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REVIEW ARTICLE

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The alternative breeding approaches for improving yield gains and stress response in pigeonpea (*Cajanus cajan*)

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Abstract

Pigeonpea is an important food legume crop of semi-arid tropical regions. Plateauing of pigeonpea yield has been worrying breeders for the past 6–7 decades. Serious breeding efforts made during this period resulted in various high-yielding and disease resistant cultivars. However, the gains in pigeonpea productivity have been modest. The authors, while reviewing this situation, conclude that long generation turnover, complexity of biological traits, low selection response and overreliance upon pedigree breeding present the key bottlenecks for this situation. In this paper, some alternative breeding approaches and technologies are suggested for the genetic enhancement of yield stability and stress response of pigeonpea.

KEYWORDS

biological constraints, genomic selection, hybrids, pedigree breeding, speed breeding, Sybrid

1 | INTRODUCTION

Pigeonpea, a high-protein pulse, evolved about 3,500 years ago from its wild ancestor *Cajanus cajanifolius* (Haines) in central India (van der Maesen, 1990; Varshney et al., 2017). Over the time a number of landraces evolved at the centre of origin and spread in different directions for cultivation. Some of the landraces were also taken to over 100 countries by traders and migrant workers (Mula & Saxena, 2010). Soon, due to its various soil ameliorating and survival properties, pigeonpea found its adaptation in subsistence agriculture throughout the tropics and sub-tropics. Globally, pigeonpea is now cultivated on over 6.99 m ha with total production of about 5.93mt and mean productivity of 852 kg/ha (FAOSTAT, 2018). Unfortunately over the decades, the global pigeonpea productivity has remained more or less stagnant around 700–800 kg/ha (Bohra, Saxena, Varshney, & Saxena, 2020); and the issue of yield plateauing still haunts both the scientists as well as policy makers. The non-availability of high yielding cultivars with stable performance, poor seed replacement ratio, inadequate cultural practices and relatively low research and development priorities are the key factors responsible for repeatedly poor harvests of pigeonpea.

A perusal of pigeonpea variety development programmes at different research centres revealed that for cultivar development the breeders not only used limited genetic variation but always relied on pedigree breeding (Kumar, Gupta, Chandra, & Singh, 2003; Naik et al., 2020; Saxena, Rathore, et al., 2018). The authors believe that these factors would have also played a role in limiting the yield enhancement efforts of this crop. In this paper, besides highlighting the key biological and plant breeding constraints, some alternative breeding approaches are also suggested which might help breeders

in developing pigeonpea cultivars with greater productivity and stability.

2 | THE POPULAR BREEDING METHODS USED IN PIGEONPEA

Pigeonpea (*Cajanus cajan* [L.] Millsp.) is an often cross-pollinated crop and the breeding methods recommended for most autogamous crops were applied for the genetic enhancement of the crop. These included direct introduction, pure line selection from germplasm, hybridization followed by pedigree selection and mutation breeding. The major plant breeding accomplishments achieved in the past are summarized in the following section.

2.1 | Germplasm selection

Initially the pigeonpea breeding activities started with the collection of landraces from farmers' fields. From these genetic stocks some promising landraces were directly released as cultivars. Besides this, some pigeonpea breeders also exploited the intra-accession variation that was present in some heterogeneous landraces and selected individual plants and advanced them through pedigree breeding. Using this approach, a number of cultivars were released and some of them are still grown by farmers. Few of these landmark varieties include C 11, T 7, BDN 1, 'Bahar', NA 1, ICP 8863 ('Maruti') and ICP 7035 etc. (Bohra, Jha, Pandey, et al., 2017). Beside this, some important sources of diseases resistance were also identified from the germplasm for example IPA 8F, IPA 9F, IPA16F and ICP 7035 for both fusarium wilt and sterility mosaic disease, 15-3-3 for fusarium wilt, ICPL 366 for sterility mosaic disease, ICP 7105 for *Alternaria* blight, PB 9 for *Phytophthora* blight and many more.

2.2 | Pedigree breeding

In the second phase of pigeonpea breeding emphasis was given to enhance specific traits through hybridization and pedigree selection. These included traits like early maturity, seed size, pod size, plant type, disease resistance and yield (Saxena, Sultana, et al., 2016). By using pedigree breeding a total of 89 pigeonpea varieties were released since 1960. Besides this, 455 advanced breeding lines were also nominated for their evaluation in the National Coordinated Trials organized by Indian Council of Agricultural Research (ICAR). These facts highlight the extensive use of pedigree method of breeding in pigeonpea. Table 1 presents a list of some of the popular pigeonpea varieties developed by pedigree and selection schemes.

TABLE 1 Some landmark pigeonpea varieties that are still preferred for cultivation despite having released more than 10 years earlier

S. No.	Variety	Release year	Pedigree	Important traits
1	ICPL 87119 (Asha)	1993	C 11 × ICPL 6	Resistant to FW and SMD
2	TJT 501	2009	ICPL 84008 × TT 6	Early maturity
3	Narendra Arhar 1 (NDA 88-2)	1997	Selection from Faizabad (Uttar Pradesh)	Compact, indeterminate
4	Malviya Chamatkar (MAL 13)	2005	(MA 2 × MA 166) × Bahar	Spreading, indeterminate, tolerant to wilt, pod borer and SMD
5	UPAS 120	1976	Selection	Early maturity
6	LRG 41	2007	Selection from Chilakaluripet in Guntur (Andhra Pradesh)	Resistant to FW and SMD
7	Jawahar Tur (JKM 189)	2007	ICPL 87119 × Plant 142	Moderately resistant to wilt, SMD and phytophthora blight
8	Maruti (ICP 8863)	1986	Selection	Indeterminate, semi-spreading, wilt resistant
9	Bahar	1986	Selection from Motihari (Bihar)	Compact, resistant to SMD
11	BSMR 736	1996	CTP 7217 × No 148	Resistant to FW and SMD

Abbreviations: FW, fusarium wilt; SMD, sterility mosaic disease.

