5 Modern Genomic Tools for Pigeonpea Improvement: Status and Prospects

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Abstract

Pigeonpea owing to its ability to sustain harsh environment and limited input/water requirement remains an excellent remunerative crop in the face of increasing climatic adversities. With nearly 70% share in global pigeonpea production, India is the leading pigeonpea producing country. Since the mid-1900s, constant research efforts directed to improve yield and resistance levels of pigeonpea have resulted in the development and deployment of several commercially accepted cultivars in India, accommodating into diverse agro-climatic zones. However, the crop productivity needs incremental improvements in order to meet the growing nutritional demands, especially in developing countries like India where pigeonpea forms a dominant part of vegetarian diet. Empowering crop improvement strategies with genomic tool kit is imperative to attain the project gains in crop yield. In the context, adoption of next-generation sequencing (NGS) technology has helped establish a wide range of genomic resources to support pigeonpea breeding, and the existing molecular tool kit includes genome-wide genetic markers, transcriptome/genome assemblies, and candidate genes/QTLs for target traits. Similarly, availability of whole mitochondrial genome sequence and derived DNA markers is immensely relevant in order to furthering the understanding of cytoplasmic male sterility (CMS) system and hybrid breeding. This chapter covers the progress of developing modern genomic resources in pigeonpea and highlights their vital role in designing future crop breeding schemes.

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5.1 Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is an important food legume crop of the semi-arid tropics (SATs). Its ability to withstand risk-prone environments and low-input conditions makes it a preferred crop for the farming community relying on subsistence agriculture (Varshney et al. 2012). Breeding through conventional means (selection and hybridization) has been fruitful, and more than 100 varieties belonging to different maturity groups have been released for commercial cultivation in India during the last 50 years (Singh et al. 2016a). These varieties adequately suit to the specific needs of the diverse agro-climatic zones. However, pigeonpea productivity that has stayed consistently low (around 700–800 kg/ha) over past several decades remains far below from the demand in global food production projected by 2050. A 70% increase in food production worldwide necessitates transformative changes in methods used for crop breeding and management (Tester and Langridge 2010). Implementation of modern genomic resources holds great promise to attend the challenge mentioned above. Collaborative research efforts have facilitated development of much needed genomic tools for pigeonpea improvement during the last ten years (Varshney et al. 2013; Bohra et al. 2014; Pazhamala et al. 2015). Like other crops, dramatic impact of evolution in sequencing chemistry was evident in pigeonpea. The modern tools established recently in pigeonpea include sequence-based molecular markers and high-density genotyping/sequencing assays, saturated genome maps and comprehensive transcript assemblies, and most importantly, candidate gene(s)/QTLs for important traits. *De novo* sequencing attempts rendered entire genome sequence of a leading variety 'Asha' available for future research and breeding.

5.2 Economic Significance and Production Constraints

Pigeonpea is largely cultivated under rainfed conditions predominantly as an intercrop with other crops like sorghum, maize, cotton, soybean, and sunflower (Sameer Kumar et al. 2016). India is the largest producer of pigeonpea contributing 3.29 Mt of the total 4.85 Mt harvested worldwide followed by Myanmar (0.57 Mt), Malawi (0.30 Mt), and Kenya (0.27 Mt) (FAOSTAT 2014). Most of the Indian population depends on pigeonpea for protein source either as split dal or as vegetable along with cereals for balanced diet (Sharma et al. 2011; Kabuo et al. 2015). According to the UN report, Indian population is expected to reach 1.69 billion in the year of 2050. Therefore, pigeonpea remains an important crop with regard to providing food and nutritional security to a large segment of India population (Abraham et al. 2014; Saxena et al. 2015).

The productivity of this crop is severely hampered by a variety of diseases (Reddy et al. 1998) and insect pests (Sharma et al. 2010). Sterility mosaic disease (SMD) presents serious threat to pigeonpea production, and up to 95% yield loss has been registered in SMD-affected pigeonpea (Reddy and Nene 1981). Kannaiyan and Nene (1981) reported that *Fusarium* wilt (FW) caused by *Fusarium udum* in pigeonpea influences different stages (from pre-pod to pre-harvest) of the crop, causing 30 to 99% yield loss. Similarly, *Phytophthora* blight of pigeonpea caused by *Phytophthora drechsleri* f. sp. *cajani* leads to potential economic loss in pigeonpea (Pande et al. 2011). Among insect pests, pod borers are reported to have devastating impact on pigeonpea production, and pod fly (*Melanagromyza obtusa*) is another important biotic factor that challenges pigeonpea cultivation (Sharma et al. 2011).

