



PROTOGYNY IS AN ATTRACTIVE OPTION OVER EMASCULATION FOR HYBRIDIZATION IN PIGEONPEA

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SUMMARY

Hybridization is the initial step for creating genetic variability in conventional breeding programmes. Pigeonpea is one of the crops in which some genetic mechanisms including protogyny tends to promote natural outcrossing. Considering these facts in view, an investigation was performed to assess the feasibility of crossing without emasculation. Sixteen crosses were made with and without emasculation during the year 2006-2007 and 2007-2008. The number of crossed pods was significantly greater under crossing without emasculation than with emasculation. "Selfs" were observed in both the schemes, which could be ascribed to chance events and could further be brought down to zero per cent by selecting appropriate buds depending on environmental conditions. The results showed that protogyny-mediated hybridization is an alternative method to crossing involving emasculation in pigeonpea.

Key words: *Cajanus cajan*, crossing, emasculation, protogyny, selfs.

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INTRODUCTION

Pigeonpea (*Cajanus cajan* L. Millspaugh) is a major food legume of the tropics and sub-tropics. Globally, it is grown in more than 80 countries of Asia, Eastern and Southern Africa (ESA), Latin America and the Caribbean on 4.86 million hectares (M ha) with an annual production and mean productivity of 4.10 million tons (Mt) and 844 kg/ha, respectively (FAOSTAT, 2011).

India has the largest acreage under pigeonpea (3.90 M ha) with a total production and productivity of 2.89 Mt and 741 kg/ha, respectively (DAC, 2011). Despite its main use as de-corticated, dried split peas (*dal*), the use of immature seeds is very common as fresh vegetable in some parts of India such as Gujarat, Maharashtra and Karnataka. Besides this, in the tribal areas of various states, the use of pigeonpea as green

vegetable is also common (Saxena *et al.*, 2010).

Pigeonpea is considered as a drought tolerant crop with a large temporal variation for maturity period. As a result, it is widely adapted to a range of environments and cropping systems (Choudhary *et al.*, 2011). Broadly, four maturity groups are recognized in pigeonpea: extra early (90 – 120 days), early (>120 – 150 days), medium (>150 – 200 days), and late (>200 – 300 days). These variations for maturity have direct relevance on the survival and fitness of the crop in different agro-ecological niches (Choudhary, 2011).

The cleistogamous flowers of pigeonpea predominantly favour self-fertilization. However, considerable extent of natural out-crossing has been reported (Saxena *et al.*, 1990). According to Onim (1981), pigeonpeas shed pollen while flowers are still in the bud stage, and they do not start germinating until the flowers start to wither 24-48 hr after their anthers dehiscence. It has also been observed that germination and development and growth of native pollen tube down the style is very slow due to the presence of weak self-incompatibility (Dutta and Deb, 1970; Choudhary, 2011) which may be more of sporophytic than gametophytic in essence, taking 54 h to reach the base of the ovary. As a result of these two mechanisms, considerable out-crossing takes place mostly due to pollen transfer by different species of honey bees (Choudhary, 2011).

Anthesis in pigeonpea usually occurs during 8.0 – 17.0 h and flowers remain open for 36 to

48 h. The stigma becomes receptive 68 h before anther dehiscence (protogyny). However, the receptivity of stigma continues for further 20 h after anthesis (Prasad *et al.*, 1977), thereby opening up avenues for formation considerable “crossed” seeds. Therefore, the presence of weak self-incompatibility (Choudhary, 2011) and protogyny (Reddy and Mishra, 1981) provides scope for crossing even without emasculation in pigeonpea. The present investigation presents the comparative results of crossing *with* and *without* emasculation and examines the efficiency of both methods in pigeonpea.

MATERIALS AND METHODS

The experimental materials comprised 12 genotypes of pigeonpea, *viz.*, ‘IPA 7-1’, ‘IPA 7-3’, ‘IPA 7-5’, ‘Kudrat 3’, ‘Bahar’, ‘NA 1’, ‘IPA 92’, ‘MAL 13’, ‘T 7’, ‘IPA 06-1’, ‘BDN 1’, ‘BDN2’, ‘Ranchi Local’ and ‘UPAS 120’. These genotypes were selected on the bases of different morphological markers (Table 1). These were “pure” lines for one or more marker traits. These genotypes are maintained at the Indian Institute of Pulses Research (IIPR), Kanpur through selfing.