TABLE 2 Yield and standard heterosis of pigeonpea hybrids as recorded in multi-location trials

Maturity group	Hybrid	Locations	Mean yield (kg/ha)	Standard heterosis (%)
Early	GTH 1	–	1,760**	42
	IPH 09-5	16	1,789**	32
	IPH 15-03	13	1595.6**	28.3
	IPH 10-02	14	1994.3**	30.4
	SKNPH 1411	10	2,016**	30.5
	PAH 5	8	1,698**	21.6
	ICPH 2433	25	2,306**	54
	ICPH 2438	25	2,127**	42
	ICPH 2363	25	2,048**	36
Medium	ICPH 3491	18	2,919**	57
	ICPH 3497	18	2,686**	44
	ICPH 3481	18	2,637**	41
	ICPH 4788	4	1624.2**	23

**Significantly different from the corresponding control hybrid/variety at $p < .01\%$.

2.3 | Mutation breeding

The initial mutation breeding research in pigeonpea was confined to determine effective doses of different chemical and physical mutagens and to record the induced genetic variation for various morphological traits. Of these, the treatments involving ethyl methane sulfonate ($C_3H_8SO_3$), fast neutrons and gamma rays were found successful in creating useful variability but their utility in breeding high yielding cultivars was limited. Success in the genetic improvement through mutations in pigeonpea has been recorded for traits such as yield, earliness, seed size and disease resistance (Pawar, Thakre, Reddy, & Bhatia, 1991). So far only five commercial pigeonpea cultivars ('Co 3', 'Co 5', 'TT5', 'TT 6' and 'TAT 10') have been bred through mutagenesis (Singh, Bohra, & Singh, 2016).

2.4 | Hybrid breeding

Unlike most pulses, pigeonpea offers a unique opportunity for exploiting hybrid vigour for yield enhancement due to a considerable extent of natural cross-pollination available in the crop. To develop hybrid technology as a first step, efficient cytoplasm-based male sterility (CMS) systems were bred using wild species as cytoplasm donors (Saxena, Kumar, Srivastava, & Shiying, 2005; Tikka, Parmar, & Chauhan, 1997) so that large quantities of hybrid seed could be produced economically. These CMS sources were used to breed male sterile lines, their maintainers and hybrid combinations in early, and medium maturing groups (Table 2). This was followed by the release of four high yielding pigeonpea hybrids (Saxena & Tikle, 2015). Most recently, an early maturing pigeonpea hybrid IPH 15–03 has been identified and released for cultivation in the North West Plain Zone (NWPZ). The large scale on-farm testing of these hybrids demonstrated that in pigeonpea this technology can help in smashing the low yield plateau. However, the hybrid technology still suffers from critical issues related to the seed quality determinations; and this constraint needs to be addressed for large-scale adoption of hybrids (Saxena, Sharma, & Vales, 2019).

3 | BREEDING ACCOMPLISHMENTS

The pigeonpea breeding accomplishments achieved in the past half century have recently been reviewed by Saxena, Sultana, et al. (2016), Saxena, Sharma, and Vales (2019). The major conclusions were (a) achieved significant success in the genetic enhancement of simply inherited traits, but the yield harvests per unit area remained more or less the same, (b) developed high yielding resistant cultivars for Fusarium wilt and sterility mosaic diseases; and this has not only reduced the yield losses but also provided stability in the production at farmers' level, (c) significantly reduced the crop maturity period from about 300 days to less than 90 days; and it has helped in diversifying cropping systems involving pigeonpea, and (iv) established hybrid technology, the first in any food legume, with an on-farm yield advantage of 30%–50% or more.

4 | KEY CONSTRAINTS ENCOUNTERED IN PIGEONPEA BREEDING

Pigeonpea breeding efforts suffered from various inherent physiological and genetic constraints and their complex interactions with environment. Some key natural and plant breeding constraints are briefly discussed herewith.

4.1 | Long generation turnover time

Breeding efforts in pigeonpea are limited by the long time it takes to complete one seed-to-seed generation. It is primarily due to very strict short-day requirement of plants to flower. Therefore, it takes about 10–12 years to breed a new cultivar. The photo-sensitivity in pigeonpea is linked to its maturity genes and hence, breeding a long duration photo-insensitive cultivar is out of the scope (Saxena et al., unpublished).

4.2 | Natural cross-pollination

The natural out-crossing in pigeonpea to the extent of 25%–30% is a common feature of this crop. It is facilitated by the presence of insects and nectar glands located at the base of flowers (Saxena, Tikle, Kumar, Choudhary, & Bahadur, 2016). Since the breeding activities are invariably performed in open fields, the crop is exposed to free insect visitations leading to undetected cross-hybridization of individual plants; and this leads to inefficiencies in pedigree breeding method by adversely affecting the breeding value of the selections. Surprisingly, in spite of knowing the ill effects of natural cross-pollination in the crop, the pigeonpea breeders always resorted to pedigree breeding while developing new cultivars.