Weak drainage system and water stagnation exert pronounced impact on pigeonpea yield (Reddy 2009). Drought and waterlogged conditions remain crucial abiotic factors that constrain pigeonpea production (Chauhan 1990). As reported by Kumutha et al. (2008), pigeonpea plants exhibit severe loss in relative water content (RWC) and chlorophyll of leaves and membrane stability index (MSI) in both roots and leaves under water-logged conditions. Based on the survey of pigeonpea grown across several locations in Kenya, substantial decrease in pigeonpea production might be credited to a variety of abiotic stresses, losses even extending up to 100% (Mergeai et al. 2001). Similarly, Mehta and Srivastava (2000) reported considerably higher reduction in pigeonpea production in India during drought years.

5.3 Taxonomy and Cytogenetics

Pigeonpea belongs to the family Fabaceae (subfamily: papilionaceae) under the tribe Phaseoleae (see Bohra et al. 2010). Divergent views were offered regarding the origin of pigeonpea with some authors favoring India (Vavilov 1951) while others advocating for Africa (Zeven and Zhukovsky 1975). On the basis of crop diversity, van der Maesen (1980) definitively suggested that India should be primary center of origin and Africa may be secondary center of origin. The close proximity of *C. cajan* and *C. cajanifolius* has been established through a wide range of diversity studies involving various wild species, which implies toward latter being the most probable progenitor of cultivated pigeonpea (van der Maesen 1980, 1990; Kassa et al. 2012). Similarly, karyotypic features including the morphology of chromosomes and the number of satellite chromosomes were reported to be strikingly similar between *C. cajan* and *C. cajanifolius* (Ohri and Singh 2002). Similar to the observation made by Roy (1933) and Naithani (1941) regarding chromosome count in pigeonpea, analysis of somatic chromosomes of ten different species including *Cajanus*, *Atylosia,* and *Rhynchosia* led authors to report 11 pairs of chromosomes in pigeonpea genome with nearly symmetric karyotypes except of *Atylosia albicans* (Pundir and Singh 1986). No significant variation in genome sizes was reported within cultivated pigeonpea, i.e., *C cajan* on the basis of flow cytometry and Feulgen densitometry (Greilhuber and Obermayer 1998).

5.4 Genomic Tools in Pigeonpea

Advances in pigeonpea genomics led a dramatic expansion in the arsenal of genomic resources that are greatly relevant to breeding (Table 5.1). In this section, we offer a brief account on these modern genomic tools and technologies.

5.5 Next-Generation Mapping Resources

A genetic population of moderate size segregating for the desired trait(s) is essentially needed to find significant associations between the DNA markers and trait(s) under consideration. Experimental populations stemming from a cross between two contrasting genotypes have been developed in pigeonpea targeting several traits such as resistance to important biotic/abiotic factors, fertility restoration, and growth habit/flowering patterns (Varshney et al. 2010; Khalekar et al. 2014; Pazhamala et al. 2015; Daspute and Fakrudin 2015). In parallel, reverse genetic tools like targeted induced local lesions in genomes (TILLING) population derived from EMS-treated 'Asha' were also reported in pigeonpea (Varshney et al. 2010). The reference mapping population in pigeonpea was generated from an interspecific cross [ICP 28 (*C. cajan*) ICPW 94 (*C. scarabaeoides*)], which eventually served for the development of reference linkage maps of moderate (SSR based) to high density (SNP based). Concomitant with the availability of high-density marker assays, a conceptual shift has been witnessed in designing the mating schemes that has paved way for the establishment of modern mapping resources involving a

