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Overview of hybrid pigeonpea seed production technology and its on-farm validation

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Abstract

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is known for its high protein grains and it occupies an important place in subsistence agriculture of tropics and sub-tropics. The major constraint of the crop has been its low productivity. The recent emergence of hybrid technology in this crop has provided a platform for breaking its decades-old low yield plateau. In the last four years three CMS-based pigeonpea hybrids with 30-50% on-farm yield advantage were released in India. To increase the national pigeonpea production, now efforts are being made to take this technology to the door steps of farmers in a big way. To achieve this, an easy and economically viable seed production technology was successfully developed. This paper, besides describing the salient features of this technology, discusses results of its on-farm seed production program. On average, hybrid yields of over 1000 kg/ha were recorded with a seed-to-seed ratio of 1: 200. In the last two seasons the adoption of hybrid technology has shown very positive response from the cultivators with its planted area stands beyond 150,000 ha mark in 2015.

Keywords: hybrids; on-farm validation; pigeonpea; seed production technology.
Abbreviations: FAO_Food and Agricultural Organization of the United Nation.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an environment-friendly crop with qualities of improving soil fertility and structure. It is an important food legume of semi-arid tropical regions of Asia, Africa, and the Caribbean islands, where it is grown on over 5 m ha (FAO 2013). However, its major share (75%) comes from India, where it is widely consumed and serves as an important protein supplement. The national demand of pigeonpea in India always scores over the production, and in spite of decades of research and development programs, the mean productivity of the crop could not cross the barrier of 800 kg/ha. To break this yield plateau ICRISAT developed a hybrid breeding technology, globally the first in any food legume. This technology is based on cytoplasmic nuclear male sterility (CMS) and natural out-crossing. In the last four years three commercial hybrids have been released in India, and these have demonstrated 30-50% yield advantage over the most popular local cultivars in farmers' fields. To enhance the adoption of these hybrids, it is necessary that quality hybrid seed is produced in large quantities and made available to the farmers at reasonable cost. The hybrid seed technology is profitable and easy to adopt. However, for its sustainability and to understand the production constraints, it was found necessary to validate this technology in famers' fields under diverse environments. Therefore, a large hybrid seed production program was undertaken in different states of India. In this paper the results of these efforts are discussed along with salient features of this technology.

The commercial hybrids

So far three commercial pigeonpea hybrids have been released. The first hybrid ICPH 2671, produced by crossing ICPA 2043 with ICPR 2671, was released in 2010. In Maharashtra state 782 on-farm trials were conducted and the hybrid recorded 35% superiority over the control. Similarly in Andhra Pradesh (399 trials, 55% heterosis), Madhya Pradesh (360 trials, 46% heterosis), and Jharkhand (288 trials, 69% heterosis), ICPH 2671 recorded encouraging performances. These four provinces are very diverse as far as their ecology is concerned, but the ICPH 2671 performed well in each state; and on average, the hybrid recorded 51% superiority over the respective controls (Saxena et al., 2013).
After the success of ICPH 2671, two more hybrids were released in India. These were ICPH 3762 in Odisha in 2014 (Saxena et al., 2014); and ICPH 2740 in Telangana (Saxena et al., 2015). The record on-farm productivity achieved by farmers indicated that pigeonpea production can be enhanced by introducing hybrid cultivation and the persistent yield plateau can be smashed.

Major components of hybrid technology

The hybrid technology is based on three major components namely, male sterility and its genetic maintenance system, stable fertility restoration, and cost effective mass pollination mechanism. In this technology the seed of hybrids and their female parents is produced on the male-sterile plants; and to achieve this, pollen from the fertile plants is transferred on to male sterile flowers by insects. A brief description of these components is discussed herein.

