Advances in Chickpea Genomic Resources for Accelerating the Crop Improvement

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Manish Roorkiwal, Ankit Jain, Mahendar Thudi and Rajeev K. Varshney

Abstract

Chickpea plays a major role in food and nutritional security worldwide. Its productivity is severely affected by various biotic and abiotic stresses; hence development of stress resilience varieties that can yield higher under stress environment remains the call of the hour. Conventional breeding approaches clubbed with the genome information, commonly known as genomic-assisted breeding (GAB) have the potential to accelerate the crop improvement efforts. In order to deploy the GAB for crop improvement in chickpea, there was need to convert an orphan crop chickpea into the genomic resource-rich crop. Advent of sequencing technology has resulted in reduction of cost and led to development of huge genomic resources in chickpea. A variety of markers have been developed, used for various mapping studies including linkage mapping and association mapping and finally deployed for developing the superior varieties using GAB approached such as marker assisted backcrossing and genomic selection. The chapter reviews the journey of chickpea status from orphan crop with almost no marker resources to a genome resource-rich crop, which are being used for achieving the genetic gains at a momentum.

6.1 Introduction

Chickpea (*Cicer arietinum* L.) is the second most important food legume with 13.98 million hectares under cultivation across 55 different countries worldwide (FAO 2014). Chickpea is a self-pollinated diploid ($2n = 16$) annual crop with genome size of ~740 Mbp (Varshney et al. 2013a). It is commonly known as gram, Bengal gram or garbanzo bean, mostly grown in arid and semiarid regions, predominantly in developing

M. Roorkiwal (✉) · A. Jain · M. Thudi · R.K. Varshney
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India
e-mail: m.roorkiwal@cgiar.org

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countries (90% of its cultivated area) (Croser et al. 2003). Chickpea is a valuable source for many important proteins, minerals, and vitamins among legumes and contributes as an important source for protein for vegetarian diet. Chickpea has one of the most balanced nutritional compositions, and its protein digestibility is the best among the cool season food legumes. Apart from human consumption, chickpea also has economic importance in animal feed as well as in herbal medicine.

Ecologically, chickpea is known as an efficient N_2-fixing system due to its capability of symbiotic nitrogen fixation and, therefore, fits well in crop rotation programs. Nearly, 90% of the crop is cultivated under rainfed condition, mostly surviving on receding soil moisture. Current global yield average of chickpea is 0.9 t/ha (FAO 2014), much lower than its estimated potential of 6 t/ha under optimum growing conditions (Singh 1985). Chickpea productivity is adversely affected by various biotic and abiotic stresses like *Ascochyta* blight (AB caused by *Ascochyta rabiei*), *Fusarium* wilt (FW caused by *Fusarium oxysporum* f. sp. *ciceris*), pod borer (*Helicoverpa armigera*), *Botrytis* gray mold (BGM), drought, and cold (Ruelland et al. 2002). Three major abiotic stresses responsible for reduction in seed yield in chickpea include drought, heat, and cold (Singh 1985; Singh et al. 1997). However, drought stands to be the major challenge in chickpea growing regions, causing a 40–50% reduction in yield globally (Ahmad et al. 2005).

Like every extensively cultivated crop, chickpea is also facing the consequences of the continuously deteriorating environmental conditions, i.e., more rigorous temperature regimes and dry soils (abiotic stress). Many physiological processes associated with crop growth and development are reported to be influenced by water deficits (Turner and Begg 1978). To counter this global phenomenon, extensive artificial irrigation is required to achieve acceptable harvest yield in many of the chickpea cultivating regions (Bakht et al. 2006). However, in the long term this practice results in increased soil salinization and therefore contributing toward declining productivity. Considering the effect of various stresses on yield, it is very important to initiate serious efforts in the direction of developing improved varieties or alternate strategies that allow sustainable chickpea production under adverse environmental conditions. Application of available approaches to improve crop productivity under adverse environmental conditions requires a better understanding of the mechanisms involved in crop's response to such stresses. Plant stress responses are generally controlled by a network of specialized genes through intricate regulation by specific transcription factors (Chen and Zhu 2004). Thus, the application of a holistic approach combining genomics with breeding and physiology, termed as genomics-assisted breeding (GAB) (Varshney et al. 2005), provides strategies for improving component traits of drought tolerance that should prove more effective and efficient than the conventional methods (Mir et al. 2012).