4.3 | Low harvest index

Harvest index is considered a good indicator of grain productivity of the crop. This parameter is closely linked to the efficiency of plants to transfer their dry matter to the developing grains. Since pigeonpea is a perennial species, it induces indeterminateness in the plants which results in the production of huge biomass and large number of flowers under optimum growing conditions. Since, only a limited amount of photosynthates is transported to the developing seeds; it results in huge flower drop, low yield and low (0.2–0.3) harvest indices (Chauhan, Johansen, & Saxena, 1995).

4.4 | Limited genetic diversity

The primary gene pool of pigeonpea germplasm includes >13,000 accessions and this collection exhibits tremendous phenotypic variability for both quantitative and qualitative traits (Bohra et al., 2010; Reddy, Upadhyaya, & Singh, 2005). The same, however, cannot be said about the diversity at molecular level (Bohra et al., 2011; Bohra, Jha, Pandey, et al., 2017; Odeny et al., 2007; Yang et al., 2006). These researchers concluded that the extent of molecular diversity in secondary gene pool is far greater than that of primary gene pool. Kumar et al. (2003) reported that during past half century only a limited proportion of germplasm from primary gene pool was used by pigeonpea breeders; and this may be one of the key factors responsible for low productivity of new cultivars. Also, for some reasons, the pigeonpea breeders in past did not exploit the genetic variability available in the secondary gene pool. This may be due to various issues like limited resources, poor success in inter-specific hybridizations and selection problems associated with presence of strong linkage drag (Saxena, Saxena, et al., 2018).

4.5 | Poor response to selection for seed yield

A perusal of the performance data generated from a number of national co-ordinated trials in India over the years. Although witnessed tremendous genetic gains through breeding with respect to simply inherited traits, but the gains with respect to productivity were far from the expectations (Green et al., 1981; Ramanujam & Singh, 1981). According to Swaminathan (1973) this failure was due to poor selection efficiency and various physiological and management limitations. Chauhan et al. (1995) viewed it as the consequence of inherently poor partitioning of carbohydrates. Green et al. (1981) postulated that in pigeonpea the genotype-environment interactions for seed yield were extremely large even at micro (single plant) level. Such interactions induce tremendous non-heritable variability among individual plants and result in poor heritability for seed yield. Besides this, huge crop biomass with tall (>2 m) canopy and long primary and secondary branches spreading in all the directions also make it difficult for breeders to exercise effective single plant selections within the segregating populations. These factors adversely affect the performance (breeding value) of the pedigree selections and limit the genetic gains especially for traits like yield.

5 | THE ALTERNATE BREEDING APPROACHES

In the past half century the breeding efforts could not help in raising the productivity level of pigeonpea and, as discussed above, there may be various reasons for yield stagnation. The authors now feel that a time has come to look beyond the traditional cultivar breeding methods and to try some alternative breeding approaches. These options, briefly outlined in the following text, may provide opportunities to some forward-looking pigeonpea breeders to break the decades-old yield plateau.

5.1 | Transform pigeonpea from an often cross-pollinated to self-pollinated crop

Natural cross-pollination is considered both a boon (for hybrid breeding) and bane (for genetic contamination) for pigeonpea breeders. The development of hybrids is of recent origin, but the purity maintenance issues are causing difficulties for over a century. Since pure line varieties dominate the scenario, their purity maintenance is important but remains an expensive business due to abundance of insect pollinators present in the nature. According to the estimates

of Green et al. (1981) it takes only 1–2 generations of open-pollination in field to destroy the potential of cultivars and selections. The logical approach to address this issue is to search for a stable genetic solution. In this context, the search of a unique genotype with cleistogamous flowers is considered a land mark (Saxena, Ariyanayagam, & Reddy, 1992). The true-breeding line was obtained in the population derived from the cross between *Cajanus cajan* and *Cajanus lineatus*. This floral trait is easy to identify, simply inherited and allows only 1%–2% out-crossing as compared to 20%–25% in the normal flower type lines (Choudhary, Bhavana, Datta, & Saxena, 2020; Saxena, Jayasekera, Ariyaratne, Ariyanayagam, & Fonseka, 1994).

Considering the difficulties faced by breeders and seed producers, the incorporation of this trait, controlled by a single recessive gene, should be given a high priority in breeding pure line pigeonpea cultivars. Recently, Yadav et al. (2019) have delineated the genomic regions responsible for the cleistogamous trait using Axiom 50K SNP array. This will allow the marker assisted breeding to transfer this trait into elite cultivars and germplasm.

5.2 | Breed genotypes with rapid seed filling rates

Srivastava et al. (2012) observed a significant variation for the time taken from flowering to maturity within a set of early maturing inbred lines which flowered more or less at the same time. Some progeny took only 31 days from flowering to maturity; while in others this period was extended by over two weeks to 48.6 days. Such differences may appear due to the presence of different genetic regulatory mechanisms which control photo-period reaction in the plants (Y. S. Chauhan; pers. com.). These genes induce indeterminateness in the plants and extend their reproductive phase, resulting in significant delays in pod setting and maturity. Pazhamala et al. (2016) using RNA sequence data generated from germination to senescence in pigeonpea revealed the presence of candidate genes such as beta-conglycinin (*C. cajan_28781*), late embryogenesis abundant protein (*C.cajan_03928*), sugar-binding proteins (*C. cajan_34645*) whose expression patterns showed marked differences from flowering to pod setting. The "rapid pod filling" is a unique and important trait for pigeonpea because it will help in developing cultivars with uniform flowering and pod maturity to facilitate easy insect control. Besides this, it will also permit the mechanised culture for economic crop production.