Male sterility

Male sterility is a situation where its male reproductive parts are either absent, aborted, or non-functional; and fail to reproduces through sexual means. This abnormality could be natural, induced by mutagens, or bred by crossing genetically diverse genotypes. This reproductive inefficiency has been exploited by plant breeders to produce hybrid seed at commercial level. In pigeonpea, so far three forms of male sterility systems differing in their genetic control have been reported. These include genetic male sterility (GMS), temperature-sensitive male sterility (TGMS), and cytoplasmic nuclear male sterility (CMS). The GMS was the first to be discovered (Reddy et al., 1978) but it could not be used for large-scale hybrid seed production due to the need of maintaining male sterility through heterozygotes. The TGMS is of recent origin (Saxena 2014) and needs a lot of research before it could be used in commercial hybrid breeding. The third form of male sterility, CMS, was developed through a cross involving a wild relative of pigeonpea and it is the most valuable product as far as commercial hybrid breeding is concern. This CMS, designated as A_4, was bred by crossing a wild relative of pigeonpea (*C. cajanifolius*) as female parent with a cultivated line as male parent (Saxena et al., 2005).

Fertility restoration

Perfect male fertility restoration of hybrid plants is important to get good yields. According to Kaul (1988) once a fertility restorer (R-) line is crossed with male sterile (A-) plant, the dominant fertility restoring nuclear gene produces certain proteins in F_1 plants and thus repairs the defective mitochondrial genome of the plant to produce male fertile hybrid plants. In pigeonpea two dominant genes (Rf_1 and Rf_2) have been identified (Saxena et al., 2011), which impart fertility restoration to the hybrid plants. In the primary gene pool the frequency of fertility restoring genes is fairly high and so far over 150 good restorers have been identified in different maturity groups (Saxena et al., 2014).

Mass cross-pollination

In most legumes, self-pollination is a common event due to their cleistogamous flower structure, but pigeonpea and a few other crops are an exception with partial natural out-crossing, often mediated by insects. Pathak (1970) identified *Megachile bicolor* and *Apis florae* as pollinating insects in pigeonpea. Subsequently, Williams (1977) identified 48 insect species foraging on pigeonpea, but the common foraging insects were *Apis dorsata* and *Megachile spp.* According to Zeng-Hong et al. (2011) *Megachile spp., Xylocopa spp., Apinae spp.,* and 16 other species visited pigeonpea flowers during flowering. Among these about half were of *Megachile spp.* Each visit to the flowers, on average, lasted for about six seconds during which not only they sucked nectar but also collected pollen grains. They also reported that the pollinating insects were more frequent on male fertile plants with a mean of 4.8 visits /10 minutes as compared to the male sterile counterparts and recorded only 2.8 visits /10 minutes. They concluded that even with 50% less insect visitations, the male sterile plants produced the cross-pollinated seed yield (384 g/plant) equal to that of more frequently visited fertile plants (357 g/plant); and hence for good pod set very high insect activity was not be essential.

Hybrid seed technology

The hybrid technology in pigeonpea is new and there are no recommended national seed standards for this product. In general, the production of hybrid seed in this crop is complex because cross-pollination is entirely dependent on certain insect species; and their population and the resultant degree of natural out-crossing are inconsistent across locations for the reasons described earlier. In this review both published and unpublished information have been gathered from different sources.

Isolation requirements

Seed production of hybrids and their parents requires quality isolation plots; for this both isolation distance and its natural habitat are important. Due to a large variation in the extent of cross-pollinations at different places (Saxena et al., 1990) even for pigeonpea inbred cultivars more than one isolation recommendations have been made in the past. For certified seed production Tunwar and Singh (1988) advised to use an isolation distance of 100 meters; while Ariyanayagam (1976) recommended 180-360 m isolation at different places in Trinidad. Agarwal (1980) and Faris (1985) respectively, recommended 200 and 300 m isolation distances. For hybrids the information generated by different research stations was used by ICRISAT to recommend a safe isolation distance. Considering various ecologies where seed production was carried out, an isolation distance of 500 m was found very safe for the production of both certified as well as breeder seed (Saxena, 2006); and so far the results are very encouraging. Besides this, the other important consideration in selecting the isolation plot, is its natural habitat. The experience gained during on-farm hybrid seed production showed that the isolation plots located near wild bushes, fruit or other flowering trees, with small water bodies produced the best results. This type of ecology helped in harbouring the pollinating insects and produced excellent hybrid yields of 1000 kg/ha or more.

