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The Chickpea Genome

Springer

Compendium of Plant Genomes

Series editor

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Whole-genome sequencing is at the cutting edge of life sciences in the new millennium. Since the first genome sequencing of the model plant Arabidopsis thaliana in 2000, whole genomes of about 70 plant species have been sequenced and genome sequences of several other plants are in the pipeline. Research publications on these genome initiatives are scattered on dedicated web sites and in journals with all too brief descriptions. The individual volumes elucidate the background history of the national and international genome initiatives; public and private partners involved; strategies and genomic resources and tools utilized; enumeration on the sequences and their assembly; repetitive sequences; gene annotation and genome duplication. In addition, synteny with other sequences, comparison of gene families and most importantly potential of the genome sequence information for gene pool characterization and genetic improvement of crop plants are described.

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The Chickpea Genome

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This book series is dedicated to
my wife Phullara, and our children
Sourav, and Devleena

Chittaranjan Kole

Preface to the Series

Genome sequencing has emerged as the leading discipline in the plant sciences coinciding with the start of the new century. For much of the twentieth century, plant geneticists were only successful in delineating putative chromosomal location, function, and changes in genes indirectly through the use of a number of 'markers' physically linked to them. These included visible or morphological, cytological, protein, and molecular or DNA markers. Among them, the first DNA marker, the RFLPs, introduced a revolutionary change in plant genetics and breeding in the mid-1980s, mainly because of their infinite number and thus potential to cover maximum chromosomal regions, phenotypic neutrality, absence of epistasis, and codominant nature. An array of other hybridization-based markers PCR-based markers, and markers based on both facilitated construction of genetic linkage maps, mapping of genes controlling simply inherited traits and even gene clusters (QTLs) controlling polygenic traits in a large number of model and crop plants. During this period a number of new mapping populations beyond F2 were utilized and a number of computer programs were developed for map construction, mapping of genes, and for mapping of polygenic clusters or QTLs. Molecular markers were also used in studies of evolution and phylogenetic relationship, genetic diversity, DNA-fingerprinting and map-based cloning. Markers tightly linked to the genes were used in crop improvement employing the so-called marker-assisted selection. These strategies of molecular genetic mapping and molecular breeding made a spectacular impact during the last one and a half decades of the twentieth century. But still they remained 'indirect' approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unraveled.

Physical mapping of genomes was the obvious consequence that facilitated development of the 'genomic resources' including BAC and YAC libraries to develop physical maps in some plant genomes. Subsequently, integrated genetic-physical maps were also developed in many plants. This led to the concept of structural genomics. Later on, emphasis was laid on EST and transcriptome analysis to decipher the function of the active gene sequences leading to another concept defined as functional genomics. The advent of techniques of bacteriophage gene and DNA sequencing in the 1970s was extended to facilitate sequencing of these genomic resources in the last decade of the twentieth century.

As expected, sequencing of chromosomal regions would have led to too much data to store, characterize, and utilize with the-then available computer software could handle. But development of information technology made the life of biologists easier by leading to a swift and sweet marriage of biology and informatics and a new subject was born—bioinformatics.

Thus, evolution of the concepts, strategies and tools of sequencing and bioinformatics reinforced the subject of genomics—structural and functional. Today, genome sequencing has traveled much beyond biology and involves biophysics, biochemistry and bioinformatics!

Thanks to the efforts of both public and private agencies, genome sequencing strategies are evolving very fast, leading to cheaper, quicker and automated techniques right from clone-by-clone and whole-genome shotgun approaches to a succession of second generation sequencing methods. Development of software of different generations facilitated this genome sequencing. At the same time, newer concepts and strategies were emerging to handle sequencing of the complex genomes, particularly the polyploids.

It became a reality to chemically—and so directly—define plant genomes, popularly called whole-genome sequencing or simply genome sequencing.

The history of plant genome sequencing will always cite the sequencing of the genome of the model plant *Arabidopsis thaliana* in 2000 that was followed by sequencing the genome of the crop and model plant rice in 2002. Since then, the number of sequenced genomes of higher plants has been increasing exponentially, mainly due to the development of cheaper and quicker genomic techniques and, most importantly, development of collaborative platforms such as national and international consortia involving partners from public and/or private agencies.

As I write this preface for the first volume of the new series "Compendium of Plant Genomes", a net search tells me that complete or nearly-complete whole-genome sequencing of 45 crop plants, eight crop and model plants, eight model plants, 15 crop progenitors and relatives, and three basal plants are accomplished, the majority of which are in the public domain. This means that we nowadays know many of our model and crop plants chemically, i.e., directly, and we may depict them and utilize them precisely better than ever. Genome sequencing has covered all groups of crop plants. Hence, information on the precise depiction of plant genomes and the scope of their utilization is growing rapidly every day. However, the information is scattered in research articles and review papers in journals and dedicated web pages of the consortia and databases. There is no compilation of plant genomes and the opportunity of using the information in sequence-assisted breeding or further genomic studies. This is the underlying rationale for starting this book series, with each volume dedicated to a particular plant.

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful both to students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is not onlyof interest for the geneticists and breeders, but also for practitioners of an array of plant science disciplines, such as taxonomy, evolution, cytology,

physiology, pathology, entomology, nematology, crop production, bio-chemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever-growing. The contents of the volumes of this compendium are therefore focusing on the basic aspects of the genomes and their utility. They include information on the academic and/ or economic importance of the plants, description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration on the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

I must confess that as the series editor it has been a daunting task for me to work on such a huge and broad knowledge base that spans so many diverse plant species. However, pioneering scientists with life-time experience and expertise on the particular crops did excellent jobs editing the respective volumes. I myself have been a small science worker on plant genomes since the mid-1980s and that provided me the opportunity to personally know several stalwarts of plant genomics from all over the globe. Most, if not all, of the volume editors are my longtime friends and colleagues. It has been highly comfortable and enriching for me to work with them on this book series. To be honest, while working on this series I have been and will remain a student first, a science worker second, and a series editor last. And I must express my gratitude to the volume editors and the chapter authors for providing me the opportunity to work with them on this compendium.

I also wish to mention here my thanks and gratitude to the Springer staff, Dr. Christina Eckey and Dr. Jutta Lindenborn in particular, for all their constant and cordial support right from the inception of the idea.

I always had to set aside additional hours to edit books besides my professional and personal commitments—hours I could and should have given to my wife, Phullara, and our kids, Sourav, and Devleena. I must mention that they not only allowed me the freedom to take away those hours from them but also offered their support in the editing job itself. I am really not sure whether my dedication of this compendium to them will suffice to do justice to their sacrifices for the interest of science and the science community.

Kalyani, India   Chittaranjan Kole

Preface

The chickpea, (also known as Bengal gram *Cicer arietinum* L.) is an important legume crop cultivated in >55 countries across the globe. Its wider adaptation to tropical, subtropical, and temperate regions climates makes it as the second most important widely cultivated cool season legume. Chickpeas are widely cultivated for seed as seeds are rich in protein, fiber, and essential amino acids hence very important in vegetarian diets in Indian subcontinent. Besides improving human health it also contributes to soil fertility through biological nitrogen fixation. Cultivated chickpea has originated in southeast Turkey, and *Cicer reticulatum* is considered as its progenitor. Desi (small seeded) and kabuli (large seeded) are two types of chickpea which occupy 85 and 15% of total cultivated area. Climate changes have been posing challenges continuously by reducing chickpea yields through abiotic and biotic stresses. Although about 350 chickpea varieties have been released using conventional breeding approaches, selection for yield *per se* has not been much rewarding in past until the use of modern breeding approaches.

During the last two decades, several crop genomes have been sequenced, which empowered scientists with new tools for developing climate smart crops. Genome sequence information also further increased our understanding of physiological responses, phenotypes and transformed genetics research and crop breeding. The year 2013 is considered as very important year for chickpea research community, as both desi and kabuli genomes have been decoded and several thousand of genetic markers, millions of genome variations that can be used for chickpea improvement have been reported. Besides, several hundreds of germplasm lines as well as release varieties of chickpea were re-sequenced to understand the genome dynamics, evolutionary history, and diversity that can be harnessed for chickpea improvement.

In view of above, this book in the genome compendium series has been planned to provide comprehensive information related to chickpea genome. The book comprises of 12 chapters that provide a detailed overview of economic importance of chickpea, botany, cytogenetics, genetics, genomics tools available and efforts toward breeding climate resilient chickpeas. In addition, the book also provides deeper insights and future perspective of using the chickpea genome information for chickpea improvement. A total of 20 authors, representing six countries from four continents have contributed eleven chapters for this volume. The editors of this volume are grateful to all

the authors for their contribution in writing chapters of high quality. We are also thankful to the reviewers for their constructive suggestions and corrections helping us in improving the quality of the chapters. The editors are also thankful to Dr. David Bergvinson, Director General, ICRISAT and Dr. Peter Carberry, Deputy Director General—Research, ICRISAT for their support. We would like to thank Prof. Chittaranajan Kole, Editor-in-Chief, Genome Compendium Series for asking us to contribute on this important subject as well as to Springer in general and Abirami Purushothaman, Shahid Mohammad, Naresh Kumar Mani, Christina Eckey, Jutta Lindenborn in particular for their help and support.

We also appreciate and recognize cooperation and moral support from our family members for sparing us precious time for editorial work that we should have spent with our respective families. RKV acknowledges the help and support of his wife (Monika), son (Prakhar), and daughter (Preksha) who allowed their time to be taken away to fulfill RKV's editorial responsibilities in addition to research and other administrative duties at ICRISAT. Similarly, MT is grateful to his wife (Jaya), daughter (Sirivarshini), and son (Vishwanath) for their support in doing editorial responsibilities in addition to research activities at ICRISAT. FM acknowledges the support and patience of his wife Linda during the writing and editing of contributions to this volume. Editors would also like to thank their friends and collaborators from chickpea research community especially Liu Xin, Xun Xu (BGI, Shenzen, China), and NP Singh (ICAR-Indian Institute of Pulses Research, Kanpur, India) for their encouragement and support in various ways.

We hope that our efforts in compiling the information on different aspects of chickpea will help the chickpea research community in enhancing better understanding about the chickpea biology and developing research strategies for chickpea improvement. This book will also benefit students, academicians, and policy makers in updating their knowledge on recent advances in chickpea research.

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The Chickpea Genome: An Introduction

Rajeev K. Varshney, Mahendar Thudi
and Fred J. Muehlbauer

Abstract

Chickpea is the second most important cool season grain legume cultivated by small holder farmers in 59 countries across the globe. Chickpea production is adversely affected by several abiotic stresses like drought, temperature extremes (high and low temperatures), salinity, and biotic stresses, e.g., insect, fungal and viral diseases. Until recently breeding for tolerance/resistance to these stresses has been challenged by lower level of natural variation and lack of genomics tools to adopt genomics-assisted breeding. Nevertheless, during recent years large-scale genomic resources like molecular markers, genetic maps, draft genome sequence of both desi and kabuli chickpea have become available as a result of partnership among different institutes and advances in sequencing technologies. The chickpea genome book provides an up-to-date account on developments made over past ten years and presents the road map for future chickpea research. This chapter introduces the book and provides brief summary of 11 chapters included in the book.

1.1 Introduction

Chickpea is the most important cool season food legume cultivated on marginal soils by resource poor farmers in the semi-arid regions of the world. Besides increasing soil fertility, it plays a key role in supplying protein requirements of human population. Chickpea or *chana* (called in Hindi) is used as an edible seed and is also used for making flour throughout the globe. In addition, it is consumed in various forms like roasted as snacks, raw, carbonized, or in broth. The acid exudates from leaves can be applied medicinally

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or used as vinegar, and its starch is suitable for textile sizing, giving a light finish to silk, wool, and cotton cloths. Archeological and botanical evidences suggest that it was used by the "hunter-gatherer societies" for eating and sustaining their communities even before 10,000 B.C (Ladizinsky 1975). Chickpea has its origin in southeastern Turkey, and after its domestication in Middle East, this crop progressed further throughout the Mediterranean region, India, and Ethiopia (Ladizinsky 1975; van der Maesan 1987). Although chickpea is cultivated in 59 countries and on about 13.98 million ha, the productivity is less than 1 ton per ha (FAO 2016; Fig. 1.1). Several abiotic and biotic stresses have been constrained to realization of production potential of chickpea. Further, recent climate changes increased the incidence of droughts, pest (pod borer), and diseases (Fusarium wilt, Ascochyta blight, dry root rot, etc.). Owing to the complexity of these stresses, breeding efforts in past were not much rewarding due to poor understanding of the genetics of the traits. For enhancing the awareness on the economic importance of pulses in food and nutritional security, Food and Agriculture Organization (FAO) has declared the year 2016 as the International Year of Pulses. Chickpea being the most important pulse crop, to meet the growing demand to reduce malnutrition, development of climate-resilient chickpea varieties with sustainable production is challenging task before the chickpea scientific community. Chickpea was

considered as an orphan crop before 2005 for not having sufficient genomic resources to take up genomics-assisted breeding which supplements the conventional breeding programs for crop improvement (Varshney et al. 2005). As a result of several efforts at national and international level, the chickpea is now considered as genomics resources-rich crop (Thudi et al. 2014; Varshney et al. 2016). This book provides an up-to-date information on different areas of chickpea including economic importance, biology, development of genomic resources, and the draft genomes of chickpea and their utilization in chickpea improvement.

1.2 Importance, Botanical Description, and Cytogenetics

Chapter 2 entitled "**Economic importance of chickpea: production, value and world trade**" written by Muehlbauer and Sarker provides information on chickpea production, value, and trade on a global, regional, and country basis to determine trends in production and product availability through domestic and international export markets. In addition, the chapter also describes the importance of chickpea in supplying human nutrition and its wide use in different dishes across the globe. In Chap. 3 entitled "**Botany of chickpea**," Sajja et al. provide insights into origin, distribution, and taxonomic classification of chickpea. Further, a detailed
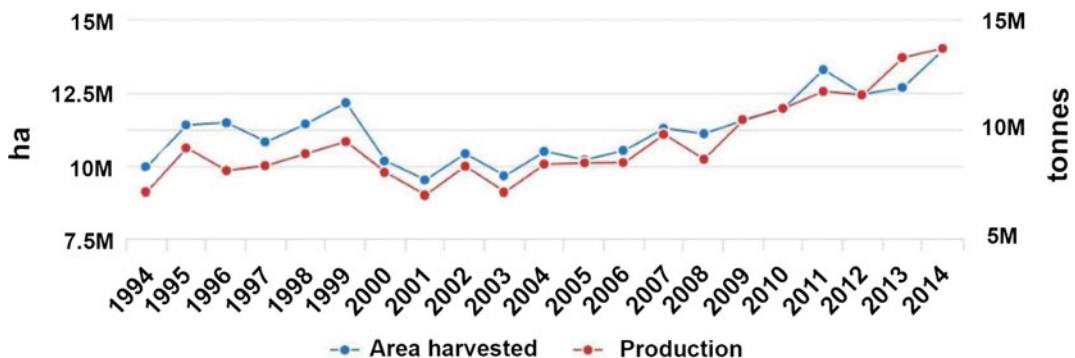
Fig. 1 Chickpea production in last two decades. The graph represents the area under cultivation in million hectares and production in million tons from 1994–2014

botanical description of the species has been provided in this chapter. Cytogenetic research is essential to establish the base information with respect to the karyotype (chromosome number, length, and morphology; and some limited descriptions based on banding) and an estimate of nuclear genome size. Efforts toward understanding the karyotypes and chromosome behavior at meiosis have been summarized by Karafiátová Miroslava and colleagues in Chap. 4 entitled "**Cytogenetics of *Cicer*.**" The chapter also emphasizes the use of flow cytometry as a means to explore the chickpea genome.

1.3 Germplasm, Genomic Resources, and Trait Mapping

Conservation of germplasm and existing variability in the germplasm lines is the key towards addressing new challenges that arise in coming future to feed the millions of people across the globe. Hari Upadhyaya and colleagues provide the details on conservation and management of germplasm resources in breeding programs in Chap. 5 entitled "**Managing and Discovering Agronomically Beneficial Traits in Chickpea Germplasm Collections**." The chapter also provides the information on the essence of developing the germplasm subsets in the form of core/mini-core collections and genotyping-based reference set to harness the existing germplasm diversity in chickpea improvement programs. In Chap. 6 entitled "**Advances in Chickpea Genomic Resources for Accelerating the Crop Improvement**," Manish Roorkiwal and colleagues summarize the development of various kinds of genomics tools like molecular markers, genetic maps, and efforts toward dissecting complex traits that hamper the chickpea production and productivity. The chapter also highlights the cost-effective utilization different marker genotyping platforms. Chapter 7 entitled "**Classical Genetics and Gene Mapping**" authored by Deokar and Taran has a major focus on linkage (and QTL) mapping and candidate

gene approaches for trait dissection, understating the genetics of the trait and validity of the genetic basis of the traits. It also provides the historical perspective of markers used for developing various kinds of genetic maps (i.e., sparse genetic maps to high-density genetic maps), and their genetic distances and traits mapped. Chapter 8 authored by Millan et al., as mentioned in the title of the chapter, has a special focus on "**Genetic Mapping and Quantitative Trait Loci**." Reduced cost of sequencing and resequencing in recent years owing to advances in NGS technologies is bringing a paradigm shift in trait mapping and breeding approaches from marker-based genotyping to sequencing-based genotyping (Elshire et al. 2011). Genotyping by sequencing, skim sequencing, and bin mapping approaches have also been summarized in this chapter.

1.4 Genome Sequence and Beyond

Chapter 9 entitled "**Requirement of Whole-Genome Sequencing and Background History of the National and International Genome Initiatives**" authored by Thudi and Varshney summarizes the background history of two independent efforts to generate draft genome sequence of kabuli and desi chickpea genomes in addition to discussing the requirements of whole-genome sequencing and its importance for crop improvement. Aamir Khan and colleagues summarize the methods adopted and the analysis tools utilized in assembling the kabuli chickpea genome in Chap. 10 entitled "**Sequencing the Chickpea Genome**."

For faster genetic gains, the use of modern breeding approaches like marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genomic selection (GS) has been proven in several crops. Srinivasan Samineni and colleagues in Chap. 11 entitled "**Impact of Genomics on Chickpea Breeding**" summarize molecular breeding efforts of developing improved lines for stresses like

drought tolerance, Fusarium wilt (FW), and
Ascochyta blight (AB) resistance. Authors have
presented utility of MABC as well as reasons for
MARS not being effective in chickpea improve-
ment. The chapter also discusses the usefulness
of multi-parent advanced generation inter-cross
(MAGIC) populations for trait dissection and
harnessing the variability in chickpea improve-
ment. Finally, Editors provide challenges and
opportunities for future chickpea research in
Chap. 12 entitled "**Future Prospects for
Chickpea Research**."

1.5 Conclusion

In summary, the volume contains useful articles
written by eminent scientists in the area of
cytogenetics, classical genetics, genomics, and
molecular breeding. The successful efforts to
breed for drought tolerance, FW, and AB through
MABC in chickpea are summarized and well
discussed. These efforts need to be extended to
other important emerging disease like dry root rot
beside enhancing stress resilience in elite culti-
vars in different chickpea growing regions of the
world. Several genomes of thousands of chick-
pea will enable estimation of genome-wide
diversity and untapped benefits of the genomic
selection (GS) initially proposed in animal
breeding for enhancing the genetic gains in

chickpea (Roorkiwal et al. 2016). Editors are
hopeful that readers will enjoy reading the book.

