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REVIEW ARTICLE

Harnessing the Potential of Crop Wild Relatives through Genomics Tools for Pigeonpea Improvement

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Cultivated pigeonpea germplasm has a narrow genetic base due to the bottlenecks caused by domestication and breeding from a small number of genotypes. Pigeonpea genetic improvement has witnessed a slow pace due to low genetic diversity and to the scarce genomics resources. To address these challenges, wild relatives of pigeonpea which represent an unexploited resource of vast genetic variation can be incorporated in breeding programmes facilitating the broadening of genetic base. Although interspecific hybridization has not been commercially successful in pigeonpea, it has played an important role in the development of the cytoplasmic male sterility (CMS) system. Recent years however have witnessed the development of genomics resources at large scale in the crop which has remained untouched with genomics revolution in the past. These resources, together with advances in genomics platform such as high throughput genotyping assays and next generation sequencing technologies and modern genetics and breeding approaches will accelerate harnessing natural variation for pigeonpea improvement.

Keywords: Wild relatives, molecular markers, linkage mapping, QTL, CMS, introgression lines.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a short-lived perennial shrub that is traditionally cultivated for grains as an annual crop in the semi-arid tropics (SAT). The SAT region is generally characterized by erratic rainfall, longer dry season, mineral deficient soils, high transpiration and unpredictable weather. Pigeonpea ranks sixth in the global grain legume production followed by beans, peas, chickpeas, broad beans and lentils. It is the second most important grain legume in India after chickpea accounting for 3.72 Mha area and 3.07 million tons of production (FAO, 2009). Pigeonpea is a vital source of protein (20–22%) especially for vegetarian diet.

Being one of the most important legume crops in rainfed agriculture, concerted research effort has been directed towards genetic enhancement of pigeonpea. Unfortunately, no increase has been witnessed in its productivity, which in the past five decades has remained stagnant at around 700 kg ha^{-1} (Figure 1) and lack of high yielding cultivars has been identified as one of the major factors explaining the yield plateau. In addition, other factors such as poor crop husbandry and prevalence of biotic and abiotic stresses in pigeonpea growing areas are also responsible for low productivity. Among biotic stresses, pests like pod borers (*Helicoverpa armigera* Hubner, *Maruca vitrata* Geyer), pod fly (*Melanagromyza chalcosoma* Spencer), and diseases like *Fusarium* wilt (*Fusarium udum* Butler), sterility mosaic disease (SMD) and *Phytophthora* blight (*Phytophthora drechsleri* Tucker) cause major yield losses every year. Furthermore, sensitivity to abiotic stresses like water-logging and salinity also reduces pigeonpea production.

Availability of adequate genetic variation in germplasm collections is a prerequisite for a successful breeding programme. Genomics tools such as molecular markers, mapping populations, genes, expressed sequence tags (ESTs) offer an attractive opportunity to exploit lots of cryptic genetic variation existing among germplasm collections through molecular breeding approaches (Varshney *et al*., 2005). However, the molecular breeding approach has not yet been initiated in pigeonpea primarily due to: (i) availability of limited genomics resources, and (ii) limited

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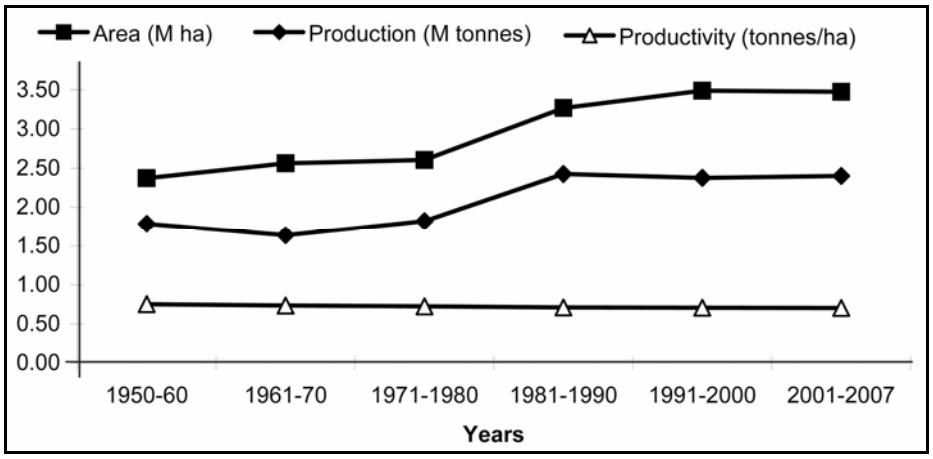

Figure 1. Area, production, and productivity of pigeonpea in the last five decades. Area under cultivation of pigeonpea has increased from 2.3 mha in 1950 to 3.53 mha in 2007 but productivity remained stagnated at around 700 kg ha^{-1}.

level of genetic diversity in a majority of elite germplasm collection. On one hand, genomics resources are being developed in pigeonpea as a part of International Initiative for Pigeonpea Genomics (IIPG, http://www.icrisat.org/gt-bt/IIPG/Home.html) and Pigeonpea Genomics Initiative (Varshney *et al*., 2010a). On the other hand, in addition to using the cultivated pigeonpea germplasm collection, efforts can be made to explore and harness the genetic variation present in crop wild relatives (CWRs) of pigeonpea. For instance, the discovery of cytoplasmic genetic male sterility (CMS) system from CWRs for producing hybrids is an elegant example of utilization of genetic variation from CWRs in pigeonpea breeding. The purpose of this article is to present a critical appraisal on potential and progress on development and utilization of genomics tools and genetic resources especially CWRs in pigeonpea improvement.

Taxonomy and gene pools of pigeonpea

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] belongs to the subtribe Cajaninae of tribe Phaseoleae under subfamily Papilionoideae of family Leguminosae. Earlier the subtribe Cajaninae had 13 genera and *Cajanus* was considered to be closely related to *Atylosia*. Later, based on various morphological, taxonomical and cytological evidences *Atylosia* was merged with *Cajanus*. Now the genus *Cajanus* comprises a total of 32 species and 11 genera are identified in the subtribe *Cajaninae* (van der Maesen, 1990) belonging mainly to India and Australia. *Cajanus cajan* represents the only domesticated species under subtribe Cajaninae. Pigeonpea has a diploid ($2n = 2x = 22$) genome with a physical size of 0.853 pg or 858 Mbp (Greilhuber and Obermayer, 1998). With the noteworthy exception of *C. kertsingii*, the only species originated in Africa, which is considered to have a haploid chromosome number of 16, i.e. $n = 16$ (Gill and Hussaini, 1986), all the wild relatives of pigeonpea carry the same chromosome number ($2n = 2x = 22$) as observed in the cultivated type.