Until last decade, chickpea was known as an "orphan crop" due to availability of limited genomic resources and hence inclination was much more toward conventional breeding approaches to increase yield (Varshney et al. 2012a). In order to generate genomic resources and deploy them for developing superior chickpea varieties using modern breeding approaches, efforts were initiated and significant progress has been made in the recent past. Using the advent of next generation sequencing (NGS) technologies, large-scale molecular markers have been developed recently. These resources have been used for constructing dense genetic maps and identification of various markers associated with traits of interest (Varshney et al. 2012b, 2015; Varshney 2016).

The chapter describes about the efforts to develop the genomic resources and deployment of these resources in breeding for enhancing the rate of genetic gain in chickpea.

6.2 Genomic Resources

Efforts to improve chickpea productivity using conventional approaches were able to enhance the yield but could not achieve the desired results

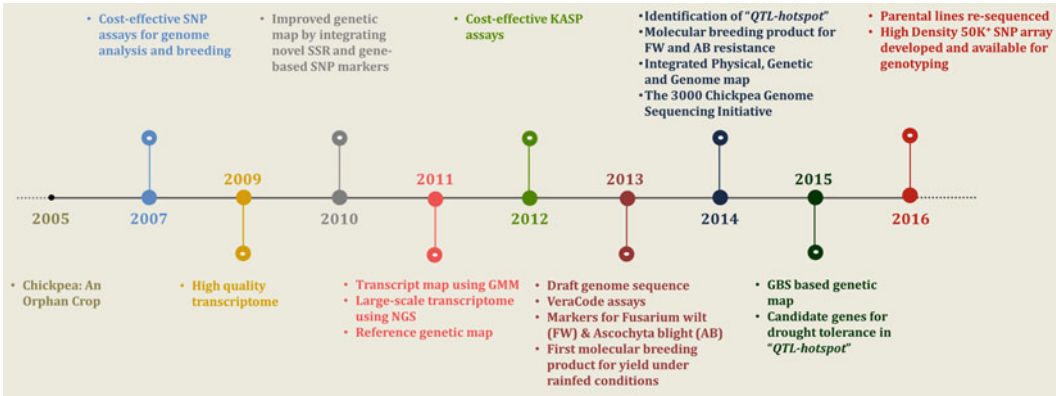

Fig. 6.1 Account of the significant accomplishments made in the field of development of genomic resources and their deployment in chickpea crop improvement

due to narrow genetic base in cultivated chickpea. Efforts at international platform were initiated to develop genomic resources. ICRISAT along with its partners accelerated the development of these genomic resources during the last few years (Fig. 6.1). These genomic resources have also been deployed in breeding using GAB and have already started to make an impact on chickpea improvement (Pandey et al. 2016). A brief update on development of different type of markers has been given below:

Isozyme markers: Isozymes are multiple forms of enzyme that differ in amino acid sequence but control different chemical reaction based on different kinetic parameters or regulatory properties. Isozymes are the form of biochemical/molecular markers that are based on the staining of proteins with identical functions with different electrophoretic movement. In the case of chickpea, isozyme markers were developed and their segregation was observed in the F_2 population derived from interspecific crosses of *Cicer arietinum* L. with *C. reticulatum* Lad. and *C. echinospermum* (Gaur and Slinkard 1990a, b). Based on isozyme profile of nine annual and one perennial species of chickpea, Kazan and Muehlbauer (1991) classified the species into four groups which was later supported by several studies (Ahmad et al. 1992; Labdi et al. 1996; Tayyar and Waines 1996). Kazan et al. (1993) with application of morphological and isozyme markers on several F_2 families supported similar mode of inheritance as obtained using morphological markers in previous studies. Low level of polymorphism was observed in most of the isozymes-based studies in the cultivated chickpea (Oram et al. 1987; Gaur and Slinkard 1990b; Ahmad et al. 1992; Kusmenoglu et al. 1992; Van Rheenen 1992; Labdi et al. 1996; Tayyar and Waines 1996).