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Economic Importance of Chickpea: Production, Value, and World Trade

2

Fred J. Muehlbauer and Ashutosh Sarker

Abstract

Chickpea is a valued crop and provides nutritious food for an expanding world population and will become increasingly important with climate change. Production ranks third after beans with a mean annual production of over 10 million tons with most of the production centered in India. Land area devoted to chickpea has increased in recent years and now stands at an estimated 13.5 million hectares. Production per unit area has slowly but steadily increased since 1961 at about 6 kg/ha per annum. Over 1.3 million tons of chickpea enter world markets annually to supplement the needs of countries unable to meet demand through domestic production. India, Australia, and Mexico are leading exporters. Chickpea is comprised of Desi and Kabuli types. The Desi type is characterized by relatively small angular seeds with various coloring and sometimes spotted. The Kabuli type is characterized by larger seed sizes that are smoother and generally light colored. Dal is a major use for chickpea in South Asia while hummus is widely popular in many parts of the world. Research efforts at ICRISAT, ICARDA, and national programs have slowly but steadily increased yield potential of chickpea germplasm.

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2.1 Introduction

This chapter examines chickpea production, value, and trade on a global, regional, and country basis to determine trends in production

and product availability through domestic and international export markets. World, regional, and country production, and demand data are reviewed to determine trends and future expectations for the chickpea crop and its importance in world trade. Data for this chapter was obtained from the FAOSTAT database provided by the Food and Agriculture Organization of the United Nations (2015) that provides country and global estimates of crop production, area harvested, yields, exports, imports, and consumption since 1961. The data is used to identify trends in overall production, yields, value, and consumption of chickpea on a worldwide basis.

2.2 Production, Productivity, and Area

Worldwide, chickpea ranks third among the pulse crops and accounting for 10.1 million tons annually. This ranking places chickpea behind beans (21.5 million tons) and peas (10.4 million tons) with mean annual production of 10.1 million tons from 2004 to 2013 (Table 2.1). Taken together, annual combined production of peas and chickpea is nearly equal to that of beans, an indication of their overall importance. These three pulses (beans, peas, and chickpeas) account for about 70% of global pulse production with chickpea accounting for approximately 17% of the total annually.

Production of chickpea in terms of harvested area from 1961 to 2013 ranged from a low of 8.9

million hectares in 1981 to a high of 13.5 million hectares in 2013 (Fig. 2.1). Earlier production trends from 1961 to 2001, in terms of area harvested, was somewhat static or slightly declining; however, yield increases began to have an impact on total production starting in the late 1900s. Steady increases in production appear in the early 2000s and continue to the present, and especially since 2004.

Chickpea yields have been steadily increasing globally since 1961 (Fig. 2.2) and trending to increases of over 6 kg/ha per annum. This positive development is likely the result and benefits of sustained research programs toward the development of improved germplasm and higher yielding varieties characterized by improved disease resistance and adaptation to environment. Also important are factors such as improved seed sources, seed supplies, and overall management practices. Expanded production in more productive environments also may account for these consistent yield increases. The expanded production in developed countries such as Australia, Canada, and the USA appears to have had a positive influence on mean yields worldwide. However, with the majority of production centered in South Asia and India in particular, production increases in South Asia have clearly had an impact globally.

Production increases have been pronounced in the most recent 10-year period (Fig. 2.3). Area harvested and tonnage produced have expanded starting in 2004 leading up to 2013 where global production reached an all time high of 13.5

Table 2.1 Mean annual global production of pulse crops 2004–2013

Pulse	Production (1000t)
Beans	21,556
Peas	10,427
Chickpeas	10,160
Cowpeas	5374
Faba beans	4156
Lentils	3982
Pigeon peas	3949
Other pulses[a]	5936
Total pulses	59,606

[a] Data for other pulses was obtained by difference between total pulse production as reported by FAOSTAT (2015) and the total of the seven pulse crops listed in this table
Source FAOSTAT (2015)

Fig. 2.1 Chickpea worldwide, area harvested (million hectares; *Filled diamond*), and production (million t; *Filled square*) from 1961 to 2013 *Source* FAOSTAT (2015)
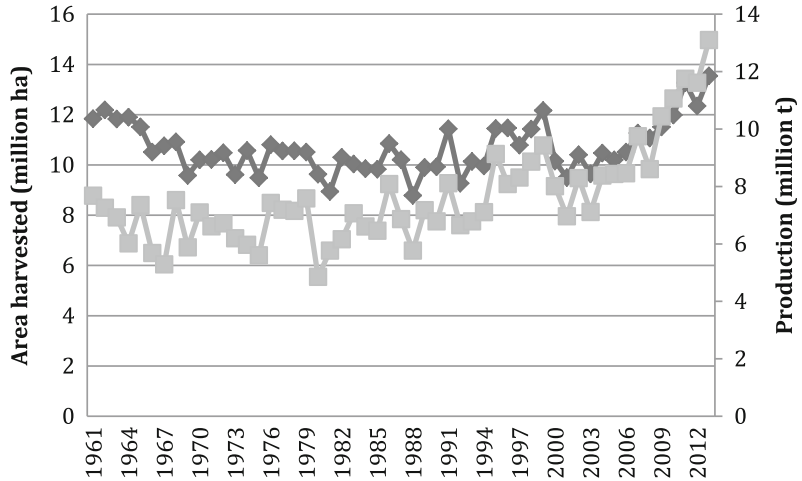
Fig. 2.2 Annual worldwide chickpea yields (kg/ha) and trend line from 1961 to 2013. *Source* FAOSTAT (2015)
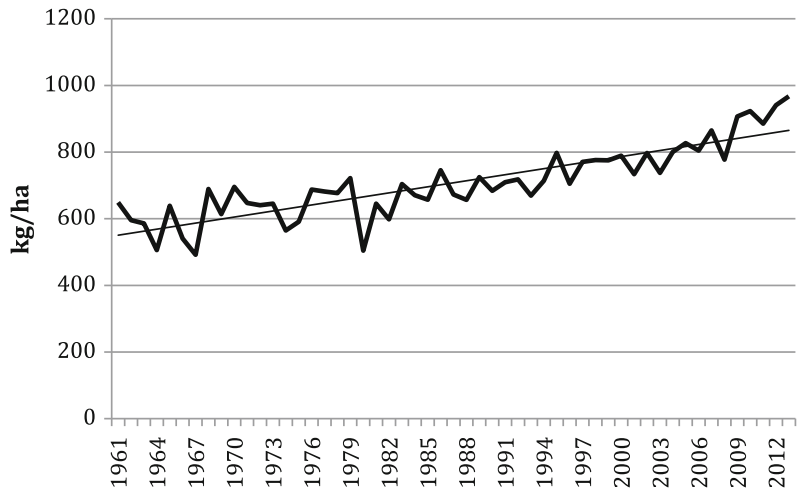
million tons from nearly 12 million hectares. Gains in yield per hectare and area harvested are positively impacting overall production.

Chickpea is produced in over 50 countries with India having the largest production and accounting for over 70% of total world production. Figure 2.4 illustrates the dominance of India in chickpea production and the relative importance of the next most important producers. Pakistan and Iran, the next most important producers, account for 10 and 5% of world production, respectively. Other major producing countries such as Turkey and Australia account for 4 and 3%, respectively; while Ethiopia having greatly increased production in recent years now accounts for over 2% of world production. Other

important producing countries include Malawi, Mexico, Morocco, and Syria.

Mean yields of chickpea have varied widely among producing countries and range from relatively low yields averaging 500–600 kg/ha in Iran, Malawi, Morocco, Pakistan, and Syria to relatively high yields in Ethiopia and Mexico (Fig. 2.5). India, the largest producer has had stable mean yields of about 900 kg/ha. The high yields in Mexico are largely due to the fact that most of the crop is irrigated and grown over the cool winter season.

Area harvested, mean yields, and total productivity of chickpea producing countries in regions of the world are shown in Table 2.2. Ethiopia has emerged as the major producing

Fig. 2.3 Chickpea production worldwide, area harvested (million hectares; Filled diamond), and tons (million t; Filled square) from 2004 to 2013. *Source* FAOSTAT (2015)
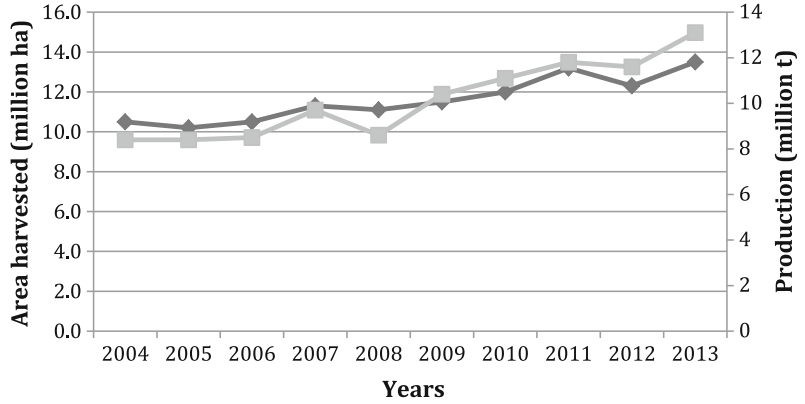
Fig. 2.4 Relative importance of the 10 leading chickpea producing countries. *Source* FAOSTAT (2015)
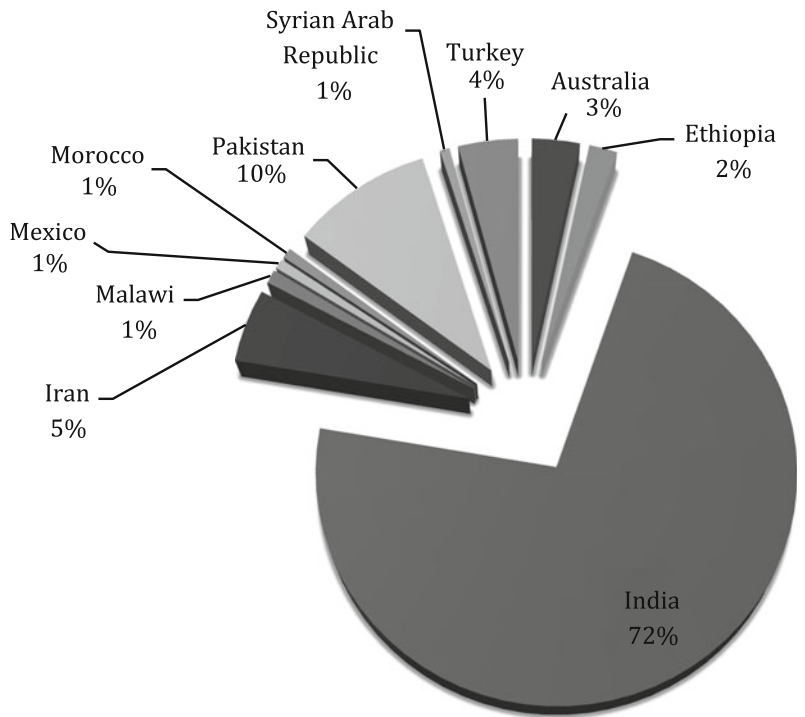
Fig. 2.5 Mean yields (kg/ha) from 2009 to 2013 for the 10 leading chickpea producing countries. *Source* FAOSTAT (2015)
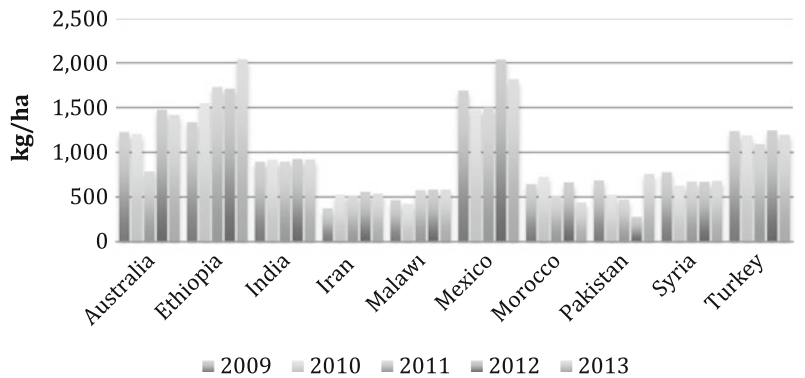
Table 2.2 Area harvested (hectares), mean yields (kg/ha), and total production (tonnes) from chickpea producing countries in important regions from 2009 to 2013

Region	Country	Area (ha)	Productivity (kg/ha)	Production (1000 tonnes)
Africa	Algeria	27,419	886	24,408
	Egypt	2671	2071	5465
	Ethiopia	202,927	1673	333,377
	Malawi	115,545	526	61,075
	Morocco	71,072	597	42,597
	Tunisia	8254	971	8003
West Asia	Iran	549,176	502	275,385
	Iraq	9790	91	894
	Israel	4250	4723	19,820
	Jordan	1153	2033	2349
	Lebanon	2766	920	2592
	Kazakhstan	25,820	527	12,960
	Syria	32,323	799	25,604
	Turkey	437,472	1191	520,935
	Yemen	19,052	2985	56,841
South Asia	India	8,634,000	910	7,858,500
	Bangladesh	7866	849	6680
	Myanmar	326,107	1414	461,708
	Pakistan	1,049,660	543	568,000
	Nepal	9037	881	7992
Asia	China	2980	3438	10,300
	Uzbekistan	2520	1460	3680
Europe	Greece	1880	1631	2960
	Italy	6622	1394	9243
	Portugal	1133	579	653
	Russia	19,920	2850	52,200
	Spain	32,323	799	25,604
North America	Canada	63,800	1947	125,080
	Mexico	92,938	1705	163,674
	USA	64,135	1740	112,364
South America	Argentina	37,800	1054	39,700
	Chile	1875	1033	1947
Australia		509,162	1222	609,402
World (total)		12,531,411	925	11,593,870

Source FAOSTAT (2015)

country in Africa, while Iran and Turkey predominate production in West Asia. Spain is the major producing country in Europe. In North America, Mexico predominates followed by USA and Canada. Most of this production is devoted to exports; however, the emergence of hummus as a popular value-added product in the USA has created domestic demand that now consumes over
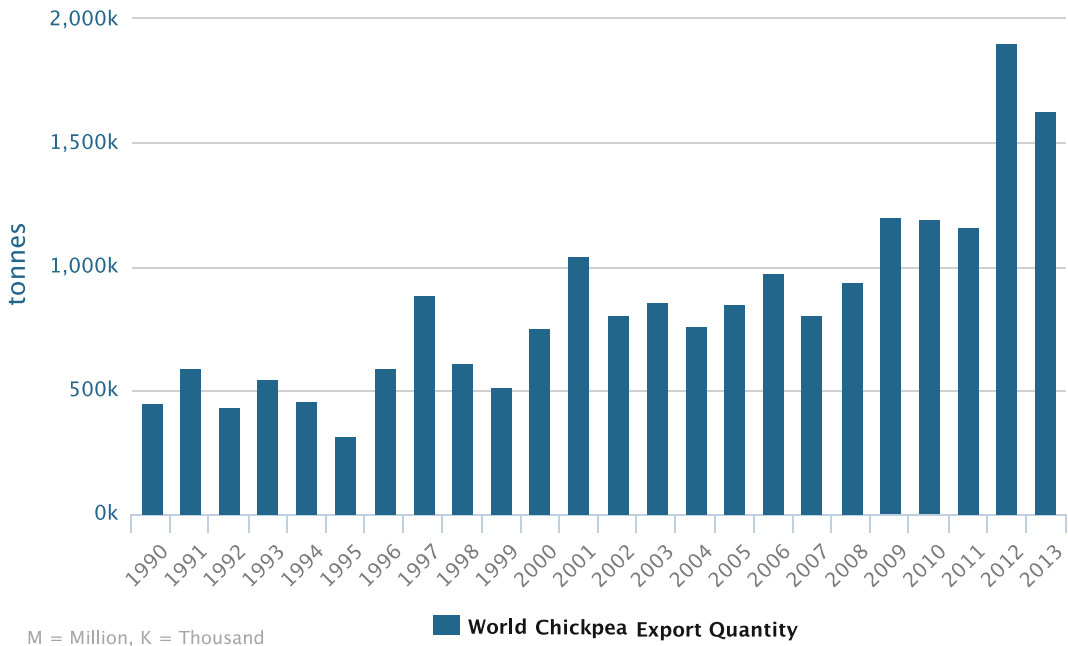
Fig. 2.6 World chickpea export quantity from 1990 to 2013. *Source* FAOSTAT (2015)

65% of production. In South America, Argentina has become a major producer. Australia has become a major producer of chickpea with most of the production being exported to South Asia to meet current demand for the commodity in India and Pakistan (Fig. 2.6).

2.3 Exports and from Which Countries

Considerable quantities of chickpea (estimated at 1.3 million tons) have entered world trade as consuming countries have been unable to meet demand through domestic production. Australia nearly meets half of world export demand and provides an over 570,000 tons to the market annually in the most recent period where data is available (FAOSTAT 2015). Exports had a value of $280 million (Australian) in 2009 (Pulse Australia 2011) and similar value in the following years up to 2013. India, while being the largest producer and importer of chickpea is also a major exporter ranking second only to Australia. Mexico, with a large production of high

quality and large seeded Kabuli types, is the third most important exporter with the commodity being exported to over 50 countries worldwide with Algeria, Turkey, and Spain their most important customers. Turkey, also a major exporter with over 40,000 tons annually, with most of the tonnage moving to neighboring countries of Iraq, Jordan, and Saudi Arabia (Table 2.3).

Table 2.3 Annual exports from major chickpea exporting countries from 2009 to 2013

Country	Exports (t)
Argentina	47,549
Australia	570,860
Canada	63,968
India	206,607
Mexico	126,710
Russian Federation	99,841
Turkey	43,638
USA	54,886
Ethiopia	56,169

Source FAOSTAT (2015)

2.4 Demand and Consumption

Chickpea is divided into two distinct classifications. The most prominent type is referred to as "Desi" and is characterized by relatively small seeds that range from light tan to black and with many variations including various markings of anthocyanin pigmentation. The relatively small seeds have rather thick seed coats and yellow cotyledons. The seeds are often decorticated (seed coat removed) and cotyledons split to form a product referred to as "dal." Dal, which can be made from most pulse crops, is used in soup making and is popular in South Asia. The less prominent type referred to as "Kabuli" is characterized by relatively large seeds that can range up to 22 mm in diameter and larger. Seed coats of Kabuli types lack pigment and are light tan and quite thin. This particular type is preferred in most markets outside of South Asia most likely because it is easier to produce and less expensive. Overall, world production of chickpea is predominated by the Desi type that accounts for 80% of production with the remaining 20% devoted to Kabuli types (Table 2.4).

2.5 Uses for Chickpea

Chickpea is a highly nutritious and an inexpensive source of protein that is estimated at 24% and ranges from 15 to 30% (Hulse 1994) depending on variety and environmental conditions (Nleya et al. 2000). Chickpea also has estimated 60–65% carbohydrates, 6% fat and is a good source of minerals and essential B vitamins. There are numerous uses for Desi and Kabuli types. They can be boiled, eaten raw as a fresh vegetable, roasted, dehulled to make dal or processed into flour that can be added to bread. Dal made mostly from Desi types, used in making a soup that is served with rice in most areas of South Asia providing a nutritious combination of a cereal grain and a pulse crop. It is well known that the amino acids of pulses, particularly those containing sulfur amino acids compensate for those that are limiting in the cereals (Table 2.5).