Table 5.1 Genomic resources in pigeonpea

Resource		Reference
High-throughput genotyping assays	GoldenGate	Kumawat et al. (2012)
	KASP	Saxena et al. (2012, 2014)
	VeraCode	Roorkiwal et al. (2013)
Modern genetic populations	MAGIC	see Pazhamala et al. (2015)
	NAM	see Pazhamala et al. (2015)
High-density genome map	910 loci (interspecific F2)	Saxena et al. (2012)
Large-scale genetic variants	SNP	Dubey et al. (2011), Singh et al. (2011), Varshney et al. (2012), Saxena et al. (2012)
	SSR	Bohra et al. (2011), Singh et al. (2011), Varshney et al. (2012)
Marker-trait associations (MTAs)	Fertility restoration (SSR)	Bohra et al. (2012)
	Fusarium wilt (SSR/SNP)	Khalekar et al. (2014), Singh et al. (2015)
	SMD (SSR/SNP)	Gnanesh et al. (2011a, 2011b), Singh et al. (2015)
	Plant type and earliness (SSR/SNP)	Kumawat et al. (2012), Geddam et al. (2014)
	Flowering pattern/determinacy (SNP)	Mir et al. (2014)
Transcriptome assemblies	4557 TACs	Raju et al. (2010)
	43324 TACs	Dutta et al. (2011)
	48726 TACs	Dubey et al. (2011)
	21434 TACs	Kudapa et al. (2012)
Mitochondrial genome sequence	545.7 Kb	Tuteja et al. (2013)
Whole genome sequences	510.8 Mb	Singh et al. (2011)
	605.7 Mb	Varshney et al. (2012)
Molecular assays to assist CMS breeding	21 SSRs	Saxena et al. (2010a, 2010b)
	42 SSRs	Bohra et al. (2011)

set of diverse founders (Bohra 2013). The two widely employed mating designs incorporating multiple parents are multi-parent advanced-generation intercross (MAGIC) and nested association mapping (NAM). These multi-parental populations are being increasingly reported across several crops like maize (McMullen et al. 2009), wheat (Huang et al. 2012; Delhaize et al. 2015), rice (Bandillo et al. 2013), pea (Tayeh et al. 2015), sorghum (Ongom et al. 2016). Availability of a reference genome sequence for 'Asha' genotype encouraged its use as a common parent in NAM scheme. Such new-generation mapping populations not only ensure the best utilization of high-throughput genotyping/sequencing platforms but also offer several advantages over conventional (biparental) mapping populations like greater resolution, allelic richness. Also, these adequately address the caveats associated with the association analysis (AA) such as the inflated rate of false positives. These advantages render these mapping populations suitable for both family-based QTL study and AA or more appropriately, the joint linkage—linkage disequilibrium (LD) analysis.

5.6 Genome-Scale DNA Markers

Diverse marker assays were employed in pigeonpea for a variety of purposes including assessment of genetic diversity, linkage mapping, and QTL analyses. The first generation of markers included restriction fragment length polymorphisms (RFLPs: Nadimpalli et al. 1992), random amplified polymorphic DNA (RAPDs: Ratnaparkhe et al. 1995) followed by the DNA markers of second generation such as simple sequence repeats (SSRs). Initially, SSR markers were developed from genomic libraries using conventional protocols (Odeny et al. 2007; Saxena et al. 2010a). A limited throughput coupled with the higher cost of the marker development and subsequent genotyping assays urgently called for DNA marker systems that are amenable in terms of throughput, cost, and accuracy. The first set of massive DNA markers in pigeonpea was reported by Bohra et al. (2011). The authors developed more than 3000 SSRs from BAC-end sequences (BESs) and successfully applied these markers in linkage analysis, hybridity testing, and other genetic analyses. The new generation of markers including diversity arrays technology (DArT) and single nucleotide polymorphism (SNP) markers extended marker coverage to genome level. The DArT assays in pigeonpea enabled assessment of the genetic variation and linkage mapping. Among the several marker systems advancing contemporarily, SNP is increasingly replacing SSRs as the DNA marker of choice. A set of 1616 SNPs designated as pigeonpea KASP assay markers (PKAMs) was subsequently used to analyze a panel of 24 genotypes and to construct a high-density linkage map (Saxena et al. 2012). Further, a subset of these PKAMs was selected on the basis of polymorphism among cultivated types, polymorphism information content (PIC) values, and assay design tool (ADT) scores, and 256 genotypes of pigeonpea reference set were analyzed using 48-plex VeraCode Assay technology on the BeadXpress platform (Roorkiwal et al. 2013). This represented an important study concerning the assessment of genetic diversity that holds greater relevance to breeder community. The 1,616 SNPs were also used to screen 184 *Cajanus* accessions (77 cultivated and 107 wild relatives from secondary and tertiary gene pool), which led to the identification of a greater number of polymorphic DNA markers (1226). Importantly, greater insights into domestication of pigeonpea were gained supporting the long-established view that *C. cajanifolius* is the closest progenitor and Madhya Pradesh is the center of origin (Saxena et al. 2014). In parallel, whole transcriptome and genome assemblies also served for the identification of large-scale DNA markers. Genetic variations were reported in the form of expressed sequenced tag (EST)—SSRs, intron spanning region (ISR) markers, and SNPs via excavating transcriptome assemblies (Raju et al. 2010; Dutta et al. 2011; Dubey et al. 2011; Kudapa et al. 2012). Likewise, genome-wide SSRs and SNPs were also recovered from whole genome sequence of pigeonpea. Increasing access to such high-throughput and cost-effective marker systems will certainly help improving the efficiency of traditional breeding methods.

