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STANDARDIZATION OF OPTIMUM ISOLATION DISTANCE FOR GENETIC PURITY MAINTENANCE IN PIGEONPEA (*CAJANUS CAJAN* L. MILLSP.) HYBRID SEED PRODUCTION

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ABSTRACT
Hybrid technology has opened a new route to breaking the yield plateau in Pigeonpea (*Cajanus cajan* (L.) Millsp.), but its commercial success depends on producing seed of high genetic purity, which is difficult in an often-cross-pollinated crop where insects carry pollen over considerable distances. Establishing an optimum isolation distance is therefore essential to limit pollen-mediated contamination and ensure genetic purity of hybrid seed. This study was undertaken to validate and optimize the isolation distance for hybrid Pigeonpea seed production under field conditions. Cytoplasmic male sterile (CMS) line served as the female parent, with its fertility restorer (R-line) as the pollen source. Field experiments were conducted during Kharif 2020-21 and 2021-22 at the Central Research Station, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. In 2020-21, the CMS line was planted at nine distances from the pollen source (200 to 600 m at 50 m intervals), and the observations were validated in 2021-22 at five distances (200 to 400 m). Seed set, seed yield per plant and test weight were recorded at different isolation distances. Flowering synchronization between parental lines the activity of insect pollinators at different times of the day were also monitored to assess their association with pollen-mediated seed set. Seed set from pollen-mediated outcrossing declined sharply with distance and was negligible beyond 350-400 m, while seed quality (test weight) remained unaffected. A 400 m isolation distance is effective for maintaining genetic purity in hybrid Pigeonpea seed production, providing a practical standard for breeders and commercial seed producers to minimize unwanted pollen contamination and ensure genetic purity.

KEYWORDS: Pigeonpea, isolation distance, hybrid seed production, pollen dispersal, genetic purity.)

1. INTRODUCTION
Pigeonpea (*Cajanus cajan* (L.) Millsp.) among the most important grain legumes of tropical and subtropical regions, serving as a major source of dietary protein for millions of people, particularly in developing countries. In India, its wide adaptability to diverse agro-climatic conditions, tolerance to drought and capacity to enrich soil through biological nitrogen fixation make it a mainstay of rainfed farming systems. Unlike most pulses, which are predominantly self-pollinated, Pigeonpea is an often cross-pollinated species, with natural outcrossing reported at 20 to 70%, mediated chiefly by insect pollinators such as honey bees (*Apis* spp.), carpenter bees (*Xylocopa* spp.) and leaf-cutter bees (*Megachile* spp.) (Saxena and Sharma, 1990; Saxena et al., 2010). Its floral morphology promotes both self- and cross-pollination, providing an excellent opportunity to exploit heterosis through hybrid breeding. The commercial release of cytoplasmic-genic male sterility (CGMS)-based hybrids has significantly strengthened the prospects of increasing Pigeonpea productivity. The same reproductive behaviour, however, makes it difficult to preserve the genetic purity of varieties and hybrids during seed production. Because pollen is carried mainly by insects rather than wind, the extent of contamination depends on pollinator abundance, foraging behaviour and the spatial separation between seed production fields. Adequate isolation distance is therefore among the most critical requirements for producing genetically pure hybrid seed.

The isolation standards currently followed for Pigeonpea seed production were framed several decades ago under production environments that differed considerably from those of today. Since then, hybrid breeding technologies, cropping systems, pollinator populations, climatic conditions and seed production practices have changed substantially and the existing recommendations have seldom been revisited. Consequently, the existing isolation distance recommendations require scientific validation under present-day production environments to ensure their continued suitability.

Several studies have reported the occurrence of pollen-mediated gene flow in Pigeonpea; however, information on the effective distance over which economically significant pollen movement occurs under field conditions remains limited. Moreover, few studies have attempted to validate existing isolation standards under practical hybrid seed production systems. Establishing scientifically validated isolation distances is essential for maintaining prescribed genetic purity standards while optimizing land use and reducing the cost of hybrid seed production. The present investigation was therefore undertaken to quantify pollen-mediated gene flow at different isolation distances under field conditions and to validate the isolation standards for Pigeonpea hybrid seed production.