Table 2.4 Major chickpea importing countries (mean imports from 2009 to 2013)

Country	Imports (t)
Algeria	69,950
Bangladesh	155,817
Sri Lanka	20,613
Egypt	20,679
France	6085
Greece	4482
India	309,536
Iran	16,786
Iraq	12,538
Jordan	31,734
Lebanon	12,794
Libya	3231
Pakistan	173,819
Portugal	13,168
Saudi Arabia	36,145
Spain	53,941
Syria	4997
Oman	2967
Turkey	22,451
United Arab Emirates	82,257
USA	21,681

Source FAOSTAT (2015)

Table 2.5 Top 25 chickpea producing countries in 2013 in terms of tons produced and value ($)

Country	Production (tons)	Value ($ Int.)
India	8,832,500	3,432,504,000
Australia	813,300	385,361,000
Pakistan	751,223	241,389,310
Turkey	506,000	189,962,230
Myanmar	490,000	135,081,110
Ethiopia	409,733	117,206,960
Iran	295,000	115,933,810
Mexico	209,941	78,157,930
Canada	169,400	78,612,320
USA	161,434	75,000,000[a]
Tanzania	110,116	51,984,950
Russian Federation	100,000	29,043,960
Malawi	67,000	28,535,690
Yemen	58,800	28,463,080
Argentina	53,500	24,566,350
Syria	53,022	23,823,550
Algeria	34,980	13,810,400
Spain	26,500	11,375,550
Israel	26,315	12,394,750
Morocco	25,003	10,723,050
Kazakhstan	14,700	1,811,340
Italy	12,077	5,555,630
Nepal	9696	4,112,620
Tunisia	7840	4,987,090
Eritrea	7150	3,185,150

Source FAOSTAT (2015); [a]Estimated value of the USA production

2.6 Conclusion

Remarkable progress has been made in the production of chickpea in the past several decades. Yields have improved considerably which is a likely consequence of sustained research efforts by the international centers, ICARDA and ICRISAT, and national research and breeding programs. Expansion of production in new regions, particularly Australia and North America, has significantly contributed to overall world production and availability of the commodity in international markets. World trade has increased markedly in the past two decades likely due to demands of an increasing population and improving purchasing power of in developing countries. The outlook for chickpea is excellent considering the excellent nutrient concentrations and food value. Expansion of production to meet expanding demand is expected to continue.

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Botany of Chickpea

3

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Abstract

Chickpea is one of the important food legumes cultivated in several countries. It originated in the Middle East (area between south-eastern Turkey and adjoining Syria) and spread to European countries in the west to Myanmar in the east. It has several vernacular names in respective countries where it is cultivated or consumed. Taxonomically, chickpea belongs to the monogeneric tribe Cicereae of the family Fabaceae. There are nine annuals and 34 perennial species in the genus *Cicer*. The cultivated chickpea, *Cicer arietinum*, is a short annual herb with several growth habits ranging from prostrate to erect. Except the petals of the flower, all the plant parts are covered with glandular and non-glandular hairs. These hairs secrete a characteristic acid mixture which defends the plant against sucking pests. The stem bears primary, secondary and tertiary branches. The latter two branch types have leaves and flowers on them. Though single leaf also exists, compound leaf with 5–7 pairs of leaflets is a regular feature. The typical papilionaceous flower, with one big standard, two wings and two keel petals (boat shaped), has 9 + 1 diadelphous stamens and a stigma with 1–4 ovules. Anthers dehisce a day before the flower opens leading to self-pollination. In four weeks after pollination, pod matures with one to three seeds per pod. There is no dormancy in chickpea seed. Based on the colour of chickpea seed, it is desi type (dark-coloured seed) or kabuli type (beige-coloured seed). Upon sowing, germination takes a week time depending on the soil and moisture conditions.

3.1 Introduction

Chickpea (*Cicer arietinum* L.) is believed to have originated in the area between south-eastern Turkey and adjoining Syria (van der Maesen

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1987) for the fact that three closely related wild annual species of chickpea, *C. bijugum, C. echinospermum* and *C. reticulatum*, are found there. Of the three, *C. reticulatum* is considered to be the progenitor of the cultivated chickpea (*Cicer arietinum*). Holm (1920) reported that the generic name was proposed by Pliny and the specific name by Dodonaeus which were accepted by Linnaeus. The Latin words *Cicer* and *arietinum* were derived from the Greek words *Kikus* meaning 'force of strength' and *Krios* referring to ram, respectively, because of the similarity between seed shape and the head of a ram (*Aries*) (van der Maesen 1987).

Common names: From its place of origin, chickpea spread in both directions—European countries in the west and up to India, later to Myanmar in the east. Several of the vernacular names were derived from its generic name, *Cicer,* the exceptions being *garbanzo* and *garabanzo* (Spanish), *garvanche, garvance* and *garavane* (French) and *Garabanze* (German). These vernacular names include *pois chiche, pois ciche, ciche, césé, céséron, cicérole, ciserolle, seses* (French); *Kicherebse, Kicher, Chicher, Chichina, Chichuria, Cicererbis, Cisa, Cyfer, Czycke* and *Keicheren, Kekeren, Keyker, Kicher(n) kraut, Kicherling, Sisern, Sekern, Venuskicher, Ziesererbsen, Zisererwedsen, Ziser* and *Ziserbohne* (German); and *Keker, kekererwt, kicher, kikkererwt* and *cicererwt, citsers, sisser* and *sissererwt* (Dutch) among others. The English word, chickpea, was actually derived from *chichpea,* referring to *Cicer-pea.* Chickpea was also described as a variety of pea which included *pois bécu, pois blanc, pois de brebis, poisbreton, pois chabot, pois citron, pois cornu, pois gris, pois pontu, pois tete de belier* (French); *Egyptian pea* (English); *hamoos pea* (Arabic); *Fontanellerbse, graue Erbsen, Malagaerbsen* (German); and *ovetche harokh* or *puzirnyi gorokh* (Russian). Some of the names refer chickpea as 'coffee pea' because of its use in preperation of coffee which includes *Kaffeeerbse deutsche* or *franzosische Kaffeebohne, deutscher Kaffee* (German); *pois cafe* (French); *kahviherne* (Finnish) and *Kaffeart* (Swedish). Chickpea is called as *nakhut, nohut* or *nut, naut* or *nohot* in Turkey, Romania, Bulgaria,

Iran, Afghanistan and in parts of Soviet Union. The Sanskrit name for chickpea is *chennuka,* and hence, the name *chana* in the Sanskrit-derived languages such as Hindi (van der Maesen 1987).

3.2 Taxonomy

Initially, chickpea was a part of the tribe Viciae, but due to its distinct characters, it was included in a new monogeneric tribe Cicereae later (Kupicha 1977). The detailed taxonomic information on chickpea (http://plants.usda.gov/core/profile?symbol=CIAR5) is given below:

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae (Leguminosae)
Subfamily	Faboideae (Papilionaceae)
Tribe	Cicereae
Genus	Cicer

The genus *Cicer* has 9 annuals and 34 perennial species. Based on the morphology, geographical distribution and lifespan, the genus *Cicer* was divided into four sections (van der Maesen 1987 & http://www.nipgr.res.in/NGCPCG/Taxonomy.html).

Section	Species included	Lifespan	Morphology
Monocicer	*C. arietinum*	Annual	Firm erect or horizontal stems Branching from base or middle
	C. reticulatum	Annual	
	C. echinospermum	Annual	
	C. pinnatifidum	Annual	
	C. judaicum	Annual	
	C. bijugum	Annual	
	C. yamashitae	Annual	
	C. cuneatum	Annual	
Chamaecicer	*C. chorassanicum*	Annual	Thin stem Creepers with small flowers
	C. incisum	Perennial	

(continued)

Section	Species included	Lifespan	Morphology
Polycicer	C. anatolicum	Perennial	Leaf rachis ends in a tendril or a leaflet. Again divided into two subsections: Nano-polycicer and Macro-polycicer. Members of Nano-polycicer subsection have creeping rhizome, short stem, imparipinnate leaves, weak and short arista. Species in Macro-polycicer subsection have non-creeping short rhizome, stems growing to 75 cm, firm arista which is longer than pedicel
	C. atlanticum	Perennial	
	C. balcaricum	Perennial	
	C. baldshuanicum	Perennial	
	C. canariense	Perennial	
	C. fedtschenkoi	Perennial	
	C. flexuosum	Perennial	
	C. floribundum	Perennial	
	C. graecum	Perennial	
	C. grande	Perennial	
	C. heterophyllum	Perennial	
	C. isauricum	Perennial	
	C. kermanense	Perennial	
	C. korshinskyi	Perennial	
	C. microphyllum	Perennial	
	C. mogoltavicum	Perennial	
	C. montbretii	Perennial	
	C. multijugum	Perennial	
	C. nuristanicum	Perennial	
	C. oxydon	Perennial	
	C. paucijugum	Perennial	
	C. rassuloviae	Perennial	
	C. songaricum	Perennial	
	C. spiroceras	Perennial	
	C. subaphyllum	Perennial	
Acanthocicer	C. acanthophyllum	Perennial	Branched stems with woody base Persistent spiny leaf rachis Spiny calyx teeth Large flowers
	C. incanum	Perennial	
	C. macracanthum	Perennial	
	C. pungens	Perennial	
	C. rechingeri	Perennial	
	C. stapfianum	Perennial	
	C. tragacanthoides	Perennial	
*C. laetum			

*Details are not available at present

3.3 Morphology

Plant: *Cicer arietinum* is a short annual herb, attaining a height of less than a metre. Depending on the angle of the branches and the soil surface, the plant assumes 'erect, semi-erect, spreading, semi-spreading and prostrate' growth habit. Branching starts from the base at ground level giving plant a bushy appearance (Fig. 3.1).

The plant surface including roots, stem, leaves and pods are pubescent, covered with glandular and non-glandular hairs. The glandular hairs secrete a mixture of acids containing malic, oxalic and citric acids. This acid mixture acts as a defence mechanism against sucking pests. The exudation from the roots helps in solubilizing the soil nutrients (Fig. 3.2).

Stem: The stem is firm due to hypodermal collenchyma, angular with ribs, straight or flexuous and pubescent. The plant produces three types of branches—primary, secondary and tertiary. The lowest nodes of the plant produce 1–8 primary branches. Alternately, the primary branches may arise from seed shoot as well. The primary branches are thick, woody with thick cuticle, and often mistaken for the main stem. The secondary branches arise from the buds on the primary branches and are comparatively thin. These branches bear the leaves and flowers. Depending on the genotype and growing conditions, tertiary branches may or may not be present. The plant grows to a height of up to 100 cm generally and occasionally reaching 150 cm depending on the growing conditions.

Leaf: The compound leaves contain 5–7 pairs of hairy leaflets per leaf, opposite or alternate, and the rachis ends in a leaflet (imparipinnate). The leaflets are oval or elliptic in shape with serrated margins. Simple leaf types also exist (Fig. 3.3).

Root: The root system is characterized by a thick tap root with several side roots developing into a robust system. The epidermis is hairy,

Fig. 3.1 Chickpea plant at
30 days after sowing
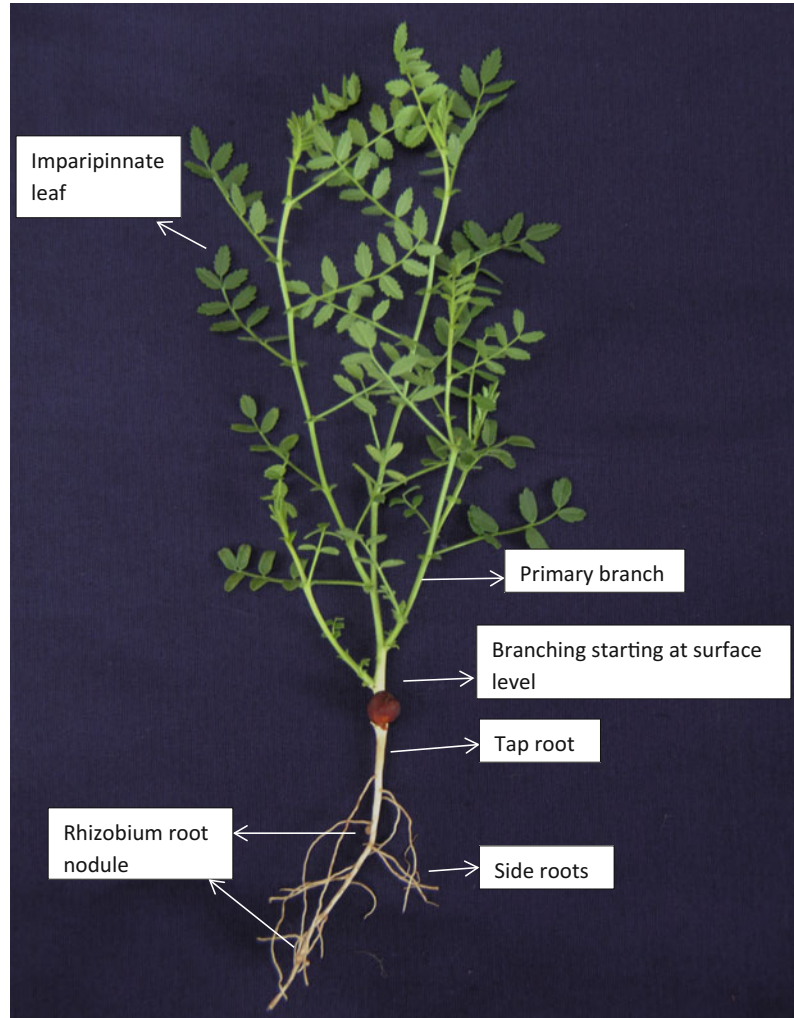
exodermis is absent, and endodermis is thin. The presence of nodules on roots indicates symbiotic relationship between chickpea and the Rhizobium bacteria (*Mesorhizobium ciceri*) leading to biological nitrogen fixation. The tap root system is so robust that it reaches more than 3 m in soil favouring the plant to survive in moisture stress conditions (Fig. 3.4).

Inflorescence: The inflorescence is an axillary raceme with generally a single papilionaceous flower though two to three flowers were also reported to occur rarely at the same node. The peduncle is 6–30 mm long, while the pedicel is 6–13 mm long. Both the peduncle and pedicel look like a single part because they are straight in

line up to fertilization, and then the pedicel bends down (Fig. 3.5).

Flower: The flower can be described as regular, bisexual, with five fused hairy sepals in a single whorl which form a calyx tube, five petals (pink, white, purple or blue in colour) in a typical papilionaceous arrangement with a big standard, two wings and two keel petals which form a boat shape, ten stamens in a diadelphous arrangement (9 stamens fused and a free 10th stamen) with orange-coloured pollen grains, linear style with globose stigma, sessile pubescent ovary containing 1–4 ovules (Cubero 1987) (Fig. 3.6).

Flowering: Commencement of flowering in chickpea is dependent on the duration of the

Fig. 3.2 Pubescence on
stem, leaves, calyx of flower
and pods of chickpea

genotype and the environment including soil and
weather (Gaur et al. 2012). Generally, flowering
starts in the range of 24 days (Kumar and Rao
1996) to 80 days after sowing and continues till
the depletion of moisture owing to the indeter-
minate growth of chickpea. When moisture
levels go down significantly, plants which bear
pods and leaves start to senesce reaching matu-
rity. Chickpea is a highly self-pollinated
crop. The anthers dehisce one day before the
flower opens ensuring self-pollination. Anthesis
continues throughout the day.

Pod: Pods start appearing about six days after
fertilization and may take up to four weeks for
completing seed development. Initially, the pod
wall starts to grow followed by the seed. The
number of pods per plant depends on the

genotype and the environmental conditions,
especially availability of moisture. The pod size
is generally in the range of 15–20 mm and may
go up to 30 mm depending on the genotype,
especially in kabuli types. Each pod contains
generally one to two seeds and rarely three
(Fig. 3.7).

Towards the end of the seed development,
leaves start to turn yellow first and then the whole
plant dries up indicating maturity (Fig. 3.8).

Seed: The shape of the seed generally
resembles a ram's (Aries) head, hence the name
'*arietinum*', while other shapes do exist such as
globular or quasi-spheric with a characteristic
beak. The surface of the seed coat may be
smooth or tuberculate. Endosperm is absent.
Seed size and colour is a varietal character and

Fig. 3.3 Leaf types in chickpea: compound leaf and simple leaf (centre)

highly influenced by environmental conditions, especially moisture availability and heat. There are two types of cultivated chickpea based on seed size and colour—desi and kabuli.

Desi type: The seeds are generally small (around 0.2 g per seed); seed coat is thick with varying colours such as cream, yellow, brown, black and green. The stem and leaves may contain anthocyanin pigmentation.

Kabuli type: The seeds are generally large (around 0.3–0.5 g per seed) to extra large (more than 0.5 g per seed); seed coat is thin and mainly cream or beige coloured, sometimes white. The plants will not have anthocyanin pigmentation (Fig. 3.9).

Seed colour in desi types assumes different shades of brown, black and green depending on the genotype, while the kabuli types have mainly beige-coloured seed. Cotyledons are mainly in three colours: cream, green or orange (Cubero 1987). Seed size exhibits huge variation starting from 0.08 g to nearly 0.8 g per seed. Generally, the kabuli types have larger seed size compared to the desi types (Fig. 3.10).

Germination: Seeds of cultivated chickpea do not exhibit any dormancy period. Seeds start to germinate within a week after sowing depending on the moisture level of the soil, temperature (28–33°C) and sowing depth (two inches). The germination is hypogeal with no hypocotyl. Plumule gives rise to a shoot bearing leaf-like scales at first and then true leaves (two pairs of leaflets and a terminal leaflet). Root growth from the radicle is much faster than above-ground shoot growth in initial stages of plant development (Fig. 3.11).

