable diploid karyotypes with a chromosome number of 14 and are usually fertile (Stubbe and Herrmann 1982). The incompatibility between the genetic components of the nucleus and cytoplasm is thought to cause bleaching of one of the two wild type plastids from the parents resulting in a variegated leaf. A similar incompatibility phenomenon has been described in *Pelargonium* (Kirk and Tilney-Bassett 1978).

Incompatibility in progeny tests of *Nicotiana* was manifested as a split corolla phenotype; corolla splitting was interpreted as an effect of quantitative genes for broader floral tubes present in the chromosomes of the recurrent parent (Sand 1968; Sand and Christoff 1973).

In our study, corolla modifications were observed without the type of male sterility described in *Nicotiana* (i.e. stigmoid anthers), but conversely, the number of ovaries and styles varied from the normal one ovary-one style to bipartate stigmas with fasciated styles to two ovaries-two styles.

Flowers of plant 15-2 separated into an ordered series of corolla splitting. This phenotypic array suggests that there exists a series of related effects, increasing quantitatively toward normal. There was also a correlation between the degree of corolla splitting and the particular pistil and stamen modification observed in the flower. The reduction in chromosome number occurring in the split corollas of 15-2 supports the contention that nucleo-cytoplasmic incompatibilities can be produced as one of the parental genomes is preferentially lost upon successive mitotic divisions, followed by the adverse reaction of the remaining chromosomes in a hybrid cytoplasm. Plant 15-2 had only the cp-DNA of the wild-type *P. parodii* parent (Clark et al. 1984), but retained restriction fragments unique to both *P. parodii* and *P. inflata* mt-DNA in varying amounts.

The progression from split corolla through ruffled sectored corollas in 15-2 along with the occurrence of distinct non-pubescent branches of tobacco-like flowers was correlated with chromosome reduction. Flowers resulting in a phenotype consisting of half-sectored and ruffled half tobacco-like flowers indicated that chromosome elimination occurred early in floral primordia development, specifically at the first few cell divisions in the floral meristem. These phenotypes too may have been conditioned by directional chromosome loss in the presence of only *P. parodii* chloroplasts.

The interaction of genic, chromosomal and cytoplasmic factors in the induction of unstable mutations in interspecific crosses parallels the germ line phenomenon of hybrid dysgenesis in *Drosophila*. Hybrid dysgenesis and transposition of p elements results from chromosomal-cytoplasmic interactions in certain interstrain crosses, and produces several aberrant traits including temperature sensitive sterility, segregation distortion, sex ratio distortion, and high frequencies of mutation, male recombination, chromosome rearrangement and non-disjunction (Engels 1981; Engels and Preston 1980; Bingham 1981; Kidwell et al. 1977). These traits are not observed in homozygous strains, but are limited to specific hybrid combinations and are conditioned by the direction of the cross. Activation of transposable elements has been determined to be the cause of the mutated phenotypes.

An analogous dysgenic mechanism functioning in the incompatible reactions in plants has been suggested (Burr and Burr 1981). The sexual cross of *P. parodii* × *P. inflata* is successful only if *P. parodii* is used as the female parent for bud-pollination. The incompatible reaction in this cross is prezygotic (Sink et al. 1978). Therefore, it is possible that dysgenic mechanisms become activated in fusion products, especially since heterokaryons and subsequent cell hybrids require co-functioning of both parental cytoplasms and nuclei. Disruption of nuclear organization upon fusion of a cell in G1 phase with cells actively synthesizing DNA, each of which is assumed to have specific organizational relationships with the nuclear membrane and to each other, may result in the induction of mutability or chromosomal rearrangements (Sved 1976). Such rearrangements could cause structural genes to be inserted near heterochromatin, resulting in partial suppression of gene activity. McClintock (1950) proposed that disturbances in the quantity and organization of heterochromatic elements could give rise to a series of changes in chromosome structure, behavior, and genic reactions that could drastically alter phenotypic expression. The frequency of transposition of controlling elements in maize has been shown to increase in tissue culture (Evola et al. 1984).

In contrast to *Petunia* somatic hybrids produced previously (Power et al. 1979) which were typically intermediate for vegetative and floral traits, and segregated as did their $2n=4x=28$ sexual counterpart, some of the hybrids in this study exhibited corolla flecking, streaking, color intensity changes and irregular segregation ratios (Schnabelrauch and Sink 1984). Thus, nuclear-cytoplasmic genomic incompatibility expressed as split corollas with the associated male sterility found in this study may limit the types of somatic fusion combinations that can be realistically attempted.