Restriction Fragment Length Polymorphism (RFLP) and Randomly Amplified Polymorphic DNA (RAPD) markers: RFLP uses difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples. RFLP includes digestion of DNA sample using restriction enzymes and separation of restriction fragments by gel electrophoresis and then hybridization with genomic DNA/cDNA probes. Subsequently, hybridization pattern is observed on x-ray film and polymorphism obtained in different banding patterns due to change in the restriction enzyme recognition site. RAPD includes differential PCR amplification of a fragment of DNAs from short oligonucleotide sequences. RAPD does not require prior sequence information, and random identical 10-mer primers are used to amplify a segment of DNA, depending on positions that are complementary to the primers' sequence.

In order to assess the polymorphism existing between *desi*- and *kabuli*-type chickpea cultivars, RFLP markers were used (Udupa et al.

1993). In another study, RFLP analysis on cultivated chickpea accessions from 11 different countries indicated three major center of diversity Pakistan-Afghanistan, Iraq-Turkey and Lebanon, and India being known as secondary center of genetic diversity previously showed lower diversity than above (Serret et al. 1997).

Furthermore, using RFLP, isozyme, and RAPD markers, an integrated genetic linkage map consisting 27 isozyme, 10 RFLP, and 45 RAPD marker loci covering 550 cM was developed in chickpea using interspecific crosses of cultivated chickpea and a closely related wild species (*C. reticulatum*) (Simon and Muehibauer 1997). In another study, RFLP and RAPD markers were used to assess the polymorphism in chickpea accessions including some of the mutants (Banerjee et al. 1999). RAPD markers were also used to identify the markers associated with fusarium resistance against race 1 and 4 using C 104 × WR 315 cross (Tullu et al. 1998). Another study using RAPD and oligonucleotide probes to assess genetic diversity among 29 elite Indian chickpea cultivars indicated narrow genetic base in chickpea (Sant et al. 1999). Similarly, genetic diversity and phylogenetic analysis across 75 chickpea accessions using 12 RAPD primer resulted in 234 polymorphic fragments (Iruela et al. 2002). Another study from Singh et al. (2003), where of 78 RAPD primers, 20 primers were found polymorphic, continues to uphold the previous hypothesis about narrow genetic base.

Amplified Fragment Length Polymorphis (AFLP): AFLP marker system effectively combines principles of both RFLP and RAPD in order to produce reproducible results (Vos et al. 1995). Genomic fragmented generated as a result of restriction digestion is ligated with primer-recognition sequences (adaptors). Selective PCR amplification of these restriction fragments using a limited set of labeled primers is separated on gel/capillaries electrophoresis. AFLP markers were utilized in assessing the genetic diversity, delineating the phylogeny of chickpea germplasm (Nguyen et al. 2004; Sudupak et al. 2004; Shan et al. 2005; Talebi et al. 2008) and construction of genetic linkage map (Winter et al. 2000).

Simple Sequence Repeat (SSR) and SNP markers: SSR (microsatellite) markers being multi-allelic and codominant in nature and SNPs owing to their greater abundance in the genome and their amenability for high-throughput genome analysis are extensively used for several genomics applications (See Varshney et al. 2007a; Singh et al. 2008; Pandey et al. 2016).

In the case of chickpea, microsatellite markers developed to date employed one of the following approaches: (i) probing the genomic libraries with oligonucleotide repeats, (ii) sequencing of microsatellite-enriched libraries, and (iii) sequencing of bacterial artificial chromosome (BAC) clones. Initially, 16 SSRs were reported by screening small insert genomic libraries with di-, tri-, and tetra oligonucleotide repeat probes to identify SSR repeats (Hüttel et al. 1999) and subsequently 174 SSRs were reported by screening size select genomic DNA libraries (Winter et al. 1999). In subsequent years, both BAC and BIBAC libraries were used for developing SSR markers by Lichtenzveig et al. (2005). In addition, as a result of concerted efforts at ICRISAT, a large number of SSR markers were developed from microsatellite-enriched libraries and bacterial artificial chromosome (BAC) clones ICC 4958 to report 311 novel SSRs (Nayak et al. 2010). Another effort by Thudi et al. (2011) sequenced 55,680 BAC clones and identified 6845 SSR motifs and designed primers for 1344 SSRs.