Fig. 3.4 Robust root system of chickpea with tap root and side roots
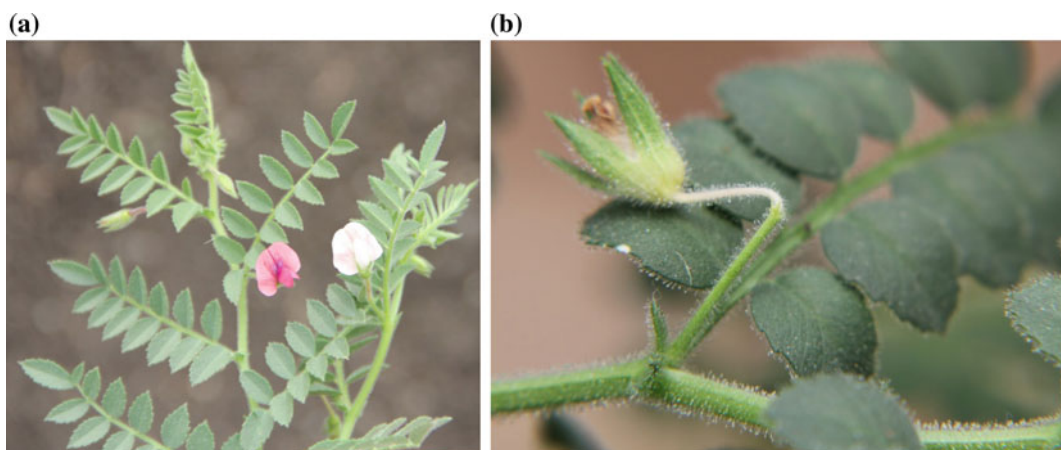
Fig. 3.5 **a** Major flower colours in chickpea: pink (left) and white (right). **b** Pedicel bending after fertilization
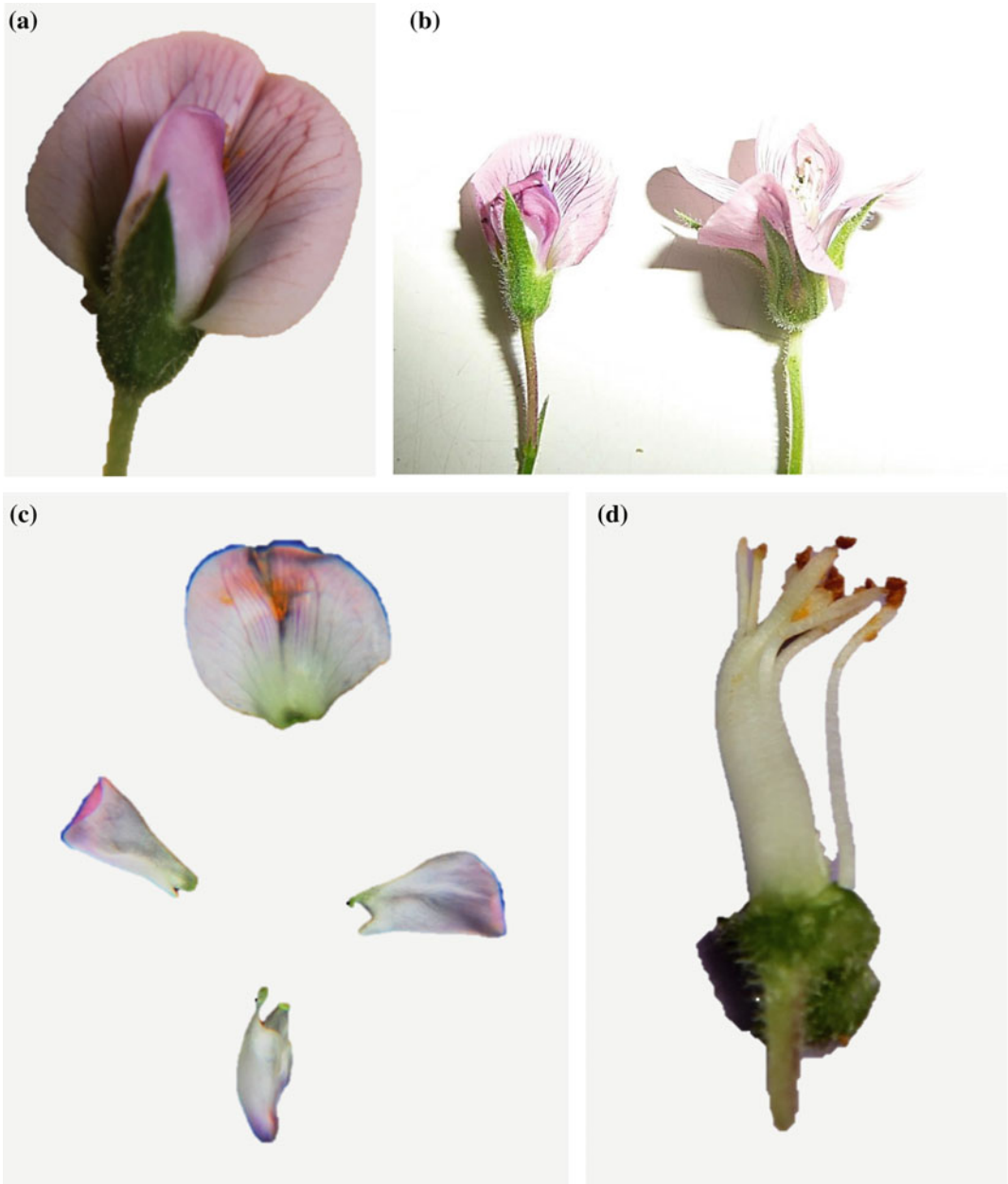
Fig. 3.6 **a** Papilionaceous flower of chickpea. **b** Normal flower (left) and open-type flower (right). **c** standard, wing and keel petals. **d** Diadelphous stamens (9 + 1)
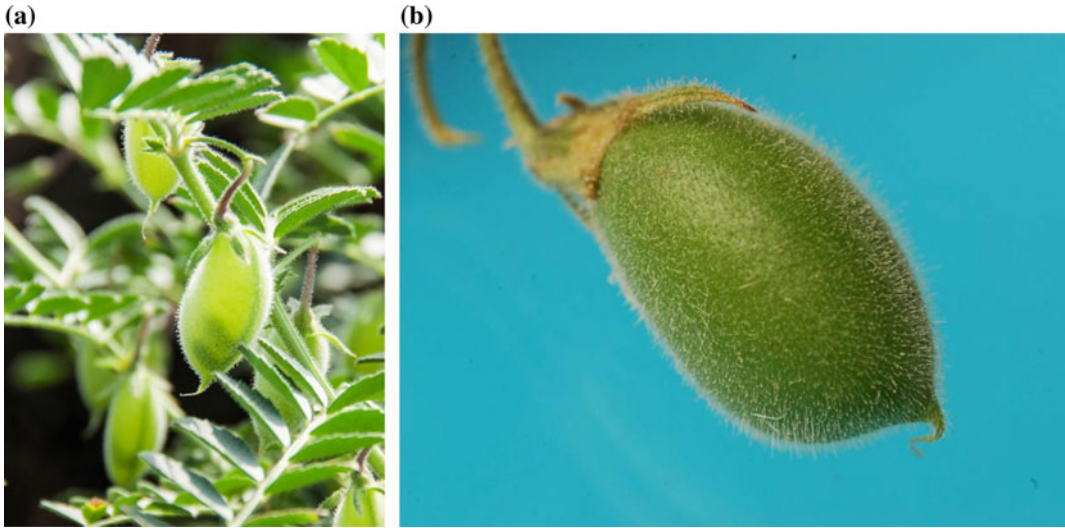
Fig. 3.7 (**a** and **b**) Immature chickpea pod

Fig. 3.8 Chickpea plants at maturity

Fig. 3.9 Desi (left) and kabuli (right) seed types

Fig. 3.10 Variation for seed size and colour in chickpea

Fig. 3.11 Seed germination, progressively, in chickpea

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Cytogenetics of *Cicer*

Miroslava Karafiátová, Eva Hřibová and Jaroslav Doležel

Abstract

Chickpea (*Cicer arietinum*) is among the most widely grown grain legumes, with the major growing area concentrated in the Indian subcontinent. The species is diploid ($2n = 2x = 16$) and is the only domesticated species in a genus, which includes over 40 annual and perennial species. The progenitor of the cultivated form is the annual species *C. reticulatum,* but both annual and perennial relatives have been considered as donors of useful genetic variation. Recent advances in genomic analysis have expanded the results of earlier cytogenetic research in the species, which established base information with respect to the karyotype (chromosome number, length, and morphology; and some limited descriptions based on banding) and an estimate of nuclear genome size. Chromosome behavior at meiosis has been characterized in a few *Cicer* species and some wide hybrids. To date, only a small number of DNA sequences have been chromosomally localized using in situ hybridization. No detailed cytogenetic map has been elaborated, and the level of knowledge regarding the long-range molecular chromosomal organization of the genome is rudimentary. A recently developed method for sorting chickpea chromosome using flow cytometry now offers a more effective means of exploring the genome.

4.1 Introduction

The genus *Cicer*, which belongs to the Fabaceae family, is the sole genus in the tribe Cicereae. Considerations of life cycle, morphology, and geographical distribution have allowed the 43 *Cicer* species to be classified into the four groups Monocicer, Chamaecicer, Polycicer, and Acanthocicer. Eight of the nine annual species (the

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exception is *C. chorassanicum*), which include the cultivated form *C. arietinum*, belong to the section Monocicer (van der Maesen 1987). Chickpea is the only cultivated *Cicer* species and, in terms of production and consumption, is among the most important grain legumes. It is important as a source of protein in the vegetarian diet, particularly in the Indian subcontinent, where the bulk of production (72% in quantity terms in 2014, according to FAOSTAT (http://faostat.fao.org/)); a further 6% of the cropping area is in western Asia. The crop is adapted to low rainfall conditions, but drought has been identified as one of the most important constraints to productivity.

The origin of chickpea has been traced to Turkey, in an area harboring most of the wild *Cicer* species, including the annual *C. reticulatum*, identified as the likely progenitor of the cultivated type (Ladizinski and Adler 1976b). Two distinct market classes are produced: The seed of kabuli types is large, non-pigmented, and smooth, while desi-type seed is rough, angular-shaped, and dark-colored. Kabuli plants lack anthocyanin pigmentation on their stem, while desi plants form pigmented stems and its flowers are pink (Pundir et al. 1985). Despite the importance of cultivated chickpea, little effort has been made to date to explore its genome at the chromosomal level, and even less with respect to that of its close relatives. Karyotypic descriptions and an estimate of the nuclear DNA amount date back at least 20 years (Ohri and Pal 1991; Ocampo et al. 1992; Galasso and Pignone 1992).

While most *Cicer* species are perennial, the cultivated form is an annual plant. The species has been described as preadapted to domestication (Ladizinsky 1979). The domestication process itself required the loss of pod shatter, a change in growth habit from a prostrate stem to a semierect to erect stem, the loss of vernalization requirement, a reduction in seed dormancy, and changes to seed size, shape, and color (Abbo et al. 2014; Gupta and Bahl 1983). The negligible economic importance of the perennial *Cicer* spp. has left these at best only superficially described, with the consequence that almost nothing is known regarding their evolution and phylogenetic relationships. At the same time, a number of both annual and perennial relatives have been exploited as donors of useful genetic variation with a view to chickpea improvement (Haware and McDonald 1992; Collard et al. 2001; Sharma et al. 2006; Singh et al. 2005).

4.2 Ploidy and Chromosome Number

The *Cicer* species are uniformly diploid, all showing a somatic chromosome number of 16 (Ladizinski and Adler 1976a; Ocampo et al. 1992). While there is no available evidence for any recent polyploidization event(s), gene copy number variation in the Fabaceae has suggested that such events have influenced the form of the chickpea genome, in particular, the legume-wide whole genome duplication predicted to have occurred about 58 million years ago (Jain et al. 2013). The rate of synonymous substitution per site per year has been estimated to be 6.05×10^{-9} (Jain et al. 2013), a frequency some 12% more rapid than is the case in *Medicago* (Young et al. 2011). The absence of any recent whole genome duplication affecting the genera *Medicago*, *Cicer*, and *Lotus* (Young et al. 2011; Sato et al. 2008; Jain et al. 2013) implies that speciation within the Fabaceae has not been driven by abrupt changes in chromosome number, but rather by chromosomal rearrangements and/or lineage-specific gene gains/losses. The diploid status of the *Cicer* species is mirrored in the genera *Lens*, *Pisum*, and *Vicia*, which belong to the related tribe Fabeae: each have a similar somatic chromosome number to that of the *Cicer* spp: in both *Lens* and *Pisum*, this is 14 (Mishra et al. 2007), while in *Vicia*, diploid species have basic chromosome numbers $x = 5$, 6 and 7 (Kaur and Singhal 2010). Species belonging to a fourth-related genus (*Lathyrus*) harbor many more chromosomes: Two *Lathyrus* species are tetraploid ($2n = 28$) and one is hexaploid ($2n = 48$) (Campbell and Clayton 1997).

4.3 Chromosome Morphology

The chickpea chromosomes are small (Fig. 4.1): The mean length of the mitotic metaphase chromosomes is around 2.2 μm (Ahmad 2000), which translates to a nucleotide content of slightly over 100 Mbp, equivalent to an eighth of the size of the average wheat chromosome (Šafář et al. 2010), but twice that of those of banana (Doležel et al. 1994). Although the somatic chromosome number of the annual *Cicer* species is invariant, there is plenty of karyological variation, and the same probably holds for the perennial species. Ahmad (2000), in a study of all nine annual *Cicer* species, recorded differences with respect to both chromosome length and the position of primary and secondary constrictions; these differences were significant enough to rule out proposing a unified karyotype across the annual *Cicer* species. Such variation supports the notion that structural alterations to the chromosomes likely have driven evolution
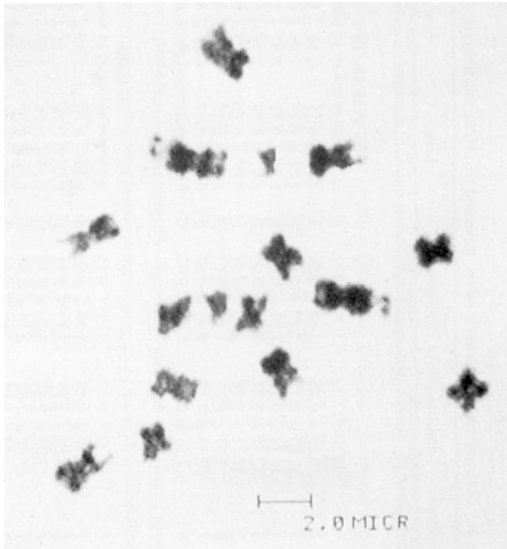
Fig. 4.1 A metaphase plate of cultivated chickpea. Copied from Venora et al. (1995) with the permission of the publisher (Karyotype of kabuli-type chickpea (*Cicer arietinum* L.) by image analysis system. Venora G, Ocampo B, Singh KB, Saccardo F. Caryologia, copyright ©University of Florence, reprinted by permission of Taylor & Francis Ltd., www.tandfonline.com on behalf of University of Florence

and speciation within *Cicer*. There is even some evidence for intraspecific karyotypic variation (Ohri and Pal 1991; Ocampo et al. 1992; Tayyar et al. 1994; Ahmad 2000; Ahmad and Hymowitz 1993; Kordi et al. 2006), since these various authors are in disagreement regarding chromosome length, arm ratio, and the position of the secondary constriction. According to Ahmad (2000), however, these discrepancies may well be artifacts arising from inconsistencies in the cytological protocols.

Chromosomes associated with the nucleolus organizing region (NOR) are readily recognized as they form a secondary constriction. Typically, only one chromosome pair in *Cicer* species shows this structure (Ohri and Pal 1991; Tayyar et al. 1994; Kordi et al. 2006). The sole exception is *C. reticulatum,* which harbors two pairs of satellited chromosomes (Ohri and Pal 1991; Ocampo et al. 1992). The silver stain assay was used by Galasso et al. (1996) to demonstrate that both NOR loci are active, albeit not equally. The conclusion was that during the evolution of *C. echinospermum* and *C. arietinum* from *C. reticulatum,* one of the two NOR loci was lost. However, Ahmad (2000) was unable to confirm the presence of two satellited chromosome pairs in *C. reticulatum* and suggested the possibility that two cytotypes of *C. reticulatum* exist, one with a single NOR locus and the other with two. Cultivated types bearing two NOR chromosome pairs have been reported in the early literature (Iyengar 1939; Kutarekar and Wanjari 1983) but have not been confirmed in more recent work. Some *C. arietinum* accessions reportedly display a tandemly arranged pair of satellites on the largest chromosome of the complement, but these only appear during late prophase/early metaphase (Meenakshi and Subramaniam 1960; Ahmad 1989, 2000; Tayyar et al. 1994; Kordi et al. 2006). The various explanations for this phenomenon have included staining artifacts (Ohri and Pal 1991; Ocampo et al. 1992; Galasso and Pignone 1992), NOR movement (Schubert 1984; Schubert and Wobus 1985), and evolutionary rearrangements (Ladizinski and Adler 1976b; Galasso et al. 1996; Kordi et al. 2006), but none of these are totally satisfactory.

The location of the secondary constriction varies among the *Cicer* species: In the annuals *C. arietinum, C. reticulatum,* and *C. echinospermum*, it is present on the longest chromosome pair, while in the others, it is associated with a medium- or a small-sized chromosome (Ahmad 2000).

With the exception of secondary constriction, chickpea chromosomes do not show marked features, which could ease their classification. Chromosome length at mitotic metaphase lies in the range from 1.32 to 3.69 µm (Ahmad 2000); three of the chromosomes are submetacentric and the others metacentric (Fig. 4.2). There is a suggestion of differences in relative chromosome length between the chromosomes of the kabuli and desi types; in the former, three of the chromosomes appear longer than their equivalents in the latter type, while the other five appear to be longer in desi types, but these differences are small, ranging from 0.2 to 0.8% of the overall relative chromosome length (Ruperao et al. 2014) (Table 4.1). Although this variability in chromosome length is consistent with the observations of Kordi et al. (2006), it is less substantial than was claimed by Ohri and Pal (1991). Only two of the eight *C. arietinum* chromosomes can be unambiguously identified based on their morphology: These are the longest submetacentric chromosome which bears the NOR, and the shortest metacentric one (Kordi et al. 2006). Except for the longest and shortest chromosomes, which are always classified as being, respectively, submetacentric and metacentric, according to Kordi et al. (2006), at least one of the six remaining chromosomes departs from the mean length and/or arm ratio assigned to the reference accession by Ahmad (2000).

Moreover, it is evident that the karyotype of cultivated chickpea is more distinctive and the differences in the length of individual chromosomes are bigger as compared to other annual species (Ahmad 2000).

Karyotype symmetry, as defined by Stebbins (1971), has some value as a descriptive parameter. The concept defines four levels of asymmetry in the placement of the centromere and three in the length of individual chromosomes. According to this system, there exist two types of asymmetry among the annual *Cicer* species (Ahmad 2000): One group clusters *C. arietinum*, *C. reticulatum*, and *C. echinospermum* and supports conclusions based on crossability, phylogenetic and genotypic (molecular marker-based) diversity analyses (Buhariwalla et al. 2005; Iruela et al. 2002; Sudupak et al. 2004).

Two chromosome naming systems have been used in *Cicer*, one based on numbers from 1 (longest chromosome) to 8 (shortest) (Ocampo et al. 1992), and the other on letters (*A–H*), where $A = 1$, $B = 2$, etc. (Galasso et al. 1996; Staginnus et al. 1999; Vláčilová et al. 2002; Zatloukalová et al. 2011). Both systems were in use until the first linkage maps were assembled, after which they were replaced by linkage group (LG) numbers. A system based on pseudomolecules has recently been proposed by Ruperao et al. (2014) (Table 4.1).

Little attempt has been made to cytogenetically characterize the perennial *Cicer* species. In 1972, van der Maesen estimated their chromosome number to be either $2n = 14$ or $2n = 16$ (van der Maesen 1972). The first description of the karyotype of a perennial *Cicer* species involved *C. anatolicum* (Ahmad 1989),
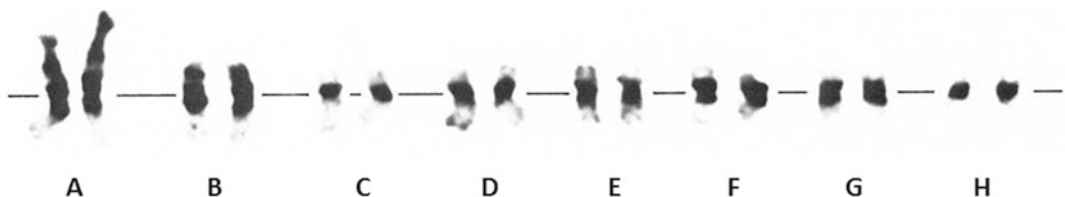
Fig. 4.2 C-banding karyotype of chickpea. Copied from Galasso and Pignone (1992) with the permission of the publisher (Characterization of chickpea chromosomes by banding techniques. Galasso I and Pignone D. Genetic Resources and Crop Evolution, copyright ©Kluwer Academic Publishers. With permission of Springer)

Table 4.1 Chickpea desi and kabuli chromosome nomenclature, their assignment to linkage groups, and individual chromosome sizes as determined from cytological data. Pseudomolecule number (Ca) corresponds to the linkage group number (LG). (Adapted from Ruperao et al. 2014)

Cicer arietinum L.					
Chromosome	Pseudomolecule	Relative chromosome length (%)		Molecular chromosome size (Mbp)	
		Desi "4958"	Kabuli "Frontier"	Desi "4958"	Kabuli "Frontier"
A	Ca5	19	19.8	164.92	174.64
B	Ca3	15.8	16.7	137.14	147.29
C	Ca6	13.3	12.9	114.58	112.9
D	Ca7	12.6	11.8	109.37	104.01
E	Ca4	11.5	11.1	99.82	97.9
F	Ca2	10.7	10.5	92.88	92.61
G	Ca1	9.9	9.4	85.93	83.91
H	Ca8	7.2	7.8	62.5	68.8
Total		100	100	867.14	882.06

establishing $2n = 16$ as the chromosome number, as is the case for the annuals. Subsequent analysis showed that the karyotype of *C. songaricum* was even more similar to that of *C. arietinum*, *C. reticulatum*, and *C. echinospermum,* at least in central and distal parts of the chromosomes.

