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# Natural cross-pollination is both a boon and bane for pigeonpea breeders

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## ABSTRACT

Natural cross-pollination in pigeonpea, mediated by insects, is a universal phenomenon with the first report appearing in 1919. Considerable information is now available on various aspects of this biological phenomenon including degree of out-crossing and pollinating agents in different parts of the world. A large variation (0-60%) has been recorded in 30 different environments across 11 countries. The role of cross-pollination in rapid deterioration of the genetic purity of cultivars and elite genetic stocks is well understood and documented by breeders and seed producers. Pigeonpea breeders have converted this constraint into an opportunity by way of visually selecting natural hybrids from landraces in farmers' fields and deriving high yielding cultivars. In a key development, the occurrence of natural cross-pollination in the wild relatives of pigeonpea has also been recorded; and in three wild species such natural hybrids have yielded valuable cytoplasmic male sterility systems. This has encouraged breeders to develop a hybrid breeding technology with natural out-crossing playing a key role in the large-scale seed production of commercial pigeonpea hybrids.


**Key words:** *Cajanus cajan*, Pigeonpea, Cross-crossing, Genetic contamination, Pollinating insects.

A. Howard, G.L.C. Howard and A.R. Khan were the first group of pigeonpea [*Cajanus cajan* (L.) Millspaugh] breeders at Indian Agricultural Research Institute, Pusa (Bihar) who, while evaluating 68 single plant progenies from field collections in 1909, observed that 61 (89.7%) of the progenies were highly variable for different morphological traits. They also noticed incredible bee activity on pigeonpea flowers and thus concluded that the reason for this variation was the uncontrolled cross-pollination caused by various insects. To confirm this hypothesis, they conducted the first ever well-designed field experiment and recorded 2.25 to 12.0% out-crossing in different progenies. From these significant observations they concluded that in pigeonpea cross-pollination is a common event therefore in this crop both the maintenance of seed purity and pure line breeding will be a difficult (Howard *et al.* 1919). These observations were further confirmed in different parts of the world; but unfortunately, none of breeders took this information seriously and continued to develop cultivars using pure line breeding with limited success. In this paper the present scenario of cross-pollination in pigeonpea has been reviewed along with its pros and cons in the modern pigeonpea breeding programmes.

## RECORDS OF CROSS-POLLINATION IN DIFFERENT ENVIRONMENTS

A large variation has been reported in the extent of natural cross-pollination from different places. A number of biological and ecological factors such as genotypes, environment and density of pollinating vectors determine the extent of out-crossing at a particular location. The information available in literature is summarized in Table 1.

Howard *et al.* (1919), were the first to record 2-12% out-crossing at Pusa (Bihar), located in Eastern India. The other reports from this region were from Ranchi (4-27%) and Bengal (30%). In central India, the first report of 3-48% cross-pollination was published from Nagpur (Mahta and Dave, 1931). Subsequently, Kadam *et al.* (1945) also reported 12-21% out-crossing at Niphad. From South India, the results were available from Coimbatore, where 13.7% out-crossing was reported (Veeraswamy *et al.* 1973) and 0-42% in Hyderabad (Bhatia *et al.* 1981). From Northern India, Bhatia *et al.* (1981) reported 10-41% out-crossing at Varanasi; while Reddi *et al.* (2001) recorded 0-21% out-crossing at Delhi. The other Asian countries from where natural out-crossing was reported include China (0-60%), Myanmar (20-40%) and Sri Lanka (1-20%). In Africa, 13-46% out-crossing was observed in Kenya, while 15-39% out-crossing was reported from Cameroon. Natural out-crossing was also reported (Table 1) from Australia (2-40%), Hawaii (6-30%) and Puerto Rico (5-6%).