A total of 16 crosses were attempted during the winter season of the year 2006-07. In all the crosses, female and male parents were qualitatively different for one or more morphological traits such as stem colour, plant type, petal (standard) colour, pod colour, and the like. Each cross was made following two schemes: one *with*

emasculating and the second *without* emasculating. In the first scheme, fully developed buds (likely to open after 2 days) were emasculated during evening hour and hybridization was performed the next morning between 9-10 h. In the second scheme, pollinations on selected buds were done on the same day during the same period. In each scheme, pollinations were performed on 100 floral buds on the same female plant (10 buds/day) for a given cross. The same pollen source was used for pollination in both the methods. Pods developed on female plants of individual cross under both the crossing schemes were counted and picked upon maturity, threshed and kept separately.

In the following year (2007-2008), seeds of the crosses of each set were sown in the field along with the respective female parents to compare individual plants of putative crosses for plant types, petal colour, pod colour and other marker traits. On the bases of morphological markers, "selfs" (if present at all) and true F_1 s were identified and tagged. Just before flowering, these plants were covered with nylon net of size $110 \times 90 \text{ cm}^2$ with 2 mm mesh size to control its pollination system, thereby ensuring 100% self-pollination. Pods from "selfs" and true F_1 's were harvested and threshed and seeds were kept separately to observe their breeding behaviour in the coming season. The same set of crosses (as attempted during the year 2006-2007) under both the schemes of crossing was attempted again during this year (2007-2008) to

confirm the results of the previous year.

During the cropping season 2008-2009, seeds from "selfs" and true F_1 s of the preceding year were sown along with the parents to compare the breeding behaviour of descendants with respect to marker traits. The same exercise as done in the year 2007-2008 for assessing the breeding behaviour of putative crosses was also repeated during this year for crosses attempted during the year 2007-2008. Seeds obtained from "selfs" and true F_1 s were kept separately and sown during 2009-2010 to confirm the results of the preceding year in the same manner as practiced during the year 2008-2009.

Statistical analysis

The statistic "t-test" was applied to compare the average difference in the pod setting between two schemes of crossing (with and without emasculating). The same statistic was also applied for test of significance of average differences in the percentage of "selfs" obtained with two schemes of crossing (with and without emasculating). The analysis was carried out using SPSS version 16.0 software.

RESULTS AND DISCUSSION

The number of crossed pods set on emasculated plants varied from 5 (BDN 1 \times Kudrat 3 and BDN 2 \times Kudrat 3) to 23 (T 7 \times Kudrat 3) and from 0 (MAL 13 \times Kudrat 3) to 12 (T 7 \times Kudrat 3) during the year 2006-2007 and 2007-2008, respectively with the mean value (over two years) ranging from 3

(BDN 2 × Kudrat 3) to 17.5 (T 7 × Kudrat 3). However, average pod setting (over two years) in the second scheme ranged from 3.5 to 22.5 for the cross ‘BDN 2’ × ‘Kudrat 3’ and ‘IPA 7-3’ × ‘IPA 7-5’, respectively (Table 2). The difference in pod setting under the two schemes of crossing was statistically significant ($t=2.33$; $P < 0.03$). Pods set under crossing without emasculation was significantly greater than that under crossing with emasculation.

