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Redefining Isolation Distance Standards for Quality Seed Production in Hybrid Pigeonpea [*Cajanus cajan* (L.) Mill sp.]

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ABSTRACT

Background: Adopting adequate isolation distances is one of the very crucial principles and practices of quality seed production. Upon research and continuous evaluation, isolation distances for each crop, kind and cultivar have been determined in almost all cultivable crops. The development of hybrids through various mechanisms has led to redefining isolation distances in a few crops like pigeonpea based upon the principles of pollination ecology and the activity of various pollinators.

Methods: Our study on the direction of male and female blocks, activity and frequency of pollinators and their effect on production potential and quality of seed was systematically conducted at various isolation distances *viz*., 100, 150, 200, 250, 300 and 400 m between female (A) and male (R) lines.

Result: Our empirical information depicts that an isolation distance of 400 m between male and female parents reduces the chances of genetic contamination and hence the isolation distance in case of hybrid seed production of pigeonpea shall be redefined as 400 m.

Key words: Hybrid seed production, Isolation, Pigeonpea, Pollinators, Quality seed, Wind direction.

INTRODUCTION

The art and science of plant breeding is the application of genetic principles and breeding methods to a heritable variation of economic value. Genetic contamination due to untoward cross pollination is one of the main factors affecting the genetic identity and purity of a variety. Hence, an ideal seed production principle is to maintain a minimum possible physical distance between the seed crop and contaminating crops and such a physical distance is called isolation distance. For almost all crops, isolation distances had been standardized. However, with the introduction of new genes and genotypes, it is necessary to redefine isolation distances in light of factors affecting outcrossing *viz.*, floral biology, plant height, style receptivity to foreign pollens, pollen viability, the activity of pollinating agents, use of barriers in seed production, *etc.*

Among the legume crops, pigeonpea [*Cajanus cajan* (L.) Mill sp.] is an important crop for subsistence agriculture due to its drought tolerance, ability to recover from the losses caused by various stresses, high-protein (20-22%) content, quality fodder and fuel wood. Predominantly, pigeonpea flowers are self-fertilized with 20 to 40% cross-fertilization. Henceforth, pigeonpea is considered as often-cross-pollinated crop whose pollination is mainly affected by insect pollinators. The prescribed minimum seed certification standard for isolation distance for pigeonpea seed production is 250 m for foundation seed and 100 m for certified class. Since outcrossing has been recorded upto 20 to 40%, there is a need to redefine isolation requirements in pigeonpea to avoid genetic contamination and deterioration of genetic purity in the seed multiplication chain.

In our present study, we studied various factors affecting cross-pollination in hybrid seed production of pigeonpea (*Cajanus cajan* L.) with a research objective to standardize the isolation distance in the foundation and certified seed production regions for maintaining the highest possible genetic purity in pigeonpea. We recommend redefining of the existing standard of 250 m to 350 meters or more. From our observations, we promulgate compulsory redefining of isolation standards of other crops wherein hybrids are very popular and have an economic impact.

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MATERIALS AND METHODS

We conducted this experiment in three (3) consecutive seed production seasons of 2019 to 2021 in the agricultural research farm of the Seed Research and Technology Centre (SRTC), Department of Seed Technology, Professor Jayashankar Telangana State Agricultural University, Hyderabad, India. We have collected pure parental lines

(ICP 2043A, ICP 2043B and ICPR 2671R lines) and hybrid seeds of pigeonpea hybrid ICPH-2671 from Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, India.

In the process of evaluating the effect of isolation distances on effective outcrossing *i.e.,* distance traveled by pollen from male parent *i.e.,* B and R Lines and receptivity of stigma of female A line affecting fertilization and development of embryo which matures to be a seed *viz.*, female line (A) or F1 hybrid seed. In the case of foundation seed production (multiplication of parental lines), we undertook the sowing of male sterile (A) and maintainer (B) lines in different blocks at a distance of 100, 150, 200, 250, 300 and 400 m. A and B lines were sown in lines at a spacing of 60 cm × 30 cm and the plots are scattered at random directions in tune with the wind for assessing the effect of biotic (insect pollinators) and abiotic (wind) pollination factors.

Similarly, for producing F_1 hybrid seeds, we planted A and R lines in different blocks at a distance of 100, 150, 200, 250, 300 and 400m and female and restorer lines were sown at 60 cm × 30 cm, following a similar design of blocks.

We followed all standard packages of practices for a successful crop stand. Regular and continuous field visits were followed for assessing the crop health in order to undertake timely effective control measures, identification of rogues and plants belonging to other distinguishable varieties (ODV) *etc*, followed by strict and scrupulous roguing for uniform and homogeneous crop stand and also observations were made to identify any pigeonpea crop of similar or different genotypes are grown in the vicinity of the experimental location.

