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Variation for natural out-crossing in pigeonpea

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Summary

Several researchers have reported a considerable degree of natural out-crossing in pigeonpea from different environments. This paper reviews the subject with respect to the variation for natural out-crossability, pollinating vectors, extent of natural out-crossing, isolation specifications, and the possible utilization of natural out-crossing in pigeonpea improvement.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a leguminous crop and its cleistogamous flowers predominantly favor self-fertilization. However, self-fertilization is not a rule in pigeonpea and a considerable degree of natural out-crossing is noticed. According to Onim (1981), pigeonpeas shed pollen while their flowers are still in the bud stage, and they do not start germinating until the flowers start to wither 24–48 h after their anthers dehiscence. Dutta & Deb (1970) studied pollen tube growth in a style pollinated with pollen from the same flower and observed it to be very slow, taking 54 h to reach the base of the ovary. Those two mechanisms provide a sufficient time gap for the foreign pollen to be introduced on to the stigma by insect pollinators before self-fertilization takes place.

The occurrence of natural out-crossing pigeonpea poses problems in developing pure lines and in maintaining the purity of released cultivars and germplasm accessions. Gupta et al. (1981a) reported that because of frequent natural out-crossing and practical difficulties in maintaining lines by selfing, the existing standard pigeonpea cultivars have become heterogeneous for several important agronomic characters, including disease resistance. A number of isolated reports of natural out-crossing in pigeonpea have been published from time to time, with a large variation in the extent of out-crossing. The findings of these studies are reviewed here and various aspects such as insect pollinators, extent of natural out-crossing, and its possible utilization in pigeonpea improvement are discussed.

Pollinating vectors

Natural out-crossing in pigeonpea takes place as a result of frequent insect visitation from one flower to another within and across the fields and, according to Onim (1981), each visit to the flower lasts between 15–55 seconds. He also observed that insect pollinators tripped almost all newly opened pigeonpea flowers by the end of the day, thereby introducing foreign pollen on the stigmatic surface.

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The activity and availability of pollinating insects is a major factor in determining the extent of out-crossing. At ICRISAT Center, Williams (1977) counted between 5500 and 107333 pollen grains on single *Xylocopa* spp. and *Megachile* spp. of insect pollinators, of which pigeonpea pollen accounted for 98 to 100%.

According to Pathak (1970), the bees *Megachile lanita* and *Apis florea,* visit open flowers and bring about cross-fertilization in pigeonpea. Williams (1977) identified 48 insect species visiting pigeonpea flowers at ICRISAT Center, while in Kenya Onim (1981) listed 24 insect species which may affect cross-fertilization in this crop. Williams (1977) reported that the main pollinating species at ICRISAT Center are *Apis dorsata* and *Megachile* spp., and that several insect species of *Vespidae, Diptera, Lepidoptera, Coleoptera, Neuroptera, Hemiptera,* and *Orthoptera* also frequently visited pigeonpea flowers but did not touch their anthers or stigma. *Apis cerana* does not forage on pigeonpea, either because the flowers are unattractive or because the insects are unable to obtain nectar or pollen from the flowers (Williams, 1977). She found that thrips (*Megalurothrips usitatus*) do carry pigeonpea pollen with them while moving from one flower to another, indicating their possible role in cross-pollination. Gupta et al (1981b) also reported the abundance of thrips activity in pigeonpea flowers; they were unable, however, to affect cross-pollination in this crop. Sen & Sur (1964) believed that thrips are responsible for both selfing and out-crossing in pigeonpea.

Extent of natural out-crossing

In pigeonpea, natural out-crossing at a particular location is determined by a combination of factors. These include the number of insect pollinators present in relation to the number of available flowers, the flowering habit of the varieties, the location of the field in relation to the insect habitat, cropping system, frequency of pesticide application, and environmental factors such as temperature, humidity, and wind velocity and direction (Bhatia et al., 1981).

For determining the degree of natural out-crossing, simply inherited traits, such as stem color (green *vs* purple), leaf type (obtuse *vs* normal), seed color (white *vs* brown), growth habit (determinate *vs* indeterminate), and flower color (yellow *vs* red) can be used. The number of plants with dominant allele observed within an open-pollinated single plant progeny of a recessive definite pure line will determine the extent of natural out-crossing that occurred in the preceding generation.