Among all the wild relatives of pigeonpea, *C. cajanifolius* (formerly *Atylosia cajanifolius*) resembles the cultivated pigeonpea in all morphological attributes except the presence of a prominent strophiole. Apart from this, these two species cross easily and provide viable F_1s, suggesting the strong genomic harmony between the two species. Hence, based on several morphological, biochemical and cytological investigations, *C. cajanifolius* was considered to be the most probable progenitor of cultivated pigeonpea (Pundir and Singh, 1985). Parsimony analysis using restriction fragment length polymorphism (RFLP) markers (Nadimpalli *et al*., 1993) and karyotype studies (Ohri and Singh, 2002) have also supported the origin of cultivated pigeonpea from *C. cajanifolius*.

On the basis of the amount of gene flow among various species, the concept of different gene pools was established by Harlan and de Wet (1971). According

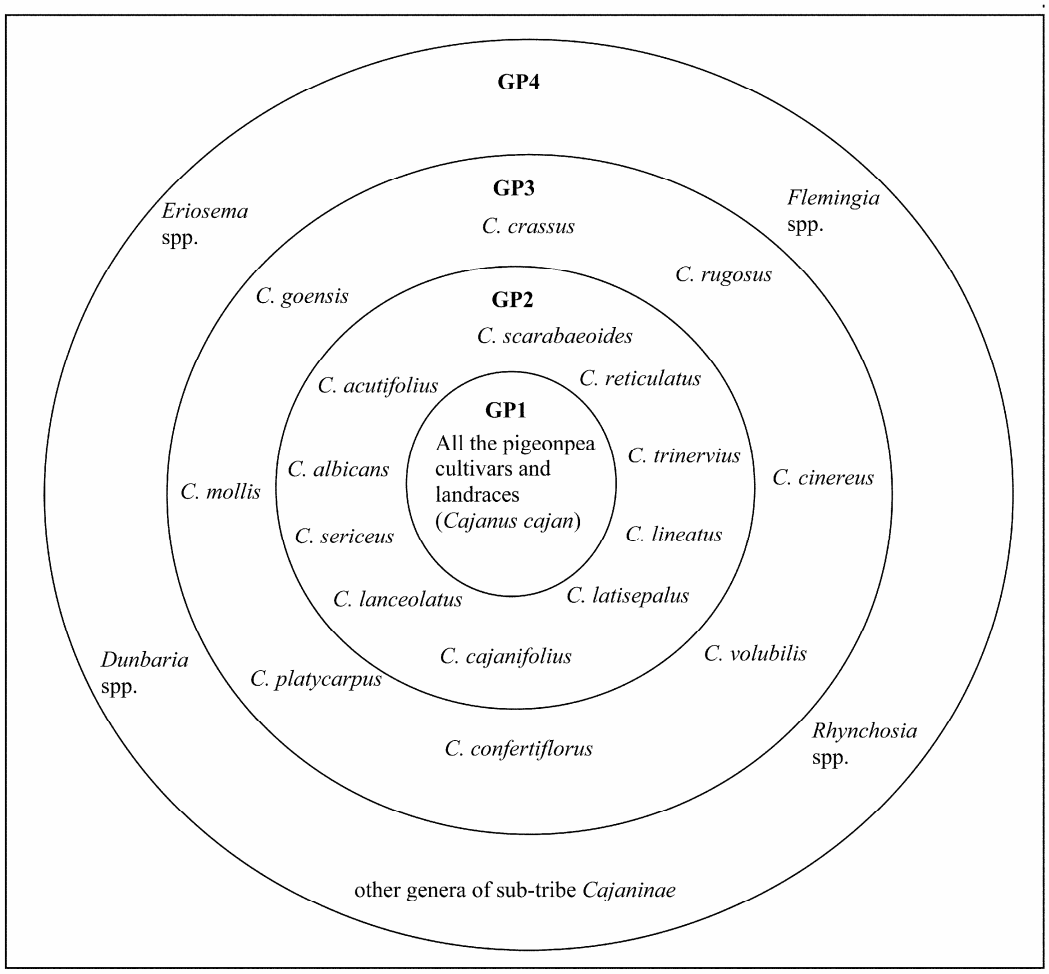

Figure 2. Genetic resource profile of pigeonpea. The entire pigeonpea germplasm comprises four gene pools (GPs) based on crossability relationships. Cultivated types are included in primary gene pool (GP1). Remaining all *Cajanus* species occupy positions in secondary (GP2) and tertiary (GP3) gene pools. Other related genera constitute quaternary gene pool (GP4) delimiting the boundary of genetic resources.

to this concept, primary gene pool (GP1) comprises the biological species which intercross easily to produce fertile hybrids. Species from the secondary gene pool (GP2) may produce partial fertile hybrids with the cultivated species, while the tertiary gene pool (GP3) comprises the species which either are not crossable with the cultivated species or crossable with the aid of embryo rescue or tissue culture techniques. The hybrids resulting from the crossing with GP3, generally have very low fertility. GP1 of pigeonpea consists of all the cultivars or landraces of *C. cajan*. All the wild *Cajanus* species are occupying positions in GP2 and GP3. In addition to this, other related genera such as *Rhynchosia*, *Dunbaria*, *Flemingia*, etc. make up the extreme outer limit for gene pool known as quaternary gene pool (GP4) (Figure 2).

A large amount of germaplam collections is present in several genebanks around the world. The largest collection, however, is with ICRISAT. The ICRISAT genebank holds an active collection of 13,632 accessions comprising 8,215 landraces, 4,795 breeding material, 555 wild relatives and 67 cultivars and advanced lines (Table 1). These 555 wild relatives of pigeonpea represent a total of 57 wild species (Upadhyaya *et al*., 2007; Saxena 2008).