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## MECHANISMS INVOLVED IN CROSS-POLLINATION

### A typical pigeonpea flower

Pigeonpea flowers with a peculiar floral morphology encourage both self- as well as cross-pollination on the same plant. Pigeonpea flowers are not truly cleistogamous and only for the first 2-3 days, the buds remain cleistopetalous

(closed). Lord, (1981) described such floral configuration as "pre-anthesis cleistogamy". The flowers have zygomorphic corolla and large petals; and their standard petal is claw-shaped at base with 14-22 mm in length and 14-20 mm in width. The wing petal is also clawed at the base. It is asymmetrically biauriculate and obovate with a straight upper margin. The keel petals are dorsally split and large (14-17 mm long and 5-7 mm wide) enough to cover both androecium and gynoecium. The style is attached to a capitate stigma; while the sessile ovary with 4-6 ovules is superior with marginal placenta. The diadelphous (9+1) anthers of pigeonpea are yellow, ellipsoid, about 1-2 mm long, tapering towards the top and flattening at the base (Reddy, 1990). According to Rao and Bahadur, (1985) the 10 stamens are initially dimorphic in height. Of these, four have short filament and the remaining six carry longer filaments. The anthers of pigeonpea are connivent; dehiscing by longitudinal slits; latrorse / introrse. The anthesis of short stamens precedes that of the longer stamens. Pollengrains of are monads/single, yellow, powdery and bi-nucleate. Floral nectaries in pigeonpea are situated at the base of perianth.

**Activation of floral nectaries and production of nectar**

Little is known about the quantity, quality and process involved in nectar production in pigeonpea. Radhika *et al.* (2010) revealed that the nectar production in *Brassica napus* was regulated by a phyto-hormone called jasmonic acid and it is produced in floral nectaries endogenously. These nectaries get activated when the floral buds start growing; and the nectar production starts with flower opening; and it peaks when the corolla is fully expanded. This process continues until the corollas wither. There are genetic differences for nectar production within a species; and this variation has been found to be related to the size and structure of the flowers (Fischer and Leal, 2006; Palmer *et al.* 2001), besides the requirement to attract the pollinators (Heinrich, 1979). According to Kumar *et al.* (2009) the process of nectar production in pigeonpea flowers remains consistent throughout the day across the entire flowering duration. They also reported that once the processes of pollination and fertilization are over, the excreted nectar is gradually absorbed back into the plant system.

**Extended receptivity of stigma and competitive advantage of hybrid embryos**

The extended period of stigma receptivity is another key factor which encourages cross-pollination in pigeonpea. Dalvi and Saxena, (2009) reported that in a healthy pigeonpea flower its stigma becomes receptive a day prior to flower opening, but under natural conditions it cannot produce pod due to non-availability of self-pollen. The receptivity of stigma peaks on the day of flower opening and at this time pollen dehiscence also takes place to affect self- fertilization in majority of the buds. Both the pollinated and un-pollinated buds are exposed for cross-pollination. Since the stigma remains active up to 2-3 days after flower opening and the nectar production continues, the nectarivore insects are attracted by large attractive flowers and affect cross-pollination while harvesting the nectar. The foreign pigeonpea pollen grains have competitive advantage over self-pollen (Mazi *et al.* 2014). These pollen grains not only germinate faster on the stigmatic surface (Reddy and Mishra, 1981) than the self-pollen, but alsotheir pollen tube grows at a faster pace to affect cross-fertilization (Dutta and Deb, 1970; Choudhary *et al.* (2012). Thus in pigeonpea,one can find both selfed as well as hybrid embryos on the same branch or plant. Besides pigeonpea, the competitive advantage of hybrid embryos over the self-pollinated embryos was also demonstrated in common bean (Kendall and Smith, 1976; Ibarra-Perez *et al.* 1999) and Faba bean (Duc and Rowland, 1990).