5.7 Molecular Linkage Maps

No genetic linkage map was reported in pigeonpea till 2011, and this lack of linkage information might be credited largely to the inadequacy of polymorphic DNA markers and the lack of mapping populations. Access to the modern marker technology like DArT enabled development of first genetic map in pigeonpea for an interspecific cross (*C. cajan* × *C. scarabaeoides*). However, the paternal and maternal specific maps could not be integrated into a single genetic map, thereby restricting its widespread use in future research work. The first SSR-based genetic map comprising 239 loci was reported for the same interspecific cross that spanned 930 cM of the pigeonpea genome (Bohra et al. 2011). In a similar fashion, genetic linkage maps were developed for cultivated crosses as well, in which the number of mapped SSR loci ranged from 59 to 140 (Gnanesh et al. 2011a, 2011b; Bohra et al. 2012). A 296-loci

(genic SNP and SSR) genetic map for cultivated pigeonpea was constructed by Kumawat et al. (2012) covering 1520 cM of the genome. Apart from these population-specific maps, the first consensus genetic map with 339 loci was synthesized by integrating marker information from six different F_2 populations (Bohra et al. 2012). Extremely low DNA polymorphism as revealed by SSRs or other earlier prevailing DNA marker systems demanded a shift toward adoption of high-throughput marker technologies such as genome-wide SNPs, and as a result of SNP markers assayed through KASP platform, a saturated genetic map was obtained for an interspecific F_2 population (*C. cajan* × *C. scarabaeoides*). The map covered a map distance of 996 cM with 910 (SNPs and SSRs) spaced at an average marker distance of 1.09 cM (Saxena et al. 2012).

5.8 Comprehensive EST Resources and Transcriptome Assemblies

Prior to the introduction of NGS techniques in pigeonpea, different research groups (Raju et al. 2010, Priyanka et al. 2010; Kumar et al. 2014) used Sanger-derived EST resources to access the transcribed regions in the pigeonpea genome. With the aim to develop and analyze ESTs responsive to FW and SMD, Raju et al. (2010) generated the first set of ESTs for maker development in pigeonpea. A total of 9,468 high-quality ESTs were obtained through sequencing 16 cDNA libraries of four pigeonpea genotypes that respond to FW (ICPL 20102 and ICP 2376) and SMD (ICP 7035 and TTB 7). The authors also found 19 and 20 genes to be differentially expressed, respectively, in FW- and SMD-responsive genotypes. Similarly, pigeonpea ESTs were characterized and the genes responsive to abiotic stress were functionally validated in *Arabidopsis thaliana* (Priyanka et al. 2010). From the cDNA libraries of drought stressed pigeonpea, 75 high-quality ESTs were obtained, of which 20 were specific to pigeonpea. Overexpression of three selected pigeonpea genes, viz. *CcHyPRP* (*Cajanus cajan* hybrid-proline-rich protein), *CcCYP* (*C. cajan* cyclophilin), and *CcCDR* (*C. cajan* cold and drought regulatory) genes, in *Arabidopsis* confirmed the plant's tolerance under abiotic stress. Kumar et al. (2014) generated 105 high-quality ESTs from the root tissues of pigeonpea genotype GRG295. Further, the expression of four transcripts, namely *S-adenosylmethionine synthetase*, *phosphoglycerate kinase*, *serine carboxypeptidase*, and *methionine aminopeptidase,* was validated through reverse transcriptase PCR.

