



Genomics, trait mapping and molecular breeding in pigeonpea and chickpea

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Abstract

Pigeonpea and chickpea are among the most important pulse crops grown in Asia, sub-Saharan Africa, Australia, Canada and Middle East. The major production of these pulses comes from India, which is also the biggest consumer and importer. However, productivity in these pulse crops is stagnant and unacceptably low for decades, mainly due to their exposure to a number of biotic and abiotic stresses in the marginal environments. Moreover, these pulses were almost untouched from the genomics interventions until early years of twenty first century. However, last ten years have witnessed significant development and deployment of genomics for crop improvement programs. At present, thousands of simple sequence repeat (SSR) markers, millions of single nucleotide polymorphism (SNP), several cost effective genotyping platforms, many dense genetic maps, draft genomes and re-sequencing data for several hundred to thousand genomes have been developed. A number of trait associated markers have been developed and are being used in developing improved lines through genomics assisted breeding (GAB).

Key words: Genomics, molecular breeding, target traits, chickpea, pigeonpea

Introduction

The pulse economy has been suffering from inactive production growth in several regions and countries. The gap between demand and supply of pulses has crossed the alarming mark in recent years. As a result it has become more difficult than the past years to provide nutritious food at affordable price in the face of nutritional and food security. Protein malnutrition is an alarming issue of concern in majority of the countries in the globe. Therefore, recognizing the importance of pulses in providing the nutritious food to the human, year 2016 has been acknowledged by

Food & Agricultural Organization (FAO) of United Nations as the International Year of Pulses (IYP). Pigeonpea (*Cajanus cajan*) and chickpea (*Cicer arietinum* L.) are the leading pulse crops grown in marginal environments in Asia and sub-Saharan Africa, though majority of the production comes from India. With high nutrition and protein values these two pulses are the default component in daily diet of most of the vegetarian families' especially in Indian sub-continent. Thus these two pulses are playing important role in ensuring nutritional food security in many developing countries in Asia. On the other hand pigeonpea is gaining importance in Africa, especially in ESA by occupying an area of about 1.14 million ha and 1.047 million tonnes of production (FAO Stat 2014). The local and particularly export demand for pigeonpea continue to rise presenting it as an economical important crop for income generation. Similarly chickpea has also gained importance in ESA, which is evident from the recent increase in productivity in Ethiopia, the sixth largest exporter of chickpea in the world (<https://www.goift.com/news/140228-pulse-feature-ethiopia-paving-the-way-for-african-chickpeas-higgins/>).

Despite the importance of these pulse crops, the productivity over past decades remained relatively low. The stagnation in productivity was mainly due to exposure of these crops to several biotic (*Fusarium* wilt (FW), *Ascochyta* blight (AB) in chickpea and FW, sterility mosaic disease (SMD) in pigeonpea) and abiotic stresses (drought in chickpea and water-logging and soil salinity in pigeonpea). This situation may be worsened in the changing climatic conditions. To address these issues, scientific community has been focusing on increasing yield in these pulses. Moreover,

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recent advances in genomics are providing better solutions to overcome the long standing constraints in yield enhancement in a number of crop species (Varshney et al. 2011). In the case of pigeonpea and chickpea genomics tools, however, could not be deployed for crop improvement until year 2005. This was mainly due to availability of limited genomic resources in these two pulses. As a result these pulses used to be called 'orphan crops'.

Last ten years have witnessed significant advancements in the genetic and genomic resources in these two pulses. Rapid advances in sequencing technologies together with active participation from different research institutions have facilitated development of large-scale genomic resources (Varshney et al. 2012a). For instance a number of genetic maps, trait associated markers, transcriptome assemblies, etc. have been developed. Another milestone in genomics research was achieved in these two pulses by decoding the genome sequences (Varshney et al. 2012b; 2013). Above mentioned advances in genomics research in these pulses has transformed their status from 'orphan crops' to 'genomic resources rich crops'. This article captures the significant achievements made in genomics research and provides glimpse on novel methodologies being used for pigeonpea and chickpea improvement programs.

