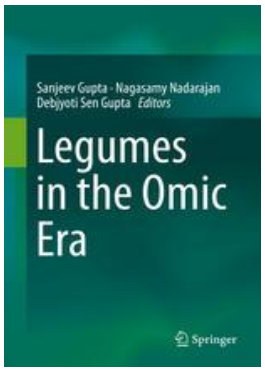

Advances in Chickpea Genomics

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Abstract

Chickpea (*Cicer arietinum* L.), the second largest consumed pulse crop of the world after common bean, is grown in over 50 countries and traded across 140 countries. The beneficial effects of chickpea on soil health and human health are well recognized. There has been a slow progress in improving average global productivity of chickpea, which continued to remain below 1.0 ton ha^{-1}. The breeding efforts in chickpea have mainly focused on improving its adaptation to different growing conditions. The changing scenario of chickpea cultivation, particularly the large shift in its area to warmer growing environments, and expected effects of climate change further impose challenges on chickpea breeding programs. After several decades of slow progress, the recent years have witnessed extraordinary growth in development of genetic (mapping populations) and genomic resources (structural and functional molecular markers, integrated genetic map, mapping of genes/quantitative trait loci, whole genome sequencing) for chickpea. Now, chickpea is one of the most advanced grain legumes in terms of availability of genomic resources. Efforts have already begun on application of genomics technologies in chickpea improvement. The coming years are expected to have an exponential growth in integration of genomics technologies in chickpea breeding programs. This book chapter provides an update on the development of genetic and genomic resources for chickpea and their current and potential uses in chickpea improvement.

Key words: *Cicer arietinum*, molecular markers, genome sequence, quantitative trait loci, marker assisted breeding

1. Introduction

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop of the world in terms of area and production. During 2010, chickpea was grown in more than 50 countries and had an area of about 12 m ha, production of 11 m tons and productivity of 910 kg ha^{-1} [1]. The major chickpea producing countries include India, Pakistan, Australia, Myanmar, Iran, Mexico, Canada and USA. The highest production and consumption of chickpea is in South Asia where India alone accounts for over two-third of the global area, production and consumption. The awareness

of health benefits of chickpea has led to considerable increase in the international trade of chickpea. Currently, chickpea is imported by over 140 countries [1].

Chickpea is known to have a diverse array of potential nutritional and health benefits. It is a good source of protein, carbohydrates, minerals and vitamins, dietary fibre, folate, β-carotene and health-promoting fatty acids [2]. Scientific studies have provided some evidence to support the potential beneficial effects of chickpea in lowering the risk of various chronic diseases such as cardiovascular diseases, type 2 diabetes, digestive diseases and some cancers [2].

Being a legume crop, chickpea is highly valued in the cropping system, particularly in rotation with cereals, for its overall impacts on soil health. There has been a large shift in chickpea area (about 3 m ha) from cooler, long growing season environments to warmer, short growing season environments during the past four decades [3]. This significant change in the chickpea growing environment and the expected impacts of climate change need to be accounted by chickpea breeding programs.

The major adaptation traits to be considered by chickpea breeding programs include phenology, plant type and resistance to key abiotic and biotic stresses prevalent in the target environment and growing conditions. Drought and heat stresses during the reproductive phase and with increasing severity towards the end of the crop season are the major abiotic stresses of chickpea as the crop is generally grown rainfed on residual soil moisture and experiences progressively receding soil moisture conditions and increasing atmospheric temperatures towards end of the crop season. Soil salinity and chilling atmospheric temperatures are also important stresses in some growing environments. Among diseases, fusarium wilt (caused by *Fusarium oxysporum* f. sp. *ciceri*), dry root rot (caused by *Rhizoctonia bataticola*), and collar rot (caused by *Sclerotium rolfsi*), are the important root diseases of chickpea in areas where the growing season is dry and warm, while ascochyta blight [caused by *Ascochyta rabiei* (Pass.) Labr.], and botrytis grey mold (caused by *Botrytis cineria* Pres.), are the important foliar diseases in the areas where the growing season is cool and humid. Pod borer (*Helicoverpa armigera* Hubner) is the most important pest of chickpea worldwide. The viral diseases, rust (caused by *Uromyces ciceris-*

arietini), root nematodes (*Meloidogyne* sp.), Phytophthora root rot (caused by *Phytophthora medicaginis*), cutworm (*Agrotis* sp.) and leaf miner (*Liriomyza cicerina*) are also important in some chickpea growing areas.

Recent advances in the development of genomic resources have made it possible to use genomics-assisted breeding for improvement of chickpea. The breeding programs will have higher precision and efficiency and thus better equipped to rapidly develop cultivars better adapted to existing and evolving growing environments and with improved nutrition quality and grain traits required by the industry and the consumers. This chapter provides an update on the progress made in development and use of genomic resources in chickpea.