Further, during recent years efforts were also made to understand the transcriptomes, gene expression profiles in various stressed plant tissues and stress responsive expressed sequence tags (ESTs) were used for candidate gene identification and develop functional markers for breeding applications. For instance, initial efforts to develop functional markers from expressed sequence tags, were made in 2005 (Buhariwalla et al. 2005). Drought and salinity responsive ESTs were used to develop 177 new EST-SSRs (Varshney et al. 2009). Similarly, several studies provided the insights into global view of transcriptome dynamics of different stress responsive

tissues (Hiremath et al. 2011; Garg et al. 2011a, b; Singh et al. 2013; Afonso-Grunz et al. 2014; Kudapa et al. 2014). Consequently, shift to study transcriptomics led to sequencing of EST libraries and resulted in flooding of EST sequences in public domains. In order to utilize the generated data efficiently and develop functional markers, screening of genic data for SSRs led to development of EST-SSR markers (Kottapalli et al. 2009; Gupta et al. 2015; Khajuria et al. 2015).

SNP markers have also become popular because of their genome-wide abundance and possibility of cost-effective high-throughput genotyping. Using *in silico* approaches, 184 putative SNPs were identified in 19 contigs constructed with 1499 ESTs generated from different *Cicer* species available in public domain (Varshney et al. 2007b). In addition, recent advances in NGS technologies enabled the generation of huge amount of sequencing data in very less time at very low cost (Thudi et al. 2012). In the case of chickpea, using Sanger sequencing technology more than 20,000 expressed sequence tags (ESTs) were generated from drought and salinity stress-challenged tissues (Varshney et al. 2009). In addition to these ESTs, NGS technologies were used for generating additional sequencing data on >20 tissues representing different developmental stages (Hiremath et al. 2011). Combined data analysis using Sanger ESTs and NGS transcripts led to generation of first transcript assembly with 103,215 tentative unique sequences (TUSs) (Hiremath et al. 2011). Analysis of these ESTs and transcript assemblies led to identification of few thousand SNPs. In addition, other sequencing approaches including Illumina sequencing of parental lines of chickpea mapping populations have identified several thousand SNPs (Hiremath et al. 2011). Similarly, allele-specific sequencing on chickpea genotypes has led to identification of ~2000 SNPs (Gujaria et al. 2011; Roorkiwal et al. 2014a). Deokar et al. (2014) have also reported 51,632 genic SNPs identified by 454 transcriptome sequencing of *C. arietinum* and *C. reticulatum* genotypes. Using genomic and transcriptomic SNPs, Gaur et al. (2015) mapped 6698 SNPs on eight linkage group spanning 1083.93 cM for interspecific RIL population. Verma et al. (2015) used genotyping by sequencing (GBS) for genotyping of intraspecific RIL population contrasting for seed traits.

Diversity Array Technology (DArT) markers: In addition to SSRs and SNPs, another marker system, DArT, has been widely used for construction of genetic maps and diversity analysis. DArT markers were marker of choice in the absence of enough genomic resources for constructing dense genetic maps and were widely used for *Triticeae* species (Neumann et al. 2011). Therefore, ICRISAT in collaboration with DArT Pty Ltd developed the DArT arrays with 15,360 clones (Thudi et al. 2011). Similar to other marker systems, DArT arrays also showed narrow genetic diversity in cultivated gene pool as compared to wild species (Roorkiwal et al. 2014b). By combining genotyping, data generated using DArTseq platform for 3000 polymorphic markers for a set of 320 chickpea lines, with multilocation phenotyping data Roorkiwal et al. (2016), estimated prediction accuracies and hence made the first attempt toward genomic selection (GS) studies.

Sequencing-based marker systems: NGS technologies offer the ability to produce huge sequence data sets at relatively low cost in less time. Availability of these low-cost sequencing technologies has enabled to map the target traits at sequencing level and replacing the traditional trait mapping approaches by sequence-based trait mapping. Sequencing technologies such as GBS, skim sequencing, and whole genome re-sequencing (WGRS) provide genome-wide large-scale marker information for high-resolution trait mapping (Pandey et al. 2016). In the case of chickpea, GBS has been used for refining the "*QTL-hotspot*" identified an intraspecific cross (ICC 4958 × ICC 1882) (Jaganathan et al. 2015). Similarly, Kale et al. (2015) used skim sequencing approach to genotype RIL population (ICC 4958 × ICC 1882) and led to identification of 84,963 SNPs,