These crosses were grown in the succeeding cropping season along with the female parents to confirm whether crosses so-derived were *true* hybrids or “selfs”. The percentage “selfs” ranged from 0.0 (IPA 92 × IPA 6-1, BDN 2 × Kudrat 3 and NA 1 × MAL 13) to 13.33 (IPA 7-3 × IPA 7-5) and from 0.0 (IPA 7-1 × IPA 7-3, IPA 6-1 × Bahar, T 7 × NA 1, Kudrat 3 × MAL 13, Ranchi Local × T 7, BDN 2 × Kudrat 3, MAL 13 × IPA 6-1 and NA 1 × MAL 13) to 20 (Bahar × Kudrat 3) in the years 2007-2008 and 2008-2009, respectively when crosses were made following emasculation. Under the second scheme of crossing (without emasculation), the range of “selfs” were observed from 0.0% (IPA 92 × IPA 6-1, T 7 × NA 1 and MAL 13 × Kudrat 3) to 22.22% (Bahar × T 7) and from 0.0% (IPA 6-1 × Bahar, IPA 92 × IPA 6-1, Ranchi Local × T 7, BDN 2 × Kudrat 3 and MAL 13 × Kudrat 3) to 25% (Kudrat 3 × MAL 13 and BDN 1 × Kudrat 3) during the year 2007-08 and 2008-09, respectively. The mean

percentage “selfs” (over two years) varied from 0.0 (IPA 6-1 × Bahar, BDN 2 × Kudrat 3 and NA 1 × MAL 13) to 14.81 (IPA 7-3 × IPA 7-5) and from 0.0 (IPA 92 × IPA 6-1 and MAL 13 × Kudrat 3) to 20.00 (Bahar × T 7) under the two schemes of crossing, respectively. The difference in mean percentage “selfs” obtained under the two schemes was statistically non-significant ($t=1.93$; $P > 0.06$). However, “Selfs” under the second system of crossing (crossing without emasculation) were numerically high (Table 3).

Selfed progenies from both “selfs” and *true* F₁s derived under both schemes of mating were assessed for their breeding behaviour. All the selfed progenies from “selfs” bred true to the type, that is, all resembled their female parents. However, hybrid progenies segregated for different marker traits such as petal colour, plant type, pod colour, stem pubescence, days to maturity, and the like (Table 4).

It is a known fact that conventional plant breeding depends on hybridization between diverse parents for creation of new genetic variability. In pigeonpea like other crops, this is accomplished by emasculating flowers on female plants followed by placing pollens from male plants onto the stigma of emasculated flowers. However, during the process much injury is caused to the ovary and style of the emasculated flowers, resulting in reduced pod setting.

Table 1. Description of pigeonpea genotypes.

Genotypes	Pedigree	Distinguishing (marker) characters
IPA 7-1	Selection from a local landrace 'Kudrat 3'	Tall and compact plant type, dorsal surface of standard petal is dark red in colour
IPA 7-3	Selection from a local landrace 'Kudrat 3'	Medium height and semi-spreading, standard petal is golden yellow, pods are green with sparse black streaks
IPA 7-5	Selection from a local landrace 'Kudrat 3'	Medium height and semi-spreading, purple pods (unripe), standard petal is dark red
Kudrat 3	A local landrace, selected from Varanasi area of U.P. (India)	Medium height and compact, dorsal surface of standard petal is pink
Bahar	Selection from a landrace of Motihari district in Bihar (India)	Compact plant type with golden yellow colour of standard petal and purple pods (unripe)
NA 1	Selection from a landrace of Faizabad district of U.P. (India)	A long-duration variety with dense red streaks on dorsal surface of standard petal with green pods
IPA 92	Selection from a local collection, '98-3'	A late pigeonpea line of spreading growth habit and green stem colour
MAL 13	(MA 2 × MA 160) × Bahar	A large seeded (14g/100 seeds), spreading, long-duration variety with constricted pods and greenish yellow standard petal
T 7	Selection from a landrace of Lucknow district of U.P. (India)	A very late (280-300 days) and tall (2.5-3.0 m) variety of long-duration pigeonpea with compact plant type and green stem colour
IPA 06-1	Selection from a landrace of Etawah district of U.P. (India)	A large seeded (> 15 g/100 seeds) pigeonpea line having purple stem colour, only 3-4 primary branches, very prominent strophiole, dark red standard and high sensitivity to low temperature
BDN 1	Selection from local landrace 'Bori' (India)	An old variety of medium maturity group (180 days) with yellow standard
BDN 2	Selection from 'Bori II - 132-A-1' (India)	Indeterminate variety of medium maturity with white seeds
Ranchi Local	A land race of Ranchi, Jharkhand (India)	A large seeded pigeonpea line of medium duration with pink standard
UPAS 120	Selection from 'P 4768'	A short-duration variety (120-150 days) with yellow standard

Table 2. Differences in pod setting on plants pollinated with and without emasculation.