2. Origin and phylogeny

Chickpea is a self-pollinated, annual, diploid (2x = 2n = 16), cool season food legume. It is considered to have originated in south-eastern Turkey and the adjoining northern region of Syria [4], because the proposed wild progenitor (*C. reticulatum*) of the chickpea and its other closely related wild species (*C. echinospermum, C. bijugum*) are found there. The genus *Cicer* includes 43 species, nine of which are annual, 33 are perennial and one with unspecified life cycle [4]. The species *C. arietinum* is the only cultivated species of this genus. Based on successes in interspecific crosses, *C. arietinum* has been placed in primary gene pool, *C. echinospermum* in the secondary gene pool and all the remaining species in the tertiary gene pool [5]. The phylogenetic relationships among nine annual species have also been studied based on allozyme polymorphism [6,7,8,9] protein banding patterns of seeds [10] and randomly amplified polymorphic DNA (RAPD) markers [11]. These studies have categorised the annual *Cicer* species into four phylogenetic groups. *C. arietinum, C. reticulatum* and *C. echinospermum* formed one group while *C. pinnatifidum, C. bijugum* and *C. judaicum* formed another group. *C. chorassanicum* was grouped with *C. yamashitae* whereas *C. cuneatum* showed the largest distance from *C. arietinum* and formed an independent group. Further, cultivated chickpea was found to be more closely related to *C. reticulatum* than *C. echinospermum*. These results were further supported by studies using molecular markers such as RAPD [12,13], amplified fragment length polymorphism (AFLP) [14,15] and simple sequence repeats (SSR) [16,17,18]. In the

process of evolution, chickpea has emerged into two distinct types; small seeded dark colored *Desi* and large seeded, cream colored *Kabuli*. About 80% of the chickpea area is under the *Desi* type and the remaining area under the *Kabuli* type.

Molecular diversity studies indicated that wild relatives of chickpea have high genetic diversity compared to its cultivated species *C. arietinum* and supports the conclusion that chickpea has a narrow genetic base [14,18]. These results indicate that the varieties currently under cultivation are closely related among themselves. Efforts should be made to widen the genetic base of the cultigen by exploiting wild species. The wild species also offer opportunities of bringing novel alleles for important traits, particularly resistance to abiotic and biotic stresses [19].

3. Genome and Genome Size

Almost all *Cicer* species have $2n=2x=16$ chromosomes. The chromosomes have been numbered from 1 to 8 in order of decreasing size of the chromosomes and the size difference between pair one and pair eight has been found to be in the ratio of 3:1 [20]. Ahmad and Hymowitz [21] recorded the total chromosome length at pachytene stage as 353.53 μm and also found that the chromosome size ranged from 30.53 to 58.05 μm. The chickpea chromosomes are small which makes the karyotype analysis difficult. The chickpea karyotype revealed from various cytological investigations has the following features: a pair of very long chromosomes, distinctly satellited and sub-metacentric; six pairs of metacentric to sub-metacentric chromosomes; and a pair of very short metacentric chromosomes (reviewed by Gupta and Bahl [22]). Both, spontaneous [23] and induced [24,25,26,27], autotetraploids have been reported in chickpea. Seed treatment with 0.1 to 0.25% colchicine for 4 hours has been found effective in inducing autotetraploidy and these autotetraploids predominantly show bivalent pairing and normal disjunction at anaphase I [28].

4. Genetic and Genomic Resources

Genetic resources, which include mapping populations, genetic stocks and breeding materials, have been developed in chickpea for use in genetic studies and breeding programs. Further, during recent years, large scale genomic resources in the form of molecular markers, genetic

linkage maps and quantitative trait loci (QTL) maps have been developed and made available to breeders for implementing integrated breeding approaches and developing cultivars more efficiently.

4.1 Mapping populations

Development of appropriate mapping population is necessary for constructing a genetic linkage map and dissecting complex traits. The first step in producing a mapping population is selecting two genetically diverse parents for one or more traits of interest. Further the parents should be genetically divergent enough to exhibit sufficient polymorphism, and on the other hand they should not be too distant that causes sterility of the progenies and expresses high level of segregation distortion during linkage analysis. A range of populations including progenies from F_2 generation, backcross (BC), recombinant inbred lines (RILs), double haploids (DH) and near isogenic lines (NILs) have been used for genetic mapping in chickpea. F_2 populations are developed by self-pollinating F_1 hybrids derived by crossing two parents, while BC population is produced by crossing F_1 to one of the parents). By repeated backcrossing for at least six generations (BC_6) with the recipient or recurrent parent, more than 99% of the genome can be recovered from the recurrent parent. Further selfing of selected individuals at BC_6F_1 or BC_7F_1 will produce lines that are homozygous for the target gene, which are considered to be nearly isogenic with the recipient parent (NILs). NILs are mainly generated for fine mapping of a QTL/ genomic region of interest. DH populations are generally developed by chromosome doubling of hapoids developed though anther culture (pollen or microspore culture) of F_1 plants. RILs are developed following single seed descent (SSD) advancement of F_2 plants by six or more generations and then developing single plant progenies. This process leads to lines that contain a different combination of linkage blocks from the original parents. Seed from RILs is predominantly homogeneous and abundant, so the seed can be sent to any lab interested in adding markers to an existing linkage map previously constructed with the RILs. Moreover, RILs can be grown in replicated trials at several locations and/or over several years making them ideal for QTL mapping. Similar types of inbred populations, such as doubled haploids, can also be used for linkage mapping with many of the same advantages of RILs. The RIL mapping populations of chickpea developed and available at ICRISAT are listed in Table 1.