Genetic resources

In early days of genomics research, there was a need to develop specialized genetic resources for trait dissection. In this respect during last ten years significant progress has been made in developing specialized genetic stocks such as bi-parental mapping populations, association mapping panels and multi-parent mapping populations segregating for several biotic and abiotic stresses as well as breeding related traits. For instance in the case of pigeonpea around 30 bi-parental mapping populations have been developed for resistance to FW, SMD, pod borer, drought tolerance, fertility restoration, plant type, earliness, water-logging etc. (Varshney et al. 2010; Pazhamala et al. 2015). Whereas, in chickpea more than 15 bi-parental mapping populations have been developed for FW (Cobos et al. 2005; Sabbavarapu et al. 2013), AB (Taran et al. 2013; Sabbavarapu et al. 2013), drought tolerance related root traits (Varshney et al. 2013), Botrytis gray mould (Anuradha et al. 2011), agronomic and yield related traits, etc. (see Thudi et al. 2014). Above mentioned populations were

used for construction of genetic maps and quantitative trait locus(QTL) analysis. In order to deploy the association analysis for detecting marker trait associations and to use available genetic diversity present in Gene banks, core collection, mini core collection (Upadhyaya and Ortiz 2001) and reference set (Glaszmann et al. 2010) were used. Further in recent years, multi-parent mapping populations such as multi-parent advanced generation inter-cross (MAGIC) and nested association mapping (NAM) are also being developed in pigeonpea and chickpea. The precisely used crossing scheme/parental lines in development of multi-parent mapping populations will not only be useful for high resolution genetic mapping of different traits but would also provide excellent breeding materials for developing superior varieties with broader genetic base in pigeonpea and chickpea.

Genomic resources

The progress made in development of large-scale genomic resources in pigeonpea and chickpea during last ten years has a catalytic effect on entire pulse community. In terms of molecular markers, thousands of simple sequence repeat (SSR) markers in both of these crops have been developed. At present, >3,000 SSR markers in pigeonpea and >2,000 SSR markers in chickpea are available (see Varshney 2016). The other type of markers such as diversity arrays technology (DArT) arrays with 15,360 features each in pigeonpea and chickpea are available (Yang et al. 2011; Thudi et al. 2011). Similarly, millions of single nucleotide polymorphism (SNP) and indel markers have been identified using a number of approaches in both pigeonpea and chickpea (Varshney et al. 2012b; 2013; Deokar et al. 2014; Das et al. 2015; Kumar et al. 2016). Further, to develop cost-effective marker genotyping assays such as Kompetitive Allele Specific PCR (KASP) assays (Saxena et al. 2012; Hiremath et al. 2012), GoldenGate assays, VeraCode assays (Roorkiwal et al. 2013) have also been developed. Very recently, 60K SNPchips have been developed for pigeonpea and chickpea using Affymetrix SNP platform.

Above mentioned molecular markers have been used for development of genetic maps and trait mapping experiments. In pigeonpea, the first genetic map was developed using DArT markers on inter-specific population (ICP 28 × ICPW 94) (Yang et al. 2011). In parallel, SSR-based genetic map with a total map length of 930.90 cM was developed using 239 SSR markers on the same inter-specific population

(Bohra et al. 2011). Further a relatively dense genetic map comprising 875 SNP loci was developed (Saxena et al. 2012). Soon after inter-specific maps, six intra-specific maps were developed. Subsequently these six intra-specific maps were used to develop the first consensus map in pigeonpea with 339 SSR loci (Bohra et al. 2012). Due to the advancement in sequencing technologies a number of populations are being genotyped at present following genotyping by sequencing (GBS) approach. This will provide much required dense genetic maps in pigeonpea. The situation in chickpea genetic mapping is relatively better than pigeonpea. A high density inter-specific genetic map was developed on population derived from ICC 4958 × PI 489 with 1,291 markers (Thudi et al. 2011). For intra-specific populations, two early generation genetic maps and a consensus map of 352 markers were developed (Varshney et al. 2014a). An integrated genetic map based on 1,007 markers (GBS based SNPs and SSRs) was developed spanning a distance of 727.29 cM (Jaganathan et al. 2015). Another sequencing based mapping approach that is skim sequencing generated >50K SNPs across the RILs derived from ICC 4958 × ICC1882 (Kale et al. 2015). These SNPs were used to develop an ultra-high density genetic map in chickpea (Bayer et al. 2015). Recently two more dense genetic maps were constructed with 2,177 and 3,625 markers in two separate populations

derived from kabuli and desi genotypes respectively, (Kujur et al. 2015). A physical map was also developed in chickpea using BAC libraries (Varshney et al. 2014b).