5.3 | Adopt speed breeding technology

As discussed earlier the breeding efforts in pigeonpea are restricted due to its long generation turnover time. Saxena, Saxena, and Varshney (2017), Saxena, Saxena, Hickey, and Varshney (2019) developed a breeding technology that can help breeders to overcome this constraint. They forced 28-day old seeds to germinate with over 95% success. To conserve genetic variability while advancing the generations, this approach was integrated with single seed descent method of breeding. Using this technology, they turned as many as four generations within a year in early maturing genotypes. This is a potential breeding tool and its integration in early maturing variety breeding programmes will not only cut down the breeding time to about three years but also save considerable resources. However, as discussed by Bohra et al. (2020), the diverse maturity groups of pigeonpea genotypes coupled with its qualitative response to photoperiod poses a unique set of challenges while implementing speed breeding technology for shortening the crop breeding cycles.

5.4 | Adopt early generation testing approach

The concept of early generation testing is not new and it is recommended for conserving resources in plant breeding. In this approach the unproductive crosses are discarded early in the breeding programme and only potential cross combinations are selected for pedigree breeding. In pigeonpea, so far only one such study has been conducted by Saxena and Sharma (1983). They studied inter-generation relationships within different crosses and concluded that on the basis of F_1 performance some low yielding crosses can be rejected safely. The unselected F_2 bulk performance was also found to be related to F_3, F_4 and F_5 bulk performances, suggesting that some more crosses can also be rejected on the basis of F_2 data. Since the shrinking resources may not allow the luxury of exercising single plant selections in large number of crosses, the early generation testing may help breeders in executing the varietal development programme with reasonable input costs.

5.5 | Integrate prebreeding in cultivar breeding programmes

Wild relatives of a cultivated species are established resource for new genes and in various crops a number of genes have been mined from wild species and used for incorporating beneficial traits. In pigeonpea, in spite of large genetic variation in the wild species (Bohra et al., 2020; Yang et al., 2006) their usage of in breeding has been limited to the development of few high protein lines and cytoplasmic nucleus male sterility systems (Saxena, Patel, et al., 2018). The widening of genetic diversity in pigeonpea can be achieved by incorporating the targeted wild species genome in breeding programmes. However, the process of gene transfers from wild to cultivated species is resource intensive and has limited probability of success. Therefore, such programmes should be designed and implemented with elaborate planning and care.

Considering the complexities and limitations of inter-specific breeding programmes, the pigeonpea breeders now undertake the entire process in two stages. The first activity, popularly known as “prebreeding”, involves the development of advanced generation breeding populations (F_5/F_6 lines) with no selection imposed. In the second phase the genetic materials are withdrawn from the bulks and used for pedigree selections as and when needed. The

derivatives from prebreeding populations can be used either for cultivar development or as parental lines for future breeding programmes. In this endeavour, the selection of trait and donor wild species should receive high priority. The next logical step should be to identify the best accession within the selected wild species because a considerable genetic variation is also present among the accessions (Mallikarjuna, Saxena, & Jadhav, 2011; Saxena et al., 1990). In order to recover the genetic background of cultivated type with reduced linkage drag, usually two backcrosses are recommended to generate prebreeding populations with high frequency of useful alleles (Sharma, 2017).Since these inbreds will serve as base materials for future breeding programmes, it would be necessary to characterize them at whole genome level using molecular markers and for key traits such as resistance or tolerance to various biotic and abiotic stresses, productivity, combining ability, etc. The concept of prebreeding is now attracting breeders and slowly it is emerging as a cost-effective crop breeding tool. Towards this end, genomics-assisted approaches like advanced backcross (AB)-QTL mapping holds particular relevance as it efficiently exploits untapped variation of wild relatives via enabling detection and introgression of exotic QTL in a single population.

5.6 | Select inbred lines from promising hybrids

Integration of two diverse nuclear genomes by crossing CMS line with a restorer produces a hybrid. Such high yielding hybrids can also be used to breed pure line cultivars. In case the hybrid performance is due to preponderance of additive genes, these provide opportunities to derive promising pure lines. This can be done by fixing the additive genetic component of total variation through pedigree selection. Saxena and Sharma (1990) while reviewing the gene action in pigeonpea concluded that in most crosses additive genetic variation played an important role in the expression of yield. This information suggested that it is possible that from some high yielding hybrids promising inbred lines, carrying positive additive alleles from both the parents, can be identified from segregating generations through pedigree breeding. In a similar exercise conducted at ICRISAT, Saxena, Chauhan, Johansen, and Singh (1992) demonstrated that some of the hybrid- derived inbred lines achieved about 70% of the realised yield of the hybrid. These inbred lines expressed 20%–25% superiority over the male parent and 15%–20% standard heterosis. Since in pigeonpea a number of high yielding hybrids are already available (Saxena, Sharma, and Vales, 2019), this breeding approach can be fruitful in breeding high yielding inbred lines which can be used as parental materials or cultivars.