For creating novel genetic variation and identification of useful allelic variants, a TILLING (Targeting Induced Local Lesions IN Genomes) population from chickpea accession ICC 4958 was developed at ICRISAT through mutagenesis by ethyl methane sulphonate (EMS). This population comprises of >5000 M2 lines which are currently being used for allele mining for various agronomically important genes. A multi-parent advanced generation inter-cross (MAGIC) population has been used to develop over 1200 lines at ICRISAT. The MAGIC population was developed from 8 parents and includes cultivars and elite breeding lines from India and Africa. Twenty-eight two-way, 14 four-way and 7 eight-way crosses were made to develop this MAGIC population. The MAGIC lines constitute a valuable genetic resource for trait mapping and gene discovery. In addition, these can be directly used as source material for development of improved cultivars [29].

4.2 Molecular markers

The genomic resources being made available for chickpea breeding community have been reviewed from time to time [29,30,31,32]. However, this chapter provides the latest developments as well as discusses the pros and cons of these marker resources in various genetic analyses. Based on the method of detection of the sequence variation, the molecular markers can be classified as hybridization based (PCR-independent), PCR dependent and micro-array based markers. RFLP markers were the first hybridization based highly reproducible, co-dominant, locus specific markers employed for plant genome analysis during 90's. The first genetic map constructed in chickpea using molecular markers included RFLP and RAPD markers along with isozyme markers [33]. Genetic diversity studies were also carried out using RFLP markers [34] and microsatellite-derived RFLP markers [35,36]. These studies showed narrow genetic variability for restriction sites in the genome of cultivated chickpea. The PCR-based marker systems are of two types – (1) non-sequence specific markers which include RAPD and AFLP markers, and (2) sequence tagged PCR-based markers which include cleaved amplified polymorphic sequence (CAPS), sequence tagged site (STS) and SSR markers. Although RAPD markers were also employed to characterize germplasm [11,13], these markers are not currently being preferred for any genetic analysis in chickpea owing to the dominant nature of inheritance

and non-reproducibility of these markers. However, utility of RAPD markers can be enhanced by converting these into more reproducible informative marker types such as sequence characterized amplified regions (SCAR). To overcome the limitations of reproducibility associated with RAPD, AFLP marker system was developed by selective amplification of DNA fragments obtained by restriction enzyme digestion. AFLP markers have been used for genetic diversity estimation in cultivated chickpea and its wild relatives in order to discover the origin and history of chickpea [14,37,38]. However, the requirement of significant technical skills, laboratory facilities, financial resources and high quality genomic DNA for complete restriction, digestion and dominant inheritance has limited the use of AFLP markers.

PCR based CAPS markers are characterized by their co-dominant inheritance and locus specific nature which are useful for genotyping applications [39,40]. In chickpea, CAPS and derived CAPS (dCAPS) markers have been developed from bacterial artificial chromosome (BAC)-end sequences [41] and expressed sequence tag (EST) sequences [42], and these markers were further integrated into composite genetic map of chickpea to study their association with disease resistance [43].

Microsatellite markers are also known as simple sequence repeats (SSRs) or sequence tagged microsatellite site (STMS), constitute tandem repeats of 1-6 bp in length [44] are advantageous over many other markers types as they are highly polymorphic and abundant, analytically simple and readily transferable [45], and show co-dominance. In chickpea genome SSRs were found to be abundant and showed moderately high level of intra-specific polymorphism when compared to other marker types [35]. About 500 SSR markers were available for chickpea earlier [46,47,48] and were used for development of genetic map [47,49]. Later, several studies reported the development of SSR markers using hybridization based microsatellite enrichment and BAC and BIBAC libraries in chickpea [48,50]. At ICRISAT, currently >2000 SSR markers are available for utilization in chickpea crop improvement [51,52,53,54].

4.3 Sequence information

Recent advances in next generation sequencing (NGS) technologies have greatly facilitated the ability to sequence the genome and transcriptomes of several plant species [55]. In case of chickpea, as on 13th November 2012, 97836 nucleotide sequences were available in the public domain (http://www.ncbi.nlm.nih.gov/nuccore?term=chickpea%20cicer) against only a limited number of expression sequence tags (ESTs) [51].

