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Role of partial cleistogamy in maintaining genetic purity of pigeonpea

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Summary

Natural out-crossing in pigeonpea (*Cajanus cajan* (L.) Millsp.) leads to genetic deterioration and in certain environments the out-crossing may be as high as 70%. Studies with partially cleistogamous lines derived from an inter-generic cross indicated that average natural out-crossing in these lines was much lower (2.5%) than that reported earlier for pigeonpea (36.5%) at ICRISAT Center. Based on seed yield and disease resistance, potential parents such as ICPL 87154 in short-duration determinate, ICPL 87018 in short-duration indeterminate, and ICPL 87159 in medium-duration indeterminate groups have been identified for introgression of the partially cleistogamous trait into pigeonpea.

Introduction

In a recent review Saxena et al. (1990) reported that natural out-crossing was a common event in pigeonpea. However, its extent varied from one environment to another, depending on the activity and availability of pollinating insects such as *Megachile* spp. and *Apis dorsata.* A considerable range for natural out-crossing in pigeonpea has been reported by researchers from India (2–70%), Kenya (12–50%), Hawaii (1–30%), Trinidad (26.4%), Australia (2–40%), Uganda (8–22%) and Puerto Rico (5–6%). This not only leads to a rapid genetic deterioration of released cultivars and genetic stocks but also influences the efficiency of pure-line breeding programs, since selfing of early generation selections is expensive and difficult. Due to limitation of resources and lack of appreciation of implications of out-crossing, breeders handle the early generation segregating populations and selections following the schemes designed for autogamous species. Recently, Singh et al. (1990) concluded that the genetic gains in pigeonpea through breeding have been limited to the incorporation of relatively simply-inherited traits such as disease resistance, seed size, plant type and maturity, and that the productivity of the crop has been static over decades. The efficiency of pedigree selections under open-pollination may be influenced adversely by natural out-crossing in the preceding generation and unconscious selection of vigorous natural hybrids. The problem of genetic contamination due to natural out-crossing can be solved by developing cultivars with traits that restrict out-crossing through floral morphological barriers or by encouraging self-pollination before the transfer of foreign pollen. Byth et al. (1982) reported one such floral modification where the petals in the floral buds were wrapped and interlocked, preventing the contact of pollen carrying insects with the stigma. Saxena et al. (1987a) revealed that the effectiveness of this trait was influenced by environmental conditions. The presence of inherent

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crossability barriers (Singh et al., 1980; Saxena et al., 1987b) can be helpful in maintaining the genetic purity of the lines. However, the incorporation of such factors in adapted cultivars will be a difficult breeding task. Saxena et al. (1992) described a partially cleistogamous floral mutant where the filament attachment is modified, wings remain enclosed within the keel and flower opening is considerably delayed. This trait, controlled by a single recessive gene, has been transferred into breeding populations and the present paper reports natural out-crossing and the agronomic performance of some partially cleistogamous lines.

Materials and methods

Indeterminate growth and normal floral morphology (di-adelphous anthers and normal keel) of pigeonpea are dominant to determinate growth and partially cleistogamy, respectively (Saxena et al., 1992). Both the traits are expressed dominantly in cultivar T.21, but recessively in the partially cleistogamous lines ICPL 87026, ICPL 87047 and ICPL 87154. Natural hybridization of the partially cleistogamous lines can readily be identified through scoring for dominant markers (indeterminate habit and normal flowers) in the F_1 generation.

In order to quantify the natural out-crossing of the three partially cleistogamous lines, each line was planted in 4 m long four-row plots. Six rows of a male sterile line ms-Prabhat (DT) were also planted in each replication to monitor the insect activity in the field. The interrow distance was 60 cm and the plant to plant distance within the row was 10 cm. Each plot was surrounded on all four sides at the same spacing by four rows of cultivar T.21. This layout was replicated four times.

The experiment was planted on 16 July 1988 on Alfisols under isolation. Adjoining the experimental area, the cultivar T.21 was grown to attract and enhance insect activity on the test lines. A mixture of Prometryn 1.5 L. (a.i.) ha^{-1} and Basalin 1.5 L. (a.i.) ha^{-1} was applied as a pre-emergence herbicide followed by one hand weeding, 40 days after sowing. Two irrigations were provided during the postrainy season. To encourage build-up of insect pollinators, no insecticide was applied during flowering and podding. At maturity, plants from the partially cleistogamous lines were harvested separately and their progenies were grown in the following rainy season. In each plant-progeny, counts were made for plants with normal and partially cleistogamous flowers at the flowering stage, and the extent of natural out-crossing was estimated as a percentage of the hybrid plants observed.