**Nectarivore pollinating insects**

The natural cross-pollination in any crop species is accomplished with the assistance of external agencies such as wind, water, or animals. In pigeonpea, the natural cross-pollination was reported in the early part of 20$^{th}$ century and no definite information was available with respect to the pollinating agents. To understand the role of wind in cross-pollinating in pigeonpea, Kumar and Saxena, (2001) conducted an experiment under controlled conditions and demonstrated that in this crop wind does not play any role in cross-pollination. Under field conditions, therefore, some other external factor is responsible for it and insects were always the prime suspect since large bright coloured flowers of pigeonpea invariably attract a variety flying insects. The first attempt to identify pollinating vectors in pigeonpea was made by Sen and Sur (1964) and they observed that thrips move freely both inside and outside the closed buds and flowers, and opined that these might be responsible for cross-pollination in pigeonpea. Williams, (1977) and Gupta *et al.* (1981) also observed high thrip activity in pigeonpea flowers, but they concluded that being tiny creatures, the thrips were unable to perform cross-pollination in pigeonpea.

Over two dozen insect species have been found to forage on pigeonpea flowers in India, China and Africa but all do not participate in cross-pollination (Williams, 1977; Onim, 1981; Pando *et al.* 2011; Zeng-Hong *et al.* 2011). In general *Megachile*, *Xylocopa* and *Apinea* species were the most frequent visitors to pigeonpea fields and they actively participated in cross-pollination in different areas. The information gathered from different places is summarized in Table 2.

**Pollen collection and cross-pollination**

According to Bahadur *et al.* (1981) the 10 stamen in a pigeonpea flower are not uniformly oriented; and on the basis of their filament length they form two whorls; the four short ones are antisepalous and responsible for self-pollination. The remaining stamen have longer filament and their pollen maturity is delayed by a couple of days and coincides with the unfolding of petals to attract insects for cross-pollination.

**Table 1:** Reports of natural out-crossing in pigeonpea across countries.

| Country | Place | Out-crossing (%) | Reference |
|---|---|---|---|
| India | Pusa | 2-12 | Howard *et al.* (1919) |
| | Pusa | 2-3 | Shaw (1932) |
| | Nagpur | 3-48 | Mahta and Dave (1931) |
| | Nagpur | 25 | Deshmukh and Rekhi (1963) |
| | Niphad | 12-21 | Kadam *et al.* (1945) |
| | Bengal | 30 | Sen and Sur (1964) |
| | Ranchi | 4-27 | Prasad *et al.* (1972) |
| | Coimbatore | 14 | Veeraswamy *et al.* (1973) |
| | Varanasi | 10-41 | Bhatia *et al.* (1981) |
| | Badnapur | 0-8 | Bhatia *et al.* (1981) |
| | Hyderabad | 28 | Sharma and Green (1977) |
| | Hyderabad | 0-42 | Bhatia *et al.* (1981) |
| | Hyderabad | 315 | Bhatia *et al.* (1981) |
| | Hyderabad | 426 | Saxena *et al.* (1987) |
| | Hyderabad | 1024 | Githiri *et al.* (1993) |
| | New Delhi | 0-21 | Reddi *et al.* (2001) |
| Kenya | Katumani | 18 | Onim (1981) |
| | Kiboko | 13 | Onim (1981) |
| | Makueni | 21 | Onim (1981) |
| | Mtwapa | 22 | Onim (1981) |
| | Kabete | 23-46 | Onim (1981) |
| Cameroon | - | 15-39 | Pando *et al.* (2011) |
| Australia | Redland Bay | 2-40 | Byth *et al.* (1982) |
| China | Guangxi | 0-60 | Yang *et al.* (2003) |
| Hawaii | - | 6-30 | Wilsie and Takahashi (1934) |
| Myanmar | - | 20-40 | Kyu Kin Lay (Pers. Comm.) |
| Puerto Rico | - | 5-6 | Abrams (1967) |
| Sri Lanka | Maha Illupallama | 1-20 | Saxena *et al.* (1994) |
| Trinidad | - | 2-40 | Ariyanayagam (1976) |
| Uganda | Kampala | 8-22 | Khan (1973) |

