Journal of Food Legumes 28(3): 190-198, 2015

Strategies for breeding, production and promotion of pigeonpea hybrids in India

KB SAXENA[1]*, IP SINGH[2], A BOHRA[3] and BB SINGH[4]

Former Pigeon Pea Breeder, ICRISAT, Patancheru; [2,3]Indian Institute of Pulses Research, Kanpur-208024; [4]Indian Council of Agricultural Research, New Delhi-111001, India

E-mail: ipsingh1963@yahoo.com

(Received: 15 December 2014; Accepted : 18 July 2015)

ABSTRACT

With the release of GTH-1 the world's first CMS hybrid in pigeonpea [*Cajanus cajan* (L.) Millsp.], the breeding of hybrids in this important pulse crop has become a reality. At present three early duration pigeonpea hybrids with yield advantage of >30% have been identified/released in India. This has been possible due to breeders' success in developing stable cytoplsmic nuclear male sterility (CMS) system and selection of stable restorers. The large-scale hybrid seed production is facilitated by insect-aided natural out-crossing and hybrid yields of 1000-1500 kg /ha have been harvested by the growers. To sustain this breakthrough in pigeonpea it is necessary to identify, evaluate and maintain the perfect CMS line (A and B lines) along with good restorers on a regular basis. In this paper, an attempt has been made to consolidate strategies related to the development of new hybrids, their seed production technology and promotion in the country.

Key words: Breeding strategies, Hybrids, Pigeonpea

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is rich in seed protein (20-22%) and its de-hulled split grains (*dal*) constitute a major supplement in the diets of many Indians. It is cultivated on 4.04 m ha (IIPR 2013) as a rainfed intercrop. To enhance its production and productivity, ICAR launched a massive breeding programme (Ramanujam and Singh 1981) and released a number varieties but the yield remained low at around 700 - 800 kg/ha. This issue of yield stagnation has been a matter of concern for a long time, particularly in view of decreasing per capita protein availability. The recent developments in hybrid pigeonpea breeding technology (Saxena *et al*. 2013, Bohra *et al.* 2010), the first in the world in any grain legume, have generated a lot of optimism among breeders to break the decades-old productivity barrier. This paper besides giving a brief overview of the evolution of hybrid technology outlines the future strategies related to breeding, production and adoption of hybrids in India. Since this technology is new, this synthesis would be helpful to pigeonpea breeders involved in the research and development of hybrids in India.

BACKGROUND INFORMATION

Pigeonpea is a partially cross-pollinated crop (Saxena *et al*. 1990) and the breeders always considered it a constraint in pure line breeding and never tried to use this trait for its genetic improvement. In the mid-seventies ICRISAT planned to convert this constraint into an opportunity for genetic enhancement of yields. In this context, the discovery of the first genetic male sterility (GMS) system (Reddy *et al*. 1978) in a germplasm accession encouraged researchers to launch breeding programmes using this male sterility system. In this endeavour, the realized levels of hybrid vigour in the experimental hybrids (Saxena *et al*. 2005a) encouraged breeders to infuse vigorous research efforts in hybrids in hybrid technology. Soon ICAR launched a hybrid breeding programme based on GMS in 1989 and hybrid ICPH 8 was released in 1991 (Saxena *et al*. 1992). This was followed by the releases of five more such hybrids; these included PPH 4 in 1994 form PAU, Ludhiana; CoPH 1 in 1994 and CoPH 2 in 1997 from TNAU, Coimbatore and AKPH 4101 in 1997 and AKPH 2022 in 1998 from PDKV Akola. These hybrids, in spite of 20-30% yield advantage in farmers' fields, could not be commercialized due to inherent seed production issues associated with GMS. This programme, however, gave two important pieces of information; (i) presence of sufficient level of exploitable hybrid vigour in pigeonpea and (ii) adequacy of the partial natural out-crossing in large-scale hybrid seed production.

Subsequently, the first cytoplasmic nuclear male sterility (CMS) system based on *C. scarabaeoides* cytoplasm (Tikka *et al*. 1997) was developed. This CMS system was found highly stable across the locations and over the years and it has been diversified with multiple disease resistance and good agronomic base (Singh *et al.* 2007, Singh *et al.* 2009). Another CMS system based on *C. cajanifolius* cytoplasm (Saxena *et al*. 2005b) was also developed, and soon the attention of breeders was shifted towards breeding CMS-based pigeonpea hybrids. Of the eight CMS systems bred so far (Saxena *et al*. 2010, Saxena 2013), only A_2 from *C. scarabaeoides* and A_4 from *C. cajanifolius* have been utilized in breeding hybrids. The utilization of A_2 cytoplasm resulted in the development of the first CMS based pigeonpea hybrid GTH 1 from SDAU, S K Nagar, Gujarat in 2004. Based on yield trials, GTH 1 (1760 kg/ha) gave 32% yield superiority over the best local variety (1330 kg/ha). In the coordinated initial hybrid trial (IHT) and advance hybrid trial (AHT) in central zone, this hybrid recorded highest yields and hence, it was released for cultivation. In spite of high performance, it could never

*Present address: 17, NMC Housing, Al Ain, Abu Dhabi

be commercialized due to problems associated with stability of fertility restoration caused by high G x E interactions (Saxena *et al*. 2004). Another A_2 CMS based short duration hybrid IPH 09-5, developed by IIPR, Kanpur, was identified in 2012 for cultivation in the North East Plain Zone. This hybrid was bred by crossing PA 163 A (female) and 261322 R (male). This hybrid (1864 kg/ha) recorded 33% superiority over the control UPAS 120 and has resistance to *Fusarium* wilt and sterility mosaic diseases. This hybrid, however, is yet to reach farmers' fields.