Distant hybridization in pigeonpea

During the course of evolution several genetic bottlenecks resulting from plant domestication, allopatric and sympatric speciation, polyploidisation and inbreeding have witnessed a random loss of alleles leading to genetic erosion (Tanksley and McCouch, 1997). The situation is exacerbated by practising breeding methods based exclusively on selection and inbreeding or mating of genetically related individuals. This has ultimately led to the availability of extremely low genetic variation in the cultivated gene pool, especially in case of self-pollinated crop (Miller and Tanksley, 1990). Therefore it is important to use CWRs in breeding programme to expand the narrow genetic base of cultivated gene pool. Such scenario was predicted a long time ago in 1970 by the great plant explorer and geneticist Jack R. Harlan as following: '*For the sake of future generations, we must collect and study wild and weedy relatives of our cultivated plants as well as the domesticated races. These sources of germplasm have been dangerously neglected in the past, but the future may not be so tolerant. In the plant breeding programs of tomorrow we cannot afford to ignore any source of useable genes*.'

The ICRISAT genebank collection of 13,632 accessions includes 555 wild accessions from 57 species (Upadhyaya *et al*., 2007; Saxena, 2008). Despite the availability of large collection, the use of germplasm in breeding programme has been very limited; therefore the concepts of core and mini core collections have been proposed to accelerate the use of germplasm in breeding programmes. Core collection is composed of 10% of entire collection but represents the maximum possible genetic diversity (~70% of total alleles) available in the germplasm collection (Brown, 1989). Similarly, the mini core collection further reduces the number of accessions to 1% of total or 10% of core collection but still represents the maximum genetic spectrum of the core collection (Upadhyaya and Ortiz, 2001) (Table 1). The core and mini core collections of pigeonpea, composed of 1,290 and 146 accessions respectively have been developed at ICRISAT (Table 1).

Table 1. Pigeonpea germplasm holdings at ICRISAT genebank.

Material	Number of accessions	Reference
[a]Active collection	13,632	Upadhyaya *et al*., 2008
Landraces	8,215	Upadhyaya *et al*., 2011
Breeding material	4,795	Upadhyaya *et al*., 2011
Wild relatives	555	Upadhyaya *et al*., 2007
Advance cultivars	67	Upadhyaya *et al*., 2010
[b]Core collection	1,290	Reddy *et al*., 2005
Breeding material	466	Upadhyaya *et al*., 2007
Landraces	810	Upadhyaya *et al*., 2007
Advance cultivars	9	Upadhyaya *et al*., 2007
Others	5	Upadhyaya *et al*., 2007
[c]Mini core collection	146	Upadhyaya *et al*., 2006
[d]Base collection	11,794	Upadhyaya *et al*., 2008

[a]Collection available for distribution to plant breeder.
[b]Represents 10% of active collection.
[c]Represents 10% of core collection or 1% of active collection.
[d]For long term storage purpose.

Conservation of CWRs in genebank provides an opportunity to minimize the risks of genetic erosion. Some of these CWRs possess the genes for resistance to several stresses as well as agronomic traits (Table 2). Therefore these genetic resources are of great interest to breeders for introgressing useful traits in breeding programmes. Several efforts have been made in the past to incorporate such economically important traits from wild relatives in the cultivated gene pool (Dundas, 1990). Some of the challenging issues such as non synchronous flowering, low rate of success in inter-specific hybridization, hybrid inviability, etc. still exist in realization of the full potential of CWRs.

The species from GP 2 which have shown crossability with the cultivated type are *C. sericeus*, *C. albicans*, *C. lineatus*, *C. trinervius*, *C. cajanifolius* and *C. scarabaeoides* (Reddy *et al*., 1981). Interspecific hybridization was successful when the cultivated type was used as a female parent but reciprocal crosses have also been achieved with *C. cajanifolius*, *C. lineatus* (Pundir and Singh, 1985) and *C. scarabaeoides* (Ariyanayagam and Spence, 1978). Some CWRs of pigeonpea like *Atylosia* and *Rhynchosia* were also used for crossing with *C. cajan* and resulting hybrids, F_2 and F_3 families were studied for physiological efficiency and agronomic superiority (Pundir and Singh, 1986). Interspecific hybridization involving *C. cajanifolius*, *C. scarabaeoides*, *C. sericeus* and *C. platycarpus* as donor parents showed successful production of hybrids from *C. cajan* × *C. sericeus* and *C. cajan* × *C. cajanifolius* while crosses with *C. scarabaeoides* and *C. platycarpus* resulted in hybrids with pods having shriveled and non-viable seeds (Yadav and Padmaja, 2002) elucidating the presence of some crossability barriers. Existence of

Table 2. Wild species harbouring genes for resistance/tolerance to various biotic and abiotic stresses and agronomically important traits.

Wild species	Important traits	Reference
C. albicans	Sterility mosaic disease resistance	Remanandan, 1981
	Pod borer resistance	Mallikarjuna *et al*., 2007
	Pod fly resistance	Sharma *et al*., 2003
	High protein content	Saxena *et al*., 2002
	Drought tolerance	Mallikarjuna, 2003
	High fruit set	Pundir and Singh, 1987
C. acutifolius	Pod borer resistance	Mallikarjuna *et al*., 2007
	Pod fly resistance	Sharma *et al*., 2003
	Salinity tolerance	Srivastava *et al*, 2006
C. lineatus	Sterility mosaic disease resistance	Remanandan, 1981
	Pod fly resistance	Sharma *et al*., 2003
	High protein content	Remanandan, 1981
	Drought tolerance	Mallikarjuna, 2003
C. scarabaeoides	Pod borer resistance	Pundir and Singh, 1987
	Pod fly resistance	Sharma *et al*., 2003
	High protein content	Saxena *et al*., 2002
	Salinity tolerance	Srivastava *et al*., 2006
	Drought tolerance	Mallikarjuna, 2003
C. sericeus	Sterility mosaic disease resistance	Remanandan, 1981
	Pod borer resistance	Mallikarjuna *et al*., 2007
	Phytophthora blight	Kannaiyan *et al*., 1981
	Pod fly resistance	Sharma *et al*., 2003
	High protein content	Saxena *et al*., 2002
	Salinity tolerance	Srivastava *et al*., 2006
	Drought tolerance	Mallikarjuna, 2003
	High fruit set	Pundir and Singh, 1987
	Bruchid and water logging tolerance	Mallikarjuna *et al*., 2011
C. volubilis (*C. crassus*)	Sterility mosaic disease resistance	Remanandan, 1981
	High protein content	Remanandan, 1981
C. mollis	High protein content	Remanandan, 1981
C. reticulatus	Pod borer resistance	Dodia *et al*., 1996
C. platycarpus	Pod borer resistance	Mallikarjuna *et al*., 2006
	Phytophthora blight	Mallikarjuna *et al*., 2006
	Salinity tolerance	Srivastava *et al*., 2006
	Early flowering	Mallikarjuna *et al*., 2006
	Photoperiod insensitivity	Mallikarjuna *et al*., 2006
	High fruit set	Pundir and Singh, 1987
R. rothii	Early flowering	Pundir and Singh, 1987
	Drought tolerance	Pundir and Singh, 1987
	Photoperiod insensitivity	Pundir and Singh, 1987
R. bracteata	Pod borer resistance	Mallikarjuna *et al*., 2007
	Pod fly resistance	Sharma *et al*., 2003
Flemingia spp.	Pod borer resistance	Mallikarjuna *et al*., 2007