**Table 2:** List of pigeonpea pollinating insects identified in different countries.

| Insect species | Location/country | Reference |
|---|---|---|
| *Apis mellifera* | India, Cameroon | Pathak (1970), Verma and Sidhu (1995), Pando *et al.* (2011) |
| *A. dorsata* | India, China | Pathak (1970), Brar *et al.* (1992), Zeng-Hong *et al.* (2011) |
| *Megachile bicolor* | India, Cameroon | Williams (1977), Brar *et al.* (1992), Pando *et al.* (2011) |
| *M. conjuneta* | India, Cameroon | Williams (1977), Verma and Sidhu (1995), Mazi *et al.* (2014) |
| *Xylocopa* (carpenter bee) | Cameroon, Kenya | Onim (1981, Pando *et al.* (2011) |
| *Bombus* (bumblebee) | Kenya | Onim (1981) |
| *Chalicodoma rufipas* | Cameroon | Mazi *et al.* (2014) |
| *C. cincta cincta* | Cameroon | Pando *et al.* (2011) |

The insects visit open pigeonpea flowers and the frequency of their visits and their foraging speed are directly related to the duration and amount of nectar produced by plants. In pigeonpea fields it has invariability been observed that the insect activity peaks in the second and third week of flowering, when plants have the maximum number of open flowers.

Saxena and Kumar, (2010) observed that on a clear sunny day the peak insect activity at ICRISAT farm occurred between 1000 and 1600 h; while in Cameroon, Pando *et al.* (2011) found that the insect activity in pigeonpea fields started at 0700 h and continued till 1600 h and it peaked between 0009 -1000 h. Onim, (1981) recorded that each insect visit to pigeonpea flower lasted for 15-55 seconds; while Pando *et al.,* (2011) recorded a high foraging speed of 10.33 flowers/min. Zeng-Hong *et al.* (2011) reported that the pollinating insects, on average, visited 4.8 flowers/10 minutes in China. Mazi *et al.* (2014) reported that bees,



**Fig 1:** A bee foraging on pigeonpea flowers.
Source: ICRISAT Photo Lab.

on average, sat on a flower for 28 seconds to collect pollen, 43 seconds to collect nectar and for 63 seconds to collect both nectar and pollen. The free stamen is provided with a groove at its base for nectarivore insects to access nectar.

During the process of foraging (Fig 1), the insects extend their proboscis to suck the nectar and during this process they brush anthers and a load of pollen get stuck on various body parts such as hairs, silk, legs, mouthparts, thorax, and abdomen. Williams, (1977) estimated that in an isolated pigeonpea field at ICRISAT, each insect had a load of about 5,500 to 107,333 pollen grains and >90% of this were from pigeonpea. The cross-pollination occurs when the pollen-laden insects forage on other flowers and rub the stigmatic surface.

### Factors encouraging cross-pollination

Pigeonpea is a peculier crop since it produces a lage number of flowers and majority of them drop, forcing plants to produce new flush of flowers. Also, being perennial and non-determinate crop, its plants continue their vegetative and reproductive stages simulteneously. They also remain in this condition until the required number of pod is set on a plant. Consequently at a given time, one can observe different sizes of floral buds, flowers and pods on the same inflorosence, branch, or plant. The extension of reproductive period, therfore, allows setting of more number of out-crossed pods on the plants.

In various field and horticultural crops certain reproductive disorders such as self-incompatibility, male sterility, *etc*. are not uncommon. In pigeonpea a number of male sterility sources have been reported (Saxena and Hingane, 2015). Besides this in some pigeonpea genotypes, Choudhary, (2011) also reported self-incompatibility and he postulated that the factors responsible for it were distributed randomly among genotypes. These situations encourage out-crossing and produce hybrid seed under natural conditions.