Another success in hybrid pigeonpea breeding was achieved when the A_4 CMS system was used to develop commercially viable hybrid technology. So far two medium duration high yielding hybrids are being cultivated on a limited scale in India. Hybrid ICPH 2671 was released in Madhya Pradesh in 2010 (Saxena *et al*. 2013) with >40% yield advantage over the control variety 'Maruti'. ICPH 2740, released in Andhra Pradesh, is suitable for deep black soils and has given very encouraging results with >40% superiority over the adapted cultivar 'Asha'. The hybrid seed production technology has also been perfected; and hybrid (A x R) yields of over 1000 kg/ha have been recorded at many places in Andhra Pradesh, Madhya Pradesh, Gujarat, and Maharashtra (Saxena *et al.* 2013). With these releases and a notable success in their commercial seed production, the CMS-based hybrid pigeonpea technology has become a reality; and to sustain the achievements of this breakthrough, it is imperative that new high yielding hybrids are released from time-to-time. To meet this goal, breeding of hybrid parents holds the key and at the national level it should be considered with priority. In this paper, therefore, the authors have outlined various strategies to (i) breed high yielding hybrids on a sustainable basis, (ii) produce their large quantities of seed and (iii) identify promotional pathways that would result in large-scale adoption of pigeonpea hybrids.

Fixing Priorities Related to Maturity and Cropping Systems

Pigeonpea is blessed with a large genetic diversity for maturity and it has almost continuous range from 90 to >300 days. This trait has allowed farmers to choose cultivars suited to their local agro-ecologies and production systems. The early types are suited to double cropping or low rainfall areas in pure stands; while medium and long duration types are adapted to rainfed intercropping systems. It is, therefore, advisable that strategies to breed hybrids for a given target region should be developed keeping in view the needs of local production system involving relevant maturity types.

Strategies for Nuclear Diversification of Hybrid Parents

In a dynamic hybrid breeding programme, induction of new hybrid parents at regular intervals is essential to produce new hybrids with greater yield and adaptation. Besides high performance, the hybrid parents should have high combining ability, stability and key market-driven traits. Such lines can either be bred or selected from the available genetic stock. The popular breeding methods recommended for breeding of inbred lines in self-pollinated crops can be used to develop new parental lines. In pigeonpea, the selection efficiency is adversely affected by natural out-crossing occurred in the preceding generation. Hence, the concerned breeder should take care of out-crossing in breeding plots. In the following text some strategies to diversify nuclear base of parents are discussed.

Search of new hybrid parents from primary gene pool

Primary gene pool of pigeonpea is rich in genetic diversity with over 20,000 accessions available with ICRISAT and ICAR gene banks, which contain tremendous genetic variability for a range of quantitative and qualitative traits. Keeping in mind the limited physical and financial resources, the breeding programme for genetic diversification of hybrid parents should be implemented in a phased manner. To start this activity, one must choose the most stable A-lines with high general combining ability and 50-60 testers, selected on the basis of objectives, cropping system and their genetic diversity. Besides germplasm, the testers can also be varieties or advanced breeding lines. In case the required genetic variability is available among old or new released varieties and advanced breeding materials, then this group of materials should be given priority over the unexplored germplasm due their high yield potential, stability and phenotypic/genotypic uniformity.

To study fertility restoration, the hybridization should be done preferably in a line x tester mating system. It is always better to organize hybridization on plant-to-plant basis and at least 3-5 single plants in each tester should be sampled randomly for this purpose. At the same time one branch of each male plant should be bagged to produce its pure seed. Based on pollen fertility/sterility data, the F_1 progenies should be classified into (i) fertility restorer (where most F_1 plants are male fertile), (ii) maintainers (where most F_1 plants are male sterile) or (iii) segregating for male sterility and fertility; such progenies arise due to heterozygosity of the tester and should be selected only if sufficient number of maintainers or restorers are not available. Recently, Saxena *et al*. (2014) reported that out of 502 testers evaluated using A_4 lines, 35.7% (179) were restorers and only 5.0% (25) maintained the male sterility. This information suggested that the frequency of fertility restoring genes in the germplasm is quite high and this resource can be exploited to selecting new male parents.

Breeding new maintainer (B-) and restorer (R-) lines

Since both the maintainers and restorers are inbred, the pedigree selection should be exercised within the segregating populations generated by crossing diverse genotypes. To breed new recombinant maintainers, a set of proven B-lines with different useful traits should be crossed in a suitable mating scheme such as half diallel, bi-parental mating etc. In the following segregating generations selection of plants with combination of desirable traits should be exercised and advanced further using pedigree method. Theoretically, all the selections from such populations should carry the recessive nuclear fertility alleles in homozygous state and will maintain male sterility when crossed with genotypes carrying sterile cytoplasm. The selected inbred progenies should be tested for their productivity, combining ability, disease resistance and other traits of importance. The top specific combiners can be absorbed directly in hybrid breeding; while the good general combiners should be maintained as genetic stocks for future use. Besides recombinant breeding, the standard backcross breeding is another approach to achieve the goal of developing new maintainer lines. Such lines should also have high *per se* performance, high combining ability, disease resistance and market-preferred traits.