out of which 76.01% were distributed over the 8 pseudomolecules. Similarly, Kujur et al. (2015) and Bajaj et al. (2015) identified >40,000 and >80,000 high-quality genome-wide SNPs using integrated reference genome- and de novo-based GBS approach from 93 wild and cultivated chickpea accessions, respectively. With the availability of large-scale SNP marker information, one of the major challenges was to use these markers routinely in breeding programs. Utilization of any marker system in breeding application is largely affected by the possibility of automation, time for data turnaround, and cost. Different approaches for deploying markers in breeding require variable number of markers, and therefore a range of genotyping platforms/systems are required. In the case of chickpea, different SNP genotyping platforms were developed to meet all needs. For instance, GoldenGate and VeraCode assays were developed in chickpea for genotyping reference set consisting of 288 of genotype with 96 SNPs (Roorkiwal et al. 2013). However, in many breeding applications, only few SNPs are required to genotype large population where GoldenGate and VeraCode assays may not be cost effective. For such applications, more than 2000 KASP markers were developed for chickpea (Hiremath et al. 2012).

High-density genotyping arrays: With the advent of low-cost NGS technologies, large-scale re-sequencing projects have been initiated and resulting in availability of millions of SNP markers in several crop plants. In order to use these ever expanding genome resources in the breeding applications, there is a need for low-cost, high-throughput genotyping platforms. Recent developments in the arrays technology have brought down the cost of high-throughput genotyping, thus making it accessible to most of the researchers and breeding communities. SNP genotyping platforms can be used for genetic diversity studies, fine mapping, association mapping, GS, and evolutionary studies. In order to exploit the available millions of SNP markers in chickpea for breeding application, efforts to develop a high-throughput SNP genotyping platform were initiated. As set of 70,463 high quality non redundant SNPs were selected using an assortment of the criterion from a pool of 4.9 million SNPs. Based on p-convert score, a set of 61,174 SNPs was selected of which 50,590 SNPs were tiled on Affymetrix Axiom array (Roorkiwal et al. 2017). These arrays are being used for genotyping breeding material and RIL population for high-resolution genetic mapping and breeding applications.

6.3 Draft Genome and Re-sequencing Efforts

Draft genome sequence serves as a base for better understanding of plants response mechanism and genetic basis for gene function. In addition, draft genome also helps for identification of large-scale markers. Reduced incurring cost of NGS and huge data output allows researcher to tap the variation prevailing in whole genome. Considering the utility of genome sequence, ICRISAT led International Chickpea Genome Sequencing Consortium (ICGSC) decoded the chickpea genome sequence. Illumina sequencing was used to sequence CDC Frontier, a kabuli chickpea variety, and ~153 Gb raw sequence data was generated. After the data cleaning, 87.65 Gb high-quality sequence data was used to assemble 544.73 Mb of genome, representing 74% of chickpea genome (Varshney et al. 2013a). In addition to draft genome, ICGSC also undertook re-sequencing of 90 cultivated and wild chickpea accessions using NGS-based whole genome re-sequencing and restriction site-associated DNA (RAD) technology (Varshney et al. 2013a).

In parallel, another effort to sequence chickpea genome targeted ICC 4958, a desi chickpea genotype for developing the draft chickpea genome assembly. NGS technology along with bacterial artificial chromosome end sequencing was used to assemble ~520 Mb of chickpea genome (Jain et al. 2013). Recently, Gupta et al. (2016) developed the draft assembly of PI 489777 that resulted in 416Mb draft genome of wild progenitor and 78% (327 Mb) of this assembly could be anchored to eight linkage groups.

Development of the draft genome assembly has been followed with efforts to improve the assemblies. Individual chromosome from both desi and kabuli varieties was isolated and sequenced using NGS-based sequencing technology to validate the desi and kabuli assemblies. Chromosomal sequencing approach could identify small misassembled region in kabuli; however, in desi a large region was found to be misassembled (Ruperao et al. 2014). In a similar manner to improve the desi assembly, Parween et al. (2015) generated additional sequence data and reported an improved assembly of ICC 4958 with 2.7-fold increase in length of pseudomolecules.