A good build-up of insect population during the flowering phase of the crop helps in cross pollination. According to Bhatia *et al.* (1981) there are certain natural factors such as temperature, humidity, wind velocity/direction and habitat which encourage natural out-crossing through influencing the build-up of pollinating insects. Besides these, the presence of alternate host plants such as specific fruit trees and legumes also help in maintaining a critical mass of pollinating insects. The presence of small water bodies, natural (ponds, rivers *etc*.) or artificial (tanks, irrigation channels, bore wells *etc*.) or water-logged/muddy paddy fields encourage harboring of the pollinating insects.

### Major constraint in maintaining genetic purity

In fact, natural out-crossing was always considered a major constraint in maintaining genetic purity of the varieties and breeding materials (Howard *et al.* 1919; Wilsie and Takahashi, 1934; Kadam *et al.* 1945). This turned out to be a serious issue due to poor seed production and delivery systems in all the pigeonpea growing countries, which forced farmers to use their own field-collected open-pollinated seed at the cost of quality and rapid loss of key traits (Gupta *et al.* 1981).

## BOON TO BREEDERS

### Boon for pigeonpea breeders

In general food legumes are considered a group of self-pollinating crops which maintain their genetic purity under natural growing conditions; but it is not entirely true and certain level of natural out-crossing has been takes place in crops like faba bean (*Vicia faba* L.), pigeonpea (*Cajanus cajan* L.), grass pea (*Lathyrus sativus* L.) *etc*. Pigeonpea breeders have been using the natural hybrids in cultivar development for a long time and more recently in hybrid breeding.

### Development of cultivars from natural hybrids within landraces

As a consequence of natural out-crossing a significant amount of gene exchange occurs within primary gene pool (Reddy *et al.* 2015). These mechanisms often lead to creation of new genetic variability in the segregating generations of the natural hybrids.

Pigeonpea breeders have grabbed this opportunity in the past and selected the heterotic segregants from within landraces and through pedigree breeding some elite cultivars were developed. According to Singh *et al.* (2016) between 1976 and 2012, a total of 86 pigeonpea varieties were released in India (Table 3). These included 33 early, 37 medium and 16 late maturing types. Further analysis of this information showed that more than one-third (32) of these cultivars originated from natural hybrids in the landraces, while the remaining were the products of deliberate hybridization and selection. In the early maturing group out of 33 cultivars released so far only six (18.18%) were selected directly from germplasm. The medium and long duration types cover majority of the pigeonpea area in India and elsewhere. The open-pollinated landraces are generally grown by farmers and this provided ample opportunity to select heterotic hybrid plants for further pedigree breeding. Almost half of the cultivars in medium (46%) and late (56.3%) groups had their origin from natural hybrids. The present day popular cultivars such as Maruti, BDN1 and LRG 30 in medium group and Gwalior-3, Bahar, and NA1 in the long duration group are the best examples



**Fig 2:** High yielding pigeonpea varieties developed from landraces (Photo: ICRISAT).




**Fig 3:** Plants of wild species *C. scarabaeoides* (right), $A_2$ cytoplasmic male sterile line (left) derived from a natural inter-specific hybrid and its anthers (center).




**Fig 3a:** Comparative plant morphology of three wild relatives of pigeonpea (left) and their natural hybrids (right).
Source: ICRISAT Photo Lab.

**Table 3:** Number of pigeonpea cultivars derived from natural hybrids identified in farmers' fields.

| Maturity group | Total released pigeonpea in India | Cultivars from natural hybrids | % of total release |
|---|---|---|---|
| Early | 33 | 6 | 18.2 |
| Medium | 37 | 17 | 46.0 |
| Late | 16 | 9 | 56.3 |
| **Total** | **86** | **32** | **37.2** |

Source: Singh *et al.* (2016).

where the selections from natural hybrid plants have made a great impact on Indian pulse scenario. Overall it is evident that the natural out-crossing in pigeonpea has been exploited very well by the breeders to the benefit Indian farmers in Asia and Africa (Fig 2).

