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SOME ASPECTS OF NATURAL OUT-CROSSING IN *CAJANUS CAJAN* (L.) MILLSP.

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SUMMARY

The extent to which natural out-crossing occurs in pigeon pea and the distance over which contamination takes place in crops planted in adjoining plots was estimated using red standard petal colour and purple blotched pods as marker genes. Out-crossing was highest (28.3%) in the row closest to the marker. As the distance from the marker increased, the proportion of out-crossing decreased. Even the plot furthest away from the marker was contaminated, but the percentage out-crossing was very low (0.7%).

A linear regression fitted to out-crossing proportion (using logit transformation) and the distance at which out-crossing took place, showed that crop rows occupying a distance of 27' between cultivars reduced out-crossing to 3%. The estimated 95% confidence limits to this distance were found to be 16' to 43'.

The genetic consequences of 28.3% out-crossing in a population was calculated. The expected homozygosity at equilibrium was 77.4%. This was reached in the sixth generation. It was suggested that segregating populations could be handled as bulk up to about the sixth generation.

INTRODUCTION

Pigeon pea is an often cross pollinated crop. Gooding (1965) found out-crossing among eight cultivars, varying from 6.2 to 79.5%. The latter value is the highest on record. This study established that varietal response to out-crossing was variable. His mean out-crossing percent, estimated from four experiments conducted over a 2-year period, was 39.6. Krauss (1932) reported that out-crossing in Hawaii was less than 1%. Bust Wilsie and Takahashi (1934) estimated 13.98 to 15.86% cross pollination in the same location. Shaw (1931) in North India, and Abrams (1967) in Puerto Rico, have also reported low percentages of 0.15 to 7.56 and 5.80 respectively. In Central India, Deshmuk and Rekhi (1962) found 25.01% natural crossing. Sharma and Green (1975) reported 27.9% out-crossing in Hyderabad, India.

The Bee, *Apis* sp., is generally believed to be the commonest cross pollinating agent [Wilsie and Takahashi (1934); Akinola *et al.* (1975)]. The smaller bees were found to transfer pollen when their heads and legs were thrust forcefully through the keel petals. The heavier bee, on the other hand, *Megachile lavata*, depresses the keel and forces out the stamen in order to harvest pollen [Mahta and Dave (1931)]. Since anthers dehisce a day before opening of the flower, cross pollination is also attributed to thrips [(*Taeniothrips distalis*) Abrams (1967), Krauss (1932), Wilsie and Takahashi (1934)].

The estimation of natural cross pollination is a prerequisite for meaningful planning of breeding procedures, for maintenance of purity of breeding material, and for seed production. Thus far, estimates of cross pollination have been attempted for the first two aims stated above. There is no reference in the literature to isolation requirements for seed production. This study was conducted with the view to assess the extent of natural cross pollination and to determine the distance between cultivars, essential to produce seed of acceptable purity.

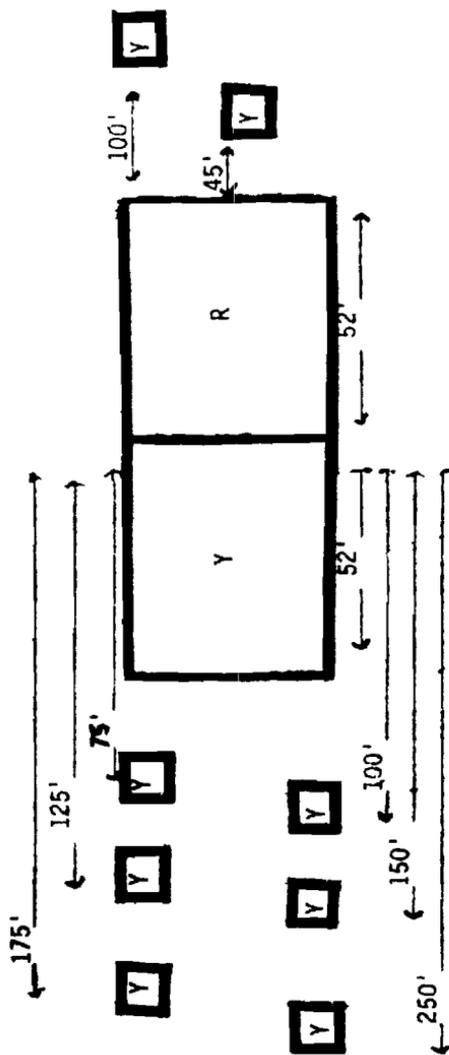
MATERIALS AND METHOD

Two semi-dwarf, fasciated, pure lines with a history of nine generations of inbreeding were selected for this study. One line bears red standard petals and purple blotched pods. The other has pure yellow standard petals and green pods. The latter two characters were reported [Krauss (1932)] recessive to red standard petal and blotched pods.