Crosses	No. of pods set on emasculated plants			No. of pods set on plants without emasculation		
	2006-07	2007-07	Mean	2006-07	2007-08	Mean
IPA 7-1 × IPA 7-3	19	4	11.5	24	12	18.0
IPA 7-3 × IPA 7-5	13	7	10.0	31	14	22.5
Bahar × Kudrat 3	11	3	7.0	13	6	9.5
Bahar × IPA 6-1	12	3	7.5	12	3	7.5
Bahar × T 7	12	8	10.0	17	12	14.5
IPA 92 × IPA 6-1	09	6	7.5	10	12	11.0
T 7 × Kudrat 3	23	12	17.5	25	13	19.0
T 7 × NA 1	07	8	7.5	09	9	9.0
UPAS 120 × Kudrat 3	18	11	14.5	29	14	21.5
Kudrat 3 × MAL 13	10	2	6.0	12	4	8.0
Ranchi Local × T 7	11	5	8.0	11	6	8.5
BDN 1 × Kudrat 3	05	5	5.0	13	8	10.5
BDN 2 × Kudrat 3	05	1	3.0	05	2	3.5
MAL 13 × IPA 6-1	13	3	8.0	17	9	13.0
MAL 13 × Kudrat 3	10	0	5.0	14	8	11.0
NA 1 × MAL 13	10	9	9.5	10	12	11.0
Mean	11.8	5.4	8.6	15.8	9.0	12.4
SE (Mean)	1.21	0.87	0.90	1.89	0.98	1.34
CD ($P = 0.05$)	2.47	1.78	1.85	3.92	2.03	2.73
t value (for differences)	--	4.22	--	--	3.16	2.33
P value for t-test	--	<0.001	--	--	0.004	0.026

Table 3. Selfs observed from crosses made with and without emasculation.

Crosses	Plants descended from crosses made with emasculation							Plants descended from crosses made without emasculation						
	2007-2008			2008-2009			All years	2007-2008			2008-2009			All years
	Total selfs	No. selfs	Self (%)	Total selfs	No. selfs	Self (%)	Total selfs (%)	Total selfs	No. selfs	Self (%)	Total selfs	No. selfs	Self (%)	Total selfs (%)
IPA 7-1 × IPA 7-3	22	2	9.1	9	0	0.0	6.5	36	3	8.3	19	3	15.8	10.9
IPA 7-3 × IPA 7-5	15	2	13.3	12	2	16.7	14.8	31	5	16.1	21	3	14.8	15.4
Bahar × Kudrat 3	16	1	6.3	5	1	20.0	9.5	13	2	15.4	11	2	18.2	16.7
IPA 6-1 × Bahar	12	0	0.0	6	0	0.0	0.0	12	1	8.3	5	0	0.0	5.9
Bahar × T 7	15	1	6.7	13	1	7.7	7.1	27	6	22.2	18	3	16.7	20.0
IPA 92 × IPA 6-1	12	0	0.0	12	1	8.3	4.2	15	0	0.0	21	0	0.0	0.0
T 7 × Kudrat 3	30	1	3.3	22	2	9.1	5.8	31	1	3.2	13	1	7.7	4.5
T 7 × NA 1	12	1	8.3	13	0	0.0	4.0	19	0	0.0	12	1	8.3	3.2
UPAS 120 × Kudrat 3	28	1	3.6	19	1	5.3	4.3	58	2	3.4	21	2	9.5	5.1
Kudrat 3 × MAL 13	11	1	9.1	4	0	0.0	6.7	14	2	14.3	8	2	25.0	18.2
Ranchi Local × T 7	13	1	7.7	7	0	0.0	5.0	15	1	6.7	6	0	0.0	4.8
BDN 1 × Kudrat 3	08	1	12.5	8	1	12.5	12.5	19	3	15.8	12	3	25.0	19.4
BDN 2 × Kudrat 3	09	0	0.0	3	0	0.0	0.0	10	2	20.0	5	0	0.0	13.3
MAL 13 × IPA 6-1	17	1	5.9	5	0	0.0	4.5	27	1	3.7	14	2	14.3	7.3
MAL 13 × Kudrat 3	16	1	6.3	--	--	----	6.3	24	0	0.0	15	0	0.0	0.0
NA 1 × MAL 13	17	0	0.0	15	0	0.0	0.0	15	1	6.7	24	2	8.3	7.7
Mean	15.8	0.9	5.5	10.2	0.6	5.9	5.7	22.9	1.9	8.2	14.1	1.5	10.7	9.1
SE (mean)	--	0.15	1.08	--	0.19	1.76	1.03	--	0.42	1.83	--	0.30	2.18	1.69
CD (P=0.05)	--	0.31	2.23	--	0.38	3.64	2.12	--	0.87	3.74	--	0.61	4.46	3.50
t value (for differences)	--	--	--	--	1.12	0.21	--	--	--	--	--	0.71	0.42	1.92
p-value for t-test	--	--	--	--	0.26	0.81	--	--	--	--	--	0.47	0.67	0.06