With an objective to exploit the germplasm wealth stored in genebank for identification of novel alleles and genetic variations, large-scale re-sequencing efforts were initiated. Large-scale germplasm resources available in genebanks provide the opportunity to address the issue of low genetic diversity (McCouch et al. 2013). Illumina HiSeq 2500 was used for re-sequencing 100 chickpea varieties released across 14 countries in last five decades. Re-sequencing data on these 100 elite varieties along with 29 earlier re-sequenced line was used for understanding the impact of breeding on genetic diversity and gain insights into temporal trends in chickpea diversity (Thudi et al. 2016). Re-sequencing data on 100 elite chickpea varieties was used for developing first-generation HapMap of chickpea. In parallel, 300 lines from chickpea reference set were also re-sequenced using whole genome re-sequencing approach. A total of 1.8 Tb raw sequence data was generated and used for aligning against reference chickpea genome to identify 4.9 million SNPs (unpublished). Re-sequencing data on 300 chickpea lines from reference set along with multi-season, multilocation phenotyping data was used for GWAS analysis for identification of markers associated with trait of interest. Very recently, ICRISAT has launched "The 3000 Chickpea Genome Sequencing Initiative" where 3000 lines from the global composite collection of chickpea from genebanks of ICRISAT and ICARDA will be re-sequenced for identification of novel alleles (Varshney 2016)".

Genetic Maps and Trait Mapping

In order to use available genomic resources for modern breeding approaches effectively, first step is to identify the markers associated with trait of interest. For identification of markers associated with trait of interest, mainly two approaches are used, namely (i) biparental mapping population-based linkage mapping and (ii) germplasm-based genome-wide association mapping (GWAS). For linkage mapping-based identification of markers associated with trait of interest, first step is to develop the genetic maps.

Beginning with the morphological markers to the next generation of markers that include DArT, SNPs, etc., wide range of marker systems have been used to generate genetic map for chickpea. Most of the genetic maps developed till date have been described in Table 6.1, and it also shows the evolution of marker system over the course of time. Current section describes some of the recently developed genetic maps briefly. Thudi et al (2011) reported a high-density genetic map developed using the interspecific mapping population (ICC 4958 × PI 489777) with 1291 loci spanning across a distance of 845.56 cM on eight linkage groups. In parallel, another effort by Choudhary et al. (2012) developed an advanced gene-rich map with 406 loci for the same population. In addition, two intraspecific mapping populations (ICC 4958 × ICC 1882 and ICC 283 × ICC 8261) segregating for drought tolerance-related root traits were also used for generation of genetic maps with comprising 241 loci and 168 loci, respectively, and a consensus genetic map comprising 352 loci was also constructed. Using extensive phenotyping, data QTL analysis was performed and 45 robust main-effect QTLs (M-QTLs) explaining up to 58.20% phenotypic variation were identified (Varshney et al. 2014a). In order to fine map these genetic maps, intraspecific mapping populations ICC 4958 ×

Table 6.1 Various genetic linkage maps generated in chickpea

S. No.	Population type	Marker type	Markers/loci mapped	Linkage groups	Map distance (cM)	References
1	Intraspecific	Morphological and isozyme markers	29	7	200	Gaur and Slinkard (1990a, b)
2	Interspecific	Morphological and isozyme markers	28	8	257	Kazan et al. (1993)
3	Interspecific	Morphological, isozyme, RFLP and RAPD markers	91	10	550	Simon and Muehibauer (1997)
4	Interspecific	STMS markers	120	11	613	Winter et al. (1999)
5	Interspecific	RAPD, ISSR, isozyme and morphological marker	116	9	981.6	Santra et al. (2000)
6	Interspecific	SSR, SAF, AFLP, ISSR, RAPD, isozyme, cDNA, SCAR and morphological markers	303	16	2077.9	Winter et al. (2000)
7	Intraspecific	STMS, RAPDs, ISSR and morphological markers	80	14	297.5	Cho et al. (2002)
8	Interspecific	55 STMS and 1 RGA markers integrated to Santra et al. (2000)	167	9	1174.5	Tekeoglu et al. (2002)
9	Interspecific	RAPD, ISSR, STMS and RGA markers	83	8	570	Collard et al. (2003)
10	Interspecific	47 R gene-specific markers integrated to Winter et al. (2000)	296	12	2483.3	Pfaff and Kahl (2003)
11	Intraspecific	STMS, RAPD, ISSR and morphological markers	125	11	33	Cobos et al. (2005)
			52	7	174.4	
			138	10	427.9	
12	Intraspecific	RAPD, ISSR, RGA, SSR and ASAP markers	230	8	739.6	Radhika et al. (2007)
13	Intraspecific	SSR and EST markers	84	10	724.4	Kottapalli et al. (2009)
14	Interspecific	STMS, RAPD, ISSR, morphological and RGA markers	169	8	751	Palomino et al. (2009)
15	Intraspecific	STMS markers	33	8	471.1	Bharadwaj et al. (2011)
16	Interspecific	STMS and cross-genome markers	555	8	652.67	Millan et al. (2010)
			229	8	426.96	
17	Interspecific	52 ICCM, 46 H-series SSR loci, 71 gene-based and 357 legacy markers	521	8	2602.1	Nayak et al. (2010)
18	Intraspecific	STMS markers	138	8	630.9	Gaur et al. (2011)
19	Interspecific	SSR, CISR, CAPS, COS-SNP, DArT, legacy markers	1291		845.56	Thudi et al. (2011)