4.4 Nuclear Genome Size

Similarly to the shortage of systematic studies on karyotype within *Cicer*, there are only a few reports on estimation of nuclear DNA content. Despite the stable chromosome number in genus *Cicer*, there seem to be remarkable differences in nuclear DNA content among its species. The first estimation of nuclear DNA amount in chickpea was reported by Bennett and Smith (1976), who gave nuclear DNA amount of 1.9 pg/2C for *C. arietinium*. In a more recent study, Ruperao et al. (2014) verified DNA amounts in chickpea using flow cytometry and estimated 2C DNA amounts of kabuli and desi types to be 1.80 and 1.77 pg, respectively. The differences in 2C amounts between four accessions of desi type were negligible. Using these values, mean nuclear 1C genome sizes of kabuli and desi types were determined as 882 and 866 Mbp, respectively. In the largest study performed so far, Ohri and Pal (1991) determined DNA content in six annual

Cicer species and five accessions of cultivated chickpea. Surprisingly, C-values of *C. arietinum* were much higher than those estimated by Bennett and Smith (1976) and Ruperao et al. (2014) (Table 4.2). Cultivated chickpea had the highest DNA amounts (2C = 3.3–3.57 pg) of all analyzed accessions. Estimates of 2C DNA content in all analyzed species ranged from 1.83 pg in *C. judaicum* to 3.57 pg in one of the cultivated chickpea accessions. DNA amount of perennial *C. songaricum* (2C = 2.72 pg) was comparable to that of *C. reticulatum* (2C = 2.66 pg) and C. *echinospermum* (2C = 2.6 pg). Some of the C value estimates were confirmed later by Galasso et al. (1996). It should be noted that both groups estimated DNA amounts using Feulgen microdensitometry and used *Vicia faba* and *Alium cepa*, respectively, as reference standards.

Clearly, there seem to be large inconsistencies in the estimates of nuclear DNA amount in *Cicer*. The reason for this is not clear, and a caution is warranted when using published data. For example, the karyotype of cultivated chickpea is similar to its wild progenitor, *C. reticulatum* (Ahmad et al. 1992; Iruela et al. 2002). Yet, the published data on 2C amounts in both species differ significantly (Table 4.2). It appears unlikely that a large change in DNA amount would occur during the process of domestication and cultivation of *C. arietinum* without marked

Table 4.2 Estimates of nuclear DNA amounts in species belonging to the genus *Cicer*

| Annual species | Reference | | | |
	Bennett and Smiths (1976)	Ohri and Pal (1991)	Galasso et al. (1996)	Ruperao et al. (2014)
C. arietinum "kabuli"	0.95	1.67	1.64	0.9
C. arietinum "desi"		1.65		0.89
C. bujungum K.H.Rech.		1.27		
C. cuneatum Hochst. Ex Rich.		1.25		
C. echinospermum P.H. Davis		1.35	1.3	
C. judaicum Boiss.		0.92		
C. pinnatifidum Jaub.& Sp.		1.28		
C. reticulatum Ladiz.		1.32	1.33	

| Perennial species | Reference | | | |
	Ohri (1999)			
C. songaricum	1.36			

changes in chromosome length and morphology. With this limitation in mind, when the available DNA content estimates are compared to recent phylogenetic data, there seems to be a positive correlation between the difference in genome size and genetic distance (Ohri and Pal 1991; Buhariwalla et al. 2005).

4.5 Longitudinal Differentiation of Chromosomes

Similarities in chromosome size and morphology do not permit identification of individual chromosomes in chickpea. However, this can be achieved after a procedure called Giemsa C-banding, which stains preferentially heterochromatin regions. When applying this method to chickpea, Galasso and Pignone (1992) and Galasso et al. (1996) observed differences in the distribution of heterochromatin along individual chromosomes. C-banding pattern included strong bands around centromeres and occasional weak banding patterns in middle and distal parts of chromosome arms (Fig. 4.2). Except for *C. judaicum* and *C. pinnatifidum*, C-banding polymorphisms have provided the means to identify each individual chromosome pair

(Tayyar et al. 1994). The use of fluorochromes differing in DNA base affinity (DAPI, Hoechst 33258, and Chromomycin A3) has revealed significant variability in heterochromatin content among the annual *Cicer* species. Tayyar et al. (1994) used these stains to arrive at an estimated heterochromatin content of 40% in most of the annual species, although the ratio rose to 60% in *C. cuneatum* and *C. bijungum*. The difference was thought to reflect a correlation between evolutionary advancement and heterochromatin reduction (Tayyar et al. 1994). However, attempts to group the species based on their heterochromatin content proved to be inconsistent with their grouping based on either crossability (Ladizinski and Adler 1976b) or alleles at isozyme (Kazan and Muehlbauer 1991) or seed storage protein (Ahmad and Slinkard 1992) loci.

The recent acquisition of the genome sequence of both the desi and kabuli types (Jain et al. 2013; Varshney et al. 2013; Parween et al. 2015) has facilitated the use of sequence-based markers to characterize the genetic diversity present both between and within wild and cultivated *Cicer* species. For example, Bajaj et al. (2015) exploited variation at >27,000 SNP loci distinguishes the cultivated type (both desi and
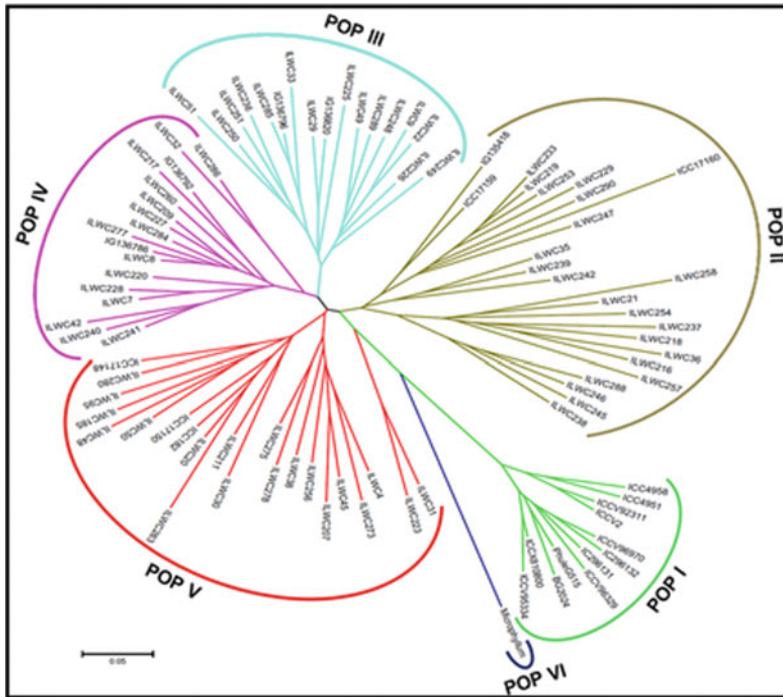
Fig. 4.3 Unrooted cladogram illustrating genetic relationships (Nei's genetic distance) among 93 wild and cultivated accessions belonging to seven *Cicer* species obtained using 27,862 genome-wide SNPs. The phylogenetic tree clearly differentiated 93 accessions into six diverse groups, which correspond to *Cicer* species and gene pools of origination. POP I consists of desi and kabuli accessions, POP II consists of the accessions of *C. reticulatum* and *C. echinospermun*, and other four distinct clades (POP III–VI) represent *C. judaicus*, *C. bijugum*, *C. pinnatifidun*, and *C. microphyllum* species. Genome-wide SNP-based molecular diversity, phylogeny, and population genetic structure among 93 wild and cultivated *Cicer* accessions by Bajaj et al. (2015), used under CC BY 4.0/excised from the original

kabuli) from accessions of *C. reticulatum* and *C. echinospermun*, and also from the more distant taxa *C. judaicus*, *C. bijugum*, *C. pinnatifidun*, and *C. microphyllum* (Fig. 4.3). Meanwhile, Kujur et al. (2015) showed that single nucleotide polymorphism (SNP)-based genotyping was able to divide a collection of cultivated germplasm into the two recognized major groups, kabuli and desi, and a detailed analysis of the SNP-based genetic diversity within these two groups has been presented by Upadhyaya et al. (2008), Roorkiwal et al. (2014), and Kujur et al. (2015).

4.6 Meiosis

Since the chromatin in a meiotic chromosome is less condensed than in a mitotic one, the former is more informative with respect to chromosome morphology and structure. As yet, however, meiotic chromosomes in the genus *Cicer* have not been systematically studied. Although Kabir and Singh (1991) observed some abnormalities, in general meiosis in cultivated chickpea was regular with eight bivalents formed in metaphase I. The character of the bivalents was more open (rod) than closed (ring), and chiasma frequency per pollen mother cell (PMC) was variable among the nine analyzed Cicer species (Ahmad and Chen 2000, Fig. 4.4). An analysis of pachytene chromosomes provided by Ahmad and Hymowitz (1993) exposed the distribution of heterochromatin along the chromosomes and confirmed that only one chromosome pair in *C. arietinum* was associated with the nucleolus; the chromosome arm carrying the NOR was highly heterochromatic, just as is the case in soybean
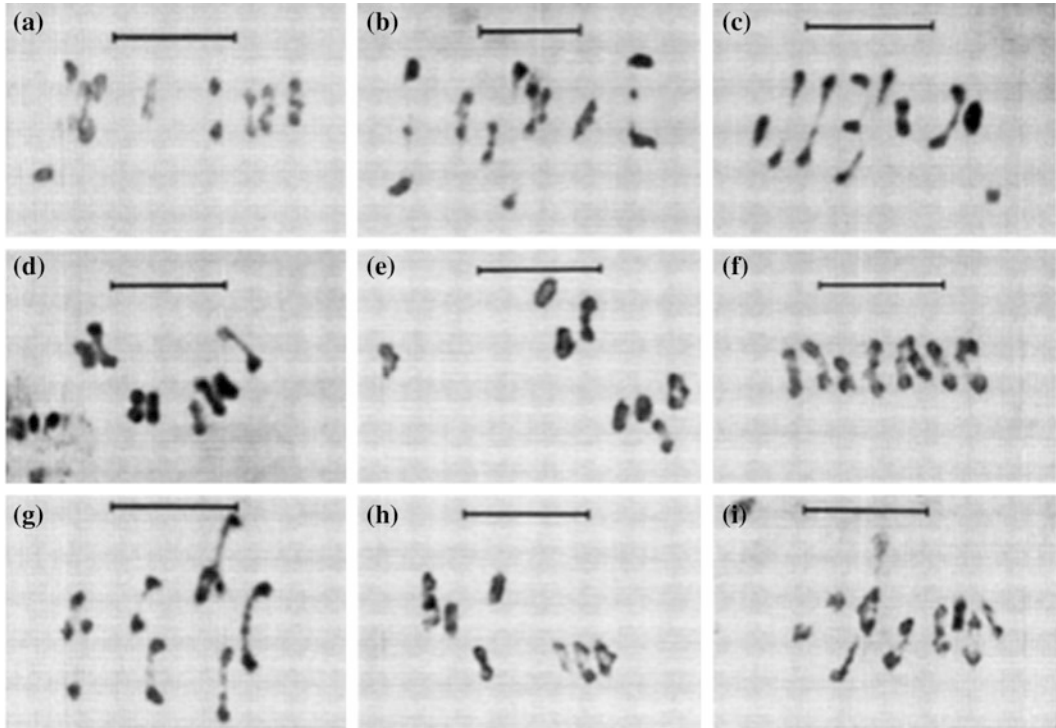
Fig. 4.4 Chromosome pairing at meiotic metaphase I in annual *Cicer* species. **a** *C. arietinum*, **b** *C. reticulatum*, **c** *C. echinospermum*, **d** *C. pinnatifidum,* **e** *C. judaicum*, **f** *C. bijungum*, **g** *C. chorassanicum*, **h** *C. yamashitae*, **i** *C.* *cuneatum*. Bar: 10 μm. The image has been taken from Ahmad and Chen (2000), with the permission of the publisher

(Singh and Hymowitz 1988), pigeon pea (Reddy 1981), and maize (McClintock 1929). The study also indicated that in the pachytene chromosome, the distinction between heterochromatin and euchromatin was clearer than in either barley (Singh and Tsuchiya 1975) or rice (Kush et al. 1984).

4.7 Molecular Cytogenetics

The elaboration of the fluorescence in situ hybridization (FISH) technique to localize specific DNA sequences on a mitotic or meiotic chromosome has generated important insights into chromosome organization in many organisms, including *Cicer* spp. The bulk of these experiments in *Cicer* has focused on the cultivated form, leaving the level of understanding of the chromosome organization in other *Cicer*

species at best only limited. The ribosomal RNA genes were the first sequences to be localized in this way (Abbo et al. 1994; Staginnus et al. 1999). While only one chromosome pair carries a visible satellite, two sites hybridize with a 45S rDNA sequence, which was interesting in light of the presence of two satellited chromosome pairs in *C. reticulatum* (Ohri and Pal 1991; Abbo et al. 1994). Two sites harboring 5S rRNA sequences have been identified, one of which lies on the same chromosome as one of the 45S rDNA sites (chromosome B) (Vláčilová et al. 2002).

About 50% of the chickpea genome comprises repetitive DNA (Jain et al. 2013; Varshney et al. 2013). Some of the sequences within this fraction can be highly informative as cytogenetic markers, especially where their chromosomal distribution is non-random (Schwarzacher 2003; Jiang and Bikram 2006). FISH based on probe sequences detecting five distinct microsatellite motifs ($(A)_{16}$,

(CA)$_8$, (TA)$_9$, (AAC)$_5$, and (GATA)$_4$), which were selected based on results of previous study (Sharma et al. 1995), unfortunately failed to produce a chromosome-specific karyotype: The distribution and intensity of the signal varied from repeat motif to repeat motif, but all five were dispersed within each chromosome (Gortner et al. 1998). As anticipated, a telomeric sequence hybridized to each of the chromosome ends, but a weaker site in the pericentromeric region of chromosome A and a major cluster on the short arm of chromosome B were also evident (Zatloukalová et al. 2011; Staginnus et al. 1999). Nevertheless, the potential of repetitive DNA sequences has demonstrated in several studies. For example, the two tandemly organized chickpea-specific repeats (CaSat 1 and CaSat 2) isolated from a genomic library by Staginnus et al. (1999) were both informative: The former defined a large cluster of sites in the subtelomeric region of both chromosomes A and B, while the latter proved to be present at each of the eight centromeres. The retrotransposon-like sequences, CaRep 1, CaRep 2, and CaRep 3, derived from different parts of a Ty3/Gypsy-like element, are dispersed throughout the genome and produce a strong FISH signal concentrated in the intercalary heterochromatin on each chromosome, but not in the pericentromeric region (Staginnus et al. 1999, 2010). A similar distribution has been reported for the CaTy sequence, which shares homology with members of the Ty1/Copia-like element family

(Staginnus et al. 2010). Only weak signal was obtained using a probe based on a chickpea LINE-like element (Staginnus et al. 2010).

FISH probes based on low or single copy sequences have been deployed in a number of plant species (Jiang et al. 1995; Lapitan et al. 1997; Zhang et al. 2004; Idziak et al. 2014). Zatloukalová et al. (2011) prepared a partial bacterial artificial chromosome library from desi chickpea genomic DNA and recovered five clones which hybridized to a single locus. One of the loci mapped to a subtelomeric region on the short arm of chromosome A, two to a subtelomeric region on each arm of chromosome B, one to one of the telomeres of chromosome E, and the last to a telomeric region on chromosome H (Fig. 4.5).

Although the number of informative FISH probes is not extensive, they are sufficient to identify five chromosomes in the karyotype. While this can provide opportunities to follow chromosome behavior during meiosis and to compare the karyotypes of cultivated and wild chickpea accessions, there is a need to elaborate additional cytogenetic markers. A possible option is to use cDNAs, since these have been successfully deployed in both barley (Karafiátová et al. 2013) and wheat (Danilova et al. 2014). The acquisition of the genome sequence means that, as has been pioneered in barley (Aliyeva-Schorr et al. 2015), it is now possible to identify in silico sequences suitable as FISH probes.
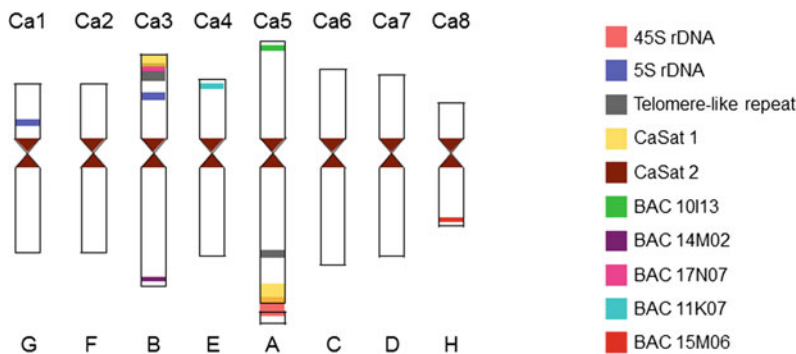
Fig. 4.5 Idiogram of *C. arietinum* desi type created using data on chromosome length by Ruperao et al. (2014) and location of a set of DNA sequences which were mapped to chromosomes using FISH (Zatloukalová et al. 2011 and unpublished data)

4.8 Chromosomal Organization at the Molecular Level

Thanks to the development of high-throughput sequencing, partial genome sequences of both desi (38.48%) and kabuli (39.37%) chickpea have been acquired (Jain et al. 2013; Varshney et al. 2013; Parween et al. 2015). The assembly of a whole genome sequence is highly revealing of chromosomal organization at the molecular level and allows for comparisons to be made of chromosome structure both within and between species (Paterson et al. 2009; Schatz et al. 2014; Schnable et al. 2009; Thiel et al. 2009). As in other plant species, the chickpea genome harbors a significant proportion of repetitive DNA, some of which is present in the form of an extended region of tandemly arranged repeats. As also suggested by the cytogenetic detection of hete-rochromatin (Staginnus et al. 1999, 2010; Zat-loukalová et al. 2011), the centromeric and pericentromeric regions are particularly repeat-rich (especially with respect to the CaSat 2 element) and gene-poor. Parween et al. (2015) showed that the mean frequency of recombina-tion in the pericentromeric region of desi is some ninefold lower than in more euchromatin-rich regions. Gene density across the desi pseudo-molecules averaged 7.07 per 100 Kbp, about double the density (3.73 per 100 Kbp) present in unanchored scaffolds, implying that the latter sequences harbor a high proportion of repetitive DNA. The current desi and kabuli assemblies represent only 24–55% of each of the eight chromosomes, and the most distal and sub-telomeric regions are mostly absent (Parween et al. 2015). Thus, it is not possible as yet to draw conclusions regarding gene density and repetitive DNA content along the full length of any of the chickpea chromosomes in the way that has been achieved in rice (Goff et al. 2002) and *Ara-bidopsis thalianan* (Schneeberger et al. 2011), for example, and even for one of the large chromosomes of wheat (Choulet et al. 2014). Nevertheless, the indications are that the desi and kabuli genomes are highly similar to one another. Ruperao et al. (2014) have suggested that apparent differences between the two assemblies

are an artifact arising from the gappiness of the sequences. Clearly, a higher quality reference genome assembly will be needed to elaborate a more precise picture of chromosome organiza-tion at the molecular level.