5.7 | Breed composite populations

The conventional pure line breeding procedures not only restrict recombination but also maintain some undesirable linkages. Pigeonpea being a partially cross-pollinated crop offers a unique opportunity to overcome these limitations by breeding composite cultivars (Khan, 1973). In this methodology the gene frequency of favourable alleles introduced from diverse sources is accumulated in a single heterogeneous population through random mating that is facilitated by natural cross-pollination. These composite populations, besides serving as a gene pool for deriving useful variability, can also be released as heterogeneous population for cultivation, especially for stressed environments. Onim (1981) implemented a population breeding programme for yield enhancement in pigeonpea and recorded 2% and 4% yield gains in each cycle of mass selection from the random mated populations. Considering the potential of this non-conventional breeding approach, it deserves consideration by pigeonpea breeders.

5.8 | Breed “Sybrid” population

As discussed above, sufficient level of hybrid vigour is present in pigeonpea, but it could not be exploited commercially due to seed quality reasons. To find a solution for this problem, Saxena (2020) designed a new breeding method, called "Sybrid". This method, an amalgam of the concepts of breeding synthetic and hybrid cultivars, allows harnessing a portion of heterosis and benefits from additive, dominance and epistatic genetic gene actions to produce more yields. “Sybrid”, however, will not be as productive as hybrid; but theoretically it is expected to be superior to inbred and synthetic cultivars in both yield and buffering ability.

In comparison to hybrids, the seed production of a “Sybrid” population is easy and involves natural cross-pollination but excludes male sterility system (Saxena, unpublished).

5.9 | Breed cultivars for major intercropping systems

Pigeonpea is a crop that is mostly cultivated under subsistence agriculture where the risk of crop failures is always high due to poor soil nutrition, various biotic and abiotic stresses, and frequent spells of droughts. Therefore, to get some sort of assurance against the crop failure risks, the farmers opt for intercropping pigeonpea with short-season cereals or legumes. At present there is no pigeonpea cultivar that has been bred specifically for any intercropping culture. The farmers therefore use those varieties which were bred under sole crop situations and this leads to their poor adaptation under intercrops. So far there is no research to define various constituent components of an ideal pigeonpea plant type that would perform well under intercropping. Saxena, Choudhary, Saxena, and Varshney (2018) reviewed this subject and concluded that for pigeonpea-cereal intercropping, a pigeonpea cultivar should have non-determinate spreading plant type and more number of long fruiting primary branches. Besides these, the traits like more pods/bunch, 5–6 seeds/pod, 12–14 g/100-seed weight, and resistance to wilt and sterility mosaic diseases, with ability to recover from various stresses are important. Breeding of an

"ideal" plant type with all these traits is not possible, but a beginning could be made by targeting a major intercrop (sorghum + pigeonpea or maize + pigeonpea) and select for some of the key traits.

5.10 | Develop cultivars with stable resistance

Pigeonpea encounters various biological (diseases and insects) and non-biological (water-logging, drought etc.) stresses during its life cycle. These stresses not only affect growth and development of crop but may result in total or partial yield losses (Choudhary, Sultana, Pratap, Nadarajan, & Jha, 2011). So far the effective resistance breeding in this crop has been limited to fusarium wilt and sterility mosaic diseases only. Considering the diversity of production environments, it is important that the cultivars maintain their productivity and resistances across the production areas. The prerequisite to achieve this is the presence of high stability in the donor germplasm. The main reasons for the breakdown of resistances are diversity (biotypes) of pathogen, genetic contamination of seed lots, or presence of extreme environmental conditions. Sharma et al. (2016) evaluated a number of pigeonpea genotypes for wilt (*Fusarium udum*) at a number of diverse locations and concluded that (a) the pathotypes present in diverse geographical origins were highly variable in terms of their virulence, (b) a considerable genotype x environment interactions existed in the expression for resistance, and (c) more than one pathotypes can co-exist at a single location. Similarly, for sterility mosaic virus three distinct strains have been reported and these have very clear geographical specificity (Sharma, Telangre, Ghosh, & Pande, 2015).

Since the genetic information on the pathotypes of the two major diseases is still inconclusive, and in most pigeonpea growing areas both the diseases co-exist, it is advisable to use the parental lines with high levels of resistances to both the diseases, viz., ICPL 20094, ICPL 20106, ICPL 20115, ICPL 20096, ICPL 20098, ICPL 20107 and ICPL 20110. These genotypes over three years have shown <10% wilt incidence at 8–9 hot-spot locations (Sharma et al., 2016); and at 4–5 hot-spot locations with respect to sterility mosaic disease (Sharma et al., 2015).

Water-logging is an important abiotic production constraint and in spite of huge losses, it was never given a priority in pigeonpea research. Recently, ICRISAT identified some genotypes with high levels of tolerance to water-logging (Sultana et al., 2013). These include ICPA/B 2043, ICPA/B 2039, ICPA/B 2047, ICPL 87119, ICPL 149 and ICPL 20125. Since the resistance to water-logging is controlled by a single dominant gene (Perera, Pooni, & Saxena, 2001; Sarode, Singh, & Singh, 2007) and its screening technology being available, the breeding of resistant pigeonpea cultivars can be undertaken with ease.

5.11 | Use of genomics-based heritability for the rapid genetic gains

Phenotypic variation is a combined expression of different heritable (additive) and non-heritable (dominance, epistasis etc.) factors and their interactions with different environmental components. The relative heritable value of a trait (heritability, h^2) determines its breeding value and genetic advance. The quantitative traits such as yield are prone to G x E interactions and their h^2 values are invariably low and enhancement of the trait value is a difficult task.