Dutta et al. (2011) sequenced transcriptomes of two pigeonpea genotypes 'Asha' and 'UPAS 120' using 454 GS-FLX pyrosequencing. The total number of transcript assembly contigs (TACs) was 43,324. Further analysis of this assembly captured more than 3,000 genic SSR markers. Moreover, primer pairs could be designed for 2,877 SSRs, and 550 (designated as ASSRs) of these SSRs provided the amplicons of expected size. Another assembly '*Cajanus cajan* transcriptome assembly' (CcTA v1) was developed by combining 454-derived 494,353 short transcript reads (STRs) for Pusa Ageti (ICP 28) with 10,817 Sanger ESTs. The assembly comprised of 48,726 (38.1%) contigs and 79,028 singletons, and N50 of this assembly was 287 bp. A search for differentially expressed TUSs resulted in the identification of 99 and 13 TUSs common to FW- and SMD- responsive genotypes, respectively. Moreover, a set of 8,137 SSRs was extracted from this assembly and a total of 12,141 SNPs were detected across different parental combinations. The most comprehensive assembly (CcTA v2) was reported recently by Kudapa et al. (2012) by combining 18,353 Sanger ESTs with reads generated from different sequencing platforms such as Illumina (128.9 million reads) and FLX/454 (2.19 million reads). The assembly composed of total of 21,434 TACs with N50 of 1,510 bp. The transcript reads assembled in CcTA v2 were generated from 16 different pigeonpea genotypes. A comparison of this assembly with soybean genome sequence permitted discovery of 10,009 ISR markers, and a subset of 116 yielding scorable amplicons was used to screen eight pigeonpea genotypes. Together with enabling

access to the functionally important segments of the pigeonpea genome, these transcriptomic tools represent an important community resource to facilitate comparative genomics and offer transferable DNA markers for cross-genera studies.

5.9 Reference Genome Sequence

Two whole genome assemblies have been reported in pigeonpea for the genotype 'Asha' by two different research groups. Varshney et al. (2012) assembled more than 70% of the entire 833 Mb genome using Illumina sequencing platform. This assembly revealed the presence of a total of 48,680 genes with a mean transcript length of 2,348.70 bp. Like other sequenced legume crops, nearly half of the pigeonpea genome was reported to contain repetitive elements (REs). New light was shed on the genetic landscape of drought tolerance in pigeonpea with detection of 111 candidate genes. Serving as a massive reservoir of the genetic markers, this assembly delivered large sets of SSRs (23,410 primer pairs synthesized of total 309,052) and SNPs (28,104). In a similar manner, Singh et al. (2011) assembled 510 Mb (nearly 60%) of the pigeonpea genome with the help of 454 sequencing system. The number of protein-coding genes in this assembly was similar to what was reported by Varshney et al. (2012); however, the average gene size was reported to be 1,170 bp. Among the total 47,004 genes contained in the genome, 1,213 were noted to be disease/defense responsive, whereas 152 genes were predicted to regulate plant's response to abiotic stress. Establishment of a reference genome sequence improves scope for future resequencing attempts and other genome-wide mapping methods including next-generation mapping (NGM). Further, decoding of the entire genome sequence of a leading pigeonpea variety will greatly assist breeders and geneticists to develop improved cultivars or hybrids, particularly to accommodate in a climate increasingly challenged by biotic and abiotic constraints. Further, coupling traditional breeding techniques to modern omics technologies will help ensure a promising future to the pigeonpea farmers and economy.

5.10 Progress Toward Finding Candidate Genes/QTL(S) Related to Target Traits

Determination of the causative gene(s)/genomic segments that explain considerable proportion of the phenotypic variation (PV) for any given trait forms a crucial step in genomics-assisted crop improvement. Examination of marker-trait associations (MTAs) entails the generation of experimental populations or an existing panel of diversified genotypes. The former is termed as family-based linkage (FBL) mapping, while the latter is known as Association Analysis (AA) (Mackay and Powell 2007).