4.4 Functional markers, ESTs, BAC Libraries

Molecular markers developed from genes/ESTs are referred as genic molecular markers (GMMs; [54] or functional markers [56]. Based on origin, genic markers are of two kinds [56]: (a) markers that are derived from polymorphisms within genes are gene targeted markers (GTMs), these markers however not necessarily involved in phenotypic trait variation, e.g. EST-based molecular markers [57]; (b) functional markers (FMs) are derived from polymorphic sites within genes involved in phenotypic expression of traits, e.g. candidate gene-based molecular markers. Functional markers can further be grouped into two subgroups depending on the involvement in the phenotypic trait variation, (i) direct functional markers (DFMs), for which the role in phenotypic trait variation is well proven, and (ii) indirect functional markers (IFMs), for which the role for phenotypic trait variation is indirectly known [56].

Few studies have been conducted on understanding the chickpea transcriptome by generating the ESTs [58,59,60,61]. Recently several EST sequencing projects have led to generation of large scale EST sequences through single pss sequencing [51,62,63,64].

Several large-insert bacterial artificial chromosome (BAC) libraries and binary BAC (BIBAC) libraries have been constructed in chickpea for marker development as well as construction of physical maps. For instance, 233 new chickpea SSR markers were developed by Lichtenzveig et al. [48] by screening the BAC library with eight synthetic SSR oligos, (GA)10, (GAA)7, (AT)10, (TAA)7, (TGA)7, (CA)10, (CAA)7, and (CCA)7. Recently a set of 1344 novel SSR markers were developed from BAC-end sequences [53]. The Chickpea Transcriptome Database (CTDB) (http://59.163.192.90:8080/ctdb/) developed at National Institute of Plant Genome

Research provides user scientists/breeders a portal to search, browse and query the data to facilitate the research on chickpea and other legumes.

4.5 Quantity trait loci (QTLs)

Understanding the genetics of complex traits like drought tolerance, *Helicoverpa* resistance and salinity tolerance will help in improving these traits through marker-assisted selection (MAS). Despite the importance of root traits in drought avoidance and availability of germplasm with prolific root systems such as ICC 4958 and ICC 8261, the breeding efforts to improve root traits have been negligible. This is because of the laborious, time-consuming and destructive methods involved in root studies. Molecular markers linked to major QTLs for root traits can greatly facilitate marker-assisted selection (MAS) for root traits in segregating generations. ICRISAT in collaboration with several partners generated > 3000 chickpea ESTs from a library constructed after subtractive suppressive hybridization (SSH) of root tissues from ICC 4958 and Annigeri to isolate and characterize root-specific genes differentially expressed between these genotypes [60,65]. This database provides researchers in chickpea genomics with a major new resource for data mining associated with root traits and drought tolerance.

A set of RILs from Annigeri × ICC 4958 cross was developed at ICRISAT and characterized for root traits [66], and SSR marker TAA 170 was identified for a major QTL that accounted for 33% of the variation for root weight and root length [67]. Based on the screening of mini-core collection, parents genetically and phenotypically more distant were identified for development of new mapping populations. These include ICC 8261 and ICC 4958 for a large root system and ICC 283 and ICC 1882 for small root systems. These two crosses were made and more than 250 RILs were developed in each cross [68]. These two mapping populations have been phenotyped and genotyped to identify additional QTLs for root traits.

Several other intra-specific mapping populations have been developed and used to identify the markers associated with traits like resistance to fusarium wilt [69,70,71,72], resistance to ascochyta blight [73,74,75), resistance to rust [76], resistance to botrytis grey mold [77], salinity tolerance [78], drought tolerance (unpublished data with ICRISAT), seed traits [79] and, for grain yield [80]. Several of these studies have been summarized in earlier reviews [29,31,42].