A large variation in the extent of natural out-crossing has been reported from various countries or locations within a country (Table 1). Howard et al. (1919) were the first to report 14% natural out-crossing in pigeonpea at Pusa Agricultural Research Institute in Bihar (India). At the same institute, Shaw et al. (1932) reported relatively less (1.6 and 3.4%) natural out-crossing in two different years. In Kanke (Bihar), Prasad et al. (1972) reported 3.8 to 26.7% natural out-crossing. In central India Mahta & Dave (1931), Kadam et al. (1945), and Deshmukh & Rekhi (1963) reported varying degrees of natural out-crossing in their experiments (Table 1). Veeraswamy et al. (1973) planted alternate rows of recessive and dominant marker genotypes and reported 13.7% natural out-crossing at Coimbatore in south India. Bhatia et al. (1981) conducted experiments on natural out-crossing at four Indian locations using a rectangular layout and reported a considerable variation from location to location in the extent of out-crossing.

Onim (1981) grew a mixture of green and purple stem lines in five environments in Kenya and reported natural out-crossing from 12.6 to 45.9% (Table 1). He also observed that the degree of natural out-crossing was positively associated with insect pollinator activity. Besides India and Kenya, a considerable degree of natural out-crossing (Table 1) has also been reported from Hawaii (Krauss, 1932; Wilsie & Takahashi, 1934), Puerto Rico (Abrams, 1967), Trinidad (Ariyanayagam, 1976), Australia (Byth et al., 1982), and Uganda (Khan, 1973).

The procedures used in estimating the degree of natural out-crossing in various studies do not permit the recovery of natural hybrids that would have resulted from out-crossing within the recessive and dominant marker populations and from recessive to dominant genotypes. If it is assumed that an equal amount of out-crossing was undetected, then the total amount of out-crossing would be considerably greater.

Variation for natural out-crossing ability

Besides the population of pollinating vectors and environmental conditions, the genetic constitution of a line also influences the degree of natural out-crossing in pigeonpea. This could be due to genetically determined morphological factors. Prasad et al. (1972) reported genotypic differences among pigeonpea cultivars in the extent of natural out-crossing. Byth et al. (1982) also observed a similar variation in two pigeonpea lines in Australia. Within the same field and same year, cvs. Prabhat and Royes exhibited >40% and <2% natural out-crossing, respectively. They attributed the reduced natural out-crossing in cv. Royes to a modification in its floral morphology, where the petals in the floral buds were wrapped and inter-locked. This prevented the contact of pollen carrying insects with the stigma of cv. Royes. On the contrary, in cv. Prabhat the bud petals were not wrapped and pollinators could work these buds with ease. An

Table 1. Percent natural out-crossing recorded in pigeonpea at various locations

Country/place	% Out-crossing		Reference
	Mean	Range	
In India			
Pusa		2.3–12.0	Howard et al., 1919
Pusa		1.6– 3.4	Shaw, 1932
Nagpur		3.0–48.0	Mahta & Dave, 1931
Nagpur	25.0		Deshmukh & Rekhi, 1963
Niphad	16.0	11.6–20.8	Kadam et al., 1945
West Bengal	30.0		Sen & Sur, 1964
Ranchi		3.8–26.7	Prasad et al., 1972
Coimbatore	13.7		Veeraswamy et al., 1973
Varanasi		10.3–41.4	Bhatia et al., 1981
Badnapur		0.0– 8.0	Bhatia et al., 1981
Coimbatore		10.0–70.0	Bhatia et al., 1981
Hyderabad	27.9		Sharma & Green, 1977
Hyderabad		0.0–42.1	Bhatia et al., 1981
Hyderabad		3.0–15.1	Bhatia et al., 1983
Hyderabad		4.0–26.0	Saxena et al., 1987a
In Kenya			
Katumani	17.7		Onim, 1981
Kibos	12.6		Onim, 1981
Makueni	21.0		Onim, 1981
Mtwapa	22.0		Onim, 1981
Kabete (low pollinators)	23.3		Onim, 1981
Kabete (high pollinators)	45.9		Onim, 1981
In other countries			
Hawaii	<1.0		Krauss, 1932
Hawaii	15.9	5.9–30.0	Wilsie & Takahashi, 1934
Puerto Rico		5.5– 6.3	Abrams, 1967
Trinidad	26.4		Ariyanayagam, 1976
Australia		2.0–40.0	Byth et al., 1982
Uganda		8.0–22.0	Khan, 1973

additional floral modification, selected from an inter-generic cross between *Cajanus cajan* (cv. ICP 6997) and *Atylosia lineata,* was also found to inhibit natural out-crossing in pigeonpea (ICRISAT, 1981). In this type of flowers, the keel petal partly surrounds the standard petal and enfolds the two wing petals, delaying flower opening until after the fertilization is complete. The mechanism that inhibits natural out-crossing in this genotype is not fully understood. However, it appears that a delay in the floral bud opening fails to attract insect pollinators, resulting in a high level of self-fertilization.