post-zygotic barriers in hybridization with *C. platycarpus* such as embryo abortion within 6 days after pollination has also been reported by Mallikarjuna and Moss (1995).

Interspecific hybridization in pigeonpea has witnessed the recovery of some novel plant types. For example three agronomic superior lines were developed utilizing a partial cleistogamous line isolated from an inter-

specific population derived from the cross *C. cajan* × *C. lineatus* (Saxena *et al*., 1998). Similarly, appearance of transgressive segregants in interspecific crosses led to the isolation a genetic dwarf progeny from crosses *C. cajan* × *C. scarabaeoides* and *C. cajan* × *C. cajanifolius*. Due to amenability to mechanical harvesting and improved harvest index (HI), these dwarf genotypes may be useful in reconstructing the ideal plant types in pigeonpea. Hybrids with enhanced pod borer resistance were also obtained from the cross between *C. cajan* and *C. acutifolius* (Mallikarjuna and Saxena, 2002). In a similar way, distant hybridization between *C. cajan* and *C. scarabaeoides* has produced a line, ICPL 87162 with increased protein content of more than 27% (Reddy *et al*., 1997).

One of the wild species from GP3, *C. platycarpus*, has been extensively attempted for trait introgression as it possesses many important traits such as extra-early flowering and maturity, photoperiod insensitivity, prolific flowering and pod setting, annuality, rapid seedling growth, salinity tolerance, resistance to phytophthora blight, cyst nematode, and *Helicoverpa* (Mallikarjuna *et al*., 2006). Successful backcross was performed by using *C. platycarpus* as a female parent and *C. cajan* as a male parent while the reciprocal cross did not generate any progeny. Hybrids between *C. platycarpus* and cultivated pigeonpea have been obtained through the use appropriate embryo rescue techniques to prevent the embryo abortion followed by chromosome doubling through colchicines treatment. Advance generation progeny lines showed insect and disease resistance and salinity tolerance (Mallikarjuna *et al.*, 2011). Hence tissue culture and embryo rescue techniques opened a new avenue for the utilization of GP3 in routine breeding programmes (Mallikarjuna and Moss, 1995; Mallikarjuna, 2007). However, successful utilization of these methods has yet to be realized in crop improvement programmes.

Advent of hybrid technology

Pigeonpea is credited to be the most suitable crop for subsistence agriculture because it is drought tolerant, needs minimum inputs, and can produce reasonable quantities of food even under unfavourable conditions. In spite of release of more than 100 pure line varieties in India, there has not been any significant improvement in the crop productivity (Figure 1). The issue of productivity plateau has been a major concern for a long time and to date it has remained a challenge. To achieve a quantum jump in yields the concept of hybrid breeding using its natural out-crossing and male-sterility systems was initiated by ICRISAT. In the last few years significant progress has been made to breed and take the hybrid technology to Indian farmers. The availability of CMS was found essential to produce commercially viable hybrid system. In pigeonpea, an exhaustive study has revealed the non existence of any source of cytoplasmic genetic male sterility (CGMS) in cultivated germplasm (Saxena, 2008); hence, plans were made to breed for this trait by placing pigeonpea genome into the cytoplasm of its wild relatives. It was expected that the interaction of such cytoplasm and nuclear genomes would produce male-sterility that would be inherited maternally. To achieve this, wild relatives of pigeonpea were crossed with the cultivated types. These interspecific crosses involving wild pigeonpea have resulted in the development of stable CMS lines (Ariyanayagam *et al*., 1993; Tikka *et al*., 1997; Souframanien *et al*., 2003). Hence, wild relatives of pigeonpea provided the last resort for the development of CGMS system which led to the paradigm shift from traditional methods of selection and population improvement to three (A, B and R) line or hybrid breeding system in pigeonpea.

Since 1981, the first attempt was made by Reddy and Faris (1981) by crossing a wild relative *C. scarabaeoides* and a cultivar, several efforts have been made in the direction of CMS development with reasonable success. So far, various wild relatives have been identified as source of CMS cytoplasms in pigeonpea (Saxena *et al*., 2010) such as:

C. cajanifolius: It is the most closely related wild species of pigeonpea and is considered as the progenitor of cultivated type that differs only by a single gene (De, 1974). The F_1 hybrid plants involving this CMS produced excellent pollen load and pod set. The A-lines with *C. cajanifolius* cytoplasm are being used extensively in hybrid breeding programmes. An efficient seed production system that could provide quality seeds at economically viable costs was also developed successfully.

C. scarabaeoides: It is another closely related wild species of pigeonpea used frequently in wide crosses and represents source of A_2 cytoplasm.

C. sericeus: A_1 cytoplasm has been derived from *C. serieus*. A_1 cytoplasm offers a potential source for the development of early maturing 'A' lines.

C. volubilis: A_3 cytoplasm, derived from *C. volubilis*, could not be employed in commercial hybrid breeding due to problem of poor fertility restoration.

C. acutifolius: Hybridization of *C. acutifolius* (male parent) with *C. cajan* (female parent) resulted in development of A_5 cytoplasm. This is the only system which was developed with cytoplasm of cultivated pigeonpea. The system is under active development and efforts are underway to identify maintainers and many pigeonpea cultivars act as good restorers.

C. lineatus: A partial male sterile plant obtained from an open pollinated population of the wild *C. lineatus* led to the discovery of A_6 cytoplasm.

C. platycarpus: Recently, a new CMS system derived from crossing of *C. platycarpus* and cultivated pigeonpea has become available (known as A_7). Efforts are being made to explore the possibilities of utilizing this system in routine hybrid breeding programme.

