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Abstract

Advances in genomics technologies, coupled with the availability of several high-throughput genotyping and sequencing platforms during recent years, provided a kick start to the adoption of modern breeding approaches to develop climate-resilient crops. Chickpea is the most important grain legume crop for global food and nutritional security in the context of population explosion and climate vagaries. During last ten years, it has transformed from orphan legume to genomics resource-rich legume like any other model legume plants. There has been a paradigm shift in the outlook of the scientific community in translating the genomic resources including the genome sequence and re-sequence information for developing superior lines with enhanced resistance or tolerance to important abiotic and biotic stresses. In addition, pan-genome and re-sequencing information of several germplasm lines will enable tailoring climate smart chickpeas. In addition, efforts to broaden the genetic base and enhanced utilization of the available trait-specific germplasm lines, multi-parent advanced generation inter-cross (MAGIC), nested association mapping (NAM) populations in breeding programs will accelerate the genetic gains at a faster pace.

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Chickpea (*Cicer arietinum* L.) is a cool season legume cultivated by resources-poor farmers in South Asia and sub-Saharan Africa. Despite its economic importance, productivity is lower than 1 ton per hectare because the crop is exposed to several biotic and abiotic stresses. Genomics research has accelerated the crop improvement in crops like rice, maize. In case of chickpea until 2005, about 150 SSR markers and sparse genetic maps were available which were of limited

usefulness for trait dissection and implementing them in breeding programs. During last decade, efforts of chickpea research community especially at ICRISAT in collaboration with several partners across the globe developed >3,000 SSRs (Nayak et al. 2010; Thudi et al. 2011; Agarwal et al. 2015), transcriptomic resources (Hiremath et al. 2011; Kudapa et al. 2014), millions of SNPs and structural variations (Varshney et al. 2013a; Thudi et al. 2016a, b). Both desi and kabuli draft genomes have been decoded (Varshney et al. 2013a; Jain et al. 2013). In addition, several genetic maps, a physical map, consensus maps and high-density genetic maps have been made available for trait dissection (Gujaria et al. 2011; Millan et al. 2010; Varshney et al. 2014b, c; Gaur et al. 2015; Jaganathan et al. 2015; Kale et al. 2015). Furthermore, the genomic regions responsible for abiotic stress (Vadez et al., 2012; Varshney et al. 2014c; Purushothaman et al. 2015; Pushpavalli et al. 2015), biotic stresses (Sabbavarapu et al. 2013) and agronomically important traits like early flowering (Mallikarjuna et al. 2017; Samineni et al. 2016), protein content (Jadhav et al. 2015) have been identified. Thus, the availability of several genomics resources and draft genomes has transformed chickpea from orphan legume to “genomics resource rich” legume crop (Varshney 2016). This provided new opportunities for accelerating genetics research and use of these resources in breeding applications for faster genetic gains.

Recent climate changes, availability of irrigation facilities encouraged farmers in north India for cultivating commercial crops such as paddy and wheat. As a result, chickpea cultivation has expanded in the southern part of India that has been exposed to more frequent droughts and thus contributing to yield losses. Chickpea is being important for food and nutritional security, development of improved lines and cultivars that adapt to new niches in the context of climate change is a prerequisite. This chapter focusses on strategies and issues that need to utilize available genomic tools together with genetic resources for enhancing the chickpea yields to meet the future demands.

12.1 Germplasm Lines Re-sequencing and Pan-genomes

The availability of draft genome sequence of both kabuli and desi chickpea genomes (Varshney et al. 2013a; Jain et al. 2013) offers novel opportunities for understanding the genome architecture and identification of genes for crop improvement. Following the draft genomes, in recent years, efforts were also made to improve the genome assemblies using sequence data from flow cytometry isolated chromosomes to identify misplaced contigs (Ruperao et al. 2014). In addition, an improved version of desi genome assembly was reported (Parween et al. 2015) and draft genome assembly of *Cicer reticulatum*, the wild progenitor of chickpea, has also become available (Gupta et al. 2017). As a single genome sequence may not be enough to explain the variation existing in >93,000 chickpea, germplasm accessions being conserved in genebanks across the world. Hence, re-sequencing of diverse germplasm lines is a necessary task ahead to understand the genome wide variations and harnessing the existing variations for designing new strategies for chickpea improvement. Towards this direction, 90 elite lines, 35 parental genotypes of mapping populations, 129 released varieties were re-sequenced (Varshney et al. 2013a; Thudi et al. 2016a, b) and efforts are underway at ICRISAT to re-sequence 3,000 germplasm lines, the composite collection.