Table 4. Breeding behaviour of selfed progenies and F₂ populations from true F₁ hybrids obtained with and without emasculation.

Crosses	Breeding behavior	
	Selfs' progenies	F ₂ populations
IPA 7-1 × IPA 7-3	All individuals of all the selfs' progeny resemble the female parent 'IPA 7-1' of the cross	All F ₂ populations segregated for standard petal colour
IPA 7-3 × IPA 7-5	All individuals of all the selfs' progeny resemble the female parent 'IPA 7-3' of the cross	All F ₂ populations segregated for colour of standard petal and pods
Bahar × Kudrat 3	All individuals of all the selfs' progeny resemble the female parent 'Bahar' of the cross	All F ₂ populations segregated for colour of standard petal and pods
IPA 6-1 × Bahar	All individuals of all the selfs' progeny resemble the female parent 'IPA 6-1' of the cross	All F ₂ populations segregated for primary branches and colour of standard petal and pods
Bahar × T 7	All individuals of all the selfs' progeny resemble the female parent 'Bahar' of the cross	All F ₂ populations segregated for days to flowering and colour of stems and pods
IPA 92 × IPA 6-1	All individuals of all the selfs' progeny resemble the female parent 'IPA 92' of the cross	All F ₂ populations segregated for plant types (erect vs. spreading) and colour of standard petal
T 7 × Kudrat 3	All individuals of all the selfs' progeny resemble the female parent 'T 7' of the cross	All F ₂ populations segregated for days to flowering and colour of dorsal petal
T 7 × NA 1	All individuals of all the selfs' progeny resemble the female parent 'T 7' of the cross	All F ₂ populations segregated for days to flowering and colour of dorsal petal
UPAS 120 × Kudrat 3	All individuals of all the selfs' progeny resemble the female parent 'UPAS 120' of the cross	All F ₂ populations segregated for days to flowering, maturity and colour of dorsal petal
Kudrat 3 × MAL 13	All individuals of all the selfs' progeny resemble the female parent 'Kudrat 3' of the cross	All F ₂ populations segregated for plant types (erect vs. spreading) and colour of dorsal petal
Ranchi Local × T 7	All individuals of all the selfs' progeny resemble the female parent 'Ranchi Local' of the cross	All F ₂ populations segregated for days to flowering and colour of dorsal petal
BDN 1 × Kudrat 3	All individuals of all the selfs' progeny resemble the female parent 'BDN 1' of the cross	All F ₂ populations segregated for days to flowering and colour of dorsal petal
BDN 2 × Kudrat 3	All individuals of all the selfs' progeny resemble the female parent 'BDN 2' of the cross	All F ₂ populations segregated for days to flowering and colour of dorsal petal
MAL 13 × IPA 6-1	All individuals of all the selfs' progeny resemble the female parent 'MAL 13' of the cross	All F ₂ populations segregated for plant types (erect vs. spreading), primary branches and colour of dorsal petal
MAL 13 × Kudrat 3	All individuals of all the selfs' progeny resemble the female parent 'MAL 13' of the cross	All F ₂ populations segregated for plant types (erect vs. spreading) and colour of dorsal petal
NA 1 × MAL 13	All individuals of all the selfs' progeny resemble the female parent 'NA 1' of the cross	All F ₂ populations segregated for plant types (erect vs. spreading) and colour of dorsal petal