The CMS-based hybrid pigeonpea technology is now ready for implementation with all its major components in place. However, considering a vast variation in agro-ecological conditions, fine tuning of the seed production technology is essential to suit local environments. The level of yield superiority observed in hybrids demonstrated that they have higher yield potential. It has been observed that the magnitude of realized heterosis for yield in pigeonpea is more or less similar to those of other crops. Therefore, it can be exploited commercially in pigeonpea since a grower-friendly mass hybrid seed production technology is now available (Saxena *et al*., 2010). It is believed that in pigeonpea, the first breakthrough in yield will come only from the hybrids. In this endeavour a good beginning has been made and soon farmers will reap the benefits of this technology. Discovery of a number of CMS sources from pigeonpea CWRs sheds a light to a very small fraction of untapped genetic variation which actually exists in several fold quanta among these CWRs.

Expanding the repertoire of genomics resources in pigeonpea

Genomics has revolutionized breeding programme in several crops such as rice, maize, wheat, barley, soybean, tomato, etc. (see Varshney and Tuberosa, 2007). However this has not been the case in pigeonpea. Limited genomics research in pigeonpea can be attributed to: (a) limited or non-availability of appropriate genomics resources, (b) a low level of DNA polymorphism in cultivated germplasm. In the past, a majority of the genomics studies were confined to diversity analysis using the first or second generation of markers such as RFLP and RAPD (random amplified polymorphic DNA). Several of these molecular diversity studies in pigeonpea have been summarized in Table 3. These diversity studies revealed the existence of high level of polymorphism among the wild types while little polymorphism was observed among the cultivated germplasm.

Among various marker systems, simple sequence repeats (SSRs) or microsatellites and single nucleotide polymorphisms (SNPs) are considered the preferred marker systems for the genetics and breeding community (Gupta and Varshney, 2000; Gupta *et al*., 2001). The first set of 10 SSR markers however became available only in 2001 (Burns *et al*., 2001). Subsequently, additional SSR markers have been generated at ICRISAT by using SSR-enriched library (Odeny *et al*., 2007, 2009; Saxena *et al*., 2010a) and about 200 SSR markers became available. Less than 10% SSR polymorphism in cultivated germplasm demanded the availability of a large number of SSR markers for developing a useful set of SSR markers for pigeonpea breeding. Therefore as a result of collaborative efforts of ICRISAT, University of California (UC)-Davis (USA), and National Research Centre on Plant Biotechnology (NRCPB) (India), a novel set of 3,072 SSR markers was developed after mining about 80,000 BAC-end sequences (BESs) (see http://www.icrisat.org/gt-bt/IIPG/Home.html; Varshney *et al*., 2010a; Bohra *et al*., 2011). In addition, high throughput (HTP) genotyping assays such as Diversity Array Technology (DArT) and GoldenGate assays for genotyping the single nucleotide polymorphisms (SNPs) have been developed. For instance, DArT arrays with about 15,360 features have been developed (Yang *et al*., 2006, 2010) and similarly, a GoldenGate assay with 768 SNP features has been developed by UC-Davis in collaboration with ICRISAT and National

Table 3. Diversity studies using molecular markers in pigeonpea.

Marker types	Experimental material	Outcome	Reference
RFLP (15 random genomic probes)	24 genotypes belonging to genera *Cajanus*, *Dunbaria*, *Eriosema* and *Rhynchosia*	Distinct clusters were obtained due to adequate RFLP polymorphism among various species. Accessions of cultivated *C. cajan* shared more DNA fragments with *C. scarabaeoides* than with *C. cajanifolius*.	Nadimpalli *et al*., 1993
RFLP (5 maize mitochondrial probes)	3 putative male sterile lines derived from the inter-specific hybridization between *C. sericus* and *C. cajan*, five *Cajanus* cultivars, one accession of *C. sericeus* and two GMS lines	All the CMS lines showed the hybridization pattern similar to that of *C. sericeus* indicating mitochondria of CMS line derived from wild parent (*C. sericeus*). The variation among different pigeonpea cultivars and GMS lines may be accounted to mitochondrial rearrangement.	Sivaramakrishnan *et al*., 1997
PCR-RFLP (4 chloroplast gene specific primers)	28 species belonging to five genera of the sub tribe *Cajaninae*; viz. *Cajanus* (15 species), *Rhynchosia* (10 species), *Dunbaria*, *Flemingia* and *Paracalyx*	Very little variation was observed in restriction patterns of five different genera indicating occurrence of limited evolutionary changes in chloroplast genome of these five genera.	Lakshmi *et al*., 2000
RFLP (3 maize mitochondrial probes)	28 accessions representing 12 species of *Cajanus* and 4 species of *Rhynchosia*. 12 species of *Cajanus* were taken from 6 sections (*Cajanus*, *Atylosia*, *Fruticosa*, *Cantharospermum*, *Volubilis* and *Rhynchosoides*)	Cluster analysis resulted in a clear-cut separation of two genera, i.e. *Cajanus* and *Rhynchosia*. Species belonging to sections like *Cajanus*, *Fruticosa* and *Rhynchodoides* exhibited section specific grouping while species like *cajanifolius*, *volubilis*, *mollis* showed discrepancy in their positions.	Sivaramakrishnan *et al*., 2002
RAPD	13 species belonging to the genera *Cajanus*, *Dunbaria*, *Eriosema*, and *Rhynchosia*	Results from cluster analysis indicated the proximity of *C. cajan* to *C. albicans*, *C. sericeus* and *C. lineatus* than *C. acutifolius*, *C. grandifolius* and *C. reticulatus*. All the *Rhynchosia* species grouped together suggesting their origin from a common ancestor.	Ratnaparkhe *et al*., 1995
RAPD (15 primer pairs)	11 cultivated pigeonpea genotypes	Potential of RAPD in discriminating varieties of distinct characters was demonstrated.	Lohithaswa *et al*., 2003
RAPD (100 primer pairs)	24 cultivated pigeonpea genotypes	Analysis resulted in separation of genotypes into distinct clusters and sub-clusters suggesting RAPD as a good marker system for diversity analysis and cultivar identification.	Choudhury *et al*., 2008
RAPD (17 primer pairs)	17 pigeonpea cultivars	Higher level of polymorphism (>80%) was observed for 50% of total markers and cluster analysis resulted in formation of two distinct groups.	Malviya and Yadav, 2010
RFLP, AFLP & SSR (3 maize mitochondrial probes, 5 AFLP primer combinations, 10 SSRs)	42 accessions representing four pigeonpea species *C. cajan*, *C. reticulatus*, *C. sericeus* and *C. scarabaeoides*	All the accessions grouped into four clusters representing four different species. Closer relationship of *C. cajan* with *C. sericeus* and *C. scarabaeoides* than *C. reticulatus* was estimated. This study added further information on inter and intra-specific variation prevailing in pigeonpea.	Aruna *et al*., 2008