To breed new recombinant fertility restoring genotypes, crosses among elite fertility restorers should be made and selection be exercised with respect to the traits of interest and its success will depend on genetic diversity available among the parental lines. To diversify the genetic base of such populations, parents with large geographic diversity may be selected. The segregating populations arising from crosses should be screened for the targeted biotic and/or abiotic stresses, besides their combining ability. In certain situations, the genetic diversity can further be enhanced through selected inter-specific or inter-generic crosses. Such populations, however, may sometimes suffer from undesirable linkage drag.

Isolating fertility restoring lines from heterotic hybrids

Crossing of two hybrid parents integrates two different nuclear genomes into one and which determines the phenotype of hybrid through various gene actions. Of these, additive genetic component of variation can be fixed through pedigree selection of fertile segregants within the hybrid populations. Saxena and Sharma (1990) reviewed gene action in pigeonpea and concluded that additive genetic variation played an important role in the expression of seed yield. This means that some inbred lines with the combination of desirable alleles of the parents can be derived. These inbreds are expected to carry positive additive genes and expected to perform better than B- or R-lines and can be used as testers in hybrid breeding. To achieve this, the best heterotic hybrid combinations should be selected for pedigree selection. A similar exercise in the GMS-based pigeonpea hybrid ICPH 8 revealed that some of the hybrid-inbred lines achieved 70-75% yield potential of the hybrid (KB Saxena, personal communication). Use of such selections in new hybrid combinations is expected to produce hybrids with productivity better than the available hybrids. On the basis of their studies Reddy *et al.* (2015) have suggested that prior to the commercialization of hybrids, assessment of their performance need to be made over seasons and locations to have information on stability in performance.

Breeding hybrid parents with naked-eye polymorphic markers

The use of distinctive morphological traits that are easy to identify by naked eye could be used as tool to ensure purity of parental lines and hybrid seed with minimum resources. These markers are popularly called as 'naked eye polymorphic markers' and these should be incorporated in hybrid parents. One such trait is 'obcordate leaf' and it is controlled by a single recessive gene (Saxena *et al.* 2011). This leaf marker expresses within a month from sowing and any off-type out-crossed seedling with dominant normal (lanceolate) leaves can be rouged easily. The hybrids developed by crossing the parents involving normal and obcordate leaf types will have normal leaves, and the sibs if any, will have obcordate leaves which can be detected at an early stage. This breeding strategy will help in producing quality seed of the hybrids and their parents with minimum resources.

Use of 'heterotic groups' in selecting hybrid parents

In the efforts to breed high yielding maize cultivars in the USA, Shull (1908) observed that the hybrid plants produced by crossing two dissimilar lines had greater plant vigour and seed yield. Richey (1922) also supported this view and reported that the crosses between geographically diverse parents manifested more hybrid vigour with respect to plants and seed yield. Subsequently, Sprague and Tatum (1942) evolved the popular concept of 'combining ability' to discriminate the hybrid parents for their ability to produce high yielding hybrids. All these ideas gradually matured into a concept of 'heterotic group'. This approach involves clustering of the parental lines on the basis of their performance in F_1 generation, origin, phenotypic or genetic diversity. According to this concept, crosses among the lines within a cluster (group) do not produce heterotic hybrids. On the contrary, crosses between the lines representing two diverse groups are likely to yield heterotic crosses. Hence, the new pigeonpea germplasm or breeding materials can be effectively used to develop heterotic groups. In pigeonpea, the first such information was recently published by Saxena and Sawargaonkar (2014). They constituted seven heterotic groups using specific combining ability effects. To overcome the effects of environment in such exercise, use of genomics tools has been suggested. Use of such heterotic groups in hybrid breeding is likely to lift the performance level of hybrids. The heterotic grouping of the parental lines will also avoid the expenses of carrying unproductive crosses in a breeding programme.

Strategies for Cytoplasmic Diversification of A-Lines

Sometimes a popular hybrid may become fragile due to the presence of some extra nuclear genes that allow invasion of certain undesirable factors, as was experienced in corn hybrids in the USA. In this case the genome of 'T cytoplasm' carried susceptible genes for southern corn leaf blight disease (Tatum 1970); and when outbreak of this disease occurred, all the hybrids based on the females carrying 'T' cytoplasm became sick and resulted in severe productivity losses. In order to overcome such potential threats, arising due to cytoplasmic uniformity among hybrids, it is essential to breed male-sterile lines with sufficient mitochondrial diversity. In pigeonpea, so far eight CMS systems (Table 1), derived from different wild relatives of pigeonpea, have been reported (Saxena *et al.* 2010, Saxena 2013), which represent a wide variation in the mitochondrial genome. Of these only two sources, derived from *C. cajanifolius* and *C. scarabaeoides,* have been used in hybrid breeding. This situation necessitates breeding of A-lines with greater cytoplasmic diversity. It should also be noted that while breeding for cytoplasmic diversity, the effect of cytoplasm on yield and other traits should also be studied. In pigeonpea, Saxena *et al*. (unpublished) reported some negative effect of *C. cajanifolius* cytoplasm on seed yield. This effect, however, was cross-specific with the maximum cytoplasmic induced yield penalty being 19.5%.