The allelic variations available in a gene of interest that may lead to desirable phenotype within a species are quite limited. Hence, Tattelin et al. (2005) proposed the concept of “pan-genome” to capture the complete gene set from different species of genera. The pan-genome is essential to fully understand the genetic control of phenotypes. Further, understanding the interconnection of genome and phenome is essential for achieving faster genetic gains in crop improvement programs. Insights into pan-genomes of several crop plants are now available for soybean (Li et al. 2014), maize (Hirsch et al. 2014; Lu et al. 2015), *Brassica oleracea* (Golicz et al. 2016), hexaploid wheat

(Montenegro et al. 2017) and a pan-genome browser was developed in case of rice (Sun et al. 2016). The draft genomes and/or re-sequence information in any species is not of much use if no biological sense is made out of the data. It is also a herculean task to store as well as to analyse the huge amount of data. The tools available for pan-genome analysis have been extensively discussed by Xiao et al. (2015).

12.2 Functional Genomics

Plant stress responses are complex and form a coordinated response network with every gene involved from recognition to signaling to direct involvement. Functional genomics facilitates understanding the stress response at the genomic level and to characterize specific genes involved in resistance to biotic and abiotic stresses in chickpea. Functional genomics approaches such as suppression subtractive hybridization (SSH), super serial analysis of gene expression (Super-SAGE), microarray and EST sequencing have been performed to identify the abiotic stress-responsive transcripts in chickpea (Molina et al. 2008; Varshney et al. 2009; Buhariwalla et al. 2005; Garg et al. 2016). In addition, sequencing and de novo assembly of chickpea transcriptome using short reads have been reported in chickpea (Garg et al. 2011a, b). Since gene expression is post-transcriptionally regulated by microRNAs, recent studies used high-throughput small RNA sequencing approach to discover tissue-specific and stress-responsive expression profile of chickpea microRNAs (Jain et al. 2014; Kohli et al. 2014). The availability of next-generation sequencing technologies accelerated the development of gene expression profiles at the whole genome level (Jain 2012; O'Rourke et al. 2014) and transcriptome sequencing as well as NGS-based large-scale discovery and high-throughput genotyping of informative markers like simple sequence repeat (SSR), single nucleotide polymorphism (SNP) in chickpea (Garg et al. 2014; Hiremath et al. 2012; Jhanwar et al. 2012; Agarwal et al. 2012; Kudapa et al. 2014; Pradhan et al. 2014; Parida et al. 2015).

12.3 Next Generation Mapping Populations

Linkage mapping studies use family-based populations like F₂, recombinant inbred lines (RILs), near isogenic lines (NILs) and double haploid populations, but alleles in these mapping populations come from only two parental lines. Hence, specialized mapping populations with a broad genetic base such as multi-parent advanced generation inter-cross (MAGIC) and nested association mapping (NAM) populations need to be developed and used. MAGIC population is generated from multiple parents of diverse origin, and the genome of the founder parents is reshuffled in different combinations (Huang et al. 2015). It serves as an important resource for high-resolution mapping and identification of target genomic regions, besides useful in the breeding programmes. A MAGIC population comprising of 1136 RILs using eight parental genotypes has been developed in chickpea. Nested association mapping (NAM), which combines the benefits of both linkage analysis and association mapping approaches, is used for high-resolution mapping of target traits. Development of NAM population is underway in chickpea to generate new breeding material with enhanced diversity. In addition, some other next-generation multi-parental populations like multiline cross inbred lines and recombinant inbred advanced intercross lines can also be developed in chickpea.

12.4 High-Resolution Mapping for Must Have Traits

Chickpea is cultivated under a wide range of agro-climatic conditions around the world and is adversely affected by diseases, insect pests, soil and environmental stresses. In addition, climatic variability and change in cultivation niches also have further implications on the cultivation of chickpea in different regions. Hence, future varieties must be able to withstand adverse and more variable conditions. Making of genetic adjustments of chickpea is needed to increase

adaptation to drought, heat stress in semi-arid areas, cold stress tolerance in the Mediterranean region, resistance to biotic stresses like *Fusarium* wilt, *Ascochyta* blight and pod borer.