(continued)

Table 6.1 (continued)

S. No.	Population type	Marker type	Markers/loci mapped	Linkage groups	Map distance (cM)	References
20	Interspecific	EST-SSR, ITP, ESTP, MtEST, gSSR and STMS markers	406	8	1497.7	Choudhary et al. (2012)
21	Interspecific	CKAM, TOG-SNP, GMM, H-series, ICCM, CAM, SSR, ISSR, SNaPshot assay-based SNP, CAPS, DArT and RAPD markers	1328	8	788.6	Hiremath et al. (2012)
22	Intraspecific	STMS, RAPD and ISSR markers	57	8	379.47	Jamalabadi et al. (2013)
23	Intraspecific	SSR and SNP markers	464	Nine LGs and three satellites	658.7	Stephens et al. (2013)
			408	Seven LGs and three satellites	752	
24	Intraspecific	SSRs, GMMs and DArT markers	241	8	621.51	Varshney et al. (2014a, b, c)
			168	8	533.06	
			352	8	771.39	
25	Intraspecific	SSR markers	23	4	690	Jingade and Ravikumar (2015)
26	Interspecific	SNP markers	6698	8	1083.93	Gaur et al. (2015)
27	Intraspecific	EST-SSR, ITP, ESTP, and genomic SSR markers	131	8	1140.54	Gupta et al. (2015)
28	Intraspecific	SNP markers	1007	8	727.29	Jaganathan et al. (2015)
29	Intraspecific	RAPD, URP, STMS and morphological markers	33	7	285.3	Karami et al. (2015)
30	Interspecific	Genic and genomic SSR, ITP and SNP markers	1697	8	1061.16	Khajuria et al. (2015)
31	Intraspecific	SNP markers	3368	8	1006.98	Verma et al. (2015)
32	Interspecific	InDel markers	1059	8	978.21	Srivastava et al. (2016)
			594	8	603.26	
			1479	8	978.61	

ICC 1882 were genotyped using GBS approach and a high-density genetic map with 1007 marker loci spanning a distance of 727.29 cM was developed (Jaganathan et al. 2015). In another effort for fine mapping, these two populations were genotyped using high-density Affymetrix SNP arrays "Axiom®*Cicer*SNP array" and dense genetic maps with more than 13,000 and 7000 markers have been generated (Roorkiwal et al. 2017). Further, two candidate genomic regions responsible for salinity tolerance have been reported using ICCV 2 × JG 11 derived RIL population (Pushpavalli et al. 2015).

In addition, two additional intraspecific mapping populations (C 214 × WR 315 and C 214 × ILC 3279) segregating for FW and AB were developed and used for QTL analysis. Two novel QTLs explaining 10.4–18.8% phenotypic variation for FW and six QTLs explaining up to 31.9% of phenotypic variation for AB were identified (Sabbavarapu et al. 2013).

Further, several transcript maps have also been developed in chickpea. A transcript map with genic molecular markers including SNP, SSR, and intron spanning region (ISR) markers has been developed on an interspecific mapping population (ICC 4958 × PI 489777) (Gujaria et al 2011). In another effort to develop a second-generation transcript map, Hiremath et al. (2012) developed a genetic map comprising 1328 marker loci including 625 novel CKAMs, 314 TOG-SNPs, and 389 published marker loci with an average inter-marker distance of 0.59 cM.