Non-genetic influences on floral biology are also likely to alter the pollination behaviour. Saxena et al (1987a) reported that in certain cases the genotypic differences observed in natural out-crossability at one location could be modified with a change in the environmental conditions. Mahta & Dave (1931) observed that the flowers of pigeonpea remained open at Pusa (Bihar) in northeastern India as long as a day and a half, but at Nagpur in central India it was normally for 6 hours. This is likely to influence the degree of out-crossing in a genotype.

In some pigeonpea genotypes, inherent crossability barriers are also reported. At ICRISAT we noticed an extremely high level of genetic purity in the open-pollinated progenies of germplasm accessions ICP 11947 and ICP 102, which was attributed to their inability to hybridize as a female parent. Singh et al. (1980) reported genetic variation for crossability in some pigeonpea lines. Saxena et al. (1987b) also reported cross-incompatibility in a pigeonpea line HPL 31. In such genotypes, foreign pollen left by the pollinators on the stigmatic surface will not result in cross-fertilization. These factors, when present in a pigeonpea line, will inhibit or reduce natural out-crossing, and such a line can easily maintain its genetic purity under open-pollination.

Isolation specifications

Natural out-crossing is the major source of varietal contamination in pigeonpea and, in order to maintain genetic purity of cultivars, seed multiplication in isolated blocks is necessary. On account of the wide range of out-crossing reported in the literature, the isolation specifications are equally variable. The FAO (Agric. Series No. 55), however, recommends an isolation in space at a minimum of 180 m to a maximum of 360 m (Ariyanayagam, 1976). For the production of foundation and certified seed of pigeonpea, Agrawal (1980) recommended isolation distances of 400 m and 200 m, respectively. Faris (1985a), however, suggested that for quality varietal seed production two pigeonpea varieties should be separated by at least 100 m, and for breeder's seed production this distance should be increased to at least 200 m. Based on out-crossing data, Sen & Sur (1964) recommended an isolation distance of 11 m while Ariyanayagam (1976) suggested that use of a crop barrier of 13 m width for maintaining the genetic purity of cultivars. Such diverse isolation recommendations could be due to the differences in the population of insect pollinators in different areas. Therefore, it is difficult to have an efficient and uniform recommendation on the isolation requirement for pigeonpea. Hence, it is imperative to determine the extent of out-crossing in an area to formulate suitable breeding and varietal maintenance programs. Pending this, the isolation specifications recommended by FAO may be followed.

Utilization of natural out-crossing in pigeonpea improvement

In recent years, there has been a growing feeling that breeding of self-pollinated crops suffers a great deal from their narrow genetic base and limited genetic recombination. The use of three-way, double-cross, and composite cross populations broaden the genetic base of the segregating populations to some extent, but owing to a predominant selfing mechanism the conservative forces of linkages restrict the reshuffling of genes coming together from diverse parents or keep it to the minimum. Therefore, random mating between selected genotypes by controlled hand pollinations has been recommended for increasing recombination in heterogenous base populations (Suneson, 1956; Jensen, 1978).

In view of partial natural out-crossing in pigeonpea, population improvement need not necessarily be confined to the various hand-pollinated intermating schemes based on self-pollinated crops. The extent of natural out-crossing in pigeonpea can be exploited with advantage by the use of suitable simple recessive markers and male sterility. Khan (1973) and Byth et al. (1981) proposed schemes for utilization of natural out-crossing in recombination breeding aimed at simultaneously creating genotypic variation and selection in pigeonpea. Green et al. (1979) used a combination of natural out-crossing and obtuse leaf recessive markers to develop dual populations in pigeonpea, as suggested by Rachie & Gardner (1975). In Kenya, Onim (1981) used natural out-crossing to develop disease-resistant, high-yielding pigeonpea composite populations. In Australia, E.S. Wallis (personal communication) used insects for mass hybridization to develop polycross populations in short-duration pigeonpea. Faris (1985b) developed six populations by using natural out-crossing for recurrent selection in pigeonpea at ICRISAT Center.