5.11 Genetic Inheritance and Gene/QTL Discovery

As described in earlier sections, FW and SMD are the major diseases that raise tremendous concerns among the pigeonpea growers. Concerning the genetic inheritance, resistance to these two important diseases was reported to be governed by recessive gene(s) with varying numbers (Odeny et al. 2009; Jain and Reddy 1995; Gnanesh et al. 2011a). However, contradictory reports showing resistance conferred by one/two dominant genes are also available (Murugesan et al. 1997, Singh et al. 2016b). In case of FW, complementary and inhibitory gene actions were noted (Ajay et al. 2013), while duplicate dominant epistasis and inhibitory epistasis (Daspute et al. 2014) are reported to play important roles in case of SMD resistance.

Several biparental mapping populations have been reported in pigeonpea that segregated for different traits. Two methods, i.e., bulked segregant analysis (BSA) and QTL mapping, were used for mapping these traits in pigeonpea. Examples of F_2-based BSA include RAPD markers for FW (Kotresh et al. 2006) and plant type (Dhanasekar et al. 2010), and AFLP marker for SMD

(Ganapathy et al. 2009) (see Bohra et al. 2014). More recent application of BSA in F_2 population (Gullyal white × BSMR 736) facilitated detection of a repulsive-phase RAPD fragment co-segregating with SMD resistance (Daspute and Fakrudin 2015). Similarly, Khalekar et al. (2014) found five SSR markers to be associated with FW resistance by analyzing resistant and susceptible bulks of an F_2 population (ICPL 87119 × T. Vishakha-1).

In addition, QTL analysis in F_2 populations unearthed putative genomic segments controlling a considerable proportion of the PV. For instance, Gnanesh et al. (2011b) analyzed F_2 population and $F_{2:3}$ families (ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035) for SMD isolates (Patancheru and Bengaluru) and detected QTLs having minor as well as major effects on the phenotype. The PV ranged between 8.3 and 24.7%. Similarly, QTL analysis performed on three F_2 populations segregating for male sterility and fertility enabled discovery of four major effect QTLs explaining PV in the range of 14.85–24.17%. Likewise, Kumawat et al. (2012) discovered QTLs for plant type and earliness from the $F_{2:3}$ population (Pusa Dwarf × HDM04-1). Of these QTLs, one could explain PV up to 51.4%. Interestingly, these QTLs were later validated in the recombinant inbred background of the population 'Pusa Dwarf × H2001-4' (Geddam et al. 2014). For instance, qSB 5.1 was found explaining 15.1% of the PV in this study (Geddam et al. 2014), while the same QTL accounted for 10.4% PV in the previous report (Kumawat et al. 2012). Once validated in different genetic backgrounds, these putative genomic regions/associated DNA markers can be immediately deployed in crop improvement schemes.

5.12 Association Analysis (AA) or Linkage Disequilibrium (LD) Mapping

Alternatively, significant MTAs are discovered using association panel, which includes genetically diverse genotypes such as elite cultivars, landraces, or wild relatives. Compared to family-based (in particular biparental) analysis, the ability of AA or LD mapping to harness historical recombination makes it more efficient strategy in terms of allelic richness and mapping resolution. Equally important, no additional efforts are needed for generating experimental material and favorably, phenotypic data are often available on the diversified panel. LD analysis can be performed at pathway level using candidate genes or at whole genome scale (genome-wide association study: GWAS). In pigeonpea, a diversity panel comprising 94 lines representing mapping parents and germplasm lines could constitute a representative panel for determinacy trait as 11 of these exhibit a determinate (DT) growth habit while remaining 83 had indeterminate (IDT) habit. A genome-scale search for MTAs was made on these 94 lines using 6,144 DArTs and 768 GoldenGate SNPs. A total of 25 markers including 6 DArTs and 19 SNPs were detected as significantly associated with the trait and the phenotypic variance (R^2) explained by these markers reached up to 14.5% (Mir et al. 2012). Later, a candidate gene-based approach was adopted to decipher the genetic mechanism that controls determinacy trait in pigeonpea. A total of 142 genotypes were investigated using seven genes specific to determinacy/flowering pattern. The study resulted in the establishment of (*Cajanus cajan* terminal flower 1) *CcTFL1* as the likely candidate gene underlying determinacy/flowering pattern. Notably, the same candidate gene was identified through single marker analysis (SMA) and composite interval mapping (CIM) analyses in an F_2 mapping population (ICPA 2039 × ICPR 2447). SIM analysis revealed that *CcTFL1* accounted for 75% PV whereas as revealed by CIM the marker interval *CcTFL1*-CcM0126 was found to control up to 90% PV for determinacy trait. A qRT–PCR experiment performed on the two contrasting genotypes, i.e., ICPA 2039 (DT) and ICPL 87118 (IDT), further validated the experimental findings (Mir et al. 2014). To make the associated SNPs user-friendly, these markers were subsequently converted to PCR-amenable assays.