The major milestone achieved in developing genomics resources in pigeonpea and chickpea through sequencing and assembling draft genomes (Varshney et al. 2012b, 2013; Singh et al. 2012; Jain et al. 2013). Using whole genome shotgun sequencing approach in pigeonpea, draft genome was assembled for one mega variety Asha (Varshney et al. 2012b) (Table 1). This draft genome captures 605.78 Mb into scaffolds representing <73% of total pigeonpea genome. Another draft genome for Asha variety was generated using 454 GS-FLX sequencing technology to assemble ~511 Mb sequence data (Singh et al. 2012) (Table 1). It is noticeable that pigeonpea was the first pulse and second legume crop after soybean for which genome sequence made available. In chickpea, a kabuli variety CDC Frontier was sequenced to develop the draft genome assembly. In chickpea draft genome, a total of 544.73 Mb genome was assembled in scaffolds representing 73.8% of the total genome (Table 1). Further, 90 accessions representing kabuli and desi types were also re-sequenced (Varshney et al. 2013). For desi type chickpea (ICC 4958) also, 520Mb covering 70% of the total genome was assembled (Jain

Table 1. Comparisons on genome assembly, gene annotation, and non-protein coding genes in *denovo* genome assemblies for pigeonpea and chickpea

	Pigeonpea		Chickpea	
	Varshney et al. 2012	Singh et al. 2012	Varshney et al. 2013	Jain et al. 2013
Number of scaffolds	1,37,542	59,681	7,163	1,81,462
Total span in genome assembly (Mb)	605.78	510.8	532.29	519.84
N50	516.06 kb (scaffolds)	4,522 bp (contig)	39.99 Mb (scaffolds)	77,313 bp (scaffolds)
GC content	32.80%	-	30.78%	26.93%
Number of gene models (protein coding)	48,680	47,004	28,269	27,571
Mean coding sequence length (bp)	959.35	268	1166.44	962
Mean number of exons per gene	3.59	4.9	4.93	4
Mean exon length (bp)	267.39	268	236.51	270
Mean intron length (bp)	536.89	288	480.43	606
Number of miRNA genes	862	100	420	60
Number of rRNA genes	329	448	478	249
Number of tRNA genes	763	671	684	627
Number of snRNA genes	363	226	647	278

et al. 2013) (Table 1). It is important to mention that above mentioned draft genomes have led the foundation of deployment of re-sequencing based strategies for trait mapping and molecular breeding in pigeonpea and chickpea. For instance in pigeonpea >1000 lines and in chickpea >3000 lines are being sequenced. Availability of the draft genome sequences and re-sequencing data on diverse lines in both the pulses have provided information on genes and alleles and opportunities to use in crop improvement programs.

Markers for target traits

Using above mentioned genetic and genomic resources, a number of economical important traits have been mapped in these two pulses. For instance, in the case of pigeonpea markers associated with FW (Singh et al. 2015), SMD (Singh et al. 2015), different agronomics traits related to plant type and earliness (Kumawat et al. 2012), cytoplasmic male-sterility (CMS) (Sinha et al. 2015) and hybrid purity assessment (Saxena et al. 2010; Bohra et al. 2011) have been identified. Efforts are also underway to identify associated markers with seed protein content, yield related traits, fertility restoration, earliness, water use efficiency, water-logging, etc. Whereas, in chickpea markers have been identified for abiotic stresses like drought and heat tolerance using both linkage and linkage disequilibrium based approaches (Varshney et al. 2014; Thudi et al. 2014). Further, markers associated with salinity tolerance (Vadez et al. 2012; Pushpavalli et al. 2015) and drought tolerance were also reported (Hamwieh et al. 2013). Markers associated with biotic stresses were also reported in chickpea for FW (Cobos et al. 2005), AB (Taran et al. 2013) and botrytis grey mould (Anuradha et al. 2011). Inheritance of seed size has been attributed to two complementary genes (Hossain et al. 2010) and two major genes by Upadhyaya and colleagues (2006) in chickpea, while quantitative nature was reported by Sharma and colleagues (2013). Markers for agronomically important traits like herbicide tolerance (Thompson and Taran 2014) and vernalization (Samineni et al. 2016) have also been reported.

Genomics-assisted breeding

Genomics-assisted breeding (GAB) thought to be almost impossible in pigeonpea and chickpea in 2005 has become now reality. This has happened because of directed and speedy progress in development of large scale genetic and genomic resources and trait

mapping studies during last ten years. Due to long generation cycle in pigeonpea the full potential of GAB has not been realized. However, a number of GAB programs have been initiated recently for introducing or combining FW and SMD resistance in targeted varieties/lines. On the other hand in chickpea GAB has produced improved lines with enhanced resistance to AB (Varshney et al. 2014c), FW (Varshney et al. 2014c) and drought tolerance (Varshney et al. 2013). Select GAB success stories in pigeonpea and chickpea have been mentioned below:

Hybrid purity assessment in pigeonpea

The emergence of hybrid technology in pigeonpea has provided a platform for breaking its decades-old low yield plateau. In the last four years three pigeonpea hybrids with 30-50% on-farm yield advantage were released in India. To increase the national pigeonpea production, now efforts are being made to take this technology to the door steps of farmers. Purity of seeds holds the key for the success of hybrids in farmers' field; any level of genetic contamination will lead to the deterioration in its performance with respect to yield and resistance to various stresses. Traditionally, the commercial producers perform Grow-out Tests (GoT) on each seed lot to assess its genetic purity. In pigeonpea, GoT approach cannot be used due to its strong photo-sensitivity and long generation turn-over time. To overcome this bottleneck, SSR based hybrid purity testing kits have been developed for quality control in hybrids and parental lines for two hybrids namely, ICPH 2438 and ICPH 2671 (Saxena et al. 2010; Bohra et al. 2011). In these purity testing kits SSR markers amplify specific alleles in parents and both alleles in true hybrids. Additionally a marker (nad7a_del) for differentiating cytoplasmic male-sterile lines from maintainer lines in A4 system has also developed and validated (Sinha et al. 2015). Above mentioned markers are being routinely used for purity assessment in hybrids and parental lines.

Molecular breeding in chickpea

In chickpea, GAB has been successfully deployed for enhancing the tolerance/resistance against abiotic and biotic stresses. In terms of abiotic stresses, terminal drought has been major production constraint and is expected to further reduce yields in the context of global climate change. In order to enhance the drought tolerance, a "QTL-hotspot" region that explained 56% of phenotypic variance for various drought tolerance component traits was introgressed into a

number of elite cultivars in India and Africa. In brief, using ICC 4958 as donor parent, this genomic region was introgressed into JG 11, a chickpea variety that is predominantly cultivated in Southern and Central India. After three generations of backcrossing followed by two generations of selfing, about 20 progenies with significantly enhanced drought tolerance were identified. Phenotypic evaluation of these lines under both rainfed and irrigated conditions indicated upto 12% and 24% increase in yield respectively compared to the recurrent parent JG 11 (Varshney et al. 2013; Fig. 1). Similarly, “*QTL-hotspot*” was also introgressed into ICCV 10, KWR 108 and DCP92-3, Pusa 362 in India, ICCV 95423 in Kenya and Ejere, Arerti in Ethiopia.

In terms of biotic stresses, molecular breeding lines with enhanced resistance to FW and AB have been developed. Using WR 315 as donor parent, marker-assisted back crossing (MABC) lines resistant to race 1 (*foc 1*) and race 3 (*foc 3*) of *Fusarium oxysporum* were developed in the genetic background of C 214 cultivar. In addition, using ILC 3279 donor parent AB resistance was introgressed into C 214 cultivar (Varshney et al. 2014c). Further, FW and AB were pyramided and evaluation of pyramided lines under controlled environmental conditions is in progress. Similarly MABC lines resistant to race 4 (*foc 4*) in the genetic background of JG 74, Phule G12 and Annigeri 1 were developed at Jawaharlal Nehru

Krishi Vishwavidyalaya (JNKKV), Jabalpur, Madhya Pradesh, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra and Agricultural Research Station (ARS-Gulbarga), Karnataka respectively. While MABC lines resistant to race 2 (*foc 2*) have been developed in the genetic background of Pusa 256 at Indian Institute of Pulses Research (IIPR), Kanpur, Uttar Pradesh.

It is very important to note that the MABC lines with enhanced drought tolerance developed at ICRISAT in the genetic background of JG 11, ICCV 10 that performed well in initial varietal trials (IVT) of All India Coordinated Research Project on Chickpea (AICRP-Chickpea) have been advanced for testing in advanced varietal trials (AVT 1) for the year 2016-17. Similarly improved lines with enhanced resistance to FW in the genetic background of Annigeri 1 and JG 14 developed at ARS Gulbarga and JNKKV, Jabalpur were also nominated for AVT 1 trials (AICRP Chickpea Annual Report 2015-16).

Future prospects

In view of the above progress made in these two important pulse crops, it is highly required to continue genomics research efforts in targeted manner. In order to achieve pulse sufficiency in India, research should be guided by farmers' needs and supported by public sectors; modern genomics approaches should be



Fig. 1. A snapshot of high performance of chickpea MABC lines as compared to JG 11 in field (Source: Pooran Gaur)

integrated with breeding (varieties/hybrids) methodologies. Other areas where research efforts should be directed include understanding host pathogen interactions, developing insect resistance cultivars in both pulses and understanding molecular basis of cytoplasmic male sterility in pigeonpea. In terms of technological advances, genome editing and establishment of doubled haploid platforms in these pulses crops may emerge as promising technologies.

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Declaration

The authors declare no conflict of interest.

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