

Effects of Enforced Self-pollination in *Petunia hybrida*

by

S. R. SREE RANGASAMY¹, R. SETHUPATI RAMALINGAM²,
P. MADHAVA MENON³ and V. S. RAMAN⁴

The self-incompatibility mechanism which promotes cross-pollination is well known in the genus *Petunia*. Of the different species of the genus, *P. hybrida* is largely grown as an ornamental annual for its wealth of variation in nature of flowers and plant form. This species is known to be of hybrid origin, the parental species being *P. integrifolia* and *P. axillaris* (Darlington and Wylie, 1955; Takehisa, 1963). Structural differentiation of the chromosomes of the genomes of the two species is considered to be still existent in the garden forms of *P. hybrida* and the heterozygosity is probably enforced through self incompatibility. Rick (1942) observed that chromosomal deficiencies and duplications can exist in this species without detriment to viability or normal development. A study of the effects of enforced self-pollination in this species would throw light on the advantages conferred by cross pollination in preventing the breakdown of the hybrid from disharmonious genetic recombinations or eliminating in breeding depression. The present study confined to a few garden forms of *P. hybrida* is aimed at a comparison of the cytological behaviour and morphological variation in the open pollinated and selfed progenies.

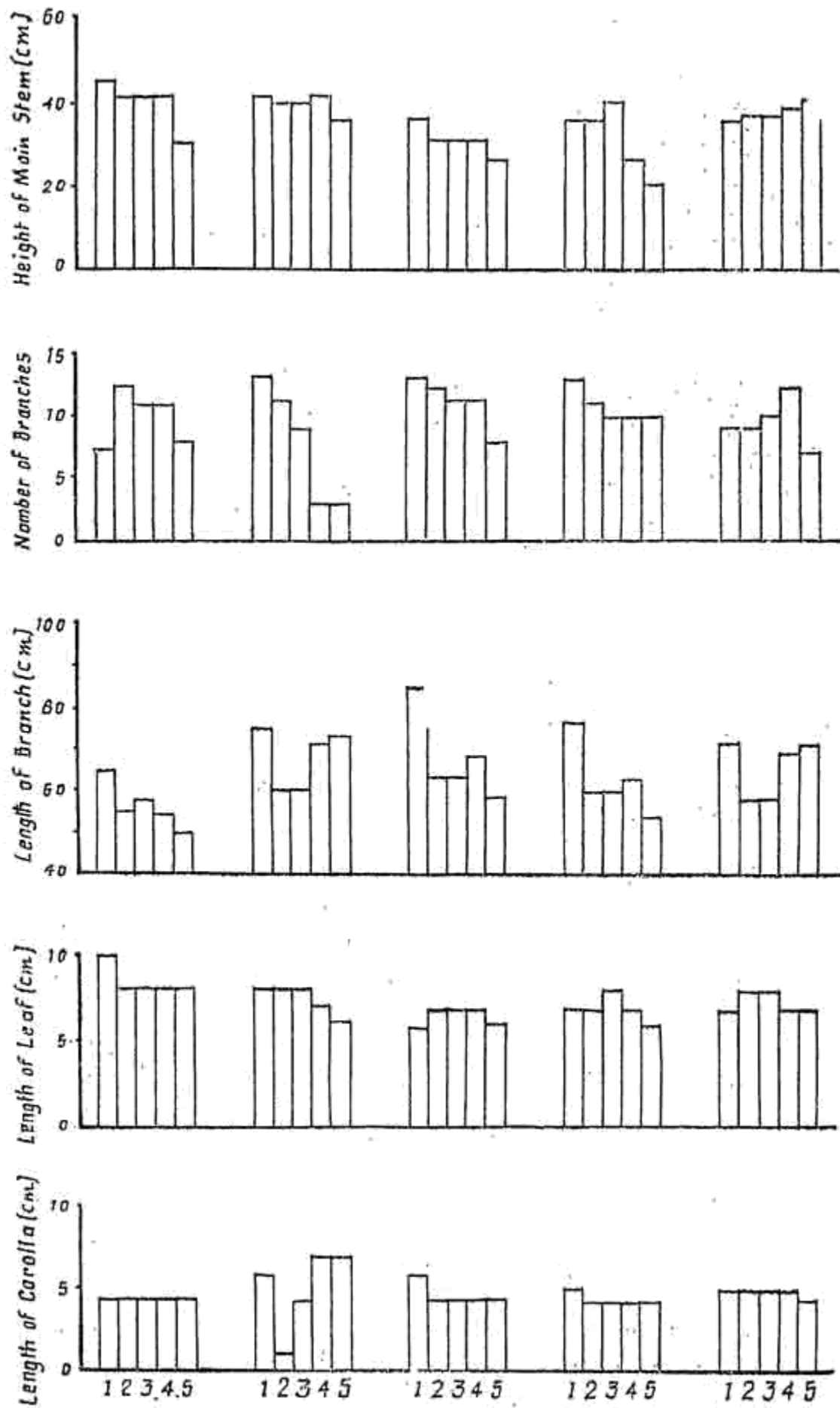
Materials and Methods: The populations of *Petunia hybrida* studied herein, comprised varieties 'Dwarf finest mixed', 'Tall single bedding finest mixed', 'Amarnath pink', 'Large flower orchid pink' and 'Large flowered special mixture'. The seeds were obtained from nursery-men at Poona and Calcutta.