We have taken data pertaining to days to flowering in both the parents, synchronization, receptivity of stigma, pollen load per unit area, the direction of the wind, effective seed filling and also seed germination parameters like seed setting percentage, seed recovery, the activity of insect pollinator at regular intervals in the morning and evening times, endosperm to embryo ratio, germination percentage, seed viability, seed vigour index. We have bagged the female panicles for affecting self-pollination so as to understand the extent of selfing. Honeybees carrying pollen from contaminator plots were recorded as pollen gatherers. The nectar collectors are devoid of pollen in their pollen baskets. The pollen gatherers and nectar collectors were identified in consultation with the entomologist. The data collected over 3 seasons were compiled and subjected to statistical analysis for interpreting the effect of physical distance between parental lines on effective seed set and finally, the seed production potential.

RESULTS AND DISCUSSION

Isolation, Pollination and seed set

The observations on plant height reveals that male line (137.5 cm) is taller than female line (125 cm) which is an ideal mode of selecting parental lines in case of hybrid seed production (Table 1). When the male plant is taller than the female, it helps in the dispersal of pollen to longer distances as well as effective pollination by abiotic (wind) as well as biotic (insect pollinators) pollinating agents. During all three (3) seasons, the male restorer line (ICPR 2671R) started flowering around 85 days after sowing while the female male sterile line (ICP 2043A) started flowering at around 81 to 82 days after sowing. There is a gap of 3 to 4 days between flower initiation between the female and male lines, thus staggered sowing of 3 to 4 days is adequate for achieving maximum pollination and seed set. A very wide range of variation in the duration of flowering was observed between male and female lines *i.e.*, the duration of flowering in the male line is for 40 days while it is around 93 to 96 days in the case of the female line. Thus, another 4 to 5 spells of staggered sowing at weekly or 10 days intervals are required for attaining maximum yields. For assessing the nature of pollination of the female line, we bagged the flowers and studied the extent of the seed set. However, we could not find seed setting and filled seeds which elucidates that pigeonpea is a completely cross-pollinated crop. We observed a drastic variation in seed set on female lines which are planted at varying distances from the male line. The highest seed set of 40.7% was observed on female plants which are 200 m away from the male block which further decreased with distance *i.e*., 32.5 at 250 m and a mere 11.2% at 300 m while there is no seed setting on female panicles observed beyond 350 m or more. Thus, it's empirical to state that an isolation distance of 400 m is an ideal one for hybrid seed production in pigeonpea. However, Ariyanayagam (1976) suggested the use of a crop barrier of 13 m widths for maintaining the genetic purity of cultivars.

Isolation and pollinator activity

We have observed the activity of pollinators *i.e*., honey bees which are major nectar collectors as well as pollinators while

Table 1: Details of flowering and seed setting in male and female lines under study.

Lines	Plant height (cm)	Days to flower initiation (days)	Days to 50% flowering (days)	Duration of flowering (days)	Selfed seed on female lines	% Seed setting
ICPR 2671R	137.5	85.0	95.0	40.0	-	
ICP 2043A-D1 (200 m)	120.6	81.0	93.0	93.0	-	40.7
ICP 2043A-D2 (250 m)	124.3	82.0	94.0	94.0	-	32.5
ICP 2043A-D3 (300 m)	125.2	81.0	94.0	94.0	-	11.2
ICP 2043A-D4 (350 m)	118.8	81.0	94.0	94.0	-	-
ICP 2043A-D5 (400 m)	122.7	81.0	94.0	94.0	-	-

the carpenter bees (*Xylocopa* sp) and leafcutter bees (*Megachile* sp) are other significant insect pollinators observed in our research farm (Table 2; Fig 1 to 3). Their activity and frequency were regularly noted down daily in the morning (9 to 10 AM) and afternoon (3 to 4 PM) which are the hours of peak flowering as well as stigma receptivity.

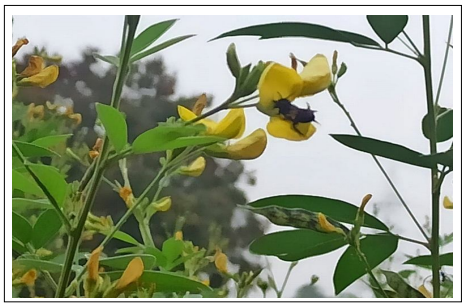

Fig 1: Leaf cutter bees (*Megachile disjuncta*) is a major pollinator and are active at morning hours.

Fig 2: Leaf cutter bees (*Megachile lanata)* is a major pollinator and are active at morning hours.

Fig 3: Carpenter bee (*Xylocopa spp*) is a major pollinator and is an obligate pollinator.

Data reveals that a maximum activity (78) of honey bees and pollen collectors is observed in the afternoon time (3 to 4 pm) as compared to 35 during the morning time (9 to 10 am). However, a similar pattern of the highest (25) activity of nectar collectors was observed in the afternoon while the lowest of 12 was observed during morning hours. It shows that both pollinators and nectar collectors prefer cool and dry conditions as compared to relatively hot and humid morning hours. Though we have collected data on pollinator activity throughout the day, we observed no pollinators between 8 am and 2 pm in a day. Our observations also depicted a variation in the activity of pollinators in the blocks of female and male parents *i.e*., highest in the vicinity of the male line and lowest in the female line. We also noted a decreasing activity of honey bees as the distance between the male and female line increases *viz*., the highest activity (16) was observed at a distance of 200 m which reduced to 13, 11 and 8 at 250 m, 300 m and 350 m, respectively. A similar pattern of reduced activity of nectar collectors was also observed *i.e.,* 12 at 200 m and 8, 5 to 3 between 250 m and 400 m, respectively.