**Development of CMS system from inter-specific natural hybrids**

Cytoplasmic nuclear male sterility (CMS), where the male sterility is maternally inherited, has been used extensively in a number of crops for commercial hybrid seed production in tandem with natural out-crossing. In pigeonpea since natural out-crossing is a common event, hybrid technology could not be developed for want of a stable CMS system. The decades long breeding efforts to develop a stable CMS ended when a natural hybrid was picked from a wild species *C. scarabaeoides* (Tikka *et al.* 1997). This natural hybrid with some unknown cultivated genotype as pollinator

**Table 4:** Seed production of pigeonpea hybrid (ICPH 2740) using CMS line through natural cross-pollination with different row ratios.

| Location | Area (ha) | Female (CMS line) | Male (pollinator) | Yield (kg/ha) |
|---|---|---|---|---|
| Rewa | 1.0 | ICPA 2047 (4 rows) | ICPL 87119 (1 row) | 1740 |
| Seoni | 1.0 | ICPA 2047 (4 rows) | ICPL 87119 (1 row) | 2500 |
| Katni | 3.0 | ICPA 2047 (3 rows) | ICPL 87119 (1 row) | 1450 |
| Jabalpur | 1.5 | ICPA 2047 (4 rows) | ICPL 87119 (1 row) | 1333 |
| Tikamgarh | 5.0 | ICPA 2047 (5 rows) | ICPL 87119 (1 row) | 3040 |

produced CMS system and designated as A2 (Fig 3). This CMS system was used to develop pigeonpea hybrids. Such natural hybrids within the population of wild species, raised from open-pollinated seed, can be easily detected due to their abnormal growth habit (Fig 3a). Besides this, the CMS systems were also bred using the natural out crosses in other wild species such as *C. lineatus* and *C. reticulatus* (Saxena, 2015). These were respectively, designated as $A_2$ and $A_6$ CMS systems.

### Development of commercial hybrid pigeonpea technology

Economically viable mass cross-pollination is the key for success of any commercial hybrid programme. In pigeonpea the hybrid technology is of recent origin (Saxena, 2015) and its natural out-crossing based large-scale seed technology is still evolving. Zheng-Hong *et al.* (2011) reported that the amount of cross-pollinated seed yield (384 g/plant) produced on the male sterile plants was similar to that of more frequently visited male fertile plants (357 g/plant). Almost similar results were also reported by Saxena *et al.* (2005). To introduce the high yielding hybrids on a large-scale, its seed production technology was first tested at research station for three years before its verification in farmers' fields using different male and female ratios. The hybrid seed yield in these on-farm trials varied from 1333 kg/ha at Jabalpur to 3040 kg/ha at Tikamgarh (Table 4). At present three commercial hybrids are being cultivated by farmers in southern and central India and their adoption is gradually increasing (Saxena and Tikle, 2015).

## CONCLUSION

It has been estimated that more than 85% of the crop species relied on ross-pollination of various degrees for their reproduction and to achieve this, they require some external pollinating agents such as animals, wind or water (Pratap, 2001). In economically important cereal crops the cross-pollination by wind is more frequent as compared to legumes, where insects play a greater role. The first and foremost benefit of natural out-crossing to breeding programmes was creation of new genetic variability, which has been exploited by pigeonpea breeders by way of breeding inbred cultivars and path breaking hybrid technology. Pigeonpea breeders, therefore, need to be aware about the ill effects of out-crossing and at the same time use it judiciously for the genetic enhancement of the crop. In pigeonpea the breeders have exploited the natural out-crossing to develop (i) high yielding inbred cultivars by selecting heterotic natural hybrid plants within landraces, (ii) cytoplasmic nuclear male sterility which provided a platform to develop a viable hybrid breeding technology and, (iii) economically viable hybrid seed production technology that helped in successful commercialization of hybrids.

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