Field plot techniques

In addition to site selection, adoption of efficient field plot techniques is also important for optimizing hybrid yields. The critical point that should be given highest consideration in the field lay out is the availability of fresh pollen for a longer period. This will ensure more visits of the pollinating insects for extended periods to enhance pod setting on the male sterile plants. In general, a row ratio of 4 female: 1 male is recommended, but it can be reduced to 3 female: 1 male, if the population of pollinating insects in the target areas is less. To ensure better pollen availability, the net seed production plot should be surrounded by a 2-3 meter belt of pollen parent; planted 2-3 weeks after the sowing of the net plot. This technique has worked well and in most places with small ($\leq$ one acre) plots. In larger production plots, a slight modification in the design has given good results. For such locations, the entire isolation plot is divided into 3-4 sub-plots; and each sub plot is treated as one unit for seed production and planted with 4 female and 1 male rows and also surrounded by a belt of male parent. The sowing of each sub-plot should be staggered by two weeks. This treatment ensures extended pollen availability and good pod set. For this planting design, however, availability of irrigation facility is essential.

Crop management

Field preparation is an important activity to assure good crop. Being a rainy season crop, it is essential to make appropriate arrangements to drain excess water and for this, ridges at the spacing of 100 cm should be made along the slope. A basal dose of 100 kg/ha of di-ammonium phosphates is recommended to provide 18 kg/ha N and 20 kg/ha P. Since genetic purity of the seed is of prime importance, the Breeder seed of highest quality should be obtained from a reliable source. The sowings should be undertaken at the time when the soil moisture is adequate for germination. The female parental seed @ 4-5 kg/ha and that of male parent @ 1-2 kg/ha should be used. To manage the weeds a pre-emergence herbicide such as Promytrine or Basaline be sprayed. For controlling pod borers (*Helicoverpa armigera)* 2-3 spraying of Dimethoate (30% EC @ 1.0 L /ha) during reproductive stage gives good results. The most important consideration in spraying is that the insecticide should not kill the pollinating insects; and for this the spraying should be organized either before 9 AM or after 5 PM. Generally three rounds of rouging are recommended to remove off-type plants. Harvesting should be organized with utmost care. All the male rows should be harvested first, removed from field, and kept aside. This should follow the easy harvesting of female rows. Threshing, cleaning, drying, seed treatment, and storage should be done as per the local practices. At every stage enough care should be taken to maintain the purity of seed.

Seed quality

Quality control through molecular markers

Purity of seeds holds the key for the success of hybrids, because any level of genetic contamination will lead to the deterioration in its performance with respect to yield and resistance to various stresses. Traditionally, the commercial producers perform Grow-out Tests (GoT) on each seed lot to assess its genetic purity. In this test simply inherited dominant morphological trait present in the male parent is used, and tracked in the hybrid plants to ensure their hybridity.

In pigeonpea, unfortunately the GoT approach cannot be used due to its strong photo-sensitivity and long generation turn-over time. Keeping in view the importance of seed quality, a molecular marker based seed quality assessment approach has now been integrated at ICRISAT in the hybrid promotional programmes. This approach has provided a better alternative to GoT in crops species such as rice (Sundaram et al., 2008), maize (Asif et al., 2009), cotton (Ali et al., 2008), safflower (Naresh et al., 2009) etc. This endeavour in pigeonpea started with the development of SSR based markers. In the very first such study, two diagnostic SSR markers were identified for purity assessment in hybrid ICPH 2438 (RK Saxena et al., 2010, 2015). Subsequently, 42 SSR markers for each of the two hybrids (ICPH 2671 and ICPH 2438) have been identified for purity assessment of hybrid seeds (Bohra et al., 2015). Four common markers namely, CcM0257, CcM1559, CcM1825 and CcM1895 have also been detected for both hybrids (ICPH 2671 and ICPH 2438) for undertaking multiplex assays. Recently seven SSR markers (CCB9, HASSR3, HASSR9, HASSR23, HASSR35, HASSR37, and HASSR43) have also been identified for distinguishing the A-/B- lines and hybrids (Bohra et al., 2015). In another major achievement, a marker gene (*nad7a_del*) derived from a mitochondrial gene *nad7a*, has also been identified to differentiate the male sterile line (ICPA 2039) from its fertile counterpart (ICPB 2039). This marker is able to detect as low as 2% admixtures of its maintainer seed in the A-line carrying A_4 cytoplasm (Sinha et al., 2015).