To evaluate the performance of advanced partially cleistogamous breeding lines and to identify the donor parents, 15 F_7 lines, 5 each in short-duration determinate, short-duration indeterminate and medium-duration indeterminate groups, were evaluated in separate trials during the 1986, 1987, and 1988 seasons. In each year the short-duration trials were grown on Alfisols while the medium-duration trial was evaluated on Vertisols along with appropriate varietal checks. Each trial was planted at the beginning of the monsoon season in randomized complete block design with four replications. In the short-duration trials the inter and intra-row spacings were kept at 30 and 10 cm, respectively, while the medium-duration materials were evaluated at row to row and plant to plant spacings of 60 and 25 cm, respectively. All the trials were grown under irrigated conditions and were kept weed-free. Endosulfan 1.5 L (a.i.) ha^{-1} was sprayed four times during the reproductive stage for effective control of the pod borer *Helicoverpa armigera*. These lines were also grown separately in wilt and sterility mosaic sick nurseries during the 1988 rainy season to assess their disease reaction. Data on 50% flowering, 75% maturity, plant height, and 100-seed mass were recorded. The medium-duration trial in 1987 failed due to severe wilt incidence.

Results and discussion

Out-crossing

Mean number of pods per plant recorded on the male fertile (41.3± 3.1) and male sterile (35.8± 7.8) plants indicated the presence of adequate pollinating vectors in the isolation block used for this study. The frequency of hybrid plants observed in progenies of the partially cleistogamous lines are given in Table 1. Overall, of the total 13605 plants raised only 394 were natural hybrids exhibiting 2.9% natural out-crossing in the partially cleistogamous lines. The coefficient of variation in the trial was high and analysis of variance showed no differences among the genotypes for the extent of natural out-crossing. ICPL 87047 had 5.88% out-crossed plants while ICPL 87154 recorded only 0.43% natural out-crossing. It was interesting to note that the variation for natural out-crossing among the replications was relatively large both in ICPL 87026 (0.35 to 4.47%) and ICPL 87047 (0.19 to 8.07%). Since the partially cleistogamous trait is stable across environments, the relatively higher values for natural out-crossing (Table 1) could be attributed to the variation in insect activity on different plants. Bhatia et al. (1981) and Onim (1981) have shown a direct relationship between the extent of natural out-crossing and insect activity. In the present experiment plants were not sprayed with insecticides and the insect activity was generally high. Though differential natural out-crossing between replications is difficult to explain, the possible explanation may lie in the plant growth variations of the test plots caused by presence/absence of weeds and unknown soil factors. It could also be that the pollinating vectors preferred to visit tall vigorous plants more frequently than the stressed plants. The out-crossing values (7–8%) observed in two replications of ICPL 87047 (Table 1) may point towards the maximum level of natural out-crossing possible in the genotypes having partially cleistogamous flowers. Nevertheless, the extents of natural out-crossing recorded in the present experiment are extremely low compared to those reported earlier (36–40%) by Bhatia et al. (1981) in the similar (adjacent plots) experimental situations at ICRISAT Center. This indicates that the cleistogamous flowers have been able to restrict natural out-crossing to a great extent and can be of use in breeding and seed production programs.

In the present experiment we used a normal flower variety as the contaminator, which permits easy foraging. Williams (1977) reported that under the ICRISAT Center environment each pollinating vector visiting normal pigeonpea flowers had 5500–107333 pollen grains attached to its body. Since in the partially cleistogamous lines the flowers have an abnormal keel petal that remains folded for a longer period and flower opening is delayed (Saxe-

Table 1. Frequency of hybrid plants observed in three partially cleistogamous inbred lines at ICRISAT Center in 1989

Line	Replication	No. of progenies grown	No. of plants		Out-crossing (%)
			Total	Hybrids	
ICPL 87026	1	78	1384	9	0.65
	2	113	1902	85	4.47
	3	46	570	2	0.35
	4	64	534	10	1.87
Total/mean		301	4390	106	2.42
ICPL 87047	1	121	1903	91	4.80
	2	42	526	1	0.19
	3	121	1550	129	7.74
	4	63	582	47	8.07
Total/mean		347	4561	268	5.88
ICPL 87154	1	87	1732	9	0.52
	2	110	1087	5	0.46
	3	84	968	4	0.41
	4	84	867	2	0.23
Total/mean		365	4654	20	0.43
Mean					2.48± 1.39

na et al., 1992), it will be interesting to study whether the foraging insects are able to collect sufficient viable pollen grains from the flowers of partially cleistogamous lines to affect natural out-crossing in the neighboring partially cleistogamous or normal flowered lines.