The emergence of different genomics technologies in recent times (Bohra et al., 2014, 2020) has opened up opportunities for rapid crop improvement, primarily due to elimination of unpredictable and complicated environment effects. These include marker assisted selection (MAS), marker assisted back-crossing (MABC), and early generation selection (EGS) etc. Besides these, the development of parallel genome sequencing and whole-genome re-sequencing (Kumar, Khan, Saxena, Garg, & Varshney, 2016; Varshney et al., 2012, 2017) has also facilitated the identification of millions of nucleotide sequence variations across the genome. Although the trait associated markers are useful in breeding, difficulties are encountered in identifying genetic markers for the quantitative traits having low heritability. There exis ts immense scope of enhancing the genetic gains by exploiting the potential of the new genomic methodologies such as estimation of breeding values based on parameters called genomic–estimated breeding values (GEBVs). The GEBVs facilitate genomic selection (GS) (Meuwissen, Hayes, & Goddard, 2001) or multi-objective optimized genomic breeding (MOOB) (Akdemir, Beavis, Fritsche-Neto, Singh, & Isidro-Sánchez, 2019). The genomics selection is already in use in various crops including pigeonpea (Bohra et al., 2020). The GEBVs that form the basis of GS rely on trait heritability, size of training population, phenotyping data collected on training population, type of DNA markers and marker-density, distributions of traits, and statistical methods etc. (Lorenz et al., 2011).

The multi-objective optimized genomic breeding (MOOB) has been proposed recently to control the rates of inbreeding and enhance multi-trait sustainable selection by reducing the effects of high selection pressure (Akdemir et al., 2019). The aim of this approach is to combine the favourable alleles from different individuals in new and superior haplotypes. The use of MOOB is advocated for defining the training population for the GS. In view of the current advances proposed to improve GS accuracy, we understand that in the case of pigeonpea MOOB can be used in defining the training population for the GS and combination of these approaches could provide the rapid and sustainable genetic gains.

6 | REJUVENATION OF HYBRID BREEDING PROGRAMME

The hybrid technology has a potential to break the decades-old yield barrier in pigeonpea. This fact has been verified by a number of research and on-farm trials conducted over the years. The results from multilocation trials reported in various ICRISAT and ICAR publications showed that the hybrids, on average, exhibited mean standard heterosis of 30%–50% (Bohra et al., 2020; Saxena, Sharma, and Vales, 2019). In spite of such high yields, the farmers are unable to reap the benefits of this technology due to difficulties encountered

in controlling the quality of hybrid seed. In the following text some methods and technologies are outlined that would help breeders to further enhance the yield, seed quality and stability of hybrids, besides refining the hybrid seed technology and make the commercial pigeonpea hybrids a reality (Figure 1).

6.1 | Use of genomics-based hybrid seed quality control

Traditionally, a Grow-Out Test (GoT) is performed to assess the purity of hybrid seeds (Pattanaik, Lakshmana Reddy, Ramesh, & Chennareddy, 2018). It involves the assessment of hybrid progeny for easily identifiable dominant morphological marker(s). Since in pigeonpea, the application GoT is not feasible due to its long generation turnover time, the genomics technologies involving molecular markers such as SSR (simple sequence repeat) and SNP (single nucleotide polymorphism) markers provide a viable option to overcome this key constraint. These markers should be able to amplify polymorphic and high quality alleles (peaks/bands) between A- and R- lines and should not show polymorphism between the A- and the B- line, (Bohra et al., 2011, 2015; Bohra, Jha, Pandey, et al., 2017; Saxena, Saxena, & Varshney, 2010). Once these markers are identified they can be used to screen DNAs of the hybrid seed along with that of A- and R- lines. When a particular seed exhibits two fragments (alleles) in its DNA, one from A- line and another from R- line, then it would be labelled as true hybrid. Seed quality control of the female parent (A-line) will involve accurate identification of A_4 cytoplasm and the nuclear genome of its maintainer (B-) line. This can be done through the use of A_4 cytoplasm-specific *nad7* derived marker (Sinha et al., 2015) and nuclear-genes specific DNA markers (Bohra et al., 2012; Saxena, Saxena, et al., 2010). This protocol is now ready for use by breeders and seed producers. The seed quality of the male parent (R-line) can also be determined by using unique fertility restoration gene (*Rf*-) specific signature markers. In this context, genomics regions controlling fertility restoration for A_4 CMS has recently been identified in pigeonpea (Bohra et al., 2012; Saxena, Patel, et al., 2018). In this seed quality testing technology a set of specific markers need to be developed for a given hybrid and its parents.

6.2 | Use naked-eye polymorphic markers for quality control of CMS lines and hybrids

The genetic purity of female parent (A- or B- lines) is important since their genetic contamination will have adverse effects on the quantum of hybrid vigour. In this context, a cost effective and simple technology such as use of ''naked eye polymorphic markers'' could be of value. The use of such distinctive morphological traits, which are easily identified by naked eye during early growth stages and not present in cultivated varieties or hybrids, could offer a great tool to ensure purity of parental lines and hybrid seed with minimum resources.