Quality control through morphological marker

To overcome the issues related to seed quality control in pigeonpea hybrids, an approach of using simply inherited recessive marker was planned. In a thorough search of germplasm a line with obcordate leaf shape controlled by a single recessive gene was selected (Saxena et al. 2011). Interestingly, this trait is expressed within 3-4 weeks of sowing. The "obcordate leaf" is a distinctive morphological marker and rare in occurrence. It is easy to identify by naked eye and hence popularly called as ''naked eye polymorphic marker''. Since pigeonpea seeds have no dormancy and there is no effect of environment on the expression of this trait, it offers a great tool to ensure purity of female parent with minimum resources. In such A-line, any out-crossed "off-type" plant with dominant normal (lanceolate) leaves can be rouged easily at seedling stage, and thus the genetic purity of the female parent can be maintained easily and economically. Also, when a restorer line with normal leaves is crossed to A-line having obcordate leaves, all the true hybrid plants will have normal leaves and any plant within hybrid population with obcordate leaves will be due to sibbing (crossed from pollen shedders) in the preceding generation. Such plants can be detected easily to assist in determining seed quality of the hybrid seed. The limitation of this approach is that any out-crossed plant in the hybrid population, arising due to pollination from any line other than male parent with normal leaves, cannot be detected. Thus, it is the best option for maintaining genetic purity of female parent.

On-farm validation of seed production technology

The two released hybrids ICPH 2671 and ICPH 2470 were chosen for on-farm validation of seed production technology by different organizations and progressive farmers. The parental seed lots used in this study were multiplied at ICRISAT with recommended isolation and cultural practices. For this study, the hybrid seed production was undertaken in farmers' fields with the help of our collaborators in the states of Andhra Pradesh and Madhya Pradesh. In Andhra Pradesh (mean 1229 kg/ha) the hybrid yields were less as compared to Madhya Pradesh (mean 2242 kg/ha). At each of the five locations in Andhra Pradesh namely; Nizamabad (1750 kg/ha), Rangareddy (1258 kg/ha), Warangal (1060 kg/ha), Nandyal (1000 kg/ha), and Manoharabad (1080 kg/ha) had relatively high temperatures and light (Alfisols) soils. In Madhya Pradesh this programme was organized by Agricultural University, Jabalpur and Agriculture College, Indore; and a total of seven locations were selected for on-farm validation, covering a wide range from north to south. The largest area (5.0 ha) was grown in Tikamgarh district and a record hybrid yield @ 3040 kg/ha was harvested. Similarly, in Seoni (1.6 ha) hybrid yield of 2500 kg/ha was recorded. These locations have good forest coverage and rainfall and achieved an amazing seed-to-seed ratio of 1: 500. The hybrid yields recorded at Indore (2267 kg/ha), Rewa (1740 kg/ha), Katni (1450 kg/ha), Jabalpur (1333 kg/ha), and Sehore (1500 kg/ha) were also very encouraging.

The seed production technology reported herein has demonstrated that, on average, the hybrid seed yields of around 1000 kg/ha can easily be achieved by seed producers. It should also be noted that most isolations in this state were large (>1.0 acre). With the recommended sowing rate of 5 kg/ha, a very healthy seed-to-seed ratio of 1: 200 can be achieved in pigeonpea; and it is much higher than rice (1:50). This suggests that now a good hybrid pigeonpea seed production technology has been developed and a quality seed chain can be established.

