#2338

Proc. IVth Int. Symp. on Pollination
Md. Agric. Exp. Sta. Spec. Misc. Publ. 1:211-218.

INFLUENCE OF INSECT POLLINATORS ON THE DEGREE OF OUTCROSSING IN PIGEON PEA IN KENYA

J.F.M. Onim, R.S. Pathak and C.L.M. Van Eijnatten

Department of Crop Science
University of Nairobi, Nairobi, KENYA

ABSTRACT

The influence of availability and activity of insect pollinators on the degree of outcrossing in pigeon pea (Cajanus cajan (L) Millsp.) were studied at six sites in Kenya. Suitability of genetic markers and methods of estimating outcrossing in pigeon pea are critically reviewed and discussed. Degrees of outcrossing varied from 25.20 to 94.50% and it is directly influenced by the number and activity of insect pollinators at each site ($r = 0.994$; $p = 0.001$). Degrees of outcrossing were not influenced by the quantity of pollen from plants with the marker gene. Implications of high degrees of outcrossing in pigeon pea are mentioned.

INTRODUCTION

According to reports from various parts of the world, pigeon pea (Cajanus cajan (L) Millsp.) outcrosses to a varying degrees. Reports from India show that outcrossing in this crop varies between 0.09 and 65% (2, 4, 6, 7, 9, 13). Pigeon pea was reported to outcross between 13.98 and 15.86% in Hawaii (15), and between 5.47 and 6.33% in Puerto Rico (1). In Uganda, outcrossing varies between 8 and 22% (8). However, an average figure has been estimated to be 20% (11).

In the past, two genetic markers have been used in studies of outcrossing in pigeon pea. The most popularly used one is flower colour (1, 15). This may not, however, be a suitable marker for such a study. Where flower colour is used in outcrossing studies in which insects are the main agents of cross-pollination, the pollinators may prefer one flower colour to another, hence the preferred flowers will be more frequently visited with a result that outcrossing will be underestimated (12). Obtuse leaf mutant has also been used as a marker (2). Normal leaf is dominant over obtuse leaf. However, this marker is also not suitable since it has undesirable association with flower morphology. Flowers of obtuse leaved plants trip naturally and are therefore more frequently visited by insect pollinators than the normal untripped flowers (14). Stem colour in pigeon pea is monogenically inherited, and purple is dominant over green (3). Because stem colour has no undesirable influence on foraging habits of insect pollinators, it can be used as a suitable marker in outcrossing studies.

The wide variation in outcrossing in pigeon pea suggests that it is influenced by one or more variable factors. Availability and activity of insect pollinators of pigeon pea at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) have been reported (14). However, the influence of insect pollinators on outcrossing in this crop has not been reported as far as we are aware. Two experiments were therefore conducted to determine the influence of insect pollinators on outcrossing in pigeon pea in two different planting arrangements at several sites in Kenya. This paper reports the results of these experiments.

MATERIALS AND METHODS

Inbred lines NPP 205/6 and NPP 199/10 with purple stems (p) and green stems (g) respectively were used in these investigations. These inbreds were selected for this study because they have the same plant height and flower colour, and they flower at the same time at the test sites in Kenya. These characteristics should be the same in the inbreds used to minimise selective foraging by insect pollinators. Two different planting arrangements were used. These will be referred to as experiments I and II.

For both experiments I and II, activity of insect pollinators at each site was determined during anthesis. A small area measuring 2m x 2m was marked at the middle of each plot and all insect pollinators visiting flowers in that area within a period of 5 minutes were carefully counted between 8.00 am and 9.00 am. Three such counts were taken at 2-day intervals for each site.

Experiment I

Three hundred seeds of each type of stem colour were mechanically mixed thoroughly before randomly planting one seed per hill at a spacing of 80cm between rows and 30cm between plants within row. Each plot was 12m x 12m and had an initial plant population of approximately 600.