The layout consisted of a centrally located rectangular block, one half of which was planted with the recessive parent and the other with the dominant marker gene. The inter and intra row spacings were 3' and 2' respectively. Each genetic line was planted in four, single plant hills across the block and 26 hills lengthwise. In addition, at known distances from the marker gene, eight smaller plots were planted with the recessive parent as shown in Figure 1. Each of these plots consisted of nine plants planted in three rows at the spacing stated before.

The experiment was planted at the University Field Station in January, 1974. Surrounding the experimental area, corn and sunn hemp were planted to attract insects. Recommended cultural practices were followed. The only exception was that insecticidal sprays for obvious reasons, were withheld. Flowering occurred in April, 1974, but it was very irregular. Hence, the plants were pruned and allowed to grow vegetatively during the long day period of 1974. During this period, two more crops of corn were grown around the experimental area. With the onset of short days, both lines flowered and produced pods. Seed was collected from individual rows across the rectangular plot and the smaller blocks separately.

Fig. 1. Layout of out-crossing experiment



Y = Yellow flower - Green pod
 R = Red flower - Blotched pod
 Spacing - 3' x 2'

In January, 1975, a randomized, complete block design, replicated three times, was planted with 32 seed samples. Two row plots spaced 3' by 2' were planted to each lot of bulk seed.

Plants with red or veined standard petals and purple blotched pods were spored as natural hybrids, while plants with pure yellow flowers and green pods were considered as self fertilized or fertilized by another member of the recessive population.

RESULTS AND DISCUSSION

1. Extent of out-crossing

The extent of out-crossing in the progeny of the recessive lines planted at known distances from the marker is expressed in percentages (Table 1). The line closest to the marker (2') showed the highest value of out-crossing at 28.3%. As distance from the marker increased, out-crossing percentage decreased, but even at the most distant rows and plots, evidence of cross pollination was seen (Figure 2). At 24' from marker, 0% was recorded; from there onwards, crop pollination was erratic. From 24' to 250' there were 21 potential points of which 12 showed no out-crossing. However, even at 250', 0.7% out-crossing occurred. The out-crossing range among the 21 points was 0 to 3.8%.

The observed out-crossing in this study was substantially lower than the mean value of 39.6% recorded by Gooding (1965). The large difference may possibly be attributed to the degree of homozygosity of the test materials used in the two studies. Gooding appears to have used material that had undergone one or two generations of inbreeding. As against this the test material selected for this study were inbred nine generations.