2. MATERIALS AND METHODS

Experimental site and plant material

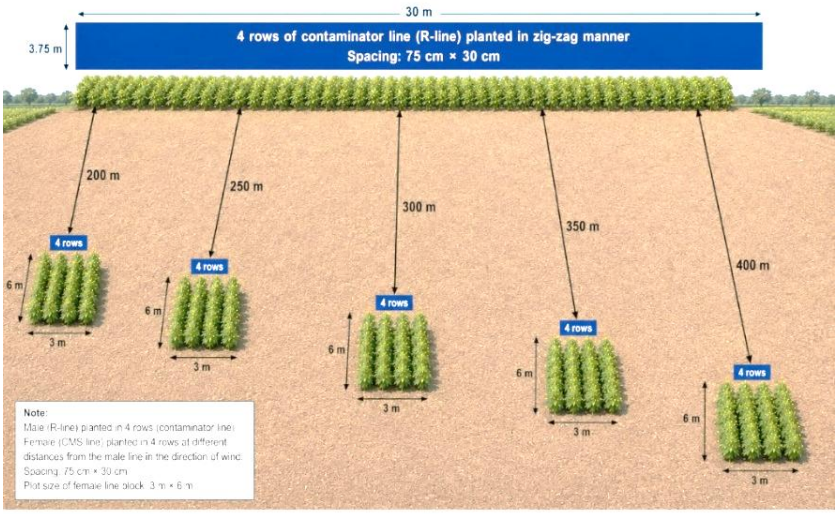
Field experiments were conducted during the kharif season of 2020-21 and 2021-22 at the Central Research Station Farm, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. The experimental site is located in the semi-arid tropics of central India, with a monsoon-dominated climate marked by hot summers, moderate rainfall and mild winters, representing a major Pigeonpea-growing agro-ecological zone. The experimental material comprised the cytoplasmic-genic male sterile (CGMS) line ICPA 2740, used as the female parent (A-line), and its corresponding fertility restorer line ICPR 2047, used as the male parent (R-line) and pollen source. Both lines were obtained from the ICAR-Indian Institute of Seed Science (ICAR-IISS), Mau, Uttar Pradesh, India, and are standard material in CGMS-based hybrid Pigeonpea seed production.

Experimental design and field layout

The pollen source/ R Lines/Male parent plot (2.25 m × 24 m in 2020-21; 3.75 m × 30 m in 2021-22) was raised on one side of the field, in line with the prevailing wind direction at a spacing of 75 cm between rows and 30 cm between plants. Plots of the A-line, each of three rows (1.50 m × 3.00 m in 2020-21; 6.0 m row length in 2021-22), were established at fixed distances from the pollen source along the same direction. In 2020-21, the isolation distances were 200, 250, 300, 350, 400, 450, 500, 550, and 600 m. and in 2021-22 they were 200, 250, 300, 350, and 400 m. To prevent contamination from external pollen, no other flowering Pigeonpea was permitted within the experimental perimeter.

Crop establishment and management

The parental lines were sown on 10 July 2020 at 75 cm × 30 cm spacing. All recommended agronomic practices were followed. Fertilizer was applied in two splits, the first as a basal dose at sowing and the second one month later. Insecticides were sprayed one month after sowing and again before flowering to control pests while avoiding any adverse effect on pollinators during flowering. Irrigation and weeding were carried out as per the recommended package of practices for Pigeonpea.



Note: AI-generated schematic illustration prepared for better understanding and visualization of the experimental layout

Assessment of pollen-mediated outcrossing

At physiological maturity, seed was harvested separately from each A-line plot at every isolation distance. Seed set, seed yield per plant and test weight were recorded. The seed from each distance was then grown out, and the occurrence of hybrid plants in the progeny was used to estimate pollen-mediated outcrossing. Per cent natural cross-pollination at each distance was calculated and used to identify the minimum isolation distance needed to maintain the prescribed genetic purity standard in commercial hybrid seed production.

Pollinator Activity

Pollinator activity was recorded at peak flowering through timed observations in two periods, 08:00-09:00 h and 15:00-16:00 h. Insects were grouped based on foraging behaviour as pollen gatherers or nectar collectors. Observations were recorded on five randomly selected plants at each isolation distance, across flowering period to capture temporal variation in pollinator visitation

3. Results

3.1 Flowering behaviour and synchronization

In both years the R-line flowered earlier than the CGMS line. In 2020-21, the R-line initiated flowering at 110 days after sowing (DAS) and reached 50% flowering at 117 DAS. The CGMS line-initiated flowering at 115-120 DAS and reached 50% flowering at 124-130 DAS, 7-13 days later than the R-line. In 2021-22 the corresponding values were 112 and 117 DAS for the R-line and 121-123 and 126-129 DAS for the CGMS line, a lag of 9-12 days. The reported flowering duration was similar for both parents (127-132 days in the CGMS line and 130-131 days in the R-line), so the flowering periods overlapped for most of the season. Bagged CGMS plants showed no selfing (0.0) at any distance, confirming complete male sterility. Any seed set on these plants therefore resulted from cross-pollination.

3.2 Plant height

The R-line was taller than the CGMS line in both years: 205.5 cm against 193.5-197.2 cm in 2020-21 (8-12 cm taller) and 215.1 cm against 208.6-212.6 cm in 2021-22 (2.5-6.5 cm taller).