In some plots it was observed that the male parent had full pod load with a few or no flowers while in the male sterile parent profuse flowering persisted with a little or no pod set. This situation arrived not due to non-synchrony of flowering between male and female parents; but it was caused by the absence of pollinating insects at the time of flowering. This is a serious concern because this situation may lead to big losses to the seed producing farmers. Such situations can be avoided by good planning i.e. not selecting a field near any of the more insect-preferred crop like sunflower, safflower, mustered etc. Further, the scheduling of insecticides for pod borer control should be around sun set or early in the morning. This will not affect the population of pollinating insects. Considering the hybrid yields and the overall economy, it seems that the state of Madhya Pradesh can become the seed production hub for hybrid pigeonpea.

Economics of hybrid seed production

Studies were conducted by MK Saxena et al. (2011) on the cost of production and profitability of hybrid pigeonpea seed production at Indore in Madhya Pradesh. They estimated that the cost of producing of one hectare of pigeonpea hybrid ICPH 2671 seed was Rs 26,395 (US $ 480), excluding the rental value of land. The labour cost was 76.9% of the total cost. This seed plot produced hybrid yield of 1440 kg/ha and yielded the net profit of Rs70, 000 (US $ 1272) / ha. Using these estimates the hybrid cost at farm gate was Rs.18.85 (US $ = 0.34) / kg.

Adoption of technology

Groups of hybrid pigeonpea growing farmers across the central India have realized that the cultivation of a hybrid crop of pigeonpea is profitable and these farmers are repeatedly harvesting yields of 2000-3000 kg/ha. This level of productivity from the cultivation of hybrids gives a net production advantage of 1000-1500 kg/ha over the local cultivars; and fetches them an additional profit of Rs 40,000-75,000 (US $ 700-1300)/ ha. In addition, it was also observed that many progressive farmers took pigeonpea cultivation beyond subsistence level and invested more resources in adopting modern production technologies to reap more profits. These farmers harvested exceptionally high yields (4000-4500 kg/ha) from hybrids with 40-50% superiority over the control (Saxena, 2015).

In fact it is just a beginning and to meet the national production deficit, the hybrid technology has to be taken to more and more farmers. To achieve this, the emphasis on seed production needs to be increased and a chain involving regular production of breeder, foundation, and certified seed will be necessary. A seed-to-seed ratio of 1: 200/300 for pigeonpea hybrids is very healthy and with a little effort a viable seed chain can be established to cover a large area under hybrid pigeonpea crop. According to the estimates of 2015, the cultivation of pigeonpea hybrid has reached 150,000 ha mark. This means that last year at least 30,000 kg hybrid seed was sold and it is a healthy sign of growth. It is also estimated that to meet the current national demand of pigeonpea and stop imports (150,000 tons/year), the introduction of hybrid cultivation on about 10-15% pigeonpea area will be sufficient.

Conclusions

Pigeonpea hybrid technology is now established with on-farm hybrid advantage of 30-50%. The large-scale hybrid seed production is no more an issue. Since the cross-pollination primarily depends on insect pollinators, the selection of production sites holds the key. During the on-farm hybrid seed production programme it was observed that pod setting on the male sterile plants was excellent when the isolation plots were located near bushes and water bodies. Such surroundings helped in harbouring the insect pollinators with hybrid yields of over 1500 kg/ha repeatedly harvested. Seed quality control is another key issue and this has been ably addressed by incorporating a naked eye polymorphic trait (obcordate leaf) in A- / B- lines. In the alternative approach, genomic science is being used to determine the genetic purity of F_1 hybrids and their parents. We believe that the temperature-sensitive male sterility system, when fully established, will make the seed production technology much easier and cost effective. Overall, the pigeonpea hybrid technology has reached a stage when it can be adopted easily. According to the information compiled by Singhal (2013) the statistics of hybrid pigeonpea with respect to on-farm heterosis, hybrid yields, and profitability are comparable with other hybrid field crops such as rice, wheat, safflower, castor etc.; and this information will raise the confidence of seed producers/companies in opting for hybrid pigeonpea business.

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