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Intraspecific gametosomatic hybridisation in *Petunia hybrida*

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ABSTRACT

Following PEG and high pH induced fusion of haploid tetrad protoplasts of a normal purple flowered variety of P. hybrida with cell suspension protoplasts of a nuclear albino mutant of the variety Blue Lace, triploid gametosomatic hybrid plants were recovered. These hybrids possessed an intermediate floral morphology and the expected chromosome number of 2n = 3x = 21. Selection was based on the fact that pollen tetrad protoplasts failed to divide in culture and that, following complementation to chlorophyll proficiency in the gametosomatic hybrid, the hybrid cells were visualised against a background of albino cells of the variety Blue Lace. The production of such gametosomatic hybrid plants in Petunia has shown that the concept of gametosomatic hybridisation can be extended to genera other than Nicotiana and that alternative selection strategies are available.

ABBREVIATIONS

BAP, 6-benzylaminopurine; IAA, 3-indole acetic acid; NAA, naphthalene acetic acid; Z, zeatin; ABN α - bromonaphthalene; MS, Murashige and Skoog (1962); MW, molecular weight; PEG, polyethylene glycol.

INTRODUCTION

There is only one previous report (Pirrie and Power, 1986) which has described the production of gametosomatic hybrid plants following the fusion, in this case, of haploid tetrad protoplasts of Nicotiana glutinosa with leaf protoplasts of a nitrate reductase deficient mutant of N. tabacum. In this system, the gametosomatic hybrid had the expected pentaploid chromosome number coupled with a predicted level of fertility.

It was anticipated that the gametosomatic hybrid produced between two very closely related species, or indeed between two varieties of the same species, would be sterile as a result of complete trivalent formation at meiosis reflecting a very high degree of homology between the respective chromosome sets.

It was important therefore to demonstrate that this would in fact be the situation in gametosomatic hybrids of closely related parents and at the same time show that alternative selection strategies can be utilised for the production of these types of hybrids. In this respect the more readily available albino mutant was chosen instead of the nitrate reductase deficient mutant. The use of two varieties of P. hybrida not only fulfills these basic requirements but provides unambiguous genetic markers at the floral level to confirm hybridity in the selected plants.

MATERIALS AND METHODS

Pollen tetrad protoplasts were isolated from greenhouse grown plants of a purple-flowered intraspecific F_1 hybrid of P. hybrida (Fig.1) (accession R51 x V23, 'Monsanto') and using the method described by Pirrie and Power (1986). Cell suspension protoplasts were isolated from albino P. hybrida cv. Blue Lace (Fig.1) (Pan American Seed Co., New York, U.S.A.); a single gene recessive mutation in this variety leading to the establishment of an homozygous albino line following selfing of the original plant. The establishment, maintenance and release of protoplasts from this nuclear albino mutant was as described previously (Power et al., 1979). Cell suspension protoplasts of albino Blue Lace were suspended at a density of 2.5 x 10^5/ml in CPW 13 M medium (Power et al., 1984) and mixed with an equal volume of tetrad protoplasts, in their respective enzyme solution and at the same density. Aliquots (1.0 ml) were placed in 5 cm Petri dishes and the protoplasts allowed to settle for 20 min. Two equally-sized drops of PEG fusion solution [22.5% (w/v) PEG, MW 6000 with 1.8% (w/v) sucrose, 1.54 g/l $CaCl_2.2H_2O$, 95.2 mg/l KH_2PO_4, pH 5.8] were added diametrically opposite so that they coalesced with the protoplasts. After 20 min. (room temp.) the PEG solution was removed by pipetting and replaced with 2.0 ml of a filter sterilised high pH fusion solution [0.05 M glycine, 1.1% (w/v) $CaCl_2.2H_2O$, 9.0% (w/v) mannitol, pH adjusted to 10.4 with NaOH] for a further 20 min. After this period protoplasts were washed twice with 10 ml of an 11% (w/v) sucrose solution containing 7.4 g/l $CaCl_2.2H_2O$ and 3.75 g/l glycine (pH 5.8) by flooding the dish and allowing the protoplasts to settle for 20 min. This was followed by two further washes with KP8 medium (Kao, 1977) whereupon the protoplasts were maintained in 3.0 ml/dish of this medium. Dishes were sealed with Nescofilm and kept at 27°C with a continuous illumination (1,000 lux; daylight fluorescent tubes). Unfused control dishes were handled in a similar way.