(*Contd*)

Table 3. (*Contd*)

Marker types	Experimental material	Outcome	Reference
SSR (20 primer pairs)	1000 accessions including 63 accessions of 7 wild species, *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. lineatus*, *C. platycarpus*, *C. scarabaeoides*, *C. sericens*	Results reports population structure and diversity in cultivated and wild species.	Upadhyaya *et al*., 2011
AFLP (14 primer combinations)	22 accessions including 20 cultivated pigeonpea, two wild accessions (*C. volubilis* and *R. bracteata*)	High level of polymorphism was observed between *C. cajan* and *C. volubilis* (62.08%) and *C. cajan* and *R. bracteata* (62.33%) while among cultivated types percentage of genetic variation was found to be low (13.28%).	Panguluri *et al*., 2006
AFLP (4 primer combinations)	41 pigeonpea varieties of African (32) and Asian (9) origin	Absence of major clustering pattern and population stratification suggested that African and Asian pigeonpea were not genetically diverse.	Wasike *et al*., 2005
DArT (≈700 markers)	96 genotypes representing 20 different species of *Cajanus*	Of the total 700 markers, only 64 were found to be polymorphic among *C. cajan* accessions supporting existence of narrow genetic base in cultivated pool. Most of the diversity was restricted to wild relatives or between the wild and cultivated species.	Yang *et al*., 2006
SSR (20 primer pairs)	15 cultivated and 9 wild relatives (*C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. latisepalus*, *C. lineatus*, *C. platycarpus*, *C. reticulatus*, *C. scarabaeoides* and *C. sericeus*)	Less diversity was detected in cultivated pigeonpea. Among different species least genetic distance and largest similarity coefficient was found between *C. cajan* and *C. cajanifolius.*	Odeny *et al*., 2007
SSR (14 primer pairs)	16 cultivated pigeonpea genotypes	Analysis separated medium and late maturing varieties into two distinct clusters.	Singh *et al*., 2008
SSR (16 primer pairs)	40 genotypes representing seven *Cajanus* species, i.e. *C. cajan*, *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. platycarpus*, *C. scarabaeoides* and *C. sericeus*	Analysis resulted in clustering of genotypes into four main groups. *C. cajan* accessions exhibited low polymorphism and the close relationship of *C. cajan* with *C. cajanifolius* was detected.	Saxena *et al*., 2010a
SSR (30 primer pairs)	32 diverse pigeonpea lines having resistance and susceptibility to fusarium wilt (FW) and sterility mosaic disease (SMD)	This study revealed the strength of SSRs in characterizing SMD and FW resistant and susceptible lines of pigeonpea. All the studied lines were used to generate a set of mapping populations segregating for SMD and FW.	Saxena *et al*., 2010b
SSR (6 primer pairs)	88 *Cajanus* cultivars (38 from east Africa and 50 from India)	Results supported the hypothesis that India is the center of origin of pigeonpea and East Africa is a secondary centre of diversity. Genetic distance between different cultivars followed the pattern of geographical proximity.	Songkok *et al*., 2010

Center for Genome Resources (NCGR). Availability of HTP marker systems such as SNP and DArT would lead to the opening of new vistas for whole genome association mapping or LD mapping and genome wide selection (GWS) in pigeonpea.

The progress of construction of genetic maps in pigeonpea has been very slow. This may be accounted to the low availability of molecular markers, non-availability of appropriate mapping population and a narrow genetic variation present in pigeonpea germ-

Table 4. Development of genomics resources in pigeonpea.

Genomics resources	Genotype used for developing genomics resources	Institutes involved in developing genomics resources	Reference
Enriched library derived SSRs			
10	ICPL 86012	University of Birmingham, UK	Burns *et al.*, 2001
20	ICP 2376	ICRISAT, India	Odeny *et al.*, 2007
73	ICP 2376	ICRISAT, India	Odeny *et al.*, 2009
23	ICPL 87119	ICRISAT, India	Saxena *et al.*, 2010a
BES-SSRs			
3,072	ICPL 87119	ICRISAT, India and University of California (UC)-Davis, USA	Bohra *et al.*, 2011
EST-SSRs			
84	ICPL 20102, ICP 2376, ICP 7035 and TTB 7	ICRISAT, India	Raju *et al.*, 2009
DArT features			
15,360	96 genotypes	Diversity Arrays Technology (DArT) Pty Limited, Australia and ICRISAT, India	Yang *et al.*, 2006, 2010
BESs			
88,860	ICPL 87119	University of California (UC)-Davis, USA and ICRISAT, India	Bohra *et al.*, 2011
Mapping populations			
25	Details available elsewhere	ICRISAT, India, Dr Panjabrao Deshmukh Agricultural University (PDAU), India, University of Agricultural Sciences (UAS), India, Indian Institute of Pulses Research (IIPR), India and Banaras Hindu University (BHU), India	Varshney *et al.*, 2010b
Tilling population			
1 (ca. >5000 mutant lines)	ICPL 87119	Banaras Hindu University (BHU), India and ICRISAT, India	Varshney *et al.*, 2010b
ESTs			
9,888	ICPL 20102, ICP 2376, ICP 7035 and TTB 7	ICRISAT, India	Raju *et al.*, 2009
75	ICP 8744	Osmania University (OU), India	Priyanka *et al.*, 2010
454/FLX Transcript reads			
494,353	Pusa Ageti	ICRISAT, India, J. Craig Ventor Institute (JCVI), USA and NCGR, USA	(Unpublished data)
SNPs			
12,141	–	ICRISAT, India and NCGR, USA	(Unpublished data)

plasm. However as a part of Pigeonpea Genomics Initiative (PGI), ICRISAT and partners have developed about 25 mapping populations segregating for various biotic and abiotic stresses (Table 4). Of these, one inter-specific (ICP 28 × ICPW 94) and one intra-specific (Asha × UPAS 120) mapping populations have been targeted for the development of reference genetic maps in pigeonpea.