In pigeonpea, obcordate leaf shape, an easily identifiable trait, is a simply inherited recessive trait and can be incorporated easily into A- and B- lines for purity maintenance (Saxena, Vales, Kumar, Sultana, & Srivastava, 2011; Saxena, Saxena, Saxena, Khandelkar, & Sultana, 2011). The hybrids derived from crosses involving obcordate leaf A-line and fertility restorer with dominant normal lanceolate leaf would have normal leaves; thus the difference between the male sterile and hybrid plants would be very clear. Therefore, it is proposed that breeding of A- and B- lines with this morphological marker can be undertaken in future hybrid breeding programmes. The hybrids derived from obcordate lines and normal leaf type restorer (R) lines will have normal lanceolate leaves, and the off-type sibs will have obcordate leaves. This particular marker expresses

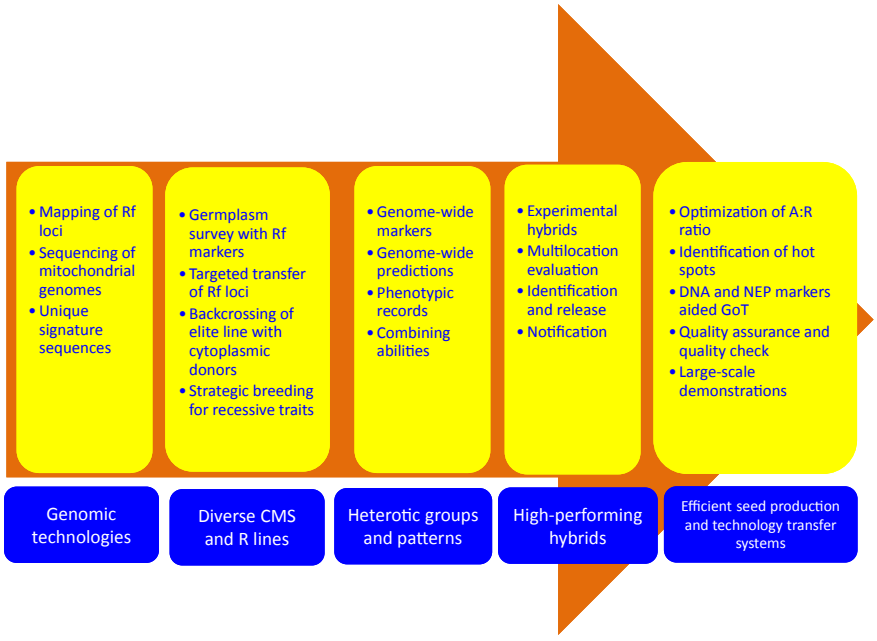

FIGURE 1 Approaches to strengthen hybrid breeding in pigeonpea. The availability of the CMS and restorer lines should be enhanced through the use of genomic technologies. Modern genomic technologies may also help developing heterotic pools and to identify the heterotic patterns for sustained gains from hybrid breeding. Equally important will be the efficiency of seed production and technology transfer systems

within four weeks after sowing and rouging of off-types within hybrid progeny can be done easily.

6.3 | Molecular tagging of fertility-restoring genes

In pigeonpea hybrids two dominant genes have been reported to control their fertility restoration (Saxena, Vales, et al., 2011) and to breed new hybrids, additional fertility restorer lines are always in demand. The breeding of new fertility restorers is time consuming, since it involves test crossing of each selection for the presence of *Rf* gene(s). The job of transferring these genes into non-restorers and selection of fertility restorers within segregating populations can be done quickly and economically using molecular marker technology. This is facilitated by conducting linkage analysis in experimental populations segregating for fertility restoring genes. In pigeonpea, Bohra et al. (2012) analysed three such mapping populations that segregated for male sterility and fertility, and QTL mapping detected four quantitative trait loci (QTL) controlling 14.8 to 24.17% phenotypic variation for fertility restoration. By using genotyping-by-sequencing (GBS) approach, Saxena, Patel, et al. (2018) recently discovered one major QTL on CcLG08 explaining up to 28.5% phenotypic variation for fertility restoration trait. The entire protocol for tagging the fertility restoring genes is now ready and breeders can start using this technology in hybrid breeding.

6.4 | Identify hybrid seed production hot spots

Since pigeonpea hybrid plants are vigorous and plastic in nature, their recommended commercial seeding rate is @ 5 kg/ha. Also, the seed producers generally harvest hybrid (A × R) yields of 1,000 kg/ha or more (Saxena, Saxena, et al., 2011). These facts mean an encouraging seed-to-seed ratio of 1:200; and it is considered quite healthy by seed production point of view. It has also been demonstrated that the hybrid yields can be increased by selecting suitable seed production sites and adoption of good crop management practices. The seed production hot spots can be identified in a region by organizing a series of small sized "pilot seed production programmes". The pod set and yield under natural conditions will indicate the presence of pollinating vectors at a particular site; and this way suitable seed production locations can be identified for good hybrid yields.

6.5 | Diversify nuclear base of hybrid parents

In a dynamic hybrid breeding programme, induction of new parental lines at regular intervals is essential to produce new hybrid products. It has been recognized that besides high per se performance, the hybrid parents should be good combiners, stable for male sterility (in female parents) and fertility restoration (in hybrids). However, their selection should be guided by breeding objectives, targeted cropping system and genetic diversity. The nuclear diversification of the male parents (R-lines) can be enhanced through screening of new germplasm, targeted breeding for fertility restoration and by converting elite maintainers into fertility restorers (Saxena, Sharma, and Vales, 2019). On the other hand the nuclear diversification of the female parents (A-lines) can be accomplished through standard backcrossing to the known maintainer lines (Bohra, Jha, Singh, et al., 2017).