Selection of donor parents

Some plant and grain characteristics of the partially cleistogamous lines are summarized in Table 2. The short-duration determinate lines had smaller seeds and low yield in comparison to the control cultivar ICPL 87. In the 1986 season, ICPL 87026 and ICPL 87047 yielded over 2 t ha^{-1}, but they failed to sustain this yield level in the succeeding years. The partially cleistogamous lines were taller than ICPL 87 and had similar flowering and maturation time. ICPL 87154 was found to be resistant to wilt disease. All the short-duration indeterminate lines flowered and matured about 2 weeks later than the control cultivar T.21. On the average ICPL 87018 and T.21 recorded similar yields but the former fluctuated considerably over seasons. ICPL 87156 showed tolerance to sterility mosaic disease but its yield was low. The medium-duration partially cleistogamous lines were inferior to check cultivar C-11 in seed size and yield. ICPL 87159 showed resistance to wilt disease. Considering yield potential, disease resistance and seed size, ICPL 87154 in short-duration determinate, ICPL 87018 in short-duration indetermi-

Table 2. Important characteristics of promising partially cleistogamous inbred lines at ICRISAT Center during 1986–1988

Line/growth habit	Days to		Plant height (cm)	100-seed mass (g)	Yield (t ha^{-1})				Disease reaction (%)[1]	
	50% flowering	75% maturity			1986	1987	1988	Mean	Wilt	Sterility mosaic
Short-duration (determinate)										
ICPL 87026	83	132	131	7.9	2.09	1.25	0.95	1.43	98	93
ICPL 87047	80	127	128	7.7	2.26	1.49	1.37	1.71	40	100
ICPL 87154	80	126	130	7.8	1.95	1.52	1.24	1.57	14	96
ICPL 87 (control)	72	125	114	10.4	1.73	2.15	2.18	2.02	–	–
SE	± 0.7	± 0.5	± 3.3	± 0.1	± 0.21	± 0.10	± 0.08	–	–	–
CV%	2.0	1.0	6.3	2.7	23.0	11.0	13.0	–	–	–
Short-duration (indeterminate)										
ICPL 87018	101	142	219	6.7	2.49	1.32	1.01	1.60	43	88
ICPL 87155	99	140	208	6.8	2.15	1.05	0.86	1.35	50	85
ICPL 87156	91	135	225	7.6	1.97	0.91	0.71	1.20	84	22
T.21 (control)	82	125	224	8.0	1.38	1.80	1.54	1.58	–	–
SE	± 0.3	± 0.4	± 3.7	± 0.1	± 0.22	± 0.10	± 0.09	–	–	–
CV%	0.7	0.6	4.2	3.4	24.3	18.4	23.2	–	–	–
Medium-duration (indeterminate)										
ICPL 87157	130	174	197	5.2	1.20	–	0.90	1.05	35	100
ICPL 87158	128	173	189	6.2	1.25	–	0.95	1.10	50	98
ICPL 87159	119	161	188	6.8	1.27	–	1.32	1.30	15	100
C-11 (control)	133	176	210	8.0	2.17	–	1.81	1.99	90	100
SE	± 0.9	± 0.9	± 4.2	± 0.1	± 0.14	–	± 0.16	–	–	–
CV%	1.7	1.3	5.3	4.9	19.9	–	27.9	–	–	–

[1]Disease sick nursery, 1988.

nate, and ICPL 87159 in medium-duration were identified as potential donor parents for hybridization. The yield data for 1986 indicated that some of the partially cleistogamous lines have yield potential similar to that of the checks but the lack of stability in their performance makes them unfit for direct use as cultivars. It will be interesting to investigate if the gradual yield decline observed in the partially cleistogamous lines was due to their poor adaptability or decreased level of heterozygosity. However, in view of the potential use of the partially cleistogamous character in maintaining the genetic purity of genotypes, the trait appears to be extremely useful. Breeders at ICRISAT Center now have started transferring this simply-inherited trait to promising disease and insect resistance sources and popular high-yielding varieties through backcrossing.

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