Table 1. List of eight CMS systems developed in pigeonpea

CMS ID	Donor species	Recipient species
A_1	*C. sericeus*	*C. cajan*
A_2	*C. scarabaeoides*	*C. cajan*
A_3	*C. volubilis*	*C. cajan*
.A_4	*C. cajanifolius*	*C. cajan*
A_5	*C. cajan*	*C. acutifolius*
A_6	*C. lineatus*	*C. cajan*
A_7	*C. platycarpus*	*C. cajan*
A_8	*C. reticulates*	*C. cajan*

Source: Saxena *et al.* (2010) and Saxena (2013)

Strategy for Exploring TGMS-Based Hybrids

Recently Saxena (2014) developed a temperature-sensitive male sterility (TGMS) in pigeonpea and it has further widened the scope of breeding hybrids. This system is based on two parents (A and R) and requires relatively less efforts and resources in breeding hybrids. Since the temperature controls the expression of male sterility and fertility, the multiplication of female parent will neither require any maintainer (B-) line nor insect pollinators. The A- line when sown in areas with < 24^{o} C will be fully fertile and produce self-pollinated seeds. On the other hand, e when it is planted in the temperature regime of >25^{o}C will remain male sterile and it can be used to produce hybrid seed with the help of insect pollinators. Further, in a big country like India the selection of seed production sites for the female parents and hybrids will not be difficult. The authors feel that the pigeonpea breeders can take advantage of this male sterility system in developing hybrids for the country, as has been demonstrated in rice by China. The temperature-sensitive male sterility gene can be transferred easily to other pure lines; and thus a large number of diverse hybrids can be produced without bothering about cytoplasmic effects and need to diversify cytoplasmic base. This will be very cost effective technology with good results.

Strategies for Breeding Hybrid Parents with Specific Traits

Disease resistance

Some testers such as ICPLs 20204, 20243, 20107 and 20186 restore male-fertility and produce heterotic hybrids; but due to lack of disease resistance inhibit their direct use in hybrid breeding. Such fertility restorers cannot be ignored either and should be improved by incorporating genes for resistance through backcrossing for use in hybrid breeding. Considering recessive nature of genes controlling resistance to diseases (Saxena and Sharma 1990), culling of the susceptible segregants should be done in F_2 generation of each backcross. Since the genes controlling resistance to wilt and sterility mosaic are independent, a large segregating population (about 2500) should be screened to recover recombinants with desirable traits and disease resistance. Perennial nature of pigeonpea helps in the selection of desirable plants; and their subsequent use in backcrossing on the regenerated (ratoon) growth (Saxena *et al.* 1976). After completing six backcrosses, about 20-25 promising progenies should be selected for final evaluation along with the recurrent parent as a control. At this time, these progenies can also be tested for their combining ability and fertility restoration in hybrid combinations. There is also an alternative to breed broad based wilt resistant pigeonpea hybrids. In this approach dominant wilt resistance genes (Saxena *et al.* 2012) are transferred to A-lines and these will produce hybrids that would be resistant to fusarium wilt. This is because the hybrids involving susceptible x resistant and resistant x resistant crosses will produce resistant hybrids. In this system promising but susceptible R-lines lines can also be used to breed resistant hybrids.

General combing ability

The general combining ability (GCA) of hybrid parents, especially A-line, plays an important role in hybrid breeding. However, breeding for such a trait is difficult. Limited studies conducted in pigeonpea have shown that high *per se* performance, high GCA, and additive genetic variances have common genetic determinants. Considering the economy of seed production and high probability of producing heterotic hybrids, it is imperative that the seed parents should have both high GCA as well as high *per se* performance. In pigeonpea, the testing of A-lines for GCA in early generations would require more resources in making crosses by hand pollinations, and their evaluation. Therefore, it is advisable that at present the emphasis in selection be given to *per se* performance of hybrid parents. This way the hybrid breeding programme can also complement the efforts of breeding pure line cultivars. The information generated from diallel or line x tester crosses can be used to substantiate the efforts of hybrid parent breeding. Patel and Tikka (2015) have reported that the specific combining ability (sca) variance was found to be more important as compared to general combining ability (gca) variance for important yield components.

Dwarfness

In pigeonpea eight dwarfing sources were identified by Sharma and Saxena (1979); of these D_1 and D_6 have similar bushy phenotype but are controlled by non-allelic single recessive genes. These lines can be used to produce both dwarf as well as tall hybrids. A dwarf hybrid can be produced by incorporating either of the dwarfing genes in both the parents. On the other hand to produce tall hybrid from dwarf parents, the breeder needs to incorporate different dwarfing (non-allelic) genes in the two dwarf hybrid parents. The tall hybrids can also be produced by crossing either of the dwarf lines with a tall line. Hence, depending on the need to produce dwarf/tall hybrids the parental materials can be identified.

High-protein

Most of the subsistence farming families depend on pulses for their protein requirements. However due increasing population pressure, reducing farm holdings and increasing cost of inputs, the per capita protein availability is on rapid decline. The high protein (up to 28%) lines derived from various inter-specific crosses (Saxena *et al.* 2002) can provide some relief to this problem. The high protein trait is controlled by additive genes and it can be transferred to hybrid parents. The hybrids developed from this materials can provide additional 500 - 600 kg/ha protein.

Water-logging tolerance

Water-logging is a serious abiotic constraint which causes huge losses each year. Sultana *et al.* (2012) reported that some of the A-lines such as ICPA 2043, 1CPA 2029 and ICPA 2047 had a good level of tolerance to water-logging. Similarly, some of the R-lines (ICPL 87119, ICPL 149, and ICPL 20125) also exhibited tolerance to this stress. Such combinations can be used to breed water-logging tolerant hybrids, as Sarode *et al.* (2007) have reported that tolerance to water logging in pigeonpea is controlled by single domonat gene, and it would be a good strategy if more A-lines are bred with tolerance to water-logging.