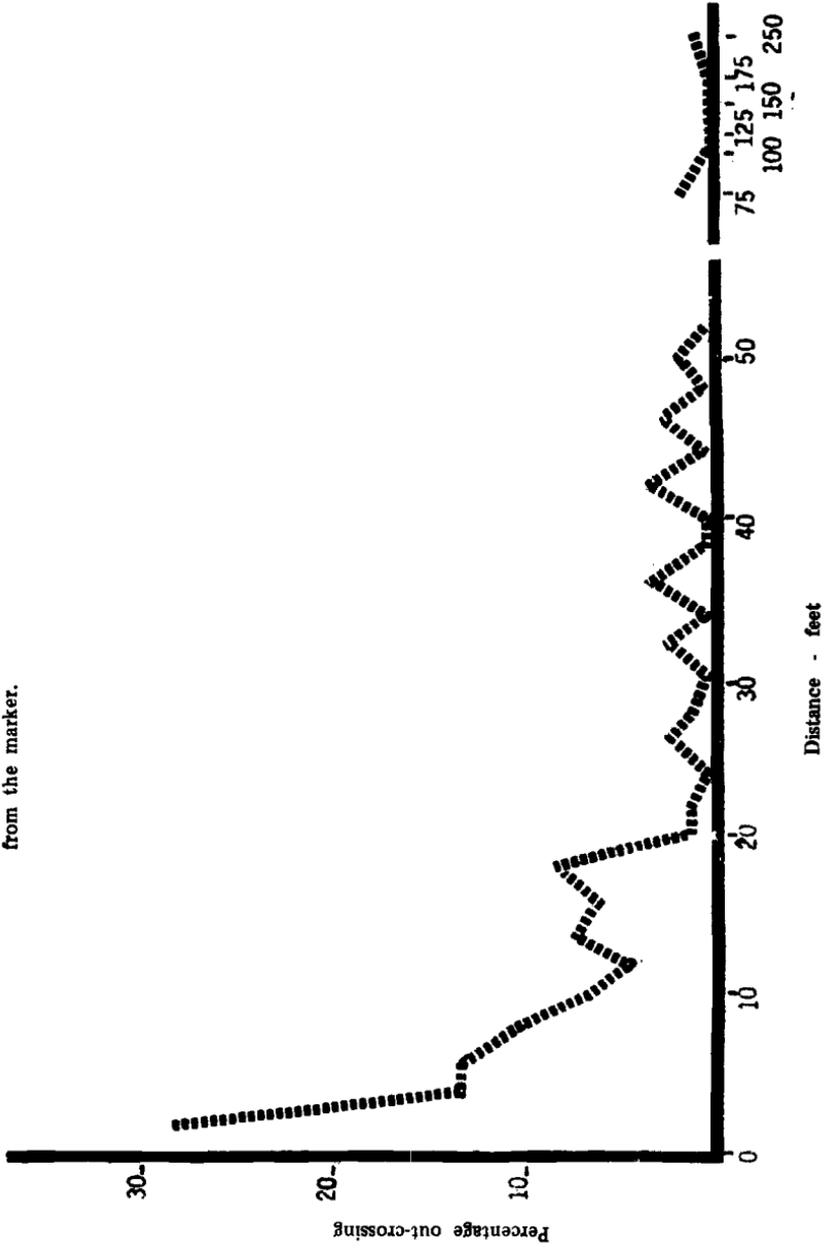
The out-crossing pattern observed (Figure 2) suggests that pollinators tend to contaminate an area in close proximity to the source of the foreign pollen.

It would appear that they seldom overfly wide sectors of intervening crop rows once they have settled down to their task. Had it been otherwise, at least a few of the rows and blocks furthest from the marker should have been contaminated as much as the rows nearer to it. The pollinators probably take short flights, visiting flowers closest to them. In so doing, they probably bring about a continuous masking or diluting effect of pollen previously acquired. Thus, the further they advance carrying foreign pollen on them, the lower would be their chance of bringing about any substantial degree of out-crossing.

TABLE 1. Out-crossing percent at known distances from marker.

Entry	Distance from marker (ft)	% out-crossing	Entry	Distance from marker (ft)	% out-crossing
1	2	28.3	17	34	0
2	4	13.7	18	36	3.8
3	6	13.4	19	38	0
4	8	10.9	20	40	0
5	10	6.6	21	42	3.4
6	12	4.7	22	44	0
7	14	7.7	23	46	2.6
8	16	6.1	24	48	0
9	18	8.5	25	50	1.6
10	20	1.7	26	52	0
11	22	1.1	27	75	1.4
12	24	0	28	100	0
13	26	2.4	29	125	0
14	28	0.9	30	150	0
15	30	0	31	175	0
16	32	2.3	32	250	0.7

Fig. 2. The out-crossing percent at known distances from the marker.



2. Purity maintenance

This pattern of visiting flowers, rather than via long random flights is an advantage to the seed producer with limited land area. A stretch of barrier rows sandwiched between varieties could effectively reduce contamination from one another. The distance required to reduce contamination to acceptable levels was estimated by fitting a linear trend to out-crossing proportion (using a logit transformation), against the distance at which out-crossing occurred. The logit transformation tends to enlarge the difference among observation points as the latter approach zero. In this instance, out-crossing ranged from 0 to 28.3%. Approximately one third of the observed values were zero.

The logit transformation is given by the formula:-

$$\lambda = \log_e \left(\frac{P}{1-P} \right)$$

Where λ = Logit Transformation

P = Proportion.

The first 26 observations representing the rows adjoining the marker were used to fit the linear regression (Figure 3). ($\hat{\lambda} = 1.571 + 0.143 D$). The estimated distance to obtain 97% purity or just 3% out-crossing was 27.0'. This distance is equivalent to approximately 13 crop rows spaced 2' apart. The estimated 95% confidence limits to this distance were found to be 16' to 43'. Thus, in a seed production programme, or purity maintenance programme, even the higher limit of 43' would not be too large a distance to be set aside for a barrier crop.

