Journal of Applied Horticulture, 15(2): 110-113, 2013

An efficient selfing technique for inbred development —A prerequisite for hybrid production in petunia

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Abstract

Petunia is one of the important bedding plants and occupies an ever increasing demand in flower seed industry. The present study was carried out to evaluate various selfing techniques to identify the efficient one for the production of large quantity of seeds in short duration to facilitate inbred development. Different selfing techniques like threading, manual pollination, bagging of single bud and bagging of multiple buds were tried in three inbred lines and also their interactions were studied. The results indicated that bagging of multiple buds took minimum days to seed set, higher pod weight and highest number of seeds/mg. Seed germination percentage was high in manual pollination followed by bagging of multiple buds. Inbred lines IIHRP-WT gave best results as compared to other inbred lines. Interaction of this inbred line with bagging of multiple buds gave good seed quality results like higher pod weight and number of seeds/mg.

Key words: Bagging, hybrids, inbreds, inbreeding depression, petunia

Introduction

Petunia is a widely cultivated genus of flowering plants belonging to the family Solanaceae, originated from South America. It is a popular bedding plant and also used in landscaping for its aesthetic value as borders, for mass planting, rock gardens, edging, and also in pots.

Seed production in flower crops have a great scope in developing countries like India, having favourable growing conditions, skilled and cheaper labour, marginal resources from small land holdings and need for crop diversification. Recently, the concept of contractual farming was set up, which is quite profitable for both foreign companies and farmers due to low volume commodity and reduced air freight charges as compared to perishable cut flowers. The major areas producing flower seeds are Ludhiana, Bangalore and Kalimpong contributing about 80-90 % of total production. Hybrid seed industry is dependent on inbred lines. Development of inbred lines is a requisite for hybrid seed production. Petunia is one such valuable crop having aesthetic value and also has importance in seed industry (Natarajan and Srimathi, 2008). The seeds which are in great demand are inbred lines, F_1 hybrids and open pollinated varieties of several annual flowers. At present, it is apparent that the share of India in world trade in respect of flower seeds is very meager. If quality seed of international standard is produced, there is tremendous potential for export of flower seeds. Seed companies are carrying out extensive breeding in annuals in India and good numbers of contract seed programmes have been organized across the country.

Hybrid development includes the cost of inbred as well as the hybridization. Inbreeding depression leading to poor seed set and reduced seed germination are problems encountered by seed industry. In this background, the present study was taken up to evaluate various selfing techniques to identify the efficient one for production of large quantity of seeds of petunia in short duration to facilitate inbred development and also for hybrid seed production.

Materials and methods

The experiment was conducted in the Division of Ornamental Crops, Indian Institute of Horticultural Research, Hessarghatta, Bangalore. The experiment was laid out in Factorial Completely Randomised Design. For the development of inbred lines, four selfing techniques *viz.,* threading, manual pollination, bagging of single bud, and bagging of multiple buds were tried. Detailed descriptions of these methods are given below.

Threading: In threading method, fully developed but unopened buds were tied at both the ends (at the pedicel end as well as at the top of the bud).

Manual pollination: In this method fully developed buds were bagged to prevent the entry of foreign pollen. Next day, the fresh pollen collected from the bagged flower was used for pollination on the stigmatic surface of the same flower and covered with butter paper bags.

Bagging of single bud: Fully developed, unopened flower buds were bagged with butter paper bags.

Bagging of multiple buds: Same aged buds of various branches were bagged together with butter paper bags.

Three inbred lines at different generations of development were used for selfing: Inbred lines, IIHRP-WT (White flower with yellow throat having plant habit as tall), IIHRP-MSd (Magenta with Semi dwarf habit) and IIHRP-WD (White with Dwarf habit). Fifteen flowers were selfed in each method and in each inbred line which represents the replications. The observations like days to seed set, pod weight (g), number of seeds per milligram, percentage of seed germination (%), percentage of seed set (%) were recorded and the data were analyzed using ANOVA.

Results and discussion

Inbred lines were not different significantly for the characters like number of days to seed set and number of seeds/mg. But the methods used and interaction between inbred lines and methods used differed significantly.

Number of days to seed set varied between 21.72days to 29.16 days (Table 1). Among the different methods used, bagging of multiple buds took minimum days to seed set (21.72 days) whereas in threading method it took maximum days to seed set (26.69 days). Minimum number of days to seed set was observed in interaction between IIHRP-WT with bagging of multiple buds (20.07 days). Interaction between IIHRP-MSd and threading method resulted in maximum number of days for seed setting (29.16 days). It may be due to more number of flowers opened at a time and the availability of pollen load in successive days allowing the flowers to get inter-pollinated resulting in quick setting of seed, and reducing the number of days taken to seed set. In rose, Sandhya (1988) reported that the duration for the full maturity of the hips was comparatively more when naturally selfed and it was supported by the findings in some other fruits (Anon., 1960) where it was stated that slower development of the embryo in the selfed ovules was due to poor development of the endosperm. The hormones that control the growth of the embryo is secreted by the endosperm and it is probable that the endosperm in the selfed ovules was unable to meet the requirements of the embryo, with the result the ovule degeneration occurred.