Strategies for using Genomic Tools in Hybrid Breeding

Markers based quality control tests

To sustain productivity of hybrids, it is essential that pure seeds are supplied to farmers in adequate quantities. In general, the purity of hybrid seed is assayed through the standard 'Grow-Out Tests' using simply inherited dominant traits. In pigeonpea this test is not possible because of its long (200 -250 days) duration and photo-sensitivity. To overcome this constraint a simple, rapid, and cost effective seed quality testing approach that is based on molecular marker assay has been developed (Naresh *et al.* 2009; Saxena *et al.* 2010). Further, to make the procedure of purity testing more cost-effective and time saving, multiplex assays based on bacterial artificial chromosome (BAC)-end derived simple sequence repeats (BES-SSRs) were developed by Bohra *et al.* (2011). Different marker groups (comprising up to eight SSR markers) were identified by choosing 42 informative BES-SSR markers for purity assessment. The multiplexed assays enable generation of robust genotyping data for multiple DNA markers from a single capillary. Concerning moderate-scale laboratory, capillary-based analysis still remains an expensive procedure. Therefore, Bohra *et al.* (2014a) recently identified a set of informative and hyper-variable SSR markers that are suitable for Agarose gel-based detection of heterozygosity and genetic purity. The SSR markers are important in genetic purity testing as it permits detection of residual heterozygosity; thereby indicate its potential implications in breeding hybrids (Saxena *et al.* 2010). With the help of DNA markers, reliable detection of off-types in the commercial hybrid seed lots can now be undertaken.

Tagging of fertility restoring genes

In pigeonpea hybrids two dominant genes control fertility restoration (Saxena *et al.* 2011). To breed new hybrids more and more restorer lines are required and breeding such lines is expensive and time consuming, since it will involve test crossing of each selection for the presence of fertility restoring genes. The recent advances made in the field of marker assisted breeding, the process of transferring *Fr* gene into non-restorers or selection of restorers within segregating populations and germplasm can be done at a faster pace and economically. Given the context, pinpointing the genomic location of gene(s)/QTL that restore fertility holds immense importance in hybrid breeding. In pigeonpea, three A_4 cytoplasm-based mapping populations viz. ICPA2039 × ICPR 2447, ICPA2043 × ICPR 2671 and ICPA 2043 × ICPR 3467 were used to elucidate the genetic makeup of fertility restoration (Bohra *et al.* 2014). As a result of QTL analysis, four candidate regions/QTLs were detected that explained the phenotypic variance in the range of 14-24%. More importantly, all these QTLs were placed on to a consensus genetic map developed from multiple mapping populations. The consensus genetic map comprised of 339 SSR loci and covered 1,059 cM of the pigeonpea genome. Notably, one of the underlying genomic regions in LG06 was found a promising genomic candidate, which can be targeted for subsequent genomics and genetics analysis like fine mapping etc. Discovery of such QTLs will not only accelerate the progress of identifying potential restorers from comprehensive germplasm collection, but also facilitate faster introgression of these fertility governing genetic factors into diverse genetic backgrounds (Bohra *et al.* 2014b).

Characterization, designation, and documentation of hybrid parents

In a hybrid parent breeding programme, the number of selections gradually decline from F_2 onwards and it ends with a hand full of lines with desirable traits. As a next step, it is essential to generate comparative data on important traits, and this is achieved by testing them together in replicated trials. Ideally, this exercise is done at more than one location for at least two years. This will take care of environment interactions to some extent. The selected hybrid parents (B- and R- lines) need to be properly designated for a permanent record. An appropriate documentation in the Gene Bank record and publication of data will help pigeonpea breeders involved in hybrid breeding. In the context of Intellectual Property Rights (IPR), it is imperative that the elite genetic materials bred at a particular location be thoroughly evaluated for at least 2-3 seasons for various distinctness, uniformity and stability (DUS) traits. The morphological traits can also be characterized at molecular level using specific DNA markers.

Selection of diverse genotypes

To reap the benefits of hybrid technology it is essential that maximum possible genetic diversity is maintained within the pool of hybrid parents. For this effective tools should be available with the breeder to measure and document the nature of variability. The measurements based on morphological traits are invariably influenced by plant phenology and certain environmental factors; and this may hide the truth about genetic variation for the traits of interest. The best option to overcome this concern is to estimate the variability based on genomics tools. This data can be used to develop heterotic groups and for selection of potential genotypes for hybrid breeding high yielding hybrids.

Stratagies for Hybrid Seed Production

The hybrid pigeonpea technology is new and there are no prescribed seed production and quality control standards. The strategies therefore have been developed on the basis of research experience gathered at different locations. For a successful hybrid seed production programme factors such as location of isolation plot, planting techniques and agronomic packages are important and strategies related to these issues are discussed here.