3. Breeding method

Assuming that 28.3% is the natural out-crossing rate in each generation, the genetic consequences of bulk population breeding was examined, in relation to breeding through 100% selfing. The level of homozygosity in each generation for the two methods was calculated using the formula [Sharma and Green (1975)]:-

$$P = P^1 \left[1 - \frac{1-h}{2} \right] + \frac{1-P^1}{2}$$

Where P is the percentage homozygosity,
 P^1 is the percentage homozygosity of the preceding generation and h is the percent selfing.

Fig. 3. The regression of out-crossing proportion to distance from marker.

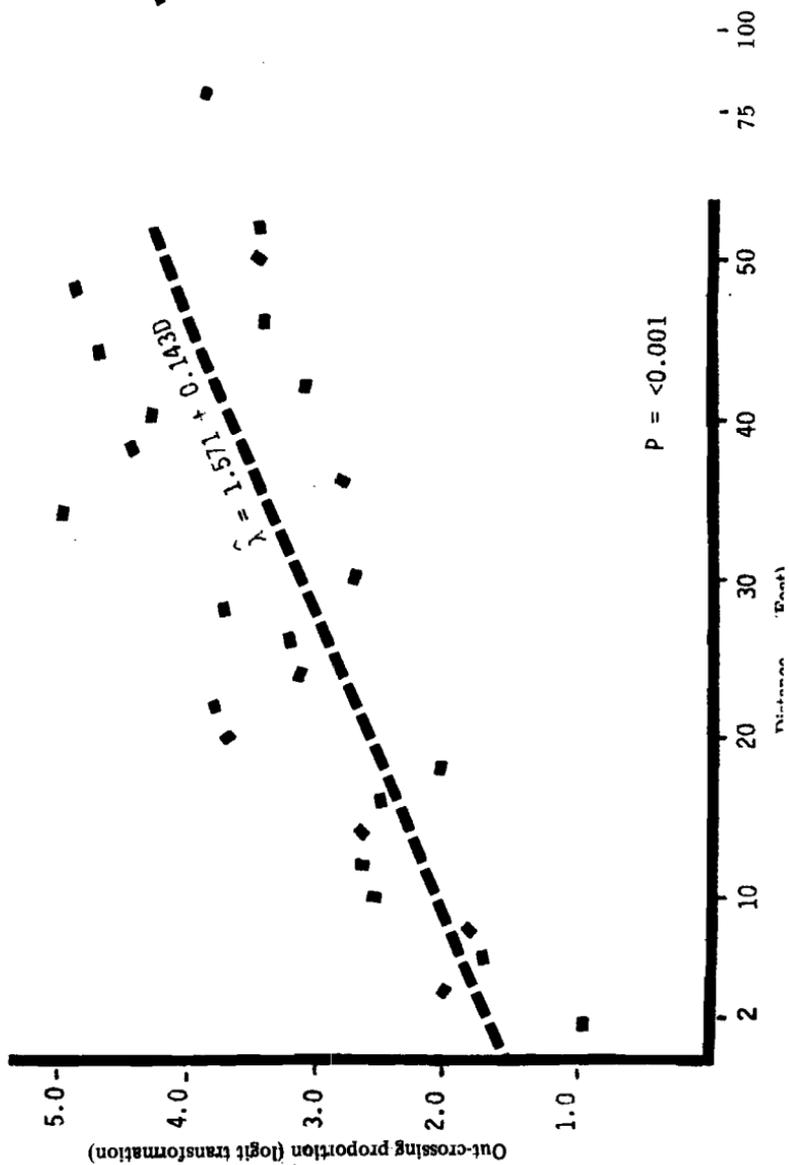


Figure 4 shows that homozygosity in a population out-crossed 28.3%, reached equilibrium from the sixth generation onwards. Out-crossing on the one hand and self pollination on the other, maintains this equilibrium. The expected homozygosity in the sixth generation is 77.4%. The corresponding figure for the 100% self pollinated population is 96.9%. Although the latter method is more efficient, considering other factors such as personnel, material, space, extensiveness of the hybridization programme etc., it might be reasonable to handle segregating generations as bulks up to about the sixth generation.

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Fig. 4. The expected average homozygosity in succeeding generations with 28.3% and 0% out-crossing.