The crop was planted at six sites in various parts of Kenya. Two plots were planted on 4.11.76 at the University Field Station at Kabete, under normal and high insect pollinator populations respectively. The crop was also planted at the following agricultural research stations: at Katumani on 9.11.76, Kampi ya Mawe on 18.11.76, Kibos on

12.11.76, and Mtwapa on 15.11.76. The high insect pollinator population plot at Kabete was situated near a 30-bee hive apiary at the edge of a forest which was well inhabited by a wide range of other insect pollinators.

At the onset of flowering in each plot, excess (p) plants or (g) plants were rogued out, leaving equal numbers of each type. The crop reached 50% flowering on January 10, 13, 14, 15, and 28 at Kibos, Katumani, Mtwapa, Kabete and Kampi ya Mawe respectively. The plots were harvested on March 3, 5, 8, 12 and 18 at Katumani, Kibos, Kampi ya Mawe, Kabete and Mtwapa respectively.

Experiment II

The aim of this experiment was to increase the quantity of pollen grains of (p) plants many times above those of (g) plants per plot, without altering the insect pollinator population at each site. Assuming that pollen yields per plant in the two inbred lines were about the same, it was decided that a ratio of 1(g) plant : 50(p)plants be used. In this experiment, a plot layout in which each (g)plant was surrounded by 3 rows of (p)plants was used. The spacing was 80 cm between rows and 40cm between plants within row.

The crop was planted in single plots measuring 15m x 15m at 3 sites in Kenya. The crop was planted on 7.4.77 at the University Field Station at Kabete, under high insect pollinator population, at Katumani on 14.4.77 and Kibos on 16.4.77. The crop reached 50% flowering on July 2, 4, and 10 at Kibos, Katumani and Kabete respectively, and it was harvested on September 15, 24 and 26 at Kabete, Katumani and Kibos respectively. Although there should have been 700 plants per plot, the ratios of (p)plants to (g)plants were 658:10 and 626:13 for Kibos and Katumani respectively. Unfortunately, pest damage was so severe at Kabete that there were not enough seeds for scoring for degree of outcrossing.

In both experiments I and II, the (g)plants were marked by means of pegs, and the plots were not sprayed against insect pests. Only pods from (g)plants at each trial site were harvested.

Outcrossing for each trial site was scored in a glasshouse at Kabete between 18.5.77 and 15.10.77. Where there was sufficient seed, upto 4 seed lots of 500 seeds each were taken from each site and were planted in 4 replicates in sand boxes. Where the seed was not sufficient, fewer replicates were used. The sand boxes were watered everyday and the degree of outcrossing was scored 14 days after planting. Scoring was done by counting the total number of seedlings in each box, then expressing the number of (p)seedlings as a percentage of the total, and this will be referred to as %(p).

RESULTS AND DISCUSSION

Experiment I

The estimates of %(p) for six sites in Kenya are presented in Table 1. These results show that %(p) varies from 12.60 at Kibos to 45.91 at Kabete under high insect pollinator population. However, according to planting arrangement used in this experiment, these %(p) values may not be accurate estimators of degree of outcrossing in this crop. The experimental model used in this study only measures outcrossing between (p)plants and (g)plants as indicated in the following equation:

$$300\ (p)\text{plants} \underset{v}{\overset{u}{\rightleftarrows}} 300\ (g)\text{plants} \quad \ldots\ldots\ldots \quad (i)$$

This equation is assuming that u = v, hence no selective foraging by insect pollinators. But since outcrossing was only measured in the (g)plants, it means that only u was determined. This is equivalent to 300 plants out of approximately 600. Moreover, equation (i) does not take into account outcrossing among 300 (p)plants or 300 (g)plants which may be presented as follows:

$$150\ (p)\text{plants} \underset{v_1}{\overset{u_1}{\rightleftarrows}} 150\ (p)\text{plants} \quad \ldots\ldots\ldots(ii),$$

and similarly for the (g)plants:

$$150\ (g)\text{plants} \underset{v_2}{\overset{u_2}{\rightleftarrows}} 150\ (g)\text{plants} \quad \ldots\ldots\ldots \quad (iii).$$

According to equations (ii) and (iii), ½ of outcrossing was not measured in each case. Hence, %(p) values which are shown in Table 1 only estimate ½ the values of actual outcrossing for each site. Because of this argument, the mean %(p) for each site should be multiplied by a factor of 2 to give the degree of outcrossing for that site. When this is done, better estimates for outcrossing of pigeon pea at each site are obtained, and these are presented in Table 2.