Moreover, the process of emasculation also entails much labour. In pigeonpea, presence of weak self-incompatibility (Choudhary, 2011) and protogyny (Reddy and Mishra, 1981) provides opportunity to perform hybridization even without emasculation. However, this operation does not completely preclude selfing. To discriminate between “selfs” and *true* hybrids, it is imperative that parents should differ for some easily identifiable marker traits. Fortunately, pigeonpea abounds in such morphological markers.

In this experiment, all the crossing parents had distinctive marker traits. When crosses were made with and without emasculation, pods formation took place in both the schemes. Pod formation under the second scheme (hybridization without emasculation) was not unexpected as Dutta and Deb (1970) also reported that emasculation may be unnecessary to perform hybridization in pigeonpea. It also substantiated earlier reports of protogyny (Reddy and Mishra, 1981) and weak self-incompatibility in pigeonpea (Choudhary, 2011). However, significant differences were observed in *per se* pod setting under the two schemes, showing unconditional advantage of crossing programme *without* emasculation over with emasculation. This could have been due to little injury to ovary in the second system of crossing, and presence of protogyny and weak self-incompatibility favouring faster germination and growth of

pollen tube in the style for foreign pollen grains.

When these putative crosses were grown for confirmation, “selfs” were observed under both schemes of crossing. However, mean percentage “selfs” was significantly higher under the scheme of crossing *without* emasculation than with emasculation during both the years. There were a few putative crosses for which no “selfs” were observed during the two years of crossing with (IPA 6-1 × Bahar, BDN 2 × Kudrat 3 and NA 1 × MAL 13) and without (IPA 92 × IPA 6-1 and MAL 13 × Kudrat 3) emasculation. There were still other cases (such as IPA 7-1 × IPA 7-3, IPA 92 × IPA 6-1, T 7 × NA 1, and the like) wherein few “selfs” were observed in one year but not in the second year under crossing *with* emasculation. Similar trends were observed for the second scheme of crossing for crosses such as IPA 6-1 × Bahar, T 7 × NA 1, Ranchi Local × T 7 and BDN 2 × Kudrat 3. Therefore, we hypothesized that IPA 92 and MAL 13 house significant protogyny, and additionally these may be having weak self-incompatibility factors with respect to the IPA 6-1 and Kudrat 3, respectively, for outcrossing to take place. It is also interesting to note that during the second year of crossing without emasculation, number of crosses without “selfs” was relatively large. This revealed that “selfs” so-obtained under emasculation method of crossing may be due to chance events that could be taken care of by selecting appropriate buds depending upon the

prevailing weather conditions, while those without emasculation may be due to lack of significant protogyny and/or presence of leaky incompatibility factors.

Selfed progenies from both “selfs” and true hybrids confirmed their classification during the next filial generations. No segregation was observed amongst progenies from “selfs”. These all bred true to their respective types. However, hybrid progenies segregated for plant types (spreading vs. compact), stem colour (green vs. purple), petal colour (red vs. yellow), pod colour (green with streaks vs. purple with streaks), days to flowering (early, medium and late), maturity period (early vs. late) and the like. It indicated that our hybrids resulting out of these two systems of crossing were indeed true crosses.

In conclusion, both systems of crossing (with and without emasculation) resulted in “selfs” and true hybrids. Crossing without emasculation resulted in increased number of crossed pods and seeds, and is, therefore, a feasible option of crossing in pigeonpea. This method also saved time and cost that would incur in emasculation. This system of hybridization thus is a feasible alternative hybridization method compared to emasculation in pigeonpea.

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