The difference in plant height between the male and female parents reflects the morphological characteristics of the parental lines. Plant height did not vary appreciably among the CGMS plots at different distances, so it is unlikely to explain differences in seed set. Saxena et al. (2010) reported that moderate differences in plant stature do not necessarily limit natural cross-pollination when flowering synchronization is maintained.

3.3 Seed set at different isolation distances

Seed set on CMS plants declined with increasing distance from the pollen source in both experimental years (Table 1). In 2020-21, seed set was 42.6, 31.22, 27.28 and 33.33% at 200, 250, 300 and 350 m, respectively, and nil at 400-600 m. For comparison, seed set in the R-line was 72.2%. In 2021-22, seed set was 40, 25 and 18% at 200, 250 and 300 m, and nil at 350 and 400 m. The decline was not strictly progressive in 2020-21, since seed set at 350 m exceeded that at 300 m. Seed set was nil at 400 m and beyond in both years, and nil from 350 m in 2021-22.

Table 1. Seed set, seed yield and test weight of the CGMS line at different isolation distances

Distance (m)	Seed set (%) 2020-21	Seed set (%) 2021-22	Seed yield plant^{-1} (g) 2021-22	Test weight (g) 2021-22
200	42.6	40	47	110
250	31.22	25	29	111
300	27.28	18	20	110
350	33.33	0	–	–
400	0	0	–	–
450-600	0	not tested	–	–

Pigeonpea is predominantly self-pollinated but exhibits natural outcrossing mediated mainly by insect pollinators. Ariyanayagam (1976) reported that natural outcrossing in Pigeonpea decreased with increasing distance and reported low outcrossing at greater distances under the conditions of his study. The present findings indicate considerably greater pollen-mediated seed setting at 200-350 m, followed by complete absence of seed setting beyond 350 m. The results further emphasize the importance of adequate spatial isolation in Pigeonpea seed production. Saxena (2006) highlighted the importance of appropriate seed-production practices for maintaining genetic purity and ensuring successful hybrid seed production in Pigeonpea. In the present study, the absence of seed setting beyond 350 m suggests that an isolation distance of 400 m provides an appropriate safety margin for minimizing unwanted pollen-mediated fertilization under the prevailing experimental conditions. Therefore, an isolation distance of 400 m may be considered for maintaining genetic purity in Pigeonpea hybrid seed production under similar environmental and pollinator conditions.

3.4 Seed yield and test weight

In 2021-22, seed yield per plant fell with distance, from 47 g at 200 m to 29 g at 250 m and 20 g at 300 m. No seed was produced at 350 and 400 m. Test weight was stable (110-111 g) at the three distances where seed was produced. This suggests that distance affected the number of seeds set rather than seed size. Test weight could not be compared at distances without seed. Thus, successful fertilization and consequent seed formation appeared to be the major factors responsible for variation in seed yield, whereas test weight remained comparatively stable and was likely influenced more by the genetic characteristics of the parental material and seed-filling conditions. The observed pattern is consistent with the findings of Saxena et al. (2005).

3.5 Pollinator activity

Honeybees and other insects visited both parental lines throughout flowering in both experimental years (Tables 2 and 3). In both years, morning counts (08:00-09:00 h) were 4-6 insects per observation for pollen gatherers and for nectar collectors. Afternoon counts (15:00-16:00 h) were 2-4. Counts showed no consistent trend with distance from the pollen source, and insects were active even in plots where no seed was set (400 m and beyond). The decline in seed set therefore cannot be explained by an absence of pollinators at the female plots. It more likely reflects the limited distance over which pollen was carried. This is consistent with reports that insects are the main agents of outcrossing in Pigeonpea (Free, 1993; Saxena et al., 2010) and that wild and managed bees visit Pigeonpea flowers and improve fruit and seed set (Pando et al., 2011). Pando et al. (2011) recorded 18 insect species visiting Pigeonpea flowers at Yaoundé, Cameroon. Among these, Chalicodoma cincta cincta was the most frequent visitor, accounting for approximately 29% of the recorded visits. Wilfredo et al. (2021) studied carpenter bee (*Xylocopa* spp.) pollination in yellow passion fruit rather than Pigeonpea. Their findings demonstrated that pollinator visitation varied during the day and contributed to reproductive success. Therefore, the study provides supporting evidence for the general importance of diurnal pollinator activity, rather than direct evidence for the specific timing of pollen dehiscence or nectar availability in Pigeonpea. Reduced pollinator activity during the afternoon may be associated with environmental conditions and changes in floral rewards; however, these factors were not directly measured in the present study and should therefore be considered as possible explanations rather than definitive causes.