4.9 Flow Cytogenetics

Flow cytometry can be highly informative with respect to chromosome size and structure (Kubaláková et al. 2003; Molnár et al. 2011; Ma et al. 2013). It supports physical mapping and whole genome sequencing, especially in the context of large genome species (Cviková et al. 2015; Raats et al. 2013; Ruperao et al. 2014; Mayer et al. 2014). Vláčilová et al. (2002) have described a protocol to synchronize cell cycle and thereby to accumulate chromosomes at mitotic metaphase in chickpea root tips and have exploited it to prepare liquid suspensions of intact chromosomes suitable for flow cytometry. The resulting flow karyotype of kabuli type comprised eight peaks, five of which were assignable using FISH to chromosomes A–C, G, and H. The other three peaks represented chromosomes D, E, and F. The purity of the single chromosome flow-sorted fractions ranged from 68% (chromo-some C) to 100% (chromosomes B and H). Applying PCR assays targeting microsatellite loci confirmed that chromosome H was equivalent to linkage group LG8, marking the first step toward integrating the chickpea cytogenetic and genetic maps. When Zatloukalová et al. (2011) flow karyotyped the desi type, both the number and positions of the peaks differed from those forming the kabuli-type flow karyotype (Vláčilová et al. 2002): Here, only six peaks were observed. This difference implied that the two genomes were distinct from one another, at least with respect to their AT/GC content, in contradiction to the conclusion reached from an analysis of the partial genome assemblies that the two genomes are highly similar (see previous section). However, the difference is in line with the suggestions of Ohri and Pal (1991) and Kordi et al. (2006), which was based on DNA amount. The lack of agreement between the kabuli- and desi-type flow

karyotypes has recently been confirmed by Ruperato et al. (2014) and is illustrated in Fig. 4.6. Of the six peaks forming the desi-type flow karyotype, four were assigned using FISH to chromosomes A, B, E, and H, and each of the other two peaks was a mixture (one of chromosomes C and D, and the other of chromosomes F and G). The purity of the flow-sorted fractions involving a single chromosome varied from 88% (chromosome A) to 98% (chromosome H). PCR-based microsatellite assays confirmed that chromosome A is equivalent to LG5, B to LG3, E to LG4, and H to LG8. Similarly, it was concluded that chromosome F is equivalent to one of LG1 and LG2, and chromosome G to the other; while chromosomes is equivalent to one of LG6 or LG7, and chromosome D to the other (Table 4.1).

Flow-sorted chromosomes are also useful as a means of validating genome sequence assemblies. Purified preparations of desi-type chromosomes A, B, and H, as well as A–C and F–H of the kabuli-type chromosomes were used by Ruperato et al. (2014) as a template for Illumina-based sequencing. When compared to the desi assembled pseudomolecules (Jain et al.

2013), some large-scale misassignations became apparent, while in the kabuli assembly (Varshney et al. 2013), a number of short defined regions were shown to have been misplaced. Thanks to the recent development of a procedure for sequencing an individual flow-sorted chromosome (Cápal et al. 2015), it has now become possible to obtain sequence from a unique chromosome, although the identity of the sequenced chromosome cannot be known a priori. A further application of flow-sorted chickpea chromosomes has been as a target for FISH. When mounted on a microscope slide, a flow-sorted preparation typically comprises thousands of chromosomes at a high level of purity and free of cell and tissue debris, which improves the robustness of the FISH assay (Vláčilová et al. 2002; Zatloukalová et al. 2011).

4.10   Induced Polyploidy

Many crop species are polyploid (Zeven 1979; Li et al. 2015); although most are allopolyploid (e.g., wheat and cotton), a few are autopolyploid
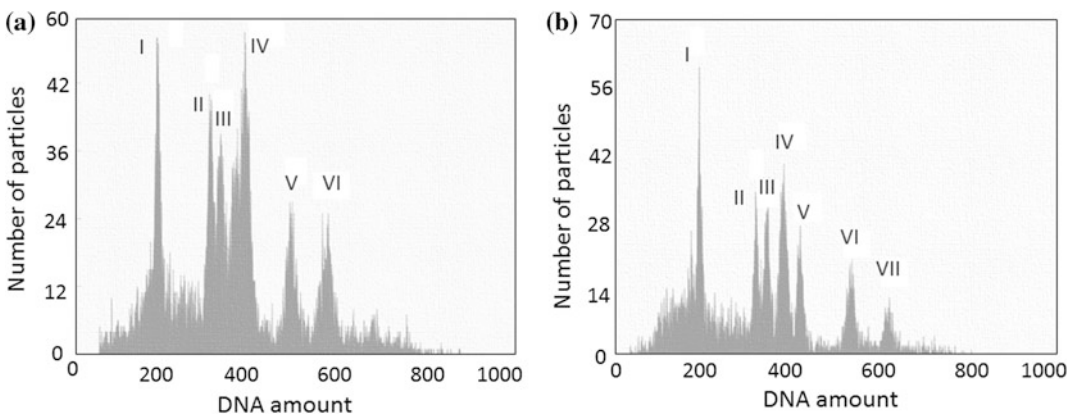
Fig. 4.6 Flow karyotype of (**a**) desi- and (**b**) kabuli-type chickpea. Liquid suspensions of mitotic metaphase chromosomes were stained by DAPI, and their relative fluorescence was analyzed using flow cytometry. Note differences in the number and position of chromosome peaks between the two chickpea types. In desi type, the flow karyotype comprises six peaks. Four peaks *I*, *III*, *V*, and *VI* represent chromosomes H, E, B, and A, respectively. Two composite peaks *II* and *IV* represent chromosomes F–G and C–D, respectively. In kabuli type, seven peaks could be resolved. Six peaks *I*, *II*, *III*, *V*, *VI*, and *VII* were assigned to chromosomes H, G, F, C, B, and A. Remaining composite peak *IV* represents chromosomes D–E

(potato and alfalfa). As increasing the ploidy level can be accompanied by improved plant performance (Ramsey and Ramsey 2014; Renny-Byfield and Wendel 2014), numerous attempts have been made to artificially induce autopolyploidy in a diploid crop species (Kinoshita and Takahashi 1969; Armstrong 1981). Sohoo et al. (1970) generated autote-traploid *C. arietinum* by treating the seedling apical meristem of both kabuli and desi types with colchicine. Although chromosome pairing at meiotic metaphase was dominated by biva-lents, seed set in the autotetraploids was only about 30% that achieved in the diploids. Never-theless, compared to their diploid progenitor, the autotetraploids did develop stronger and deeper roots, tougher stems, thicker pods, and bigger seeds. On the other hand, their germination was slow, and because of their reduced fertility, their grain yield was compromised (Sohoo et al. 1970; Pundir et al. 1983). In some induced autote-traploids, selection in subsequent generations has been able to restore fertility (Stebbins 1950), but the literature does not report any attempt to pursue this strategy in chickpea. The ability to reduce the ploidy level from diploid to haploid has been exploited as a means to rapidly fix a genotype via subsequent chromosome doubling, an approach which has been commercially exploited in a number of crop breeding pro-grams, notably in barley (Forster et al. 2007), rice (Jiang et al. 2014) and eggplant (Rotino 2016). Haploids can be induced from either the micro-spore (androgenesis) or the megaspore (gyno-genesis). The former approach typically relies on the in vitro culture of immature anthers. A first attempt to develop in vitro anther culture in chickpea was reported by Khan and Gosh (1983), which was followed by improvements in proce-dures to promote somatic embryogenesis and regeneration (Altaf and Ahmad 1986; Bajal and Gosal 1987; Huda et al. 2001; Vessal et al. 2002). Full protocols for the production of dou-bled haploid lines via androgenesis have been documented by Grewal et al. (2009) and Pan-changam et al. (2014).

4.11   Wide Hybridization

There has been continued interest in the potential of wide hybridization as a means to improve chickpea. Targets for introgression have included disease resistance, stress tolerance, yield poten-tial, and end-use quality. Post-fertilization incompatibility barriers are responsible for the relatively poor rate of success in producing wide hybrids. These include the presence of translo-cation differences between the parental genomes, leading to meiotic irregularities and a subsequent loss of fertility; cytoplasmic incompatibility; chromosome elimination and loss; excessive seed dormancy; and hybrid breakdown (Bassiri et al. 1987; Stamigna et al. 2000; Ahmad and Slinkard 2004). Although the fertilization process itself is relatively unhindered, the hybrid embryo often aborts within a few days. Attempts to deploy embryo rescue to circumvent this problem have not met with a great deal of success, and levels of efficiency are low (Verma et al. 1995; van Dor-restain et al. 1998; Mallikarjuna 1999).

While no published examples of a successful hybrid between chickpea and one of the peren-nial *Cicer* species exist, hybrids with several of the annual ones have been attempted (Croser et al. 2003). Hybrids are formable between *C. arietinum* and either *C. reticulatum* or *C. echi-nospernum* (the two species most closely related to the cultivated type), but their fertility is vari-able (Ladizinski and Adler 1976b; Singh and Ocampo 1993). If *C. arietinum* × *C. echi-nospernum* are highly sterile, *C. arietinum* × *C. reticulatum* F_1s are fertile and their meiosis is relatively regular, what tends to support the notion that *C. reticulatum* is the progenitor of the cultivated form (Ladizinski and Adler 1976b). The occasional meiotic irregularities observed in F_1 pollen mother cells comprise univalents and quadrivalents. According to Jaiswal et al. (1987), these hybrids flower early, have a high yield potential, and are better able to tolerate low temperatures than *C. arietinum*. The level of crossability between *C. arietinum* and *C. echi-nospernum* is low; the plants develop normally,

form six bivalents and two quadrivalents at meiosis, and are only partially fertile (Ladizinski and Adler 1976b). The presence of a quadrivalent suggests that the chromosomes involved have suffered a reciprocal translocation. A few interesting introgression events have been identified among the offspring of these two wide hybrids (Jaiswal et al. 1987; Singh and Ocampo 1993). A number of attempts to use either *C. bijungum* or *C. pinnatifidum* as a parent have failed (Singh et al. 1994, 1999; Verma 1990). However, the *C. arietinum* × C. *judaicum* hybrid was feasible (Verna et al. 1995); the resulting plants formed a high number of branches and pods and yielded well (Singh et al. 1994; Verma et al. 1995). In contrast, Ladizinski and Adler (1976b) did succeed in crossing *C. arietinum* with each of *C. judaicum*, *C. pinnatifidum*, and *C. bijungum*; meiotic pairing in each of these hybrids comprised mostly bivalents, with rare univalents, but the plants were all sterile. Recently, Abbo et al. (2011) described successful cross between annual *C. cuneatum* and perennial *C. canariense* with 50% pollen fertility and intermediate look of hybrid plants.

The outcomes of wide hybridization experiments led Ladizinski and Adler (1976b) to assign each of the annual *Cicer* species as a member of either the crop's primary genepool (*C. reticulatum*), its secondary genepool (*C. echinospernum*), or its tertiary genepool (*C. judaicum*, *C. pinnatifidum*, *C. bijungum*). The updated scheme suggested by Croser et al. (2003) matches the set of phylogenetic relationships derived by Buhariwalla et al. (2005) from a SNP-based analysis of genotypic diversity. Based on hybridization, Ladizinski and Adler (1976a, b) assigned all annual Cicer species to three crossability groups according to the classical definition as proposed by Harlan and de Wet (1971). More recently, this system was revised by Croser et al. (2003) Newly, primary genepool comprises *C. arietinum* and *C. reticulatum*, secondary genepool *C. echinospernum* only, and its tertiary genepool all remaining annual (and probably all perennial) Cicer species. This grouping correlates with genetic diversity of wild annual Cicer species (Buhariwalla et al. 2005).

4.12 Conclusion

Progress in chickpea cytogenetics has been slower than in many of the agriculturally important crops. There remain major knowledge gaps regarding chromosome structure both in the cultivated form and in its near relatives within the genus *Cicer,* and whether chromosome organization differs between the various *Cicer* species is quite unknown. Meiotic chromosome behavior in wide hybrids and their offspring are at best sketchily described. A major advance in filling these gaps should follow from the acquisition of the chickpea genomic sequence, the development of molecular cytogenetics technology, and the use of flow cytometry to apportion the nuclear genome into its component chromosomes. The probability is that in the near future, the chickpea community will be in a position to better utilize the full range of genetic diversity present in the genepool and thereby to support the breeding of improved cultivars of chickpea.

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Managing and Discovering Agronomically Beneficial Traits in Chickpea Germplasm Collections

5

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Abstract

Access to crop biodiversity is foremost to address new challenges to agricultural production. The chickpea genetic resources maintained at ICRISAT genebank have been characterized for morpho-agronomic traits. The passport and characterization data were used to form representative subsets: core/mini core collections and genotyping data of composite collection was used to form reference set. The chickpea core and mini core collections, respectively, consist of 1956 and 211 accessions, while reference set 300 accessions. These subsets are ideal genetic resource to dissect population structure and diversity, identify new sources of variations, mine allelic variation, and conduct association genetics to identify QTLs associated with agronomic traits which upon validation may be used in applied breeding. Using mini core collection, a number of accessions with early maturity, high yield and large seed size, seed nutritional traits, and stress tolerance were identified, including some with high yield and multiple stress tolerance. A systematic program is underway to introgress wild *Cicer* gene(s) to enhance levels of resistance and to broaden the genetic base of cultigen genepool.

5.1 Introduction

Plant genetic resources are the basic raw materials and their judicial use in crop improvement is one of the most sustainable ways to conserve valuable genetic resources for future and simultaneously increase agricultural production and food security. Key to successful crop improvement is a continued supply of genetic diversity including new or improved variability for target traits. International Crops Research Institute for the Semi-Arid

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Tropics (ICRISAT) has global responsibility to collect, conserve, maintain, characterize, evaluate, document and distribute the wealth of genetic variation of its six mandate crops and five small millets. The germplasm collections maintained in genebanks are the source for detailed characterization of agronomically beneficial traits such as tolerance to abiotic and biotic stresses, yield, nutrition, and grain quality. In this chapter, we report the current status of chickpea germplasm of both cultivated and wild types preserved in RS Paroda genebank at Patancheru, India and the major activities carried out by the genebank scientists to promote use of germplasm in breeding and genomics of chickpea.

5.2   Global Chickpea Genetic Resources

Global collection of chickpea genetic resources consists of 99,877 accessions conserved across 120 national/international genebanks in 64 countries. Of these, 1476 accessions are of wild *Cicer* types. Table 5.1 represents the list of major genebanks holding chickpea collections greater than 1000 accessions. Together, they contain 87,341 accessions, 98.3% cultivated and 1.7% the wild *Cicer* types. ICRISAT genebank currently holds 308 wild *Cicer* accessions representing eight annuals and 10 perennial species (Table 5.2).

Germplasm conservation at ICRISAT: Currently ICRISAT genebank holds the largest chickpea germplasm collection (20,764 accessions) representing 59 countries of origin.

New collection assembled: Between 2004 and 2012, a total of 3332 accessions from USDA-USA (2098), ICARDA Syria (900), Nepal (265), Azerbaijan (44), and Bulgaria (25) were incorporated into the ICRISAT collection.

5.3   Conservation and Regeneration

Conservation: The base germplasm collection at ICRISAT is conserved under long-term storage conditions (−20 °C), while the active collection is maintained under medium term storage at 4 °C

and 30% RH. The moisture content during seed processing for storage as active and base collections should be 10% and 6–7%, respectively. About 350 g of chickpea seed per accession is preserved in aluminum screw cap containers as the active collection. Vacuum-sealed standard aluminum foil pouches are used for conserving 200 g of seed per accession in the base collection (Upadhyaya and Gowda 2009). As a safety backup, seed samples of 16,996 chickpea accessions were deposited in Svalbard Global Seed Vault, Norway.

Regeneration: Chickpea is a cool-season grain legume crop grown in climates ranging from semi-arid tropics to temperate environments. Seed regeneration is an important aspect of management of genetic resources. Reproductive biology largely influences procedures used for regeneration. Chickpea being self-pollinated (unlike cross-pollinated species) can be easily regenerated under field conditions.

Chickpea accessions are regenerated when seed quantity in the active collection is less than 75 g or when viability falls below 85%; whereas accessions in the base collection are regenerated when seed viability falls below 90%. An adequate quantity of seeds should be used for regeneration to maintain as much of the original variation as possible within an accession. At ICRISAT, two four-meter rows containing approximately 80 plants are grown and harvested to provide regenerated seed of each accession. Accessions being regenerated are regularly monitored during the cropping season to detect and eliminate off-type plants. Data on discrete phenotypes such as growth habit, flower and seed color and seed shape is recorded during regeneration and compared to previously generated passport information to ensure integrity of each accession (Upadhyaya and Gowda 2009; http://cropgenebank.sgrp.cgiar.org/index.php/crops-mainmenu-367/chickpea-mainmenu-360/regeneration-mainmenu-374). Wild *Cicer* species are regenerated in a glasshouse under extended light (18 h) conditions. Seeds are scarified before planting to overcome dormancy due to hard seed coat.

Table 5.1 List of genebanks holding more than 1000 chickpea germplasm accessions

Institute	Wild Cicer		Cultivated	Total
	Species	Accession		
Australian Temperate Field Crops Collection (ATFCC), Horsham Victoria, Australia	18	246	8409	8655
Institute of Biodiversity Conservation (IBC), Addis Ababa, Ethiopia			1173	1173
Institute for Agrobotany (RCA), Tápiószele, Hungary	5	9	1161	1170
International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India	19	308	20,456	20,764
National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India	10	69	14,635	14,704
National Plant Gene Bank of Iran, Seed and Plant Improvement Institute (NPGBI-SPII), Karaj, Iran			5700	5700
Estación de Iguala, Instituto Nacional de Investigaciones Agrícolas (INIA-Iguala), Iguala, Mexico			1600	1600
Plant Genetic Resources Program (PGRP), Islamabad, Pakistan	3	89	2057	2146
N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR), St. Petersburg, Russian Federation			2767	2767
International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syrian Arab Republic	11	540	15,194	15,734
Plant Genetic Resources Department, Aegean Agricultural Research Institute (AARI), Izmir, Turkey	4	21	2054	2075
Institute of Plant Production n.a. V.Y. Yurjev of UAAS, Kharkiv, Ukraine			1760	1760
Western Regional Plant Introduction Station, USDA-ARS, Pullman, USA	21	194	7844	8038
Uzbek Research Institute of Plant Industry (UzRIPI), Botanica, Uzbekistan			1055	1055
Total		1476	85,865	87,341

(http://www.fao.org/wiews-archive/germplasm_query.htm?i_l=EN)

5.4 Characterization and Evaluation

Characterization refers to recording easily-observed and highly heritable characters expressed in all environments; while evaluation of agronomically beneficial traits often requires more extensive screening tests and data recording. Accessions are usually grown in augmented block designs using standard checks at every 10 or 20 accessions and characterized following chickpea descriptors (IBPGR, ICRISAT, and ICARDA 1993). To date, about 98% of chickpea germplasm has been characterized for morpho-agronomic traits, 63% for seed protein content, and 35% for biotic stresses, namely fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri* (Padwick) Snyd. & Hans.), ascochyta blight (*Ascochyta rabiei* [Pass.] Labr.), botrytis gray mold (*Botrytis cinerea* Pers. Ex Fr.) and colletotrichum blight (*Colletotrichum dematium* (Pers ex Fr) Grove). All the characterization and evaluation data can be accessed through http://genebank.icrisat.org/. Large range variations among entire collection accessions were noted for both quantitative and qualitative traits (Table 5.3, Figs. 5.1 and 5.2).