Selfing was resorted to by adopting the 'bud pollination' technique. The technique consisted of making lateral incisions on petals of flower buds 2 to 4 days before normal opening and emasculating the buds and dusting the stigma with pollen from just dehisced anthers from the same plant. The bud pollinated flowers were then labelled and covered with paper bags. Seeds were collected from matured capsules. Seeds were also collected from capsules resulting from open pollination on the same plants. Data were collected from self and open pollinated progenies in each variety for two generations.

Cytological studies were made with young flower buds fixed in Carnoy's fluid 6:3:1 (alcohol, chloroform and acetic acid) mixture. The flower colour grades were decided by a comparison with the colour chart.

1. Assistant Cytogeneticist, 3. Reader in Genetics, 4. Cytogeneticist, Agrl. College and Research Institute, Coimbatore and 2. Lecturer in Cytogenetics, Agrl. College, Madurai.

FIG. 1. Diagram showing the mean performance of the five quantitative attributes in the open and self-pollinated population of *Petunia*.



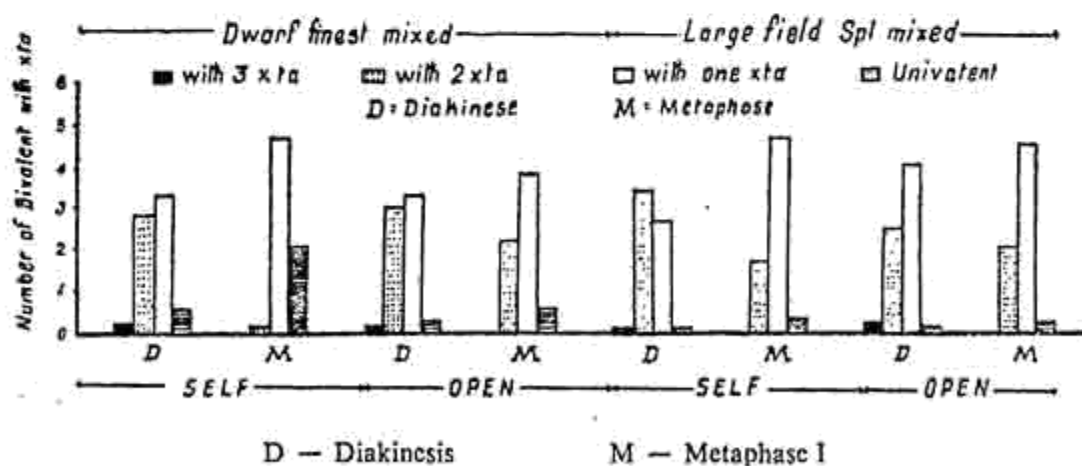
A - Amarnath Pink; B - Dwarf Finest Mixed; C - Large flower Orchid Pink;
 D - Large flower Special Mixture; E - Tall Single Bedding Mixed
 1 - Original population; 2 - & 4 - Open pollinated first & second generation &
 3 & 5 - S_1 & S_2 population.