The estimated values for outcrossing in pigeon pea shown in Table 2 range from 25.20% at Kibos to 94.50% at Kabete under high insect pollinator population. These values are quite high when compared to most of the values reported in literature. The main reason for these differences may lie in the methods used by various people for estimating outcrossing in this crop. It would seem that the majority of the methods used have underestimated outcrossing in pigeon pea. However, the values reported in this paper point to the possibility that this crop may be improved by population improvement methods like an open-pollinated crop (8). This approach was attempted in our pigeon pea improvement project at Katumani. By employing 4 cycles of Stratified Mass Selection (SMS) according to Gardner (5), progress per cycle of selection of 3.12, 8.83, 10.57 and 11.74 for number of pods, total dry matter yield, number of leaves and leaf area per plant respectively were realised (10). This good response to SMS supports the higher estimates of degrees of outcrossing which are shown in Table 2.

Table 1: Estimates of %(p) from six sites in Kenya when the number of (p)parents and (g)parents are the same

Site	Replicate	No. of seedlings			Replicate	Site mean
		Total	(g)	(p)	%(p)	%(p) ± SD
Katumani	1	394	309	85	21.57	
	2	376	316	60	15.96	
	3	347	291	56	16.14	
	4	395	327	68	17.22	17.72±2.62
Kibos	1	441	387	54	12.24	
	2	342	293	49	14.33	
	3	334	298	36	10.78	
	4	391	340	51	13.04	12.60±1.47
Kampi ya Mawe	1	405	320	85	20.99	
	2	269	220	49	18.22	
	3	400	322	78	19.50	
	4	377	282	95	25.20	20.98±3.03
Mtwapa	1	414	320	94	22.71	
	2	391	308	83	21.23	21.97±1.05
Kabete (Low pollinator pop)	1	142	122	20	14.08	
	2	89	60	29	32.58	23.33±13.08
Kabete (High pollinator pop)	1	258	143	115	44.57	
	2	218	115	103	47.25	45.91±1.90

Table 2: The estimated degrees of outcrossing at six sites in Kenya

Site	Site mean %(p)	Estimated degree of outcrossing (%(p)x2)%
Katumani	17.72	35.44
Kibos	12.60	25.20
Kampi ya Mawe	20.98	41.96
Mtwapa	21.97	43.94
Kabete (Low pollinator pop)	23.33	46.66
Kabete (High pollinator pop.)	47.25	94.50

Counts of insect pollinators which visited $4m^2$ of the crop in a period of 5 minutes during anthesis between 8.00am and 9.00am, and their relationship with estimated degrees of outcrossing are presented in Table 3.

Table 3: The relationship between insect pollinator activity and degrees of outcrossing at six sites in Kenya

Site	No. of pollinators/ $4m^2$ in 5 min. (Mean ± SD)	Estimated degree of outcrossing %
Katumani	4.00 ± 2.00	35.44
Kibos	3.00 ± 1.00	25.20
Kampi ya Mawe	7.33 ± 2.08	41.96
Mtwapa	8.67 ± 3.79	43.94
Kabete (Low pollinator pop.)	9.00 ± 1.00	46.66
Kabete (High pollinator pop.)	22.00 ± 3.61	94.50

Table 3 shows a wide range of insect pollinator activity varying from 3.00 visits in $4m^2$ of crop area in 5 minutes at kibos to 22.00 at Kabete under high insect pollinator activity. There is a close positive relationship between insect pollinator activity and the degree of outcrossing at each site ($r = 0.994$; $p = 0.001$). This close relationship shows that the number of insect pollinators in an area is the major determinant of the degree of outcrossing in pigeon pea.

Experiment II

The %(p) values for experiment II are presented in Table 4.