5. Genome Mapping

5.1 Physical mapping

As mentioned above, large scale genomic resources like molecular markers and genetic linkage maps were developed during recent past. Although QTLs for different traits were identified (Table 2), the markers were not close enough for their effective use in molecular breeding. In this context, genome-wide physical maps have been used in several species to effectively integrate genomic tools for marker-assisted breeding, high-resolution mapping and positional cloning of genes and QTL [81]. In addition physical maps will also enable desirable genome sequencing and comparative genomics. Despite these advantages, a genome-wide physical map has not been developed for chickpea. However, recently a BAC/BIBAC based physical map was developed; three large contigs closely linked to QTLs contributing to ascochyta blight resistance and flowering in chickpea were identified [82]. However, a genome-wide physical map is essential for genomics research, cloning candidate genes and enhancing molecular breeding. Towards development of genome-wide physical map, in chickpea in collaboration with National Institute of Plant Genetic Research (NIPGR), New Delhi (S Bhatia and A K Tyagi) and UC-Davis, USA (MingCheng Luo), two new BAC libraries were constructed using *Hin*dIII and *Eco*RI restriction enzymes employing pCC1BAC Epicentre vector in DH10b. A total of 96,768 clones from both the libraries that cover ~15.7 X genome were fingerprinted. In addition clones from BAC library developed by Thudi et al. [53] and NBS-LRR genes were also fingerprinted and used for developing the physical map as a result chickpea physical map was developed spaning an estimated 574 Mb (http://probes.pw.usda.gov:8080/chickpea/). Genetic map positions for 245 BES-SSR markers permit an initial integration of BAC contigs with the chickpea genetic map. Efforts are underway to define the minimum tiling path (MTP) based on the available physical mapping data, which will facilitate either BAC-end or pooled BAC-sequencing of MTP clones. The resulting integrated genetic and physical map is expected to enhance genetics and genomics research and breeding applications in chickpea. The integration of physical map with genetic maps has been reported earlier in different plant species including some fruit trees such as peach [83], papaya [84], apple [85]. The framework physical map serves as a valuable resource for

various other studies such as effective positional cloning of genes and quantitative trait locus (QTL) fine-mapping.

5.2 Genetic mapping

The first linkage map of chickpea was reported in 1990 and consisted of 26 isozyme and three morphological trait loci [86,87]. Several additional isozyme loci and morphological trait loci were mapped in the subsequent studies [33,88,89]. The use of DNA markers in gene mapping greatly accelerated progress in development of a detailed genetic map of chickpea. A linkage map of DNA markers was first published in 1997 which contained 10 RFLP and 45 RAPD markers [33]. These maps were developed by using F_2 mapping populations. The first map using RILs was developed in 2000, which consists of 118 STMS, 96 DAF (DNA amplification fingerprinting), 70 AFLP, 37 ISSR (inter simple sequence repeats), 17 RAPD, 2 SCAR, 3 cDNA and 8 isozyme markers [90]. All these earlier studies used interspecific mapping populations because of limited polymorphism observed for then available markers in the cultivated chickpea. Availability of additional markers made it possible to use intraspecific segregations in linkage studies. A molecular map based on intraspecific cross (kabuli-desi cross) was developed and used to tag genes for resistance to Fusarium wilt. Two SCAR markers and two RAPD markers [91] were found associated with resistance to race 1 and one ISSR marker with resistance to race 4 [92]. The genes for resistance to races 4 and 5 were found to be linked and located close to one STMS and one SCAR marker [90].

As a result of concerted efforts of ICRISAT in collaboration with several partners across globe, large-scale markers resources are now available for chickpea Employing these marker resources both intra and inter-specific maps have been developed. A set of interspecific RILs from *C. arietinum* (ICC 4958) × *C. reticulatum* (PI 489777) cross has been used as reference mapping population for chickpea. Nayak et al. [52] developed a comprehensive map of this reference population with 521 loci that mainly comprised of SSR markers developed from microsatellite enriched library. Further, this map was integrated with BES-SSRs, DArT and gene-based markers by Thudi et al. [53], which comprised of 1291 loci. An advanced gene-rich map of chickpea comprising of 406 loci (including 177 gene-based markers) spanning 1,497.7 cM genetic distance has been developed for this reference population [93]. Recently, Hiremath et al.

[94] developed large-scale KASPar assays for SNP genotyping and developed a genetic map comprising 1328 marker loci including novel 625 CKAMs (Chickpea KAspar Assay Markers), 314 TOG-SNPs and 389 published marker loci for this reference population. The summary of genetic maps developed in chickpea is illustrated in Table 3.

6. Comparative and functional genomics

The advances in next-generation sequencing technologies facilitated the sequencing of trancriptomes as well as the genome of several crop plants. In this context understanding the gene function is of great importance. Recently several genes/ESTs involved in various stress responses have been identified based on transcriptomic and proteomic studies [51,95,96,97,98,99] . However, limited efforts have been made on gene discovery and only a few candidate genes cloned and functionally validated [100,101,102,103]. Several functional genomics studies have been performed in chickpea to identify the abiotic stress-responsive transcripts by approaches such as suppression subtractive hybridization (SSH), super serial analysis of gene expression (SuperSAGE), microarray, and EST sequencing [51,60,98]. The salt stress transcriptomes of roots and nodules studied by Molina et al. [99] by using deep SuperSAGE provided deep insights into the first molecular reactions of a plant exposed to salinity. By studying two chickpea varieties (BGD 72 and ICCV 2) for differences in transcript profiling during drought stress treatment by withdrawal of irrigation at different time points, Jain and Chattopdhyay [64] reported that most of the highly expressed ESTs in the tolerant cultivar predicted that most of them encoded proteins involved in cellular organization, protein metabolism, signal transduction, and transcription. Deokar et al. [104] in addition to studying the genes that are up- and down-regulated in a drought-tolerant genotype (ICC 4958) under terminal drought stress and a drought susceptible genotype (ICC 1882), also studied the gene expression between the bulks of the selected RILs exhibiting extreme phenotypes. Garg et al. [105] reported the sequencing and *de novo* assembly of chickpea transcriptome using short-read data.