Isolation distance

In pigeonpea there is no wind pollination (Kumar and Saxena 2010) and a number of insects are responsible for out-crossing (Onim 1981). Dalvi and Saxena (2009) also reported that the extended stigma receptivity after flower opening encourage cross pollination. Since the population of insect pollinators is not uniform at different locations, under natural field conditions a considerable extent (0-70%) of out-crossing has been reported (Saxena *et al*. 1990).The recommended isolation distance (100 m) for pure line cultivars cannot be adopted for hybrid seed production and it needs research because under different environments, the flying insects can carry pollen grains for variable distances. The unpublished information generated by ICRISAT from different locations over years showed that an isolation distance of 500 m has worked well and pending the official recommendations by appropriate authority, it may be followed for multiplying seeds of hybrids and their parents.

Seed production sites

The other important consideration in commercial hybrid seed production is the habitat of the seed production sites. Attempts should be made to select the isolation plots in areas which are infested with wild bushes, fruit or other trees that generally harbour the insects. It is also been observed that if the seed production plots are located near water bodies, the pollinating insects are attracted which help in enhancing natural out-crossing and good hybrid seed yields. Such seed production 'hot spots' can be identified by organizing a number of small-sized 'pilot seed production plots (Table 2). The pod set under natural conditions will indirectly indicate the presence of pollinating vectors at a particular site; and this will help in identifying

Table 2. Some hot spots identified for hybrid seed production in pigeonpea

State	Location	Area sown (ha)	Yield (kg/ha)
Madhya Pradesh			
	Tikamgarh	5.0	3040
	Seoni	1.0.	2500
	Indore	1.5	2267
	Rewa	1.0	1740
	Katni	3.0	1450
Andhra Pradesh			
	Nizamabad	1.0	1750
	Medak	1.0	1250
	Medchal	1.4	1214
	Warangal	1.2	1063
	Nalgonda	2.0	1000
Maharashtra			
	Risod	1.0	1000
Gujarat			
	Ahmedabad	1.0	1063

suitable locations for commercial seed production. A record of such locations and productivity should be maintained and shared with seed producing agencies.

Field plot techniques

In addition to site selection, the adoption of efficient field plot techniques is also important to optimize the hybrid yields. The main focus in designing the layout should be to make fresh flowers available for extended periods. This will ensure more visits of the pollinating insects and enhanced pod setting. 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. It is also recommended that in a large seed production programme at a given site, the entire plot should be divided into 2-3 sub plots. The planting in each sub-plot should be staggered by two weeks. This will ensure extended pollen availability and good hybrid yields.

Strategies for Promotion of Hybrids in India

Local versus wide adaptation

Adaptation of cultivars to a set of given environments is a complex issue since it involves interaction of cultivars with various environmental factors at different stages of growth. These factors could be biotic, abiotic, or edaphic in nature. The concerned breeder needs to make a decision about the areas of adaptation for hybrids. Initially, when a few hybrids are available these should be tested for their adaptation at representative multi-locations. These hybrids may be classified on the basis of their performance. Some hybrids will perform well across locations (*i.e.* wide adaptation) and a few at certain locations (*i.e.* specific adaptation). As the hybrid programmes will grow in size and numbers, more breeders will get involved in hybrid breeding activity. This may lead to breeding of more specifically adapted hybrids and may bring laurels to the country with self-sufficiency in pigeonpea production.

Human resource development

For promotion of any technology involvement of expert hands and minds will bring a lot of difference in technology transfer. Since hybrid pigeonpea involves special attention in various seed production activities, a thorough training of seed producers is essential. Such training programmes should be organized once before planting for selecting the best possible fields with appropriate isolation and local ecology that would harbour pollinating insects. The other training programmes should cover various aspects of crop husbandry, especially insect management without harming pollinating insects. Rouging is another area where field training of seed growers will be of immense value.

Ensure sufficient quality seed

For enhancing adoption of hybrids the basic requirement is to make available high quality seed at reasonable rates. To achieve this, an efficient seed chain need to be developed to cater the needs of breeder, foundation, and certified seeds. Fortunately, the seed-to-seed ratio for pigeonpea hybrids is high (1: 200 to 1: 300) and this will ensure large area coverage. However, a good planning would be required for quality control and timely supply of seed to seed distributors and growers. Initially, it is also important that seed distributors should be given enough information about the product.

Strategy for area expansion

The expansion of pigeonpea area under hybrids is critical keeping in view the national interest. Since this is a multi-facet issue, several agencies dealing with production, marketing, distribution, and extension need to work together. Hence a strong coordinating unit is a must to develop links with different disciplines.

CONCLUSIONS

As a result of huge investment of scientific and financial resources for over four decades, a breakthrough in pigeonpea breeding technology was achieved and considering the yield advantages the prospects of hybrid technology are bright. To sustain the benefits of this technology, it is essential that both long as well as short term strategies dealing with research and development at both regional as well as national levels are designed and implemented. The key for its success is the efficiency with which the coordination among various disciplines is affected. In this context, the role of both public and private agencies could be very important. The promotion of hybrid technology will not only raise the national pigeonpea production by about 30%, but also reduce the imports and save valuable foreign currency. With hybrid (A x R) yields of 1000-1500 kg/ha and seed rate of 5 kg/ha, the seed-to-seed ratio for hybrid pigeonpea is reasonably high and ranges from 1:200 to 1: 300. This means that to cover 10% (=400,000 ha) of the national pigeonpea area with hybrids, only 2000 ha of certified seed production programme would be required. This would add 20,000-30,000 tonnes (@ 25% hybrid advantage) of additional grain to the country's pigeonpea production. It does not appear to be a difficult task, but to achieve this, a firm strategy and commitment to promote the hybrid technology are required.