Effects of Enforced Self-pollination in Petunia hybrida 559

Observations: Morphology: The population derived from self and open pollination of the same individuals exhibited distinct variation for growth habit, height of main stem, hairiness of plant parts, texture of leaves and also for the grades of flower colour. Data on the metric characters such as height and thickness of main stem, number and length of laterals, size of leaf and flower are gathered and the mean performance of the open and self pollinated population for two generations is presented in Fig. 1.

Cytology: Seven bivalents were regularly observed at diakinesis, of which one is nucleolar. Seldom two bivalents were also noticed to be associated with nucleolus. The bivalents did not differ much in their size. Occasionally univalents ranging from 2 to 4 were also noticed due to early disjunction of bivalents. The plant 2 of 'Dwarf finest mixed', however, showed $1_{IV}+5_{II}$ and $1_{III}+4_{II}+3_1$ respectively in two out of 50 p. m cells studied. At M_1 all the bivalents congressed and oriented normally. In addition to bivalents, univalents were also observed at M_1 mostly due to the precocious separation in one to as many as three bivalents. The chiasma frequency was scored at mid-diakinesis and M_1 . The number of chiasmata per bivalent ranged from 1 to 3 at diakinesis. The univalents whenever occurred were considered as pairs possessing 'O' chiasma. The number of chiasmata per p. m cell ranged from 4 to 15 at diakinesis and 1 to 13 at M_1 . A reduction in the frequency of chiasmata per cell was noticed from diakinesis to metaphase in all cases (Fig. 2).

FIG. 2. Selfmean chiasma frequency in the open and 'populations' of 'Dwarf finest mixed' and 'Large flowered special mixed' varieties. of *Petunia*.



The chromosome association ranged from 7_{II} to $3_{II} + 8_1$ in all the varieties studied (Table 1). Among the p. m cells examined at diakinesis and M_1 , cells with 7_{II} were most frequent followed by cells with 2, 4, 6 and 8 univalents. The precocious separation was noticed in 1 to 3 bivalents in all the varieties examined. The normal separation ranged from 14 to 43.5%

TABLE 1. Chromosome association in different varieties of *Petunia hybrida*.

Chromosome association at diakinesis and metaphase-I	Dwarf finest mixed-pink		Dwarf finest mixed-white		Large flowered special mixture (open)		Tall single bedding-white		Large flowered special mixture (self)	
	D	M	D	M	D	M	D	M	D	M
7	—	14	20	11	46	36	39	24	44	34
6	2	11	12	14	4	7	1	1	4	16
5	4	5	2	17	—	3	—	—	—	—
4	6	3	6	17	—	4	—	—	2	—
3	8	2	—	12	—	—	—	—	—	—
1	—	—	2	—	—	—	—	—	—	—
—	1	—	—	—	—	—	—	—	—	—
Mean	0.04 _{IV} ⁺	5.9 _{II} ⁺	6.15 _{II} ⁺	4.9 _{II} ⁺	6.9 _{II} ⁺	6.5 _{II} ⁺	6.97 _{II} ⁺	6.96 _{II} ⁺	6.8 _{II} ⁺	6.7 _{II} ⁺
	0.02 _{III} ⁺	2.2 _I	1.7 _I	4.2 _I	0.16 _I	1.0 _I	0.03 _I	0.04 _I	0.4 _I	0.6 _I
	6.5 _{II} ⁺									
	0.8 _I ⁺									

D - Diakinesis

M - Metaphase-I

of cells in different varieties. Thirty five percent (with a range of 26 to 49 %) of p. m cells showed precocious separation of one bivalent and precocious separation of two were noticed in 25 % of cells. Only 5 % of cells on an average showed 3_{II} disjoining precociously.