7. Progress towards whole genome sequencing and data mining

In recent years, genome sequencing has become very popular in the area of plant genomics and breeding as it offers three fold advantages: a) enables us to understand plant genome structure

and dynamics of molecular evolution, b) enable identification of genes and functional elements and help in annotation of completed genome, and c) provide the genomic tools and platforms for gene mapping, gene isolation and molecular breeding. Further, information gained from sequenced genomes, coupled with genetic association studies, may allow us to identify key genes/quantitative trait loci and networks in the other species. Such information can be very useful for molecular breeding programmes in order to develop improved varieties/ hybrids. Several crop plant genomes have already been sequenced for instance rice [106,107], sorghum [108], using Sanger sequencing. Further, a number of plant genomes were sequenced using NGS technologies, for example cucumber [109], castor [110], cannabis [111], date palm [112], cacao [113] and pigeonpea [114].

A draft genome sequence of chickpea has been published recently which consists of about 738-Mb draft whole genome shotgun sequence of kabuli chickpea variety CDC Frontier [115]. The sequence contains an estimated 28,269 genes. In addition, resequencing and analysis of 90 cultivated and wild genotypes from ten countries was published and targets of both breeding-associated genetic sweeps and breeding-associated balancing selection were identified. Candidate genes were identified for disease resistance and agronomic traits, including traits that distinguish desi and kabuli chickpea. The chickpea genome sequencing work was carried out by the International Chickpea Genome Sequencing Consortium (ICGSC) led by ICRISAT. This ICGSC involved 49 scientists from 23 organizations in 10 countries. This is a landmark milestone in chickpea genomics and will pave the way for more rapid progress towards integrating physical and genetic maps and genomics-assisted breeding of chickpea.

8. Use of genomic resources in molecular breeding

The large scale genomic resources developed during recent years are currently being employed for accelerating the molecular breeding programs in chickpea. For instance, a genomic region controlling root traits and several other traits related to drought tolerance contributing >30% phenotypic variation identified in the Phase I of the Tropical Legume (TL-I) project of Generation Challenge Programme (GCP) has been intogressed into three popular chickpea varieties, JG 11 and KAK 2 from India and Chefe from Ethiopia. Phenotypic evaluation of these lines is underway in India, Kenya and Ethiopia. ICRISAT and its partners in India, which include

Indian Institute of Pulses Research (IIPR), Kanpur and Indian Agricultural Research Institute (IARI), New Delhi are introgressing this genomic region to additional chickpea cultivars under a project funded by the Department of Biotechnology, Government of India. Similarly, ICRISAT's partners in Ethiopia (Debre Zeit Agricultural Research Center, Debre Zeit) and Kenya (Egerton University, Njoro) are introgressing this genome segment to elite lines/cultivars of these countries under phase 2 of TL-I project (Table 4).

In addition, race specific resistance to fusarium wilt is being introgreesed through MABC into selected Indian chickpea cultivars under Accelerated Crop Improvement Programme (ACIP) project sponsored by Department of Biotechnology, Government of India. These efforts are being led by ICRISAT and being carried out in partnership with Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur; Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri; IIPR, Kanpur; and Agricultural Research Station (ARS), Gulbarga. ICRISAT is pyramiding resistances for *foc1* and *foc3* from WR 315 and 2 QTLs for Ascochyta blight resistance from ILC 3279 line into C 214. JNKVV, MPKV, ARS-Gulbarga are transferring resistance to *foc4* from WR 315 genotype in leading varieties namely JG 74, Phule G12 and Annigeri-1, respectively, while IIPR is engaged in introgressing resistance to *foc2* in Pusa 256. A range of backcross progenies followed by both foreground selection and background selection has been generated by these institutes.

A marker-assisted recurrent selection (MARS) program is also in progress at ICRISAT, India and Egerton University, Kenya for accumulating favorable alleles for yield under moisture stress conditions. MARS is a modern breeding approach that enables increasing frequency of several beneficial alleles having additive effect and small individual effects in recurrent crosses [116]. While several multi-national companies are using MARS in crops like maize and soybean, only a few public sector institutes have started to use MARS in crops likes wheat [117], maize [118]. At ICRISAT four superior desi genotypes based on their performance have been selected ICCV 04112, ICCV 05107, ICCV 93954 (released as JG 11 in India) and ICCV 94954 (released as JG 130 in India) and two crosses were made by using elite and elite lines (JG 11 × ICCV 04112 and JG 130 × ICCV 05107). The F_3 plants were genotyped and $F_{3:5}$ progenies were evaluated at three

locations (Ethiopia, Kenya and India) under rainfed and irrigated conditions. To pyramid superior alleles of the favorable QTLs identified based on F_3 genotyping data and F_5 phenotyping data, a set of eight lines were selected for each cross using OptiMAS 1.0. It is anticipated that at the end of the project, RC_3F_4 progenies will be available for evaluation at multi-locations. Recently, IARI, New Delhi and IIPR, Kanpur have also initiated MARS in chickpea for Pusa 372 × JG130 and DCP92-3 × ICCV 10 crosses, respectively. These efforts are expected to develop superior lines with enhanced drought tolerance.