To reap the benefits of this technology, the quality of hybrid seed is prime key. At present there are no prescribed seed standards for hybrids in pigeonpea. This issue must be addressed at national level and the information must be made available to all the seed producers. The field oriented standard grow-out test is not possible in pigeonpea and to address this issue, a genomic based alternative has already been invented. The extensive on-farm testing of the hybrids in states of Madhya Pradesh, Maharashtra, Karnataka, Gujarat, Telangana, Andhra, and Odisha have given very positive signals to the farmers about high yields of hybrids. Now the issues related to seed availability need attention from all the corners. Since the hybrid technology in pigeonpea is new, it is essential to convince the public and private seed companies about the financial viability of the hybrid technology. Therefore, besides interacting with them, on-farm seed production programmes should be organized with them at strategic locations under the supervision of experts. This will develop confidence among seed producers and help in the promotion of hybrids.

REFERENCES

Bohra A, Dubey A, Saxena RK, Penmetsa RV, Poornima KN, Kumar N, Farmer AD, Srivani G, Upadhyaya HD, Gothalwal R, Ramesh R, Singh D, Saxena KB, KaviKishor PB, Singh NK, Town CD, May GD, Cook DR and Varshney RK. 2011. Analysis of BAC-end sequences (BESs) and development of BES-SSR markers for genetic mapping and hybrid purity assessment in pigeonpea. BMC Plant Biology **11**:56.

Bohra A, Mallikarjuna N, Saxena K, Upadhyaya H, Vales MI and Varshney RK. 2010. Harnessing the potential of crop wild relatives through genomics tools for pigeonpea improvement. Journal of Plant Biology **37**:1–16.

Bohra A, Saxena RK, Gnanesh BN, Saxena KB, Byregowda M, Rathore A, Kavikishor PB, Cook DR and Varshney RK. 2012. An intra-specific consensus genetic map of pigeonpea [*Cajanus cajan* (L.) Millsp.] derived from six mapping populations. Theoretical and Applied Genetics **25**:1325-38.

Bohra A, Saxena RK, Saxena KB, Sameerkumar CV and Varshney RK. 2014b. Advances in Pigeonpea Genomics, In: Legumes in the Omic Era. S. Gupta, N Nadarajan and DS Gupta, Eds. Springer, pp 95-110.

Bohra A, Singh IP, Yadav AK, Pathak A, Soren KR, Chaturvedi SK and Nadarajan N. 2014a. Utility of informative SSR markers in the molecular characterization of cytoplasmic genetic male sterility-based hybrid and its parents in pigeonpea. National Academy Science Letters DOI 10.1007/s40009-014-0288-6.

Dalvi VA and Saxena KB. 2009. Stigma receptivity in pigeonpea (*Cajanus cajan* (L.) Millsp.). Indian Journal of Genetics and Plant Breeding **69**: 247- 249.

IIPR. 2003. Project Coordinator's Report. All India Coordinated Research Project on Pigeonpea, Indian Institute of Pulses Research, Kanpur. Pp 49.

Kumar RV and Saxena KB. 2010. First report of wind pollination in pigeonpea. Indian Journal of Genetics and Plant Breeding **61**: 279 -280.

Aresh V, Yamini KN, Rajendrakumar P, and Dinesh KV. 2009. EST-SSR marker-based assay for the genetic purity assessment of safflower hybrids. Euphytica **170**: 347- 353.

Patel PT and Tikka SBS. 2015. Combining ability of CMS lines and pollen fertility restorers in pigeonpea [*Cajanus cajan* (L.) Millsp.] Journal of Food Legumes **28**:11-25.

Ramanujam S and Singh SP. 1981. Pigeonpea breeding in the All India Coordinated Programme. In: Proceedings of the International Workshop on Pigeonpeas. Vol. 1. ICRISAT, Patancheru, India, 502324. Pp 403 – 414.

Reddy BVS, Green JM and Bisen SS. 1978. Genetic male sterilityy in pigeonpea. Crop Science **18**: 362-364.

Reddy VG, Jayalakshmi V and Sreenivas T. 2015. Studies on fertility restoration and extent of heterosis in CMS based pigeonpea hybrids. Journal of Food Legumes **28**:26-30.

Richey FD. 1922. The experimental basis for the present status for corn breeding. Journal American Agronomy **14**: 1-17.

Sarode SB, Singh MN and Singh UP. 2007. Genetics of waterlogging in pigeonpea (*Cajanus cajan* (L.) Millsp.) Indian Jounal of Genetics and Plant Breeding **67**: 264-265.

Saxena KB. 2008. Genetic improvement of pigeonpea - a review. Tropical Plant Biology **1**:159–178.

Saxena KB. 2013. A novel source of CMS in pigeonpea derived from *Cajanus reticulatus*. Indian Journal of Genetics and Plant Breeding **73**: 259-263.

Saxena KB. 2014. Temperature-sensitive male sterility system in pigeonpea. Current Science **107**: 277-281.

Saxena KB, Chauhan YS, Johansen C and Singh L. 1992. Recent developments in hybrid pigeonpea research. In: New Frontiers in Pulses Research and Development. Proceedings of National Symposium. 10-12 November 1989: Directorate of Pulses Research. Kanpur 208024, India.Pp.58-69.

Saxena KB, Kumar RV and Rao PV 2002. Pigeonpea nutrition and its improvement. Journal of Crop Production. **5**: 227- 260.