As a result of using the above mentioned genomics resources, the first generation maternal and paternal genetic maps have been developed by using DArT markers on ICP 28 × ICPW 94 population (Yang *et al.*, 2010). Subsequently, by using genomic DNA-derived SSR and BES-SSR markers, a reference genetic map comprising 239 marker loci has also been developed (Bohra *et al.*, 2011). SNP genotyping of the mapping population has facilitated integration of 628 SNP loci with BES-SSRs and the present map has 833 SSR and SNP loci (unpublished data). Other mapping populations are also being phenotyped for the traits of interest to the breeders and also being genotyped with the available polymorphic markers. It is anticipated that several genetic linkage maps would be available

soon that will help in the identification of QTLs (quantitative trait loci)/genes associated with the trait of interest to breeder.

In terms of transcriptomic resources, a total of 25,132 pigeonpea ESTs have become available in public domain as on July 30, 2010 (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). A majority of ESTs have been generated from sterility mosaic disease (SMD) and fusarium wilt (FW) challenged tissues at ICRISAT (Raju *et al*., 2010). Next generation sequencing (NGS) technologies, such as 454/FLX and Illumina/Solexa sequencing result in generation of massive sequence data (Varshney *et al*., 2009). For instance, 454/FLX sequencing of a normalized cDNA pools coming from >20 different developmental tissues/stages of Pusa Ageti genotype provided a total of 494,353 short transcript reads (STRs). Assembly of these STRs, together with Sanger sequence reads, defined 127,754 tentative unique sequences (TUSs) comprising 79,028 singletons and 48,726 contigs. Similarly, RNA sequencing of 12 parental genotypes using Illumina/Solexa sequencing provided a set of ~20 million tags. With an objective to identify SNPs, these reads were aligned to the set of TUSs mentioned above by using Alpheus pipeline at NCGR. As a result, >10,000 SNPs were identified among 6 different combinations.

Availability of these transcriptomics data for pigeonpea in combination with transcriptome/whole genome sequence data for other legume species like soybean (*Glycine max*), Medicago (*Medicago truncatula*) and lotus (*Lotus japonicus*) would allow establishing anchor points between pigeonpea and other legume species. Furthermore, this will help in the development of orthologous and cross genera transferable markers in pigeonpea.

Molecular breeding approaches for harnessing natural variation

In terms of quantitative genetics, genetic gain or response to selection ($R = h^2\sigma pi/L$) is a function of heritability (h^2), selection intensity (i), phenotypic variation for the trait (σp) and length of cylcle (L). Heritability refers to the proportion of trait which will be transmitted to the next generation while selection intensity is the proportion of the plants selected from the base population to produce progeny (Moose and Mumm, 2008). Use of molecular markers in breeding programmes promises to enhance genetic gain through increasing the selection intensity (i) and reducing the length of selection cycle (L). In order to exploit the immense potential of germplasm collection, several molecular breeding approaches have been proposed for trait introgression utilizing genomics resources (Figure 3).

As mentioned earlier, CWRs possess genes or QTLs for several useful traits (Table 2). In addition to the interspecific hybridization barriers, progress of QTLs/genes introgression from CWRs in cultivated lines, however, is hindered due to presence of undesirable genomic fraction harbouring various deleterious alleles collectively known as 'linkage drag'. Availability of molecular markers and genetic maps can help in the identification of rare recombinant events leading to the breakage of linkage thus reducing the amount of deleterious alleles in the new genetic background. One of the modern breeding approaches used for systematic introgression of donor genome is marker assisted backcrossing (MABC). MABC relies on the selection for the precise donor genomic fraction through the use of tightly linked or flanking markers to the target locus/QTL (foreground selection) accompanied with a parallel 'background selection' utilizing the markers covering the whole genome (unlinked to the target loci) to maximize the recovery of recurrent parent genome (Varshney *et al*., 2010c). Identification of markers tightly associated with the QTLs/genes of interest is a prerequisite for undertaking MABC programmes. Trait mapping in pigeonpea for various biotic and abiotic stresses is underway and would subsequently lead to availability of markers linked with the trait of interest, to facilitate trait introgression through MABC.

Advanced backcross QTL (AB-QTL)

Since QTL mapping and trait introgression are two independent events, the introgressed trait might not be expressed due the interactions with the new genetic background. To overcome this situation a new approach was proposed by Tanksley and Nelson (1996) which results in simultaneous identification and introgression of favourable alleles of QTLs into elite cultivar. In this scheme, QTL analysis is undertaken in advanced generations such as backcross 2 (BC_2) and backcross 3 (BC_3) and selection is practiced against