6.6 | Diversify cytoplasmic base of CMS lines

The unique three-parent hybrid breeding systems sometimes become fragile due to invasion of certain undesirable genetic factors associated with specific cytoplasm as it was experienced in corn hybrid programme that was based on a single "T cytoplasm". This cytoplasm had genes for the susceptibility to southern corn leaf blight disease (Levings, 1993); and consequently, all the hybrids made on this male sterile lines carrying this cytoplasm were knocked down by the blight disease. In order to overcome such potential threats arising due to cytoplasmic uniformity, it is essential to breed female parents with diverse cytoplasm base. In pigeonpea so far nine CMS systems derived from different wild species have been reported (Bohra, Jha, Premkumar, Bisht, & Singh, 2016; Saxena, 2013; Saxena, Sultana, et al., 2010). Although these male-sterility systems represent a wide cytoplasmic variation but so far only two of them derived from *C. cajanifolius* and *C. scarabaeoides* have been used in hybrid breeding. Therefore, in future breeding programmes more emphasis should be given to develop male sterile lines with different cytoplasm sources.

6.7 | Breed CMS lines with dominant wilt resistance genes

Fusarium wilt is the most common pigeonpea disease across all the cultivation areas. Breeding of wilt resistant inbred cultivars using sick-nursery approach and recessive resistance genes has been quite successful (Saxena, 2008). However, breeding of wilt resistant pigeonpea hybrids using these sources is rather cumbersome and resource intensive as all the three hybrid parents (A, B, R) need to carry the recessive resistance alleles. This situation can be eased if a dominant gene for wilt resistance is incorporated in the female parents. This will enhance the scope of breeding wilt resistant hybrids. Since the crosses made on such females using resistant or susceptible restorers will produce only resistant hybrids. In this context, it may be noted that Saxena et al. (2012) have already identified a genotype (ICPL 87119) which not only restores full pollen fertility in hybrid combinations but also carries a pair of dominant wilt resistance alleles. Pigeonpea breeders can make use of this resource in breeding wilt resistant inbred parental lines and hybrids.

6.8 | Establish and use heterotic pools

The concept of "heterotic pool" involved formulation of groups of parental lines for facilitating crop breeding, and producing high yielding hybrids and inbred cultivars. This tool is used to discriminate germplasm/parental lines on the basis of their combining ability, origin or genetic divergence that is measured through some logical statistical or genomics tools. In pigeonpea, Saxena and Sawargaonkar (2014) made the first such attempt and formulated seven heterotic groups using multi-location specific combining ability data. They also confirmed that heterosis for seed yield was much greater when the parental lines representing the two diverse heterotic groups were crossed. The use of marker technologies such as SSRs and SNPs to select diverse parental lines for hybrid breeding is considered more effective as it eliminates the effects of environment and genotype × environment interactions in the formation of heterotic groups (Aguiar, Schuster, Amaral Junior, Scapim, & Vieira, 2008; Mudaraddi & Saxena, 2015). With the advancement in high-density parallel genotyping technologies, genome-wide predictions or GS have recently assumed greater significance to establish heterotic groups and identify high-yielding heterotic patterns for sustained yield gains in different crops including pigeonpea (Bohra et al., 2020). The formation and use of heterotic groups with respect of new germplasm or breeding materials can be effective in conserving resources by eliminating some undesirable parental lines from breeding programme and for producing high yielding pigeonpea hybrid combinations.

6.9 | Use environment-sensitive male sterility in hybrid breeding

Environment-sensitive male sterility is a unique system where the expression of male sterility and fertility in the plants is controlled by environmental factor. Under this system the male sterility expresses only under specific environment such as low or high temperature, short or long photo-period, variable light intensity, and or soil-borne stress (Kaul, 1988). Recently, in pigeonpea also an environment-sensitive male sterility system was bred by Saxena (2014). In this system the male sterility is expressed only when exposed to high (>25°C) temperature regime (Pazhamala et al., 2020). In this environment, such male sterile plants can be used to produce hybrid seed with assistance from insect pollinators. Interestingly, when the same male sterile (A-) line is exposed to low (<24°C) temperatures, its anthers start producing fully fertile pollen grains and self-pollinated seeds (as in normal cultivars). This means that the seed of the male sterile line can be multiplied like a normal fertile inbred line without any maintainer (B-) line. The development of this unique male sterility system in pigeonpea has opened up options for breeders to develop two-parent (A- and R-) hybrids.

7 | CONCLUDING REMARKS

Scores of farmers in the tropics and sub-tropics earn their livelihoods through subsistence agriculture and pigeonpea is considered as its important crop component because, it helps to provide nutrition to the farming families as well as their agricultural lands. To meet the requirements of ever increasing population and limited land area, the productivity enhancement research and development efforts in pigeonpea deserve high priority. Considering the limitations of traditional breeding methods in increasing the crop productivity, some alternative breeding approaches are suggested; and these may provide viable options to pigeonpea breeders in developing new cultivars. The use of new breeding tools will reduce the dependency on pedigree breeding and help in producing high yielding inbred cultivars and hybrids. The authors have identified some priority research areas such as (a) incorporation of high self-pollinating trait, (b) reduction in maturity and diversity of cropping systems, (c) enhance the genetic diversity, (d) incorporate stable disease resistances, (e) commercialize hybrids, and (f) increased use of genomic tools for improving breeding efficiency and seed quality control.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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