The MAGIC population developed at ICRISAT (described in section 4.1) also provided breeding materials for direct use in chickpea breeding programs. ICRISAT has shared F_4 seed from 4-way and 8-way crosses with several institutes in South Asia and sub-Saharan Africa. The plant breeders can select promising plants at their locations and develop progenies for further evaluations. Several heat tolerant progenies have been developed from MAGIC population at ICRISAT.

9. Conclusions

Rapid advancements in development of chickpea genomic resources during the past decade have made it possible to initiate genomics-assisted breeding in chickpea for improvement of its adaptation to abiotic and biotic stresses. MABC lines, in which a genomic region that controls root traits and several other drought tolerance related traits was introgressed, are already under field evaluation. Several other projects on marker-assisted breeding of chickpea are in progress and elite lines being developed from these projects are expected to be available for field evaluation in coming years. The year 2013 began by adding a landmark milestone in chickpea genomics as the draft genome sequence of chickpea genome was published on 27 January 2013. The information revealed by the draft genome sequence will further boost efforts on development of genomic resources and their applications in chickpea improvement. Integrated breeding approaches would improve speed, precision and efficiency of ongoing breeding efforts of chickpea improvement in development of cultivars better adapted to existing and evolving

growing environments and cropping systems and with grain and nutritional quality preferred by the industry and the consumers.

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Table 1: List of chickpea RIL mapping populations developed and available at ICRISAT

RIL Population	Cross	Generation	No. of RILs	Segregating traits
ICCRIL01	ICCV 2 × JG 62	F_{10+}	573	Fusarium wilt (FW) resistance, botrytis gray mold (BGM) resistance, *Helicoverpa* resistance, salinity tolerance
ICCRIL02	Annigeri × ICC 4958	F_{10+}	257	Root traits
ICCRIL03	ICC 4958 × ICC 1882	F_{10+}	264	Root traits
ICCRIL04	ICC 283 × ICC 8261	F_{10+}	281	Root traits
ICCRIL05	ICC 506-EB × Vijay	F_{10+}	328	*Helicoverpa* resistance
ICCRIL06	ICC 3137 × IG 72953	F_6	241	*Helicoverpa* resistance
ICCRIL07	ICC 995 × ICC 5912	F_{10+}	240	Protein content
ICCRIL08	ICC 6263 × ICC 1431	F_8	266	Salinity tolerance
ICCRIL09	ICCV 2 × JG 11	F_8	280	Salinity tolerance
ICCRIL10	JG 62 × ICCV 05530	F_{10+}	315	Ascochyta blight (AB), BGM and FW resistance
ICCRIL11	Pb 7 × ICCV 04516	F_8	127	AB resistance
ICCRIL12	ICC 4567 × ICC 15614	F_8	296	Heat tolerance
ICCRIL13	ICC 4567 × ICC 1356	F_8	291	Heat tolerance

Table 2: Summary of trait mapping for biotic, abiotic and agronomically imporatnat traits in chickpea

Traits studied	QTL/genes	Markers linked	References
Biotic stress			
Resistance to *fusarium* wilt	*Foc0*	RAPD, SSR	[119]
	Foc1	SSR	[120]
	Foc2	SSR	[120]
	Foc3	SSR	[71,120]
	Foc4	SSR	[71,72]
	Foc5	SSR	[120]
Ascochyta blight	QTL	RAPD	[121]
	Ar19	RAPD	[122]
	QTL*ar2b*	SSR	[70]
	QTL*AR3*	SSR	[73]
	QTL*ar1*	SSR	[123]
	QTL*ar2*	SSR	[123]
	QTL	SSR	[74]
	QTL	SSR	[124]
Botrytis grey mould	QTL	SSR	[77]
Resistance to rust	*Uca1/uca1*	SSR	[76]
Abiotic stress			
Salinity	QTL	SSR	[78]
Root weight; root length	QTL	SSR	[67]
Root traits	QTL	SSR	Varshney et al. Unpublished
Drought tolerance score	Q3-1	SSR	[80]
Canopy temperature differential	Q1-1	SSR	[80]
Agronomic and yield			
Plant growth habit	*Prostrate*	SSR	[124]
	Hg/hg	RAPD	[120]
Days to flowering	Q3-1	SSR	[80]
	QTL	SSR	[124]
	QTL	SSR	[124]
	DF3	SSR, RAPD	[120]
Flowering time	*Efl1,Efl2*	-	[124]
Days to maturity	Q3-1	SSR, RAPD	[80]
Seed coat thickness	QTL_{Tt}	*SSR, morphological*	[120]