Advances in chickpea genomics and availability of genome sequences (Jain et al. 2013; Varshney et al. 2013a; Gupta et al. 2017) and re-sequencing data from hundreds of germplasm lines in chickpea have offered a different kind of marker genotyping platforms. For instance, large-scale SSR markers (Nayak et al. 2010; Thudi et al. 2011), VeraCode assays (Roorkiwal et al. 2013) and KASPar assays (Hiremath et al. 2012) have become available for genotyping germplasm collections and mapping populations. Genotyping of different populations with above-mentioned marker systems, however, is an expensive and time-consuming business. Furthermore, for undertaking association mapping, there is a need to genotype populations with high-density markers. In this direction, Axiom[®] arrays comprising 50 K single nucleotide polymorphism (SNP) markers have been developed in chickpea. These arrays have been proven very useful for generating large-scale polymorphisms in bi-parental mapping populations (Roorkiwal et al. unpublished). In addition, genotyping by sequencing and skim sequencing-based bin mapping approaches were adopted for fine mapping the traits (Jaganathan et al. 2015; Kale et al. 2015). Nevertheless, unlike genotyping the entire population, approaches like sequencing bulk segregant analysis (BSA-Seq) and QTL-Seq approaches have been deployed to identify the causal SNPs and candidate genes in legumes including chickpea (Singh et al. 2015, 2016; Pandey et al. 2017). We believe that in coming years trait mapping can be faster by using QTL-Seq approaches and use of MAGIC population, NAM with high-density arrays like Axiom[®] will help fine map the QTLs.

12.5 Next Generation Breeding

Development of large-scale genomic resources in chickpea (Varshney et al. 2012) and availability of pedigree information combined with

optimized precision phenotyping methods make it possible to undertake new generation of breeding approaches in chickpea. Some of the genomics assisted breeding approaches like marker-assisted backcrossing (MABC) have been successfully employed to introgress disease resistance (Varshney et al. 2014a) and drought tolerance (Varshney et al. 2013b) into elite cultivars of chickpea. Marker-assisted recurrent selection (MARS) is another breeding approach proposed for pyramiding of superior alleles at different loci/QTLs in a single genotype (Bernardo and Charcosset 2006) is also being initiated to assemble favourable alleles for drought tolerance in chickpea (Thudi et al. 2014b). In addition, Advanced backcross (AB-QTL) analysis is another useful approach to introgress desired QTL or a gene especially from wild/exotic species (Tanksley and Nelson 1996) that can be developed in chickpea.

12.6 Genomic Selection

Genomic selection (GS) is a novel approach that predicts the breeding values of a line based on historical phenotyping data and the genotyping data. For addressing complex traits controlled by many small effect QTLs, genome-enabled selection of genotypes based on their breeding value (i.e. the genomics estimated breeding values) has potential relevance (Meuwissen et al. 2001). GS utilizes genome wide markers data along with phenotypic data to increase the accuracy of the prediction of breeding and genotypic values. This has become feasible due to the availability of a large number of SNP discovered by various NGS approaches and cost-effective genotyping platforms available in chickpea (Hiremath et al. 2012; Varshney et al. 2012). Genomic selection has been successfully used in animal breeding for predicting breeding values (Hayes et al., 2009) and also in crop plants like oil palm (Wong and Bernardo, 2008) and maize (Zhao et al. 2012). Recent study showed that genomic-enabled prediction as a promising avenue for improving yield in chickpea (Roorkiwal et al. 2016).

In addition to the above, we believe that diagnostic markers associated with must have traits can be used in an early generation in chickpea breeding programs which we call as “early generation selection (EGS)”. Right now, diagnostic markers are being used in EGS for drought tolerance, Fusarium wilt and Ascochyta blight in chickpea. We believe that in coming years, we will have more markers for must have traits and all loci. In summary, we need to adopt MABC approach for elite varieties deficient of one or two traits. For normal breeding, we propose to use diagnostic markers for EGS for target trait improvement and genomics selection approach for multiple traits. We envisage the use of a combination of EGS, GS and genome editing in chickpea in coming years.

12.7 Conclusion

As evident from different chapters of the book, we got large-scale germplasm and genomic resources for trait mapping, etc. It is high time to use the markers in regular breeding programs. We believe that combination of EGS and GS will accelerate genetic gains in breeding programs.

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