Besides genetic recombination, a substantial level of natural out-crossing in pigeonpea offers a unique opportunity for commercial exploitation of hybrid vigour. A heterosis breeding program in pigeonpea, based on natural out-crossing coupled with genetic male sterility, was initiated at ICRISAT Center, and high-yielding, drought-tolerant hybrids have been developed (Saxena, et al., 1986). The success of pigeonpea hybrids has enhanced the possibility of breaking the productivity threshold levels of pigeonpea.

The pigeonpea plant thus offers an opportunity to (i) manipulate the variation in its floral biology towards self-pollination and to handle such populations for improvement and maintenance by conventional breeding methods, and (ii) manipulate the variation towards out-crossing and handle the improvement of populations, through heterosis breeding, using genetic male sterility, cytoplasmic male sterility (to be searched in pigeonpea) and male gametocides (Kaul & Singh, 1976). However, for utilizing natural out-crossing for assured gains in pigeonpea yield through population breeding and hybrids, the breeder should determine the level of out-crossing at a particular location to develop efficient breeding and seed production strategies.

References

Abrams, R., 1967. Studies on natural cross-pollination in pigeonpea (*Cajanus cajan*). J Agril Univ Puerto Rico 51(1): 1–4.

Agrawal, R.L., 1980. Seed Technology. Oxford & IBH Publication Co., New Delhi, India, 685 p.

Ariyanayagam, R.P., 1976. Out-crossing and isolation in pigeonpeas. Trop. Grain Legume Bull 5: 14–17.

Bhatia, G.K., S.C. Gupta, J.M. Green & D. Sharma, 1981. Estimates of natural cross-pollination in *Cajanus cajan* (L.) Millsp. Several experimental approaches. Proc Intl Workshop on Pigeonpeas, 15–19 Dec 1980. ICRISAT Center, Vol 2: 129–136. Patancheru, A.P. 502 324, India. International Crops Research Institute for the Semi-Arid Tropics.

Byth, D.E., E.S. Wallis & K.B. Saxena, 1981. Adaptation and breeding strategies for pigeonpeas. Proc Intl Workshop on Pigeonpeas, 15–19 Dec., 1980. ICRISAT Center, Vol 1: 450–465. Patancheru, A.P. 502 324, India. International Crops Research Institute for the Semi-Arid Tropics.

Byth, D.E., K.B. Saxena & E.S. Wallis, 1982. A mechanism for inhibiting cross-fertilization in pigeonpea (*Cajanus cajan*(L.) Millsp.). Euphytica 31: 405–408.

Deshmukh, N.T. & S.S. Rekhi, 1963. Study of natural crossing in pigeonpea (*Cajanus cajan* (L.) Millsp.). Proc Bihar Acad Agric Sci 8–9: 135–139.

Dutta, P.C. & Deb Arati, 1970. Floral biology of *Cajanus cajan* (L.) Millsp. var. bicolour D.C. (Papilionaceae). Bull Bot Soc Beng 24(1/2): 135–145.

Faris, D.G., 1985a. Production of quality breeder's seed. Paper presented at the AICPIP. Kharif Pulses Workshop, TNAU, Coimbatore, India. 16–19 May 1985, 4 p.

Faris, D.G., 1985b. Pigeonpea population breeding at ICRISAT. Paper presented at the AICPIP. Kharif Pulses Workshop, TNAU, Coimbatore, India. 16–19 May 1985, 12 p.

Green, J.M., D. Sharma, K.B. Saxena, L.J. Reddy & S.C. Gupta, 1979. Pigeonpea Breeding at ICRISAT. Presented at the Regional Workshop on Tropical Grain Legumes, University of the West Indies, 18–22 June 1979. St. Augustine, Trinidad, 28 p.

Gupta, S.C., L.J. Reddy, D. Sharma, J.M. Green, A.N. Murty & K.B. Saxena, 1981a. Maintenance of pigeonpea cultivars. Proc Intl Workshop on Pigeonpeas, 15–19 Dec. 1980, ICRISAT Center, Vol 1: 295–302. Patancheru, A.P. 502 324, India. International Crops Research Institute for the Semi-Arid Tropics.