The three inbred lines, methods and their interactions differed significantly for pod weight (Table 2). Among the inbred lines, IIHRP-WT resulted in highest pod weight (0.149 g) and the inbred line IIHRP-WD got least pod weight (0.105 g). Where as in methods used, bagging of multiple buds recorded higher pod weight (0.252 g) and the lowest pod weight was observed in bagging of single bud (0.046 g). Among the interaction effects, inbred line IIHRP-WT with bagging of multiple buds gave the highest pod weight (0.343 g). It is due to the availability of more number of flowers at the same time and higher nutrient flow within these flowers. Incase of lower pod weight, high level of inbreeding depression during embryo development and seed maturation leading to reduction in fruit weight has been reported in many gymnosperms (Stevens and Bourgourd, 1988; Karoly, 1991; Husband and Schemske, 1995).

Table 1. Number of days to seed set as influenced by inbred lines, selfing methods and their interaction effects in petunia

Inbred lines (A)	Days to seed set	Selfing methods (B)	Days to seed set	Inbred line × Selfing method (A×B)	Days to seed set
A1-IIHRP-WT	23.32 (4.83)	B1-Threading	26.69 (5.17)	A1 × B1	26.01 (5.10)
				A1 × B2	24.00 (4.90)
				A1 × B3	23.40 (4.84)
A2-IIHRP-MSd	24.30 (4.93)	B2-Manual pollination	22.62 (4.76)	A1 × B4	20.07 (4.48)
				A2 × B1	29.16 (5.40)
				A2 × B2	23.71 (4.87)
A3-IIHRP-WD	22.47 (4.74)	B3-Bagging of single bud	22.55 (4.75)	A2 × B3	23.50 (4.85)
				A2 × B4	21.16 (4.60)
				A3 × B1	25.00 (5.00)
		B4-Bagging of multiple buds	21.72 (4.66)	A3 × B2	20.25 (4.50)
				A3 × B3	20.79 (4.56)
				A3 × B4	24.01 (4.90)
F-test	NS (2.37)		5.81*		3.10*
SEm±	0.06		0.07		0.13
CD@5%			0.22		0.38

* = Significant at P=0.05. NS= Non significant. Note: values in brackets are square root transformants.

Table 2. Pod weight (g) as influenced by inbred lines, selfing methods and their interaction effects in petunia

Inbred lines (A)	Pod weight (g)	Selfing methods (B)	Pod weight (g)	Inbred line × Selfing method (A×B)	Pod weight (g)
A1-IIHRP-WT	0.149	B1-Threading	0.081	A1 × B1	0.102
				A1 × B2	0.081
				A1 × B3	0.069
A2-IIHRP-MSd	0.112	B2-Manual pollination	0.109	A1 × B4	0.343
				A2 × B1	0.070
				A2 × B2	0.146
A3-IIHRP-WD	0.105	B3-Bagging of single bud	0.046	A2 × B3	0.036
				A2 × B4	0.195
				A3 × B1	0.072
		B4-Bagging of multiple buds	0.252	A3 × B2	0.099
				A3 × B3	0.031
				A3 × B4	0.219
F-test	4.01*		44.49*		3.58*
SEm±	0.012		0.014		0.024
CD@5%	0.033		0.038		0.065

Number of seeds/mg varied between 4.2-8.0. Highest number of seeds/mg was observed in bagging of multiple buds (8.0) followed by manual pollination (7.6). Lowest number of seeds/mg (4.2) was observed in threading method (Table 3). Among the interaction effects, inbred line IIHRP-WT with bagging of multiple buds resulted in maximum number of seeds/mg (10.2).

There was a significant difference among the different methods used, but the inbred lines and interaction effects did not differ significantly for seed germination (Table 4). Germination percentage varied from 38.88 to 86.44 %. Seeds collected from manual pollination gave maximum germination percentage. In threading method, poor germination percentage was observed (38.88 %). Viable and good quality seeds were obtained from manual pollination, whereas natural pollinations produced seeds of poor quality which affected the germination adversely. During manual pollination the chance of ovule getting fertilised is much higher. Seed germination is also dependent on harvesting, the seeds obtained from first harvestings had more germination capability than later harvestings (Natarajan and Srimathi, 2009). Compared with selfing, outcross-pollination enhanced germination rate. Reduced rate in natural selfing was due to the action of different deleterious loci (Juan *et al*., 2010).

The seed set percentage varied with inbred lines and selfing methods as shown in (Fig.1). Cent percent seed set was observed in the inbred line IIHRP-WT with bagging of multiple buds and also in the inbred line IIHRP-MSd with manual pollination. Among all inbred lines, threading method gave lower seed set percentage. It may be due to lack of pollen movement to stigmatic surface, abortion of ovules occurred after self fertilization and also lack of nutrients supply to the developing embryos along with some environmental factors like high temperature which leads to poor seed set. The effect of temperature on self-fertility has been demonstrated in *Petunia integrifolia* (Dana and Ascher, 1985), *Lycopersicon peruvianum* (Williams and Knox, 1982). Increase in the frequency of deleterious recessive genes as a result of inbreeding greatly reducing seed set was reported in three species of *Vaccinium* (Hokanson and Hancock, 2000) and also in black spruce trees (Johnsen *et al*., 2003).