References

Barbier M, Dulieu H (1980) Effects genetiques observes sur des plantes de Tabac regenerees parter de cotyledons par culture in vitro. Ann Amelior Plant 30:321–344

Bingham P (1981) A novel dominant mutant allele at the white locus of *Drosophila melanogaster* is mutable. Cold Spring Harbour Symp Quant Biol 45:519–525

Burr B, Burr F (1981) Transposable elements and genetic instabilities in crop plants. Stadler Symp 13:12–18

Clark E, Schnabelrauch L, Hanson MR, Chinnici J, Sink KC (1984) Differential fate of plastid and mitochondrial genomes in *Petunia* somatic hybrids. Mol Gen Genet (in press)

Clayton E (1950) Sterile tobacco. J Hered 41:171–175

Engels W (1981) Germline mutability in *Drosophila* and its relation to hybrid dysgenesis and cytotype. Genetics 98: 565–587

Engels W, Preston C (1980) Components of hybrid dysgenesis in a wild population of *Drosophila melanogaster.* Genetics 95:111–128

Evans D, Bravo JE, Kut SA, Flick CE (1983) Genetic behavior of somatic hybrids in the genus *Nicotiana: N. otophora* + *N. tabacum* and *N. sylvestris* + *N. tabacum.* Theor Appl Genet 65:93–101

Evola SV, Burr FA, Burr B (1984) The nature of tissue culture induced mutations in maize. Plant Mol Biol Conf, Jerusalem, Israel

Frearson EM, Power JB, Cocking EC (1973) The isolation, culture and regeneration of *Petunia* leaf protoplasts. Dev Biol 33:130–137

Hayward C, Power JB (1975) Plant production from leaf protoplasts of *Petunia parodii.* Plant Sci Lett 4:407–410

Keller WA, Melchers G (1973) The effect of high pH and calcium on tobacco leaf protoplast fusion. Z Naturforsch 28:737–741

Kidwell M, Kidwell J, Sved J (1977) Hybrid dysgenesis in *Drosophila melanogaster:* a syndrome of aberrant traits including mutation sterility and male recombination. Genetics 86:813–833

Kirk JTO, Tilney-Bassett RAE (1978) The plastids. Elsevier/ North-Holland Biomedical Press, New York

Krishnamurthi M (1974) Notes on disease resistance of tissue culture sub-clones and fusion of sugar cane protoplasts. Sugarcane Breeder's Newslett 35:24–26

Larkin PJ, Scowcroft WR (1981) Somaclonal variation: a novel source of genetic variability from cell cultures for plant improvement. Theor Appl Genet 60:197–214

Matern U, Strobel G, Shepard J (1978) Reaction to phytotoxins in a potato population derived from mesophyll protoplasts. Proc Natl Acad Sci USA 75:4935–4939

McClintock B (1950) The origin and behavior of mutable loci in maize. Proc Natl Acad Sci USA 36:344–355

Meins F (1983) Heritable variation in plant cell culture. Ann Rev Plant Physiol 34:327–346

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Power JB, Berry SF, Chapman JV, Sink KC, Cocking EC (1979) Somatic hybrids between unilateral cross-incompatible *Petunia* species. Theor Appl Genet 54:97–99

Sand S (1968) Genetic modification of cytoplasmic male sterility in tobacco. J Hered 59:175–177

Sand S, Christoff G (1973) Cytoplasmic-chromosomal interactions and altered differentiation in tobacco. J Hered 64: 24–30

Scandalios JG (1970) Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem Genet 4: 297–320

Schnabelrauch LS, Kloc-Bauchan F, Sink KC (1981) The use of Pectinol-AC for protoplast isolation from suspension cultures of a *Petunia inflata* albino mutant. Plant Mol Biol Rep 2:75–76

Schnabelrauch LS, Sink KC (1984) Genetics of stable and unstable traits in petunia somatic hybrids. Genetics (in preparation)

Shepard JF, Bidney D, Shahin E (1980) Potato protoplasts in crop improvement. Science 208:17–24

Shepard JF (1982) The regeneration of potato plants from leaf-cell protoplasts. Sci Am 246:112–121

Sink KC, Power JB, Natarella NJ (1978) The interspecific hybrid *Petunia parodii* × *Petunia inflata* and its relevance to somatic hybridization in the genus *Petunia.* Theor Appl Genet 53:205–208

Stubbe W, Herrmann RG (1982) Selection and maintenance of plastome mutants and interspecific genome/plastome hybrids from *Oenothera.* In: Edelman M, Hallick RB, Chua N-H (eds) Methods in chloroplast molecular biology. Elsevier Biomedical Press, New York, pp 149–165

Sved J (1976) Hybrid dysgenesis in *Drosophila melanogaster:* a possible explanation in terms of spatial organization of chromosomes. Aust J Biol Sci 29:375–388

Thomas E, King PJ, Potrykus I (1979) Improvement of crop plants via single cells in vitro – an assessment. Z Pflanzenzücht 82:1–30