Table 4: Estimates of %(p) when the number of (p)parents is in excess of (q)parents 50 times

Site	Replicate	No. of seedlings			Replicate %(p)	Site Mean %(p) ± SD
		Total	(g)	(p)		
Katumani	1	410	343	67	16.34	
	2	364	300	64	17.58	16.96±0.88
Kibos	1	402	343	59	14.68	
	2	379	328	51	13.46	14.07±0.86
Kabete (High Pollinator pop.)	-	-	-	-	-	-

Although the data presented in Table 4 is limited, these site mean %(p) values are very similar to those presented in Table 1 for Katumani and Kibos. However, since the (p) pollen was 50 times more abundant in experiment II as compared to experiment I, much higher %(p) estimates were expected.

Insect pollinator activity counts during anthesis in this experiment were 5.33, 4.33 and 24.67 for Katumani, Kibos and Kabete under high pollinator population respectively. Since the insect pollinator activity at these sites were quite similar for experiments I and II, these results are interpreted to mean that it is not the abundance of pollen from other plants in a pigeon pea population that determines the degree of outcrossing, it is the abundance and activity of insect pollinators which influence it directly.

The high estimates of outcrossing reported in this paper imply that the breeding of pigeon pea and maintenance of its cultures should be reconsidered, and the crop should be treated largely as an open-pollinated one.

REFERENCES

1. Abrams, R. 1967. Studies on natural cross pollination in pigeon pea (*Cajanus cajan*). J. Agric. Univ. PR, 57(1): 1 - 3.

2. Anonymous, 1974. Pigeon pea breeding. Ann. Rept. 1974-75 of ICRISAT, pp 158 - 168.

3. D'Cruz, R., and Deokar, A. B. 1970. Genetic studies in pigeon pea - I. N. green x red grained. Res. J. Mahatma Phule Agric. Univ. 1:44 - 53.

4. Deshmukh, N. T., and Rekhi, S. S. 1962. Study of natural crossing in pigeon pea (*Cajanus cajan* (L) Millsp.). Proc. Bihar Acad. Agric, Sci. 8 - 9: 135 - 139.

5. Gardner, C. O. 1961. An evaluation of effects of mass selection and seed irradiation with thermal neutrons on yield of corn. Crop Sci. 1: 241 - 245.

6. Howard, A., Howrard, G. L. C., and Khan, A. R. 1919. Studies in the population of Indian crops. I. Mem. Dept. Agric. Indian Bot. Ser. 10: 195 - 200.

7. Kadam, B. S., Kulkarni, R. M. and Patel, S. M. 1945. Natural crossing in *Cajanus cajan* (L) Millsp. in the Bombay-Deccan. Indian J. Genet. 5: 60 - 62.

8. Khan, T. N. 1973. A new approach to the breeding of pigeon pea (*Cajanus cajan* Millsp.). Formation of composites. Euphytica 22(3): 373 - 377.

9. Mehta, D. N., and Dave, B. B. 1931. Studies in *Cajanus indicus*. Mim. Dept. Agric. Indian Bot. Ser. 19: 1 - 25.

10. Onim, J. F. M. 1978. Pigeon pea breeding. The 3rd Progress Rept. for IDRC (Mimeographed) pp. 7 - 17.

11. Pursglove, J. W. 1968. Tropical Crops. Dicotyledons 1. Longmans, pp 236 - 241.

12. Rawal, K. M., Bryant, P., Rachie, K. O., and Porter, M. 1978. Cross pollination studies of Male-Sterile Genotypes in cowpeas. Crop Sci. 18(2): 283 - 285.

13. Shaw, F. J. F. 1932. The breeding of Cicer and Cajanus. Bull. Imp. Bur. Pl. Genet. 7: 11 - 16.

14. Williams, I. H. 1977. Behaviour of insects foraging on pigeon pea (Cajanus cajan (L) Millsp.) in India. Trop. Agric. 54(4): 355 - 363.

15. Wilsie, C. P. and Takahashi, M. 1934. Natural crossing in pigeon pea. J. Agric. Res. 49(10): 923 -927.

This work was supported by IDRC funds