All pulse crops are open-pollinated to varying degrees and the extent of pollination depends upon the wind and pollinator activity, Abrol *et al.* (2012) reported that pigeonpea, blackgram, greengram, beans, peas and cowpeas get benefitted from bee pollination to a varying range from 1,100% in beans to 600% in kidney beans. Thus, they observed a tremendous increase in yields of pulse crops which are pollinated by insect pollinators (Pando *et al.,* 2011) also reported that the majority of pollination in pigeonpea occurs due to pollinator activities. Various studies were carried out to investigate the interactions between the plant species and the *Megachile* bees because they affect pollination as a part of their adult foraging activity on the flowers and leaf-cutting activity on the leaves during regular visits. Smith (1879) revealed that *Megachile* spp, which are solitary leafcutter bees interact with almost 30 plant species for nectar as well as pollen and 19 plant species for leaf pieces from 17 plant families. However, these bees prefer plants from the Leguminosae family for both provision and leaf pieces. Similarly, Wilfredo *et al.* (2021) reported that natural pollination during their foraging behavior by carpenter bees (*Xylocopa* spp.) was also reported in many crops including pulse crops. From 7:00 AM to 5:00 PM, carpenter bees were spotted visiting the flowers, with 1:00 PM seeing

Table 2: Observations on pollinator activity at different isolation distances.

	Honey bees		Nectar collectors	
	FN (9-10 am)	AN (3-4 pm)	FN (9-10 am)	AN (3-4 pm)
ICPR 2671R	35	78	12	25
ICP 2043A-D1 (200 m)	8	16	4	12
ICP 2043A-D2 (250 m)	11	13	6	8
ICP 2043A-D3 (300 m)	4	11	4	5
ICP 2043A-D4 (350 m)	3	8	3	5
ICP 2043A-D5 (400 m)	4	6	0	3

Table 3: Observations on seed quality parameters in male and female lines.

Isolation distances/ Parental lines	Seed set %	Seed yield/ plant (g)	Test weight (g)	Germination (%)	Total seedling length (cm)	Seedling vigor index
ICPR 2671R	82	38.22	11.40	99	16.60	16.43
ICP 2043A-D1 (200 m)	41	28	11	68	11	7.62
ICP 2043A-D2 (250 m)	27	13	10	51	10	5.05
ICP 2043A-D3 (300 m)	6	2	10	45	6	2.84
ICP 2043A-D4 (350 m)	-	-	-	-	-	-
ICP 2043A-D5 (400 m)	-	-	-	-	-	-

the most activity. At 11:00 AM, when flower opening was at its busiest, the lengthiest mean time between trips to the flowers was also noted. Carpenter bee flight patterns likewise showed random distances, regardless of flower distances, according to Wilfredo *et al*. (2021).

Seed and seed quality parameters

We have taken observations on various seed and seed quality parameters like seed set (%), seed yield per plant (g), test weight (g), seed germinability and seedling vigour index which have illuminated a conspicuous variation in relation to the distance between the male and female blocks (Table 3). A mean 82% seed set was observed on male panicles across the three (03) seasons while a highest of 41% seed set was observed on female lines planted at 200 m away from the male block and a decreasing trend of seed set was observed at 250 m (27%) and 300 m (6%) and there was no conspicuous seed set beyond 300 m. Almost similar observations were recorded in the case of seed yield per plant with a maximum seed yield of 38.22 g/plant was observed on male plants and pertaining female lines recorded a highest of 28 g at 200 m, 13 g at 250 m and a mere 2 g at 300m isolation distance. Our observations on various parameters of germinability *viz*., germination percentage, viability and endosperm to embryo ratio revealed that those stigmas that received abundant pollen load affected more successful pollination and seed development while those ovules deprived of adequate pollen load affected the lowest pollination, fertilization and seed development and the data establishes aforesaid facts *viz.*, 68% on the female lines which is 200m away from the male block and 51% and 45% on female lines which are 250m and 300m, respectively away from the male lines. Similar observations had been observed in the case of total seedling length and seedling vigor index which indicates the ability of seeds to establish a healthy and successful crop stand in field conditions. The seedling vigor index showed a diminishing trend in seeds harvested from lines away from male lines.

CONCLUSION

Maintenance of genetic identity and purity are crucial in the quality seed production of pigeonpea. We considered both biotic and abiotic pollinating agents for understanding the distance pollen could travel to affect pollination and fertilization on the female parent and our findings confirmed that a minimum distance of 300 m isolation distance between A and B lines of ICP 2043 is ideal for avoiding contaminations in the seed production of pigeonpea.

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Conflict of interest: None.

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