Table 2. Pollinator activity (number of insects per observation) on the R-line and CGMS line at different isolation distances, Kharif 2020-21, Dr. PDKV, Akola

Parental line / distance	Honeybee/Other pollinators			
	Pollen gatherers		Nectar collectors	
	FN (08:00-09:00 h)	AN (15:00-16:00 h)	FN (08:00-09:00 h)	AN (15:00-16:00 h)
R-line (male)	4	3	5	4
CGMS line at different distances from Male Line				
D1 (200 m)	5	3	4	2
D2 (250 m)	6	4	5	3
D3 (300 m)	5	2	5	3
D4 (350 m)	4	3	6	2
D5 (400 m)	5	2	4	3
D6 (450 m)	6	3	5	2
D7 (500 m)	4	2	4	2
D8 (550 m)	--	--	--	--
D9 (600 m)	--	--	--	--
Mean of CGMS plots (D1-D7)	5.0	2.7	4.7	2.4

Table 3. Pollinator activity (number of insects per observation) on the R-line and CGMS line at different isolation distances, Kharif 2021-22, Dr. PDKV, Akola

Parental lines	Honeybee/other pollinators			
	Pollen gatherers		Nectar collectors	
	FN (08:00-09:00 h)	AN (15:00-16:00 h)	FN (08:00-09:00 h)	AN (15:00-16:00 h)
R-line (Male)	5	4	6	4
CGMS line at different distances from Male Line				
D1 (200 m)	6	3	4	2
D2 (250 m)	5	2	6	3
D3 (300 m)	6	4	5	3
D4 (350 m)	4	2	4	2
D5 (400 m)	5	3	5	4
Mean of CGMS plots (D1-D5)	5.2	2.8	4.8	2.8

Note: FN = forenoon; AN = afternoon. Values are the number of honeybees and other insect visitors observed on ten randomly selected plants at each distance.

4. DISCUSSION

Seed set on the male-sterile CGMS line declined with distance from the pollen source in both years. The decline in seed set with increasing distance from 42.6% at 200 m to 0% beyond 400 m, and in 2021-22 already at 350 m. Because bagged CGMS plants set no seed, this seed set can be attributed to insect-mediated cross-pollination. The pollen source therefore acted as a proxy for any foreign pollen source, and the results indicate how far effective pollen flow extended from it. The distance at which seed set reached zero differed between years: 400 m in 2020-21 and 350 m in 2021-22. Seed set was still 33.33% at 350 m in 2020-21, so the last distance with seed set in that year was only 50 m short of the recommended 400 m. Pollen movement over these distances probably varies with pollinator populations, flowering overlap and weather, and the one-year difference shows that this variation is real. A 400 m distance therefore gives a margin at the edge of the observed range rather than a large safety buffer.

Both parents flowered over nearly the same period, with the CGMS line reaching 50% flowering 7-13 days after the R-line. The R-line's long flowering period meant pollen was available while the CGMS line was receptive. Pollinators were more active in the morning than in the afternoon, and they were present at all distances. Together, these results suggest that the distance pollen travels, not pollinator availability, limits contamination. Temperature and floral rewards were not measured, so we cannot say what reduced afternoon activity.

Test weight was similar at the three distances that produced seed. Seed size therefore seems to depend on the genotype and seed-filling conditions more than on the pollen load. Distance influenced the number of seeds set (seed set and yield per plant), which is the practical concern for seed producers. Taken together, the results support 400 m as an isolation distance for CGMS-based hybrid Pigeonpea seed production under conditions similar to these. This is a practical, evidence-based recommendation that [compare with the isolation distance in the current Indian Minimum Seed Certification Standards, with citation].

5. CONCLUSIONS

Isolation distance was a decisive factor in controlling pollen-mediated contamination in hybrid Pigeonpea seed production. Seed set on the male-sterile line declined with increasing distance from the pollen source in both years and fell to nil at 400 m and beyond (and at 350 m in 2021-22). Test weight was not affected at the distances where seed was produced. Pollinators were active at all distances, with higher counts in the morning. An isolation distance of 400 m is therefore recommended for commercial hybrid Pigeonpea seed production under comparable agro-climatic and pollinator conditions.

An isolation distance of 400 m is therefore recommended for commercial hybrid Pigeonpea seed production under comparable agro-climatic and pollinator conditions. This technology was recommended for release at the 37th Annual Group Meeting of the All India Coordinated Research Project (AICRP) on Seed (Crops), 2022, and was certified by ICAR-Indian Institute of Seed Science, Mau, in 2023. Its adoption can help maintain genetic purity and strengthen the quality assurance framework for hybrid Pigeonpea seed production.

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Conflicts of Interest: The authors declare no conflicts of interest.

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