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The osmotic pressure of the protoplast culture medium was progressively reduced by adding after 7 days, 1.0 ml/dish of K8 medium (Kao, 1977), and after 14 days a further 1.0 ml of K8 medium to each dish having first removed 1.0 ml of the old medium. This latter step was repeated weekly until after 4 weeks, green colonies (3-4 mm diam.) which had appeared in the fusion dishes were transferred to MSP1 medium [MS medium with 2.0 mg/l NAA, 0.5 mg/l BAP, 3% (w/v) sucrose, 0.8% (w/v) Sigma agar, pH 5.8] with 5,000 lux continuous illumination (daylight tubes). After a period of continued growth (3-4 weeks) the selected calluses were transferred and subcultured monthly on MSZ regeneration medium [MS medium with 1.0 ml/Z, 3% (w/v) sucrose, 0.8% (w/v) agar, pH 5.8]. Regenerated shoots, which had appeared after 4-7 months were detached from the callus and rooted after 2 weeks in MSP2 medium [MS medium with 0.1 mg/l NAA, 3% (w/v) sucrose, 0.8% agar, pH 5.8]. Putative gametosomatic hybrid plants were grown on in E.F.F. soil-less compost (E.F.F. Products Ltd.) under conditions of high humidity (2 weeks) and finally flowered in the open greenhouse

Lateral shoots (5 cm) of the regenerated plants were detached and rooted (after 2 weeks) in hydroponic tanks which contained quarter-strength Hoaglands (1950) salts solution plus 1.0 mg/l IAA. Roots for somatic chromosome number determinations were pretreated with a saturated aqueous solution of ABN (3h; 4°C), fixed in acetic alcohol (6h; room temp.), hydrolysed with 1N HCl (6 min.; 60°C) and stained in Schiff's reagent.

RESULTS AND DISCUSSION

These experiments were repeated ten times (with 4 fusion-treated dishes/experiment) of which seven were negative. The remaining three experiments gave several green calluses, two of which underwent organogenesis on MSZ medium to give a total of 20 plants. All control dishes were negative. The regenerated plants all had flowers that were intermediate with respect to colour distribution, and colour intensity (Fig.1) and a reduced petal fringing; a feature unique to the Blue Lace parent (Fig.1). A random sample of plants taken from different experiments all had a chromosome number of $2n = 3x = 21$. This was the expected situation since both parental varieties were $2n = 2x = 14$. Pollen of all plants was inviable as judged by staining in acetocarmine and the plants could not be reciprocally back-crossed to either parental variety or indeed other varieties of *P. hybrida*.

The production of the gametosomatic hybrids in *Petunia* has shown that an albino mutant can be substituted for the previously used nitrate reductase deficient mutant as described earlier for *Nicotiana* (Pirrie and Power, 1986). Complementation to chlorophyll proficiency in the gametosomatic hybrid can therefore be effected by the fusion of a non-dividing pollen tetrad

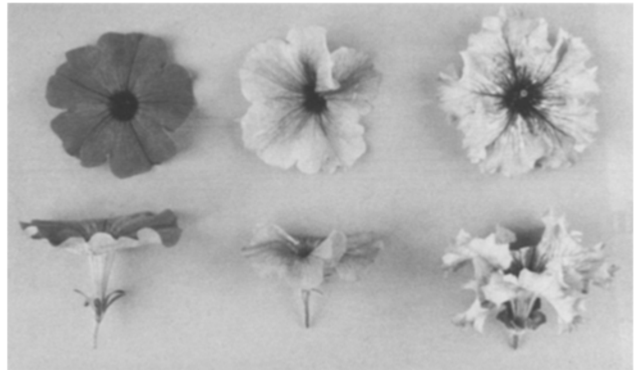

Fig.1. Flowers of (left to right) *P. hybrida* variety 'Monsanto' ($2n = 2x = 14$) the gametosomatic hybrid ($2n = 3x = 21$) and Blue Lace ($2n = 2x = 14$). Flower colours, as determined using the Royal Horticultural Colour Charts, were for Monsanto, cyclamen-purple 74A and for the gametosomatic hybrid and Blue Lace, cyclamen-purple 87D (light regions) and 83A (dark regions). (Mag. x ½).

protoplast system (wild-type) with an actively dividing nuclear albino somatic protoplast system. Hybrids can be readily visualised against the background of colourless, dividing cells from the unfused or homokaryon protoplasts of the albino parent. This approach is therefore likely to be of general applicability not only for the production of gametosomatic hybrids but, provided the parent species are not closely related, the generation of fertile interspecific gametosomatic hybrids such as those of species in the genus *Nicotiana*.

In this respect preliminary experiments, based on an identical protocol as described here but having substituted *P. hybrida* cv. Blue Lace for a nuclear albino mutant of *P. parviflora*, have shown that fertile interspecific gametosomatic hybrids can be produced between these two sexually incompatible *Petunia* species.

REFERENCES

Hoagland DR, Arnon DT (1950) Calif Agr Exp Sta Cir p 347.

Kao KN (1977) Mol Gen Genet 150: 225-230.

Murashige T, Skoog F (1962) Physiol Plant 15: 473-497.

Pirrie A, Power JB (1986) Theor Appl Genet 72:48-52.

Power JB, Berry SF, Chapman JV, Sink KC, Cocking EC (1979) Theor Appl Genet 55: 97-99.

Power JB, Chapman JV, Wilson D (1984) Laboratory Manual: Plant Tissue Culture, University of Nottingham, UK.