Table 5.2 Status of wild *Cicer* species accessions conserved at ICRISAT genebank

Species	No. of accessions	Chromosome number $(2n)$[a]	Country of origin
Annual			
C. bijugum	49	16	Iraq, Syria and Turkey
C. chorassanicum	4	16	Afghanistan
C. cuneatum	5	16	Ethiopia
C. echinospermum	18	16	Turkey
C. judaicum	70	16	Afghanistan Ethiopia, India, Israel, Jordan, Lebanon, Morocco, Syria, and Turkey
C. pinnatifidum	42	16	Ethiopia, Israel, Lebanon, Syria, and Turkey
C. reticulatum	36	16	Turkey
C. yamashitae	7	16	Afghanistan
Perennial			
C. anatolicum	3	14, 16	Turkey
C. canariense	1		Spain
C. floribundum	1		Turkey
C. macracanthum	5		Pakistan
C. microphyllum	52		India and Pakistan
C. montbretii	2	16, 24	Turkey
C. multijugum	1		Russian Federation
C. nuristanicum	2		Pakistan
C. pungens	9	14	Afghanistan
C. rechingeri	1		Afghanistan
Total	308		

[a]Saxena, M.C. & K.B. Singh (eds) 1987, pp 1–34. The Chickpea. C.A.B International, Wallingford, Oxon, UK

5.5 Documentation

Documentation of information on germplasm collections is critical for efficient genebank operations. Information on passport, characterization, inventory (season and location seeds produced, seed viability and date germination test conducted, date seed stored, moisture content, quantity and availability of seeds, and 100-seed weight) and distribution of each accession is maintained at ICRISAT using Genebank Information Management System (GIMS). GIMS is a standalone facility developed internally to meet the demands of genebank documentation activities at ICRISAT. Passport information regarding chickpea germplasm stored at ICRISAT genebank can be browsed through http://genebank.icrisat.org/ and http://www.genesys-pgr.org.

5.6 Access to the Collection

All the FAO-designated germplasm are available to researchers after signing with ICRISAT the Standard Material Transfer Agreement of International Treaty on Plant Genetic Resources for Food and Agriculture. To date, ICRISAT

Table 5.3 Range and mean as observed for quantitative traits in entire collection as well in desi, Kabuli and pea-type chickpea accessions evaluated at Patancheru, India

Character	# accessions characterized	Entire		Desi		Kabuli		Pea	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
Plant height (cm)	20,219	14.0–105.7	39.2	14.0–80.8	36.7	15.0–96.6	45.9	19.0–105.7	41.3
Plant width (cm)	20,287	13.3–210.8	45.1	13.3–187.0	43.2	14.0–187.0	51.1	19.0–210.8	43.7
Basal primary branches number	20,284	0.2–15.7	2.8	0.2–15.7	2.8	0.7–11.2	2.8	1.0–6.0	2.7
Apical primary branches number	20,284	0.0–12.0	1.6	0.0–12.0	1.6	0.0–7.8	1.5	0.0–8.0	1.7
Basal secondary branches number	20,284	0.0–15.2	3.2	0.0–15.2	3.3	0.0–13.7	3.1	0.0–10.7	3.0
Apical secondary branches number	19,982	0.0–28.2	4.7	0.0–28.2	4.9	0.0–21.0	4.1	0.0–15.2	4.5
Tertiary branches number	20,283	0.0–30.8	1.3	0.0–30.8	1.2	0.0–24.8	1.6	0.0–13.0	1.1
Days to flowering	20,339	28–152	62	28–152	61	31–102	66	39–100	62
Flowering duration	18,319	11–104	33	13–104	34	11–99	33	14–93	36
Days to maturity	20,217	70–196	116	70–196	114	77–164	123	86–173	117
Pods per plant	20,210	2.0–263.0	42.0	2.0–263.0	46.7	2.0–202.0	32.4	5.0–155.0	37.7
Seeds per pod	20,151	1.0–3.2	1.2	1.0–3.0	1.3	1.0–2.9	1.1	1.0–3.2	1.2
100 Seed weight (g)	20,285	1.0–71.1	17.9	1.0–53.6	14.4	1.7–71.1	27.5	2.7–59.9	18.7
Seed yield (kg ha^{-1})	20,167	22–5130	1250	41–5130	1314	22–3464	1079	39–3840	1217
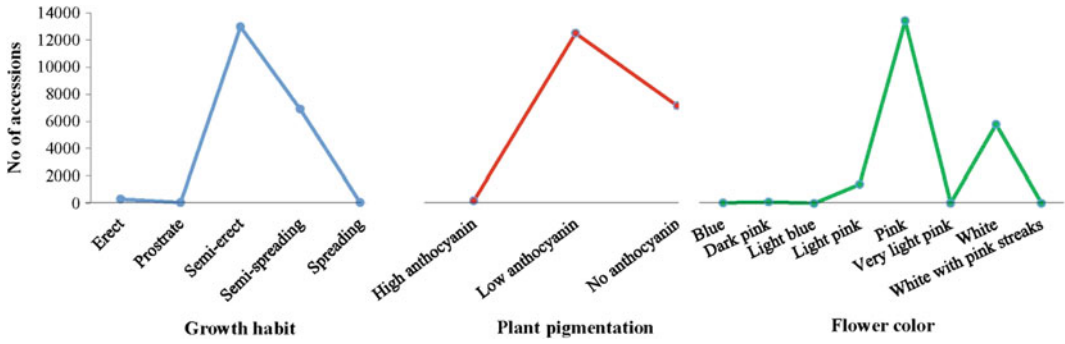
Fig. 5.1 Variation in growth habit, plant pigmentation, and flower color as observed in entire collection of chickpea germplasm maintained at ICRISAT Genebank
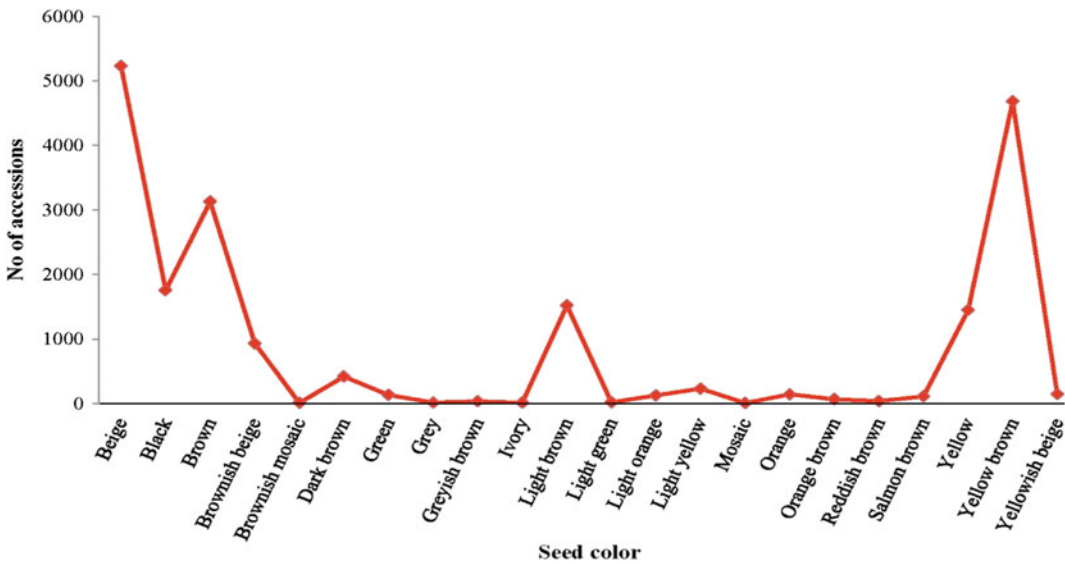
Fig. 5.2 Variation in seed color as observed in entire collection of chickpea germplasm maintained at ICRISAT Genebank

genebank has distributed 350,958 chickpea germplasm samples to researchers in 88 countries, with Indian NARS (27%) and ICRISAT scientists (56%) being the largest beneficiary, and 87 other countries receiving the remaining 17% of seed samples distributed.

5.7 Global Crop Register for Chickpea

Germplasm register provides better knowledge on unique holdings in addition to passport information on collections, and an opportunity to improve

accuracy of the existing data on germplasm collections. Access to the crop-specific accession based information on germplasm collections would benefit the germplasm user community. The 'Global Crop Register for Chickpea' was developed by ICRISAT for priority collections and collections maintained in common (GPG2 Activity 3.3) at different genebanks. This chickpea crop register is a searchable database via Web site through which the global chickpea data of ICRISAT (20,602 accessions), ICARDA (13,818 accessions), and USDA-ARS (12,426 accessions) could be cross-referred through ICRISAT

chickpea data set to identify unique chickpea collections maintained by each genebank.

5.8 Forming Representative Subsets for Trait Discovery and Utilization

Representative subsets: Both conventional core and mini core collections and genotype-based reference set have been formed to enhance greater utilization of chickpea genetic resources in breeding and genomics. The core collection consists of 1956 accessions (Upadhyaya et al. 2001), while mini core collection 211 accessions (Upadhyaya and Ortiz 2001). For forming genotype-based reference set, a global composite collection (3000 accessions) was formed composed of 80% landraces, 9% advanced breeding lines, 2% cultivars, 1% wild species, and 8%

accessions of unknown origin (Upadhyaya et al. 2006). This composite collection was then molecularly profiled using 50 SSR markers and the genotyping data on 48 SSRs was statistically analyzed to form a reference set comprised of 300 genetically most diverse accessions, capturing 78% allelic diversity of the 1683 composite collection alleles (Upadhyaya et al. 2008). Mini core collection is an ideal resource for allele mining, association genetics, mapping and cloning of genes, and applied breeding for the development of elite genetic materials/cultivars.

Stress tolerance: Researchers at ICRISAT and elsewhere have extensively evaluated mini core collection and reported a number of accessions with multiple stress tolerance (Table 5.4), for example, ICC 6874 and ICC 14402 resistant to drought, salinity, heat, fusarium wilt, and legume pod borer; ICC 12155 to drought, salinity, heat, fusarium wilt, and botrytis gray mold;

Table 5.4 Sources of resistance to abiotic and biotic stress using mini core collection in chickpea

Stress	Summary of stress resistant germplasm	References
Abiotic stress		
Drought	ICC# 283, 456, 637, 708, 867, 1205, 1422, 1431, 1882, 2263, 2580, 3325, 4495, 4593, 4872, 5337, 5613, 5878, 6874, 7272, 7323, 7441, 8261, 8950, 10399, 10945, 11121, 11944, 12155, 12947, 13124, 14402, 14778, 14799, 14815, 15868, 16524, 16796	Reviewed in Upadhyaya et al. (2013)
	ICC 7571	Kashiwagi et al. (2013)
Salinity	ICC# 283, 456, 708, 867, 1431, 2263, 2580, 3325, 4495, 4593, 4872, 5613, 5878, 6279, 6874, 7272, 7441, 8261, 9942, 10399, 10945, 11121, 11944, 12155, 13124, 14402, 14778, 14799, 15868, 16524, 16796	Reviewed in Upadhyaya et al. (2013)
Heat	ICC# 283, 456, 637, 708, 1205, 1882, 2263, 4495, 5613, 5878, 6874, 7441, 10945, 11121, 11944, 12155, 13124, 14402, 14778, 14799, 14815, 15868	
Chilling stress	Reproductive stage: ICC 16348 and ICC 16349	Kumar et al. (2011)
Biotic stress		
Fusarium wilt	ICC# 1710, 1915, 2242, 2277, 2990, 3325, 4533, 5135, 6279, 6874, 7184, 7554, 7819, 9848, 12028, 12037, 12155, 13219, 13441, 13599, 13816, 14199, 14402, 14831, 15606, 15610	Reviewed in Upadhyaya et al. (2013)
Dry root rot	ICC# 1710, 2242, 2277, 11764, 12328, 13441	
Ascochyta blight	ICC# 1915, 7184, 11284	
Botrytis gray mold	ICC# 2990, 4533, 6279, 7554, 7819, 9848, 11284, 11764, 12028, 12037, 12155, 12328, 13219, 13599, 13816, 14199, 15406, 15606, 15610	
Legume pod borer	ICC# 3325, 5135, 6874, 14402, 14831, 15406, 15606	
Herbicide	ICC# 2242, 2580, 3325	

ICC 3325 to drought, salinity, fusarium wilt, and herbicide; ICC 6279 to salinity, fusarium wilt, and botrytis gray mold, and ICC 2580 to drought, salinity, and herbicide. More importantly, many of these accessions on average produced about 1.5 t seed yield ha^{-1} (Upadhyaya et al. 2013), thereby agronomically comparable with controls.

The physiological basis of stress tolerance revealed that conservative water use (i.e., less during the vegetative growth stage could keep more soil water available during reproductive growth) in addition to drought avoidance root traits are associated with improved grain yield under drought stressed environments in chickpea (Zaman-Allah et al. 2011; Purushothaman et al. 2013; Kashiwagi et al. 2013). In addition, canopy temperature depression (CTD) is positively associated with grain yield and biomass, and thereby a cooler canopy temperature at mid reproductive stage can be used as selection criterion for drought tolerance (Purushothaman et al. 2015). For heat stress, researchers used heat tolerance index as measure of tolerance to identify heat stress tolerant chickpea germplasm (Krishnamurthy et al. 2011; Devasirvatham et al. 2015). Phenology is negatively correlated with grain yield at high temperature, while plant biomass, pod number, filled pods/seeds plant^{-1} are positively correlated. Upadhyaya et al. (2011) evaluated 35 early maturing germplasm accessions for their tolerance to heat stress and identified tolerant lines. Cold tolerant chickpeas are less affected due to stress-related leaf injury but showed greater ascorbic acid and proline than cold-sensitive germplasm (Kumar et al. 2011). Further, they detected higher activity of enzymes related to carbohydrate metabolism such as β-amylase, invertase, and sucrose synthase in cold tolerant than cold susceptible germplasm.

Early maturity: Early maturity helps chickpea to avoid terminal drought and heat stress, and thereby diverse sources of early maturity germplasm are needed to enhance chickpea adaptation in the subtropics. Upadhyaya et al. (2007) detected substantial genetic variation for days to flowering and maturity among 28 early maturing desi chickpea germplasm across five environments. ICC# 11040, 11180, 12424, 16641, and 16644 were earliest to mature, similar to or earlier than controls (Harigantars and ICCV 2), and produced about 23% more seed yield than the mean of the controls. ICC# 16641 and 16644 also showed higher 100-seed weight than controls (Annigeri and ICCV 2). Further analysis delineated this germplasm into three clusters, with maturity the main basis of delineation of the first cluster from others, while pod yield and its associated traits the basis for delineation of the second cluster from the others. Seed size is an important trait in Kabuli chickpea, and those with a 100-seed weight of >40 g fetch higher market price, largely because of consumer preference. When evaluated large-seeded Kabuli germplasm lines, Gowda et al. (2011) identified a few large-seeded high-yielding lines such as ICC 17109 and ICC 17452 (100-seed weight >50 g) with moderate stable seed yield across environments. Thus, these germplasm would be useful in breeding broad-based, early maturing and high-yielding cultivars.

Seed nutritional traits: Widespread micronutrient malnutrition results in an enormous negative socio-economic impact on the society, and crop biofortification is an ideal approach to minimize the adverse impact of micronutrient malnutrition. Similarly, protein is an important nutrient. Research to date suggests adequate genetic variation for seed iron (Fe), zinc (Zn), and protein among chickpea mini core accessions, i.e., from 54 to 76 ppm Fe and 31 to 59 ppm Zn and from 19.2 to 23.7% protein. Accessions with highest seed Fe, Zn and protein content could be used in breeding programs. Diapari et al. (2014) reported eight SNP loci associated with Fe and/or Zn concentrations in chickpea. One SNP on chromosome 1 is associated with both Fe and Zn. Three and two SNPs on chromosome 4, respectively, are associated with Zn and Fe. Two additional SNPs, one on chromosome 6 and the other on chromosome 7, were also found associated with high Fe and Zn concentrations, respectively. These SNPs after

validation could be used in marker-assisted breeding to enhance seed nutritional value (Fe and Zn) of chickpea.

5.9   Broadening Cultigen Genepool Using Wild *Cicer* Species

Species description and trait discovery: The genus *Cicer* comprises 43 wild species (35 perennials and 8 annuals) and a cultivated chickpea (*C. arietinum* L.), which were grouped into three gene pools based on crossability with chickpea. The primary gene pool consists of chickpea and the progenitor species, *C. reticulatum*, which is freely crossable with chickpea. The secondary genepool consists of *C. echinospermum*, a species that is crossable with chickpea, but with reduced fertility of the resulting hybrids and progenies. The remaining six annuals and 35 perennial species form the tertiary genepool, which require specialized techniques for gene transfer into the cultivated genetic background (reviewed in Sharma et al. 2013). *Cicer* species have shown a very high level of resistance to ascochyta blight, botrytis gray mold, fusarium wilt, and pod borer and tolerance to drought, cold, and heat stress (reviewed in Sharma et al. 2013). An accession from *C. echinospermum* showed reproductive tolerance at lower temperature (10 °C) under field conditions, which commenced podding earlier and yielded more than Rupali, the most productive chickpea. Further, when this accession was evaluated under controlled environment conditions, pollen germination, viability and frequency on the stigma surface, and pod set in relation to cultivated chickpea were unaffected by low post-anthesis temperatures (13/7 °C). *Cicer echinospermum* is therefore considered a good source of low temperature tolerance (Berger et al. 2012) and supports previous observations about freezing tolerance in *C. echinospermum* accessions (Saeed et al. 2010). *Cicer reticulatum* and *C. pinnatifidum* are also reportedly resistant to drought and heat stress (up to 41.8 °C) (Canci and Toker 2009).

Pre-breeding: It refers to the development of intermediate products with specific characteristics and minimum linkage drag that breeders can use in their breeding programs to develop productive cultivars. Ascochyta blight and botrytis gray mold are two devastating diseases in chickpea. Crosses involving cultivated and wild *Cicer* (*C. echinospermum*, *C. reticulatum*, and *C. pinnatifidum*) resulted in progenies with resistance to ascochyta blight and/or botrytis gray mold (Ramgopal et al. 2013; Kaur et al. 2013). Further, *C. reticulatum* contributed resistance to cyst nematode, *C. echinospermum* to cold tolerance, and *C. reticulatum* and *C. echinospermum* to early maturity and large seed weight in chickpea (reviewed in Sharma et al. 2013). Pod borer (*Helicoverpa armigera*) is one of the major constraints to chickpea production, with very low level of resistance in cultivated germplasm. *C. reticulatum* is reported resistant to pod borer. At ICRISAT, work is in progress to develop pre-breeding populations including resistance to biotic stresses such as botrytis gray mold, dry root rot, and pod borer using cultivated chickpea and *C. reticulatum*, and *C. echinospermum* accessions following simple and complex crosses (Shivali Sharma, ICRISAT, person. commun.).