In case of bagging of multiple buds, all the flowers were in contact

Table 3. Number of seeds/g as influenced by inbred lines, selfing methods and their interaction effects in petunia

Inbred lines (A)	Number of seeds/mg	Selfing methods (B)	Number of seeds/ mg	Inbred line × Selfing method (A×B)	Number of seeds/mg
A1-IIHRP-WT	7.3 (2.5)	B1-Threading	4.2 (1.8)	A1 × B1	3.9 (1.8)
				A1 × B2	6.4 (2.3)
				A1 × B3	8.7 (2.8)
A2-IIHRP-MSd	6.3 (2.3)	B2-Manual pollination	7.6 (2.6)	A1 × B4	10.2 (3.2)
				A2 × B1	3.2 (1.6)
				A2 × B2	10.1 (3.2)
A3-IIHRP-WD	6.0 (2.2)	B3-Bagging of single bud	6.3 (2.2)	A2 × B3	5.4 (2.0)
				A2 × B4	6.7 (2.3)
				A3 × B1	5.6 (2.1)
		B4-Bagging of multiple buds	8.0 (2.7)	A3 × B2	6.4 (2.4)
				A3 × B3	4.9 (1.9)
				A3 × B4	7.0 (2.5)
F-test	NS (1.47)		6.26*		NS (2.97)
SEm±	0.13		0.16		0.27
CD@5%	0.38		0.44		0.77

* = Significant at *P*=0.05. NS= Non significant. Note: values in brackets are square root transformants.

Table 4. Percentage of seed germination as influenced by inbred lines, selfing methods and their interaction effects in petunia

Inbred lines (A)	Germination (%)	Selfing methods (B)	Germination (%)	Inbred line × Selfing method (A×B)	Germination (%)
A1-IIHRP-WT	62.16 (52.41)	B1-Threading	38.88 (38.43)	A1 × B1	38.00 (37.93)
				A2 × B1	38.00 (37.88)
				A3 × B1	40.66 (39.48)
A2-IIHRP-MSd	61.83 (52.39)	B2-Manual pollination	86.44 (68.44)	A1 × B2	84.66 (66.83)
				A2 × B2	86.00 (68.16)
				A3 × B2	88.66 (70.33)
A3-IIHRP-WD	65.00 (54.37)	B3-Bagging of single bud	58.22 (49.63)	A1 × B3	60.00 (50.66)
				A2 × B3	52.66 (46.40)
				A3 × B3	62.00 (51.82)
		B4-Bagging of multiple buds	68.44 (55.73)	A1 × B4	66.00 (54.23)
				A2 × B4	70.66 (57.12)
				A3 × B4	68.66 (55.84)
F-test	NS (1.33)		121.11*		NS (0.78)
SEm±	0.98		1.13		1.96
CD@5%			3.31		

* = Significant at *P*=0.05. NS= Non significant. Note: values in brackets are arc sign transformants.

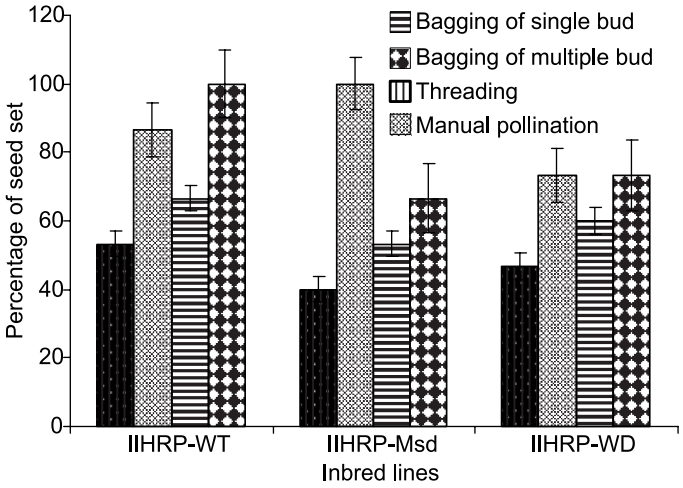

Fig. 1. Percentage of seed set as influenced by selfing methods

with each other in which the pollen grains from anthers were very close to stigmatic surface and were capable of pollinating more stigmas that favoured complete pollination. Manual pollination also resulted in cent percent seed setting. It is due to application of pollen to the stigmatic surface which enhanced the seed setting. It was also indicated by Shivaraju *et.al.* (1987) in sunflower.

Based on the above findings, it is concluded that seed industry needs appropriate techniques for production of seeds in short duration and in large quantity. Present study has resulted in identifying appropriate techniques for selfing. Bagging of multiple buds reduces the time and produces good quality seeds by which we can save the labour cost also and can produce large quantity of seeds.

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Received: November, 2012; Revised: February, 2013; Accepted: April, 2013