Gupta, S.C., M.V. Reddy & Y.L. Nene, 1981b. Role of thrips in pollination. Intl Pigeonpea Newsletter (ICRISAT) 1: 13.

Howard, A., G.L.C. Howard & A.R. Khan, 1919. Studies in the pollination of Indian crops. I. Memoirs, Dept Agric India (Botanical series) 10: 195–200.

ICRISAT, 1981. ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Annual Report 1979/80, pp. 101–102. ICRISAT, Patancheru, A.P. 502 324, India.

Jensen, N.F., 1978. Composite breeding methods and the DSM system in cereals. Crop Sci 18: 622–626.

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

Kaul, C.L. & S.P. Singh, 1967. Effects of some growth-regulators with gametocidal properties on *Cajanus cajan* (L.) Millsp. Ind J Agric Sci 37: 69–76.

Khan, T.N., 1973. A new approach to the breeding of pigeonpea (*Cajanus cajan* Millsp.). Formation of composites. Euphytica 22: 373–377.

Krauss, F.G., 1932. The pigeonpea, its culture and utilization in Hawaii. Bull. Hawaii Agric. Expt. Sta. 64: 1–46.

Mahta, D.N. & B.B. Dave, 1931. Studies in *Cajanus indicus*. Memoirs, Dept Agric India (Botanical Series) 19: 1–25.

Onim, J.F.M., 1981. Pigeonpea Improvement in Kenya. Proc Intl Workshop on Pigeonpeas. 15–19 Dec. 1980, ICRISAT Center, Vol 1: 427–436. Patancheru, A.P. 502 324, India. International Crops Research Institute for the Semi-Arid Tropics.

Pathak, G.N., 1970. Red gram. In: Pulse Crops of India. Indian Council of Agricultural Research 14–53.

Prasad, S., R. Prakash & M.A. Hassan, 1972. Natural crossing in pigeonpea (*Cajanus cajan* (L.) Millsp.). Mysore J Agric Sci 6: 426–429.

Rachie, K.O. & C.O. Gardner, 1975. Increasing efficiency in breeding partially outcrossing grain legumes. Proc Intl Workshop on Grain Legumes. 13–15 Jan 1975, ICRISAT Center, pp. 285–297. Patancharu, A.P. 502 324, India. International Crops Research Institute for the Semi-Arid Tropics.

Saxena, K.B., D.G. Faris, L.J. Reddy, D. Sharma, B.V.S. Reddy, S.C. Gupta & J.M. Green, 1986. Prospects for hybrid pigeonpeas. In: B. Napompath & S. Subhadrabandhu (Eds), New Frontiers in Breeding Researches Proc SABRAO Fifth Intl Congress, 25–29 Nov. 1985. pp. 379–388.

Saxena, K.B., D. Sharma, & D.G. Faris, 1987a. Ineffectiveness of wrapped flower in inhibiting cross-fertilization in pigeonpea. Euphytica 36: 295–297.

Saxena, K.B., B.K. Durga & L. Singh, 1987b. A case of cross incompatibility in pigeonpea. Intl Pigeonpea Newsletter (ICRISAT) 6: 33–34.

Sen, S.K. & S.C. Sur, 1964. A study on vicinism in pigeonpea (*Cajanus cajan* L.) Millsp. Agriculture Louvain (2) 12: 421–426.

Sharma, D. & J.M. Green, 1977. Problems and prospects of upgrading yield levels in pigeonpea. Proc Third Intl Congress SABRAO 2: 6–10.

Shaw, F.J.F., 1932. The breeding of *Cicer* and *Cajanus*. Bull Imperial Bureau of Plant Genet 7: 14–16.

Singh, B.V., M.P. Pandey & B.P. Pandya, 1980. Pod set in pigeonpea. Ind J Genet 40 (3): 568–572.

Suneson, C.A., 1956. An evolutionary plant breeding method Agron J 48: 188–191.

Veeraswamy, R., G.S. Palaniswamy & R. Rathanaswamy, 1973. Natural cross-pollination in *Cajanus cajan* (L.) Millsp and *Lablab niger* Medikus. Madras Agric J 60 (9–12): 1828,

Williams, I.H., 1977. Behaviour of insects foraging on pigeonpea (*Cajanus cajan* (L.) Millsp.) in India. Trop Agric 54: 353–363.

Wilsie, C.P. & M. Takahashi, 1934. Natural crossing in pigeonpea. J Agric Res 49 (10): 923–925.