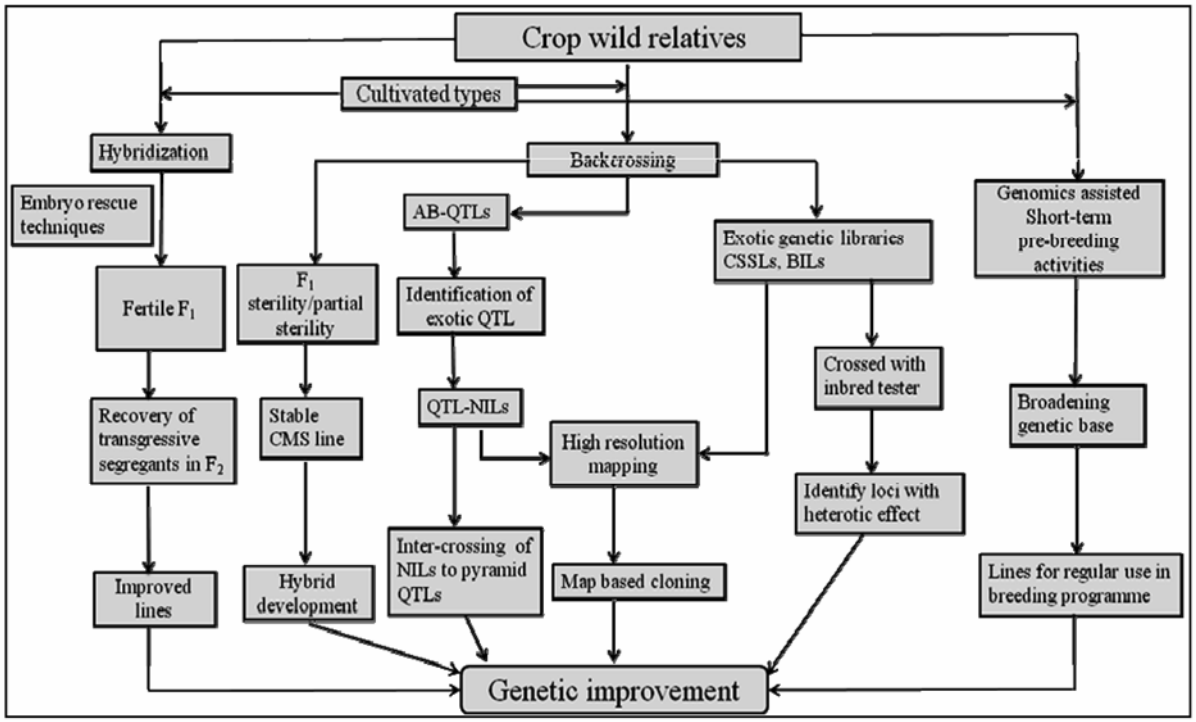

Figure 3. A holistic approach combining genomics with incorporation of wild relatives in pigeonpea genetic improvement. Genomics tools such as molecular markers provide systematic introgression of wild genome leading to the generation of introgression lines (ILs). ILs like NILs, CSSLs, BILs, etc. provide detailed dissection of complex traits facilitating positional cloning of underlying genes. Inter-specific hybridization or backcrossing may result in generation of partial sterile/sterile or fully fertile F_1s. Partial sterile/sterile F1s can lead to development of stable CMS line through further backcrossing while fully fertile F_1s may offer transgressive segregation on selfing. In addition, genomics assisted germplasm enhancement or pre-breeding would be useful in broadening the genetic base of pigeonpea.

the undesirable donor traits like shattering etc. Advanced generations like BC_2 and BC_3 possess an equal statistical potential for the detection of QTLs as the early generations. In addition, QTL analysis in advanced generations helps in identification of QTLs with additive effects only, reducing the scope for epistatic interactions.

Once a desirable allele of the QTL is identified, it can be fixed by one or two generation of selfing resulting in generation of near isogenic lines (NILs) or more appropriately QTL-NILs. These QTL-NILs can be crossed in different combinations to pyramid the QTLs/genes harboured by the individual NIL. AB-QTL method has some disadvantages also such as the investment proceeds without the prior knowledge of the QTL effects of donor genome and maintaining sufficiently large size of the backcross population to allow minimum loss of favourable alleles and to map the QTLs precisely, sometimes becomes cumbersome (Varshney *et al*., 2005). Nonetheless, AB-QTL holds strong promise in exploiting the naturally occurring variation in a species where a little variability is existed in the cultivated germplasm. Some efforts have been initiated at ICRISAT to harness potential of AB-QTL approach.

Introgression lines

Primary mapping populations such as F_2 and RILs routinely used for the identification of QTLs, can only locate a QTL to a genomic region (confidence interval) and these confidence intervals are seldom less than 5 cM or often 30–50 cM in case of these populations showing limited genetic resolution (Tanksley, 1993). Hence, some advanced backcross lines such as introgression lines (ILs) are required to eliminate the 'background genetic noise' for fine mapping of QTL

as well as for the detection of QTLs with smaller effects. Identification of the precise position of the 'wild or exotic QTLs' associated with superior performance is of paramount importance to exploit the 'exotics' in a progressive way. For this purpose several strategies have been proposed based on ILs such as backcross inbred lines (BILs), chromosome segment substitution lines (CSSLs), stepped aligned inbred recombinant strain (STAIRS), etc.

CSSL comprised of a set of ILs, each of which carries a different homozygous chromosomal segment of donor genome in an elite genetic background, eliminating the possibility for nonallelic or epistatic effects mediated by the other part of donor genome. A complete set of CSSLs or BILs provides a full coverage of wild donor genome. Low or high resolution mapping (depending on the average introgressed segment size) of the trait through CSSL is facilitated directly through the comparison of individual IL with the other ILs or recurrent parent and if a significant difference is observed between the two, it can be concluded that the phenotype is associated with the donor genomic fragment. CSSLs represent a dynamic resource for map based cloning of the QTL/gene underlying trait of interest.

Another approach, STAIRS, is a further modification of chromosome substitution lines where chromosome substitution strain (CSS) is crossed with the recurrent parent and BC_2 is subsequently selfed to obtain a set of homozygous single recombinant lines (SRLs) which together constitute STAIRS (Koumproglou *et al*., 2002). Hence, STAIRS offers a detailed step by step dissection of the genomic region associated with the complex trait. All these various types of introgression lines constitute an 'immortal' source represented as 'exotic genetic library' which can be utilized for the exploitation of genetic variation which has been lost under domestication (Zamir, 2001). These homozygous lines can be crossed to a tester and the resultant F_1 would provide an idea about the heterotic effects associated with introgressed segment leading to the simultaneous discovery of the loci involved in the phenomenon of heterosis or hybrid superiority. More detailed study of epistasis can be performed with the help of reciprocal IL crosses. The loci involved in epistatic interactions would result in a 'knocked out phenotype' in the reciprocal cross. Apart from their direct use for identification of QTLs, CWRs together with genomics tools can also be targeted for the pre-breeding activities thus providing huge scope for 'genomics assisted germplasm enhancement'.

Towards harnessing the full potential of CWRs through genomics tools

Although it is known for quite a long time that CWRs have useful genes/QTLs for several useful traits, the progress on use of such germplasm in breeding has been quite limited in pigeonpea. Use of CMS from CWRs for development of hybrids with higher productivity is an excellent example of utilization of CWRs for breeding purposes. The availability of large scale genomics resources coupled with advances in the area of wide hybridization and cell biology offer possibilities to utilize CWRs in a very systematic way to detect and transfer the QTLs/genes for traits of interest in breeding programmes. For instance, high-density genetic maps are being developed based on interspecific mapping populations that will provide QTLs and markers for several important traits. Similarly, some efforts have been made to develop the AB-QTL populations that will provide the useful introgression lines in addition to QTLs. In summary, recently developed genomics resources and use of modern breeding approaches are expected to realize the full potential of CWRs for pigeonpea improvement.

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