5.13 Reference Genes to Predict Response Under Abiotic Stresses

More recently, attempts were made to find a set of 'reference' genes to support analysis of gene expression in pigeonpea under the conditions challenged by abiotic stresses, in particular drought, heat, and salt stress (Sinha et al. 2015a, 2015b). Expression variation was measured for ten pigeonpea-specific genes orthologous to commonly used housekeeping genes (*EF1α, UBQ10, GAPDH, 18SrRNA, 25Sr RNA, TUB6, ACT1, IF4α, UBC,* and *HSP90*). The root, stem, and leaf tissues of the popular genotype 'ICPL 87119' were used for the analysis. As a result of quantitative assessment of gene expression, stable genes were obtained with regard to drought (IF4α and TUB6: Sinha et al. 2015a), heat (*UBC, HSP90, GAPDH*: Sinha et al. 2015b), and salt (*GAPDH*, *UBC, HSP90*: Sinha et al. 2015b) stress, which could act as internal control for expression studies in pigeonpea, as specific reference genes are required for specific species for given stress conditions.

5.14 Next-Generation Trait Mapping

Unlike the conventional mapping approaches involving multiple steps, analysis of mapping population with NGS facilitates discovery and mapping of genetic variants in an instantaneous fashion, and importantly, nucleotide level resolution can be achieved (Schneeberger 2014). In the context, various strategies have been proposed in recent years that effectively integrate high-throughput genotyping/sequencing protocols into mapping schemes (Varshney et al. 2013, Bohra and Singh 2015). In pigeonpea, NGS-based QTL Seq of pooled samples (susceptible and resistant extremes) of a recombinant inbred population (ICPL 20096 × ICPL 332) coupled with the resequencing data generated for four genotypes ICPL 20097, ICP 8863, ICPB 2049, and ICPL 99050 enabled discovery of four non synonymous (ns) SNPs each for FW (four candidate genes) and SMD (three candidate genes). Furthermore, expression study using qRT-PCR helped substantiate the robustness of the candidate genes that condition resistance against these two important diseases, i.e., '*C.cajan*_03203' for FW and '*C.cajan*_01839' for SMD (Singh et al. 2015).

5.15 Genomics to Underpin Cytoplasmic Genetic Male Sterility (CMS)-based Hybrid Breeding

Heterosis breeding constitutes an important approach for pigeonpea genetic improvement. Discovery of a stable CMS system in pigeonpea has offered time-saving and cost-effective ways to harness hybrid vigor. As reviewed recently by different researchers (Saxena et al. 2015; Bohra et al. 2016), deployment of genomic tools can help improving efficiency of CMS-based crop breeding programmes.

5.16 Mitochondrial Genome Sequence and Derived Molecular Tools

CMS offers a unique opportunity not only to understand the cytoplasm and nucleus interaction but also to breed hybrids with improved performance. CGMS is a maternally inherited trait, and the factors conditioning male sterility are known to reside within the mitochondrion. These male sterility-inducing causative genes or open reading frames (*orfs*) have been identified in various crops in which CMS system exits (Touzet and Meyer 2014; Horn et al. 2014; Hu et al. 2014). In order to identify these causative *orfs*, sequencing the whole genome of the mitochondrion remains an attractive strategy. In pigeonpea, mitochondrial genome sequence was established with master circle covering length of 545 Kb (Tuteja et al. 2013). The mitochondrial genome harbored a total of 51 genes, of which 34 were found to be protein coding. Sequence comparison of mitochondrial genomes between A and B lines