Seed size	QTL_{SW1}	SSR	[120]
Seed/pod	*Spp*	RAPD, SSR	[125]
Double podding	*Sfl*	SSR, RAPD	[125]
Harvest index	Q1-1	SSR	[80]
	Q3-1	SSR	[80]

Table 3: Summary of genetic maps developed for chickpea

Mapping population	No. of loci mapped	Types of markers	Genetic map length (cM)	References
ICC 4958 × PI 489777	1328	SSR, CKAM, TOG-SNP, DArT	789	[94]
ICC 4958 × PI 489777	406	EST-SSRs, intron targeted primers (ITPs), expressed sequence tag polymorphisms (ESTPs), and SNPs	1,498	[93]
ICC 4958 × PI 489777	1063	SSR and SNP	1,809	[3]
ICC 4958 × PI 489777	1291	SSR, SNP, DArT	846	[53]
ICC 4958 × PI 489777	300	SSR, CISR, CAPS	767	[54]
ICCV 2 × JG 62	138	STMS	631	[19]
ICC 4958 × PI 489777	521	SSR, RAPD, AFLP, RGA	2,602	[52]
Five narrow crosses (Desi × Kabuli types)	229	STMS, RAPD, cross-genome markers	427	[126]
Five wide crosses (*C. arietinum* × *C. reticulatum*)	555	STMS, RAPD, cross-genome markers	653	[126]
ICC 4991 × ICCV 04516 (F2)	84	SSRs	724	[75]
JG 62 × Vijay (RIL), Vijay × ICC 4958 (RIL)	273	RAPDs and ISSRs)	740	[125]
ILC72 × Cr5-10	89	RAPDs, ISSRs, STS	-	[127]
Hadas × Cr205 (RIL)	93	SSRs, CytP450 markers	345	[128]
WR315 × C104	102	ISSR, STMS, RAPD, STS	-	[71]
ILC 1272 × ILC 3279	55	SSRs	-	[70]
ICC 12004 × Lasseter (F_2)	69	SSRs, RGAs, ISSRs	-	[129]
Lasseter × PI 527930 (F_2)	83	RAPDs, SSRs, ISSRs, RGA	-	[130]

C. arietinum × *C. reticulatum* (F_2)	296	47 defense response gene markers to the map of Winter et al. 2000	-	[131]
C. arietinum × *C. echinospermum* (F_2)	83	SSRs, RAPDs, ISSRs and RGA	-	[130]
ICCV 2 × JG 62 (RIL)	103	SSRs, RAPDs, ISSRs, morphological	-	[132]
ICC4958 × PI 489777 (RIL)	56	SSRs and RGA	1,175	[69]
C. arietinum × *C. reticulatum* (F_2)	117	SSRs and RGA	-	[133]
FLIP 84-92C × PI 599072 (RIL)	144	RAPDs, ISSRs, morphological, isozyme	-	[134]
C. arietinum × *C. reticulatum* (F_2)	116	marker loci RAPDs, ISSRs, isozyme, and morphological	-	[134]
C. arietinum × *C. reticulatum* (F_2)	354	SSRs, DAF, AFLPs, ISSRs, RAPDs, isozyme, cDNA, SCAR and morphological	2,078	[90]
C. arietinum × *C. reticulatum* (F_2)	120	STMS	-	[47]
C. arietinum × *C. reticulatum* (F_2) ; C. arietinum × C. echinospermum (F2)	91	morphological, isozyme, RFLPs and RAPDs	-	[33]
C. arietinum × *C. reticulatum* (F_2) ;C. arietinum × C. echinospermum (F2)	28	morphological and isozyme	-	[88]
C. arietinum × *C. reticulatum* (F_2)	29	morphological and isozyme	-	[86,87]

Table 4: Details of MABC progenies being developed by introgression of genomic region controlling root traits and other traits involved in drought tolerance from ICC 4958 into chickpea cultivars

Organization	Cross	Current status
EIAR, Ethiopia	Ejere × ICC 4958	BC_3F_3
	Arerti × ICC 4958	BC_3F_3
EU, Kenya	ICCV 97105 × ICC 4958	BC_3F_3
	ICCV 95423 × ICC 4958	BC_3F_4
ICRISAT, India	ICCV 10 × ICC 4958	BC_3F_4
IIPR, India	DCP92-3 × ICC 4958	BC_2F_1
	KWR108 × ICC 4958	BC_2F_1
IARI, India	Pusa 362 × ICC 4958	BC_3F_1