Saxena KB, Srivastava DP, Chauhan YS, and Ali M. 2005a. Hybrid pigeonpea. In: Advances in Pigeonpea Research. Indian Institute of Pulses Research, Kanpur 208024, India. Pp. 99-133.

Saxena KB, Kumar RV, Srivastava N and Shiying B. 2005b. A cytoplasmic-nuclear male-sterility system derived from a cross between *Cajanus cajanifolius* and *Cajanus cajan*. Euphytica **145**: 289-294.

Saxena KB, Kumar RV, Saxena RK , Sharma M, Srivastava RK, Sultana R, Varshney RK, Vales MI, and Pande S. 2012. Identification of dominant and recessive genes for resistance to Fusarium wilt in pigeonpea and their implication in hybrid breeding. Euphytica **145**: 289-294.

Saxena KB, Kumar RV, Tikle AN, Saxena MK, Gautam VS, Rao SK, Khare D, Chauhan YS, Saxena RK, Varshney RK, Reddy BVS, Sharma, D, Reddy LJ, Green JM, Faris DG, Mula M, Sultana R, Srivastava RK, Gowda CLL and Sawargaonkar SL. 2013. ICPH 2671 - The world's first commercial food legume hybrid. Plant Breeding **132**: 479-485.

Saxena KB, Kumar RV and Bharathi M. 2014. Studies on fertility restoration in pigeonpea. Euphytica **196**: 127-135.

Saxena KB and Sharma D. 1990. Pigeonpea Genetics. In: The Pigeonpea. Nene YL, Hall SD and Shiela VK, Eds. CAB International Wallingford, UK. Pp. 137- 158.

Saxena KB and Sharma D. 1995. Sources of dwarfism in pigeonpea. Indian Journal of Pulses Research **8**: 1- 4.

Saxena KB, Sharma D and Green JM. 1976. Pigeonpea rationing - an aid to breeders. Tropical Grain Legume Bulletin **4**: 21.

Saxena KB, Singh L and Gupta MD. 1990. Variation for natural out-crossing in pigeonpea. Euphytica **46**: 143-148.

Saxena KB, Sultana R, Mallikarjuna N, Saxena RK, Kumar RV, Sawargaonkar SL and Varshney RK. 2010. Male sterility systems in pigeonpea and their role in enhancing yield. Plant Breeding **129**: 125-134.

Saxena KB, Sultana R, Saxena RK, Kumar RV, Sandhu JS, Rathore A, Kavi Kishor PB and Varshney RK. 2011. Genetics of fertility restoration in A_4-based, diverse maturing hybrids of pigeonpea [*Cajanus cajan* (L.) Millsp.]. Crop Science **51**: 574-578.

Saxena KB and Sawargaonkar SL. 2014. First information on heterotic groups in pigeonpea (*Cajanus cajanus* (L.) Millsp.) Euphytica **200**: 187-196.

Saxena KB, Tikka SBS, and Mazumder ND. 2004. Cytoplasmic genic male sterility in pigeonpea and its utilization in hybrid breeding programme. In: Pulses in New Perspective. Ali M, Singh BB, Kumar S, and Dhar, V. Eds. Indian Institute of Pulses Research, Kanpur 208024, India. Pp 132-136.

Saxena KB, Vales MI, Kumar RV, Sultana R and Srivastava RK. 2011. Ensuring genetic purity of pigeonpea hybrids by incorporating a naked-eye polymorphic marker in A and B lines. Crop Science **51**: 1564-1570.

Saxena RK, Saxena KB, Varshney RK. 2010. Application of SSR markers for molecular characterization of hybrid parents and purity assessment of ICPH 2438 hybrid of pigeonpea. [*Cajanus cajan* (L.) Millspaugh]. Molecular Breeding **26**: 371-380.

Shull GF. 1908. The composition of a field of maize. Report American Breeders' Association **4**: 296-301.

Singh BB, Singh IP and Kumar S. 2007. Diversification and evaluation of CMS lines in pigeonpea *(Cajanus cajan)*. Journal of Food Legumes **20**: 201-202.

Singh F, Singh IP and Majumder ND. 2009. Cytochemical analysis and stain development in pollen grains of parental lines and hybrids based on cytoplasmic genetic male sterility system in pigeonpea (*Cajanus Cajan*). Indian Journal of Agricultural Sciences **79**: 709-714.

Singh IP, Singh BB, Ali Iqrar and Kumar S. 2009. Diversification and evaluation of cytoplasmic nuclear male sterility system in pigeonpea (*Cajanus cajan* L. Millsp.) Indian Journal of Agricultural Sciences 79: 291-294.

Sprague GJ and Tatum LA. 1942. General and specific combining ability in single crosses of corn. Journal of American Agronomy **34**: 923-932.

Sultana R, Vales MI, Saxena KB, Rathore A, Rao S, Rao SK, Mula M, and Kumar RV. 2013. Waterlogging tolerance in pigeonpea (*Cajanus cajan* (L) Millsp.): genotypic variability and identification of tolerant genotypes. Journal of Agriculture Science **151**: 659-671.

Tatum LA. 1971. The southern corn leaf blight epidemic. Science **173**. Doi: 10.1126/ science 173. 3991.39.

Tikka SBS, Pawar LD, Chauhan RM. 1997. First record of cytoplasmic-genic male sterility system in pigeonpea [*Cajanus cajan* (L.) Millsp.]. Gujarat Agricultural University Research Journal **22**: 160-162.