At A_I 92 to 95 % of the cells showed normal separation in all the varieties and the rest showed unequal separation of 8-6 chromosomes. In 28.0 % of cells at A_I, 1 to 4 (1-2_{II}) laggards were noticed and 6.9 % of cells showed one bridge. At A_{II}, 46.2 % of cells was normal, laggards (1 to 3) and bridges (1-2) were noticed in 45 % and 2.2 % cells respectively. In six percent of cells multipolar spindles were seen. In the quartet cells, 3 to 10 % showed micronuclei. Pentads, one to two were noted in 3 to 18 % of cells.

Pollen fertility varied from 68.7 to 96.8 %. The varieties that showed tetrad abnormalities were, however, more sterile. The pollen diameter ranged from 27.2 to 37.4 μ with a mean value of 34.4 μ .

Discussion: The meiotic behaviour of the open pollinated progenies of the three varieties studied indicated abnormalities which would point to structural heterozygosity, which however can be considered to be not of a high order. The pollen sterility, however, has been found to vary in different plants with a range of 4 to 32 %. It could be that cryptic structural differences causing inhibition of gametophyte development may be present, which however can not be easily distinguished from genes with the same effect, the relative constancy in most of the floral and vegetative characteristics in the garden varieties does suggest that vigorous selection and inbreeding in small populations have favoured the attainment of homozygosity for these features. The residual heterozygosity could therefore, be of significance, if at all, in their effects on quantitative variation and cytological behaviour.

A comparison of the open pollinated and self pollinated progenies of individual plants for the five varieties, do not, however, indicate any marked reduction in vigour as may be expected in a naturally cross pollinated species (Table 1). The range in variation was also not high in quantitative attributes in the selfed progenies compared to open pollinated ones. Meiotic instability noticed in the selfed progenies was not significantly different in degree from that in open-pollinated lines. However, a greater variation in chiasma frequency towards positive and negative directions could be noticed (Fig. 2). It is evident, there from, that enforced self-pollination do not lead to inbreeding depression, nor to any breakdown of the progenies due to disharmonious genetic recombinations. For production of more uniform lines, than obtainable in commercial seed, self-pollination can be resorted to without any deleterious effects.

Summary: A comparison of progenies raised from self and open pollinated seeds of *Petunia hybrida* was made to bring out the effects of self-pollination on vigour. There was no significant reduction in vigour in self-pollinated progenies. Variation in respect of eleven characters taken into consideration was noticed almost to the same extent in both the populations. The cytological abnormalities pointing to structural differences of a small order was common to both the open pollinated and selfed progenies. The advantages of out-breeding usually noticed in other plants by way of heterosis was not observed in this species, though self-incompatibility promoted out-breeding.

REFERENCES

- Darlington, C. D. and A. P. Wylie. 1955. *Chromosome atlas of flowering plants*. George Allen and Unwin Ltd., London.
- Rick, C. M. 1942. Cytogenetic consequences of X-ray treatment of pollen in *Petunia*. *Bot. Gaz.*, 104 : 528-39.
- Takehisa, S. 1963. The karyotype of *Petunia hybrida* and the differential chromosome condensation. *Jap. J. Genet.*, 38 : 237-43.

YOUR ATTENTION PLEASE

1. The annual subscription to the Madras Agricultural Journal including postage is Rs. 15/- (Inland) and \$ 3-00 (Foreign). The price of a single copy is Re. 1-25.
2. The Journal is published every month.
3. Complaints about the non-receipt of the Journal should reach the Secretary, The Madras Agricultural Journal, Agricultural College and Research Institute, Coimbatore-3 within 15th of the next month. Otherwise requests for copies will not be complied with.
4. Change of address should be intimated promptly. Otherwise complaints about non-receipt of the journals on that account will not be attended to.