A physical map based on finger printing of more than 70 K clones was developed for the reference genotype ICC 4958 (Varshney et al. 2014b). In addition to linkage mapping approach, efforts to map the markers using GWAS were able to identify several markers associated with traits of interest. Recently, Thudi et al. (2014) undertook a comprehensive association mapping analysis using whole genome scanning and candidate gene-based approach, which led to identification of 312 markers significantly associated with drought and heat response in chickpea. Another effort to map the markers using GWAS used the WGRS data on 300 lines from chickpea reference set and multi-season, multilocation phenotyping data for identification of several markers associated with yield and yield-related traits (unpublished). In summary, in addition to genetic maps for dissecting the complex traits, the integrated physical map with genome maps can be utilized for QTL cloning.

6.4 Molecular Breeding

With the availability of large-scale genomic resources and markers associated with trait of interest, next step is to use this information for accelerating the crop improvement program to enhance the rate of genetic gain. In chickpea efforts to use the markers in breeding have been focused on marker-assisted backcrossing (MABC) and now being shifted to GS. MABC has been successful for addressing the simple traits, while for addressing the complex traits where trait is controlled by several small effect QTLs, MABC is not that effective. GS approach using genome-wide marker profile has been suggested as a potential breeding approach for developing superior lines to address such complex traits (Meuwissen et al. 2001).

In chickpea, MABC efforts focused on introgression of QTL(s)/genomic region(s) responsible for yield under rainfed condition and disease resistance. As part of trait mapping, a genomic region on LG04 was identified as "*QTL-hotspot*" explaining up to 58% phenotypic variation for several root traits that control the yield under rainfed condition. Efforts to introgress this genomic region into elite chickpea genotype JG 11 were initiated using MABC approach as described by Varshney et al. (2013b). Introgression lines generated after three backcross and two rounds of selfing (BC_3F_3) showed improved performance with 12% (under rainfed) to 24% (under irrigated) higher yield. After multilocation field evaluation, 10 introgression lines have been identified as superior and are being sent for AICRP trial for release in India. Inspired by success of JG11+, efforts have already been initiated to introgress this genomic region in several other elite chickpea varieties. In addition, similar

efforts to introgress the genomic region were also initiated by Indian Agricultural Research Institute (IARI, New Delhi) and Indian Institute of Pulse Research (IIPR, Kanpur) and their introgression lines are under field evaluation. Similar efforts were also undertaken for introgressing the FW and AB resistance in elite chickpea cultivar C 214 using MABC. Introgression lines developed in the background of C 214 have shown enhanced resistance for FW and AB (Varshney et al. 2014c). Currently, efforts are underway to pyramid FW and AB resistance in same genotype of C 214 background through intercrossing of introgression lines.

In addition to MABC, ICRISAT also initiated efforts to deploy the GS in the chickpea breeding program. For this, a set of 320 elite chickpea lines was selected and genotyped using DArT markers. This set was phenotyped at Patancheru and New Delhi for two seasons for yield and yield-related traits. Phenotyping data along with genome-wide marker profile data was used with six statistical GS models to estimate the prediction accuracies (Roorkiwal et al. 2016).

6.5 Conclusion

Chickpea was earlier known as "orphan crop" because of limited availability of genomic resources, but recent efforts have transformed it to a genomic resource-rich crop. Last decade has witnessed tremendous growth in establishment of genomic resources for chickpea and utilization of these genomic resources in enhancing the chickpea productivity. Focus has never been limited to developing genomic resource, but to deployment of developed genetic resources in crop improvement programs leading to enhancement of chickpea production. Availability of whole genome sequence and different re-sequencing efforts has allowed the development of high-throughput genotyping platform, one such being Axiom® *CicerSNP* array (Roorkiwal et al. 2017). In order to deploy these genomic resources in chickpea breeding, MABC is being routinely used for developing superior varieties by targeting simple traits. Recently, GS has also gained momentum with its capability to target complex traits and ICRISAT has initiated deployment of GS in chickpea. As mentioned above, narrow genetic diversity is one of the major factors, restraining the efforts for enhancing the chickpea productivity. ICRISAT has also started toward developing the multi-parent advanced generation intercross (MAGIC) population for addressing the issue of narrow genetic diversity. Similarly, nested association mapping (NAM) population are also being developed. In summary, chickpea crop improvement is moving toward integrating modern genomics approach with existing breeding programs for enhancing chickpea yield.

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