facilitated detection of 13 chimeric *orfs*, which could be considered as putative genomic candidates inducing CMS in pigeonpea. These chimeric *orfs* are outcome of extensive genomic rearrangement events that occur in mitochondria. However, the exact genomic segment causing CMS remains to be pinpointed. An elaborated study involving interaction of both *Rf* and *orf* is warranted in order to generate greater insights into CGMS phenomenon. More recently, examination of expression profiling of 34 protein-coding genes from mitochondria led to the identification of nine differentially expressed genes, of which *nad4L* and *nad7a* also showed SNP and InDel, respectively. A PCR-based marker developed by targeting *nad7a* would prove helpful in identification between A and B lines (Sinha et al. 2015c). Survey of this mitochondrial genome further provided a set of 24 SSR markers (Khera et al. 2015).

5.17 Molecular Assays for Genetic Purity Assessment

In addition to mitochondrial-specific DNA markers, SSR marker from genomic libraries and BESs were used in CMS breeding schemes. Through enabling the assessment of genetic purity of hybrids and their parents, these offer valuable tools to support procedures to perform grow out test (GoT). Based on the SSR analysis of hybrid (ICPH 2438) carrying A_4 cytoplasm and its parents (ICPA 2039 and ICPR 2438), a set of 21 SSR markers was found to be informative because these SSRs generated monomorphic fragments between ICPA 2039 and ICPB 2039, and at the same time, these markers successfully discriminated ICPA 2039 and ICPR 2438. Further, out of these 21 SSR markers, two SSRs (CCB4 and CCttc006) were found to be diagnostic while testing the genetic purity of CMS-based hybrid ICPH 2438. Similarly, a total of 42 SSR markers were identified for facilitating purity testing of two hybrids ICPH 2671 and ICPH 2438. Importantly, SSR protocols were optimized to accommodate up to eight SSR markers in a multiplex (Bohra et al. 2011). More recently, seven SSR markers (CCB9, HASSR3, HASSR9, HASSR23, HASSR35, HASSR37, and HASSR43) were obtained by screening an A_2-cytoplasm-derived hybrid IPH 09-5 and its parents (PA 163 A and AK 261322) with 66 hyper-variable and informative SSRs (Bohra et al. 2015). Likewise, assaying CMS line (GT 288 A), restorer (GTR 11), and derived hybrid (GTH 1) with 40 SSRs facilitated identification of one DNA marker (CcM0030), which could be informative while assessing the heterozygous nature of hybrid GTH 1 (Patel et al. 2012).

5.18 Prospects for Fast-Track Trait Introgression and Molecular Breeding

The genomics-enabled crop improvement is at its initial stage in pigeonpea. The progress in last 10 years has been satisfactory in terms of generation of valuable genomic tools. This period represents 'development' or 'training' phase of the molecular breeding in which important marker-trait associations (MTAs) are established for downstream selection procedures or prediction models are trained for genomic selection (GS) (Nakaya and Isobe 2012; Bohra 2013). The real potential of genomics-assisted breeding will start unleashing once we enter into the 'breeding phase.' For traits that lie under the control of major effect QTL/gene, marker-assisted backcrossing (MABC) will be the most appropriate strategy for defect elimination, thus precisely improving an otherwise elite cultivar for the trait under consideration. At the same time, advanced backcross (AB)-QTL offers exciting avenues for detection as well as transfer of the traits. Example includes advanced segregating generations derived from wide crosses involving *C. scarabaeoides* as the wild donor (Varshney et al. 2013). By its virtue, AB-QTL seeks unexploited wild gene(s)/allele(s) that are usually absent in cultivated gene pool. Further, implementation of genome-wide approaches like GWAS and MAGIC/NAM in light of the NGS advances is likely to expand the array of robust genomic

segments associated with the trait along with guiding the community for prioritizing the candidate genes. Growing adoption of schemes like GS will help to curtail the cost and time invested in repeated phenotypic screening.

5.19 Conclusion

Nutrient-dense crops like pigeonpea remain important when viewed from the point of food security and subsistence farming. New scientific interventions are needed to be in place in order to meet the challenges that the current agriculture faces worldwide. In recent years, genomics-assisted breeding emerges as a promising approach for accelerating crop production per unit area. In the context, an attractive collection of genomic tools is now available to exercise genomics-assisted crop improvement. Importantly, CMS-based hybrid breeding technology will also be informed greatly by the current genomic developments.

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