5.10   Conclusion

Climate change and variability are likely to constrain chickpea production worldwide. Significant progress has been achieved since the formation of core and mini core subsets in identifying accessions with agronomically beneficial traits (early maturity, seed size, seed yield, seed nutritional traits, and stress tolerance) for use in chickpea breeding and genomics. Several sources of resistance to stress have been identified among wild Cicer accessions and efforts are on to develop pre-breeding populations (intermediate products) at ICRISAT and elsewhere to facilitate greater access and use of such lines in chickpea breeding. The chickpea researchers can access germplasm by signing material transfer agreement with ICRISAT.

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Advances in Chickpea Genomic Resources for Accelerating the Crop Improvement

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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

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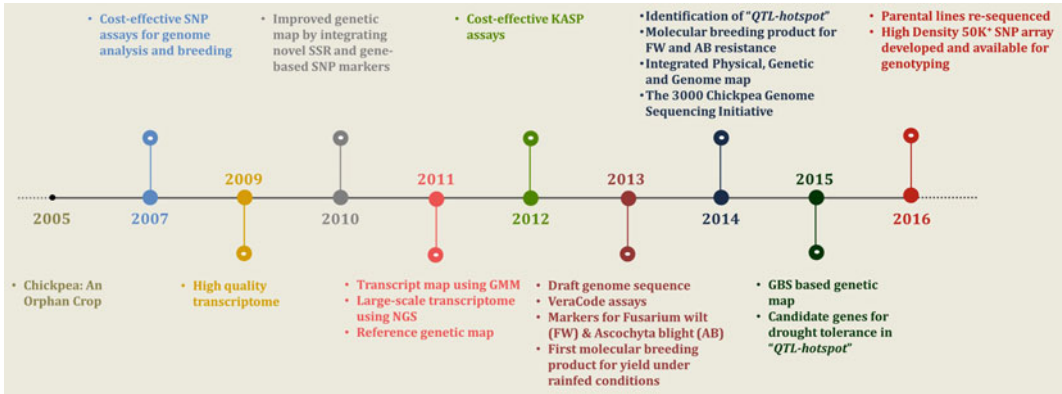
Fig. 6.1 Account of the significant accomplishments made in the field of development of genomic resources and their deployment in chickpea crop improvement

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 416 Mb 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)

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$_3$F$_3$) 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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Classical Genetics and Gene Mapping

7

Amit Deokar and Bunyamin Tar'an

Abstract

The search for the genetic factor associated with many important traits in chickpea has been focused on two major techniques, linkage (and QTL) mapping and candidate gene approach. Linkage mapping is the process of systematically scanning the plant genome of both intraspecific (within a species) and interspecific (across different species) populations segregating for the trait of interest using variable also known as polymorphic DNA segments or single nucleotide whose position on the genome is known, as such they are collectively called as genetic markers. Using those populations, researchers can identify genetic regions associated with or linked to the trait by observing that the affected lines share certain marker variants (i.e., alleles) located in those regions more frequently than would be expected by random chance. These regions were then often being isolated for further analysis and characterization of the responsible genes. Linkage mapping techniques have already resulted in the identification of several potential DNA regions that may contain important genes for plant and seed morphology, flower and seed color, disease resistance, and other important traits in chickpeas such as double podding, nodulation, and resistance to herbicide. The primary advantage of linkage mapping is that researchers do not need prior knowledge of the physiology underlying the traits. The candidate gene analysis will then enable the researchers to examine the validity of the genetic basis of the traits. This chapter discusses examples of linkage and gene mapping in chickpea and some potential candidate genes underlying the traits.

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7.1 Linkage Mapping and Candidate Gene Analysis

Genetic mapping is one of the important tools in the genetic study and provides insight into the structural organization of an individual genome, and wide application in crop improvements through genes and quantitative trait locus (QTL) mapping. In the early phase of chickpea genomic studies, due to the limited genetic diversity detected in the cultivated gene pool and/or lack of marker system that can effectively detect the genetic polymorphisms, forced the researchers to use interspecific crosses to generate linkage maps. However, with the advent of genetic markers and high-throughput genotyping systems, these limitations had been overcome and several high-density, saturated intraspecific genetic linkage maps have now been available (Deokar et al. 2014; Kujur et al. 2015). These high-density saturated maps could potentially be used in fine-scale gene and QTL mapping, map-based cloning of important agronomical and physiologic traits, and also whole-genome assembly process.

By the availability of highly saturated genetic maps with gene-based markers (functional markers), candidate gene analysis can serve as an effective strategy for rapid gene discovery. Candidate genes are the gene associated with variation in trait and with known or predicted function based on their involvement in biochemical or regulatory pathways associated with the trait of interest.

Candidate gene mapping in chickpea has been initiated in the early 2000s with the genetic mapping of resistance gene analogs (RGAs) as a candidate resistance gene and analyzed their association or co-localization with the QTLs for fusarium wilt and Ascochyta blight (Huettel et al. 2002; Tekeoglu et al. 2002). Moreover, the genetically mapped candidate genes would be a more effective choice than the gene/QTL flanked anonymous markers for marker-assisted selection (MAS) as the candidate gene-based markers are derived directly from the protein-coding region of a gene and thus are in complete linkage with the allele associated with the trait of interest. In

this section, we summarized the progress and current status of linkage mapping and candidate gene analysis in chickpea.

Early Generation Linkage Maps

The early generation linkage maps of chickpea were based on morphological markers, and later isozyme markers were added to the maps. Due to limited variation for morphological and isozyme markers within the cultivated chickpeas (*Cicer arietinum* L), interspecies mapping populations derived from crosses between *Cicer arietinum* and *Cicer reticulatum* have been used to develop chickpea genetic maps with greater number of markers (Tuwafe et al. 1988; Gaur and Sltinkard 1990). In the very first attempt to generate linkage map for chickpea, 13 isozymes and three morphological markers were mapped into seven linkage groups (Gaur and Slinkard 1990). With the advent of restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers, genetic maps with a higher number of markers were generated (Simon and Muehlbauer 1997; Idnani 1998). These maps were successfully used to study homology between chickpea, pea, and lentils and identified five conserved genomic regions (Simon and Muehlbauer 1997). Eventually, further genetic diversity analysis revealed that the polymorphism among chickpea accessions detected by RFLP and RAPD markers was as low as that detected by isozyme markers (Sant et al. 1999). Microsatellite or simple sequence repeats (SSR) marker has significantly changed the practical utility of DNA-based markers and eventually becomes the marker of choice in several plant species including chickpea (Gupta and Varshney 2000). A total of 174 microsatellite markers were reported by Winter et al. (1999), and a genetic map of 120 SSR markers consisted of 11 linkage groups covering 623 cM was reported. Eventually, with the integration of different kind of genetic markers, a genetic linkage map comprised nine linkage groups, included one morphological, nine isozymes, 17 ISSR, and 89 RAPD markers covering 981.6 cM with an

average intermarker distance of 8.4 cM was developed (Santra et al. 2000). A more comprehensive genetic map was developed by Winter et al. (2000), comprises of 303 markers with 6.8 cM average intermarker distance. Tekeoglu et al. (2002) further integrated 50 SSR markers and a resistance gene analog (RGA) locus into the linkage map reported by Santra et al. (2000) and improved the map resolution to an average intermarker distance of 7.0 cM. This was the first time a candidate gene associated with disease resistance was genetically mapped in chickpea. Further Huettel et al. (2002) identified 13 different RGAs from *Cicer* species, and genomic organization and map position of these candidate genes were studied using CASP and RFLP analysis. Six polymorphic RGAs were genetically mapped to LG 2, LG3, LG 5, and LG 6 of an interspecific map derived from ICC 4958 (*C. arietinum*) × PI489777 (*C. reticulatum*). Rajesh et al. (2002) genetically mapped RGA protein kinase gene (RGAPto-kin1) on chickpea LG 5 of another interspecific mapping population derived from FLIP 84-92C (*C. arietinum*) and PI 599072 (*C. reticulatum*).

Until early 2000, several linkage maps with a large number of molecular markers and mapped QTLs were available for chickpea; however, their potential utility was limited due to the use of interspecific (cultivated and related wild species) rather than intraspecific populations for linkage mapping. The genetic maps derived from interspecific cross may not represent the true recombination distance and map order of the cultivated genome (Flandez-Galvez et al. 2003), which was further illustrated by Radhika et al. (2007) through comparison of interspecific and intraspecific maps. Additionally, the markers mapped in the initial interspecific linkage maps were also found less polymorphic in the cultivated chickpea lines and limit their utility in marker-assisted selection for disease resistance (Tekeoglu et al. 2002). Additionally, complex composition of the interspecific mapping population, relatively low resolution, and a high number of unallocated markers and accessibility of a large number of polymorphic SSR markers leads to new intraspecific linkage maps. Cho

et al. (2002) developed the first intraspecific linkage map of chickpea based on a cross of ICCV 2 and JG 62 using 80 (55 STMS, 20 RAPDs, 3 ISSR, and 2 morphological) markers. A more comprehensive intraspecific map with 66 markers comprised of 51 chickpea-STMS, three ISSR, and 12 RGA was developed by Flandez-Galvez et al. (2003) and consisted of eight linkage groups that spanned 534.5 cM with an average marker density of 8.1 cM. Four out of 12 RGAs were clustered on LG 3, and the remaining were located on LG 1, LG 2, LG4, and LG6. In an effort to genetically map the candidate genes involved in plant defense response, 47 novel genes were integrated into an intraspecific map. These genes were distributed throughout the genome, with 3–5 genes per linkage group (Pfaff and Kahl 2003). In addition to RGAs, Abbo et al. (2005) incorporated Cytochrome P450-based markers (CytP450) in an intraspecific genetic map consisting of nine linkage groups and a total length of 344.6 cM. An intraspecific genetic linkage map derived from desi × kabuli cross was developed using flower color, double podding, seed coat thickness and resistance to fusarium wilt race 0 (*foc-0*) and 160 molecular markers (Cobos et al. 2005). Another genetic map derived from desi × kabuli cross was developed using 144 SSR markers and flower color as a morphological marker (Tar'an et al. 2007). Till the end of 2007, several intra- and interspecific genetic linkage maps were reported in chickpea. Radhika et al. (2007) generated a composite integrated intraspecific genetic linkage map by integrating two intraspecific individual genetic maps. The integrated genetic map consisted of 230 molecular markers (44 RAPDs, 16 ISSRs, 165 SSRs, 2 RGAs, 1 ASAP) and two morphological markers (double podding and seeds per pod) grouped into eight linkage groups. The integrated map covered 739.6 cM with an average intermarker density of 3.2 cM. Similarly, a composite integrated interspecific genetic linkage map was generated by integrating two interspecific individual genetic maps (Palomino et al. 2009). This composite integrated interspecific genetic linkage map consisted of 169 markers grouped into eight linkage groups and

spanned over 751 cM. Six RGAs were also incorporated into this composite integrated interspecific genetic linkage map using cleaved amplified polymorphism sequence (CAPS) and derived CAPS (dCAPS) marker system. Using similar marker system, Nayak et al. (2010) developed an interspecific genetic map with 71 gene-based SNP markers along with 450 SSR markers. The mapped candidate genes include P40, chitinase, NBS-LRR resistance gene homologs (RGH), which further used as anchor points to detect synteny between chickpea and Medicago genome. Millan et al. (2010) generated a consensus genetic map by integrating ten different genetic maps derived from five wide interspecific crosses (*C. arietinum* × *C. reticulatum*) and five narrow intraspecific (Desi × Kabuli types) crosses. Millan et al. (2010) also report the differences in marker order between interspecific and intraspecific crosses.

Functional or Transcript Linkage Maps

With the availability of large-scale mRNA sequences as ESTs, an approach to direct gene mapping as EST-SSR and EST-SNPs markers has been successfully used to generate functional or transcript maps in chickpea. First, large-scale interspecific transcript linkage map of 126 gene-based markers (along with 174 SSR markers), spanned about 767 cM with an intermarker distance of 2.5 cM, was published by Gujaria et al. (2011). Using the same interspecific mapping population and a new set of EST-derived genic molecular markers (GMM), along with previously published 108 markers, a transcript map of 1,498 cM with an average intermarker distance of 3.7 cM was reported by Choudhary et al. (2012). Gaur et al. (2012) generated a saturated genetic linkage map with 1063 SNP markers that were mapped onto eight linkage groups spanning 1809 cM with an average intermarker distance of 1.7 cM. Hiremath et al. (2012) added 317 tentative orthologous genes (TOGs)-SNPs and reported an improved interspecific genetic map with an average intermarker distance of 0.6 cM. Further, Thudi et al. (2011)

added SSR markers from BAC-end sequences and diversity Arrays Technology (DArT) arrays and reported a comprehensive interspecific genetic map with 1291 markers. This comprehensive interspecific genetic map was further used to anchor 347 Mb of the first draft version of CDC Frontier chickpea genome (Varshney et al. 2013).

Recent advances in genotyping and sequencing technologies have offered large-scale simultaneous genome-wide SNP discovery and genotyping, which leads to development of several high-density, sub-centimorgan genetic maps in chickpea (Deokar et al. 2014; Saxena et al. 2014; Jaganathan et al. 2015; Kujur et al. 2015; Gaur et al. 2015). Recently, a high-density interspecific chickpea genetic linkage map using 834 candidate gene transcription factors (TFs)-derived SSR and SNP markers was reported (Saxena et al. 2014). This map comprising of eight LGs spanned a total map length of 949 cM with an average intermarker distance of 1 cM. The mapped transcription factors are known to be involved in the various developmental process and biotic and abiotic stress tolerance in chickpea. The TFs derived SSR and SNP markers associated with QTL can be potential candidates for marker-assisted genetic improvement in chickpea.

The availability of whole-genome sequences and genetic maps with sequence-based SNP markers has facilitated anchoring of QTL associated markers and positioning the QTLs on physical map to identify the underlying candidate genes. Anchoring of the whole-genome assemblies is another potential important application of high-density genetic linkage maps. High-density sequence-based genetic maps can facilitate anchoring of de novo assembled sequences and orients and order the small scaffolds into pseudo-chromosomes (Fierst 2015). Inconsistency between the marker in genetic map and assembled sequences can indicate a potential error in assembly. Deokar et al. (2014) generated a high-density intraspecific genetic map using 1336 Restriction-site Associated DNA Sequencing (RAD-Seq) genotyping-by-sequencing (RAD-Seq GBS) and Illumina® GoldenGate SNP genotyping

assays. This genetic map enabled alignment of 215 unplaced scaffolds and also provided potential information for improvement of the existing CDC Frontier v1.0 chickpea reference genome assembly. Gaur et al. (2015) reported a high-density interspecific genetic linkage map of 6698 markers distributed on eight linkage groups spanning 1084 cM with an average intermarker distance of 0.16 cM. This map has significantly improved the anchoring of both desi (ICC 4958) and kabuli (CDC Frontier v 1.0) draft genome assembly.

7.2 Gene Mapping

Genetic linkage maps were effectively used for mapping genes and QTLs associated with several important agronomical and physiologic traits. In chickpea, several monogenic traits were mapped as morphological markers, such as flower and seed coat color. Most of the earlier studies of traits such as seed size, flowering time, and resistance to fusarium wilt were measured as qualitative traits and genetically mapped as a single gene. However, in the course of time, these traits were also analyzed as quantitative traits and mapped as QTL. Interestingly, some of these QTLs reported earlier were also located in the vicinity or overlap with the single gene locus reported earlier for the same trait. For example, fusarium wilt resistance for race 0 and 1–5 was found in the cluster on LG2, and QTLs for the fusarium wilt resistance were also mapped to the same location (Cobos et al. 2009). Here we summarized genetic mapping of some of the qualitative traits.

Plant Growth Habit (Hg)

Chickpea has a short stature with morphological variations for most of its traits (Ladizinsky and Alder 1976). A determinate growth habit would be desirable especially in areas where growing conditions often lead to excessive vegetative growth. Through mutation breeding, van Rheenen et al. (1994) identified a mutant with determinate growth habit. Further study indicated that

the inheritance followed a digenic model with epistatic effects. The authors proposed DT as the allele conditioning for the determinate growth habit.

Based on the angle of the branches from the vertical axis, the chickpea plant could be erect to semi-erect and spreading with a few or highly branched (van der Maesen 1972). Erect to semi-erect are among the main selection criteria for the new cultivars especially in the production areas where mechanization facilities are available. Earlier study by Singh and Shyam (1959) reported that plant growth habit is inherited as a single gene. Prostrate growth habit was dominant over semi-erect growth habit. For the first time, growth habit gene (Hg) was genetically mapped using an intraspecific cross between PI 489777 (C. reticulatum) and PI 489776 (Cicer echinospermum). The Hg gene was found linked with an isozyme marker 6-phosphogluconate dehydrogenase (Pgd-c) and separated by 18 map units (Kazan et al. 1993). The prostrate growth habit gene of pea was also found linked with Pgd-c locus, indicated conserved linkage group in pea and chickpea (Kazan et al. 1993). Further Winter et al. (2000) mapped the isozyme marker 6-phosphogluconate dehydrogenase on LG3 of the same interspecific cross. Cobos et al. (2009) using another intraspecific linkage map developed for a cross between ICCL 81001 (C. arietinum) and Cr5-9 (C. reticulatum) mapped the Hg locus on LG3. A major gene for plant growth habit (prostrate) was mapped between two SSR markers, TA34 and TA142 on LG3 of an interspecific cross between cultivated chickpea line ICC 3996 with semi-erect growth habit and ILWC 184 (C. reticulatum) line with prostrate growth habit (Aryamanesh et al. 2010). All these reports indicated that the gene for growth habit (Hg) has been constitutionally mapped on LG3 in different genetic backgrounds. Recently, Ali et al. (2015) analyzed the plant growth habit using an intraspecific ILC72 (C. arietinum) × Cr5-10 (C. reticulatum) and interspecific (WR315 × ILC3279) recombinant inbred lines (RILs) and identified the Hg1 locus on LG 3 same as reported previously. However, one new locus associated with growth habit

(Hg2) was also identified on LG1 flanked by SSR marker TA1 and TA8. This newly reported plant growth habit gene (Hg2) together with previously reported Hg1 gene indicated that the growth habit in chickpea might be regulated by two major genes (see Chap. 6).

Leaf Morphology

Leaf types in chickpea are governed by two genes "ml" and "sl" which regulate the leaf types through supplementary gene actions (Pundir et al. 1990). Dominant ml gene (ml + sl/.sl) produces multi-pinnated leaves, recessive ml gene (ml./ml.), irrespective of the state of the second gene produces simple leaves, whereas both the genes in dominant form (ml + sl +/…) produce normal leaves. Normal leaf type was dominant over all other leaf types (Atanasova and Mihov 2006; Danehloueipour et al. 2008). Leaf morphology gene was the first trait to be tagged with another morphological marker flower color. Two non-allelic leaf morphology genes filiform (fil) and simple leaves (slv) were found in linkage with white flower color gene "w2" and root nodulation gene rn3, respectively (Davis 1991). The "w2" and "fil" were separated by 5 and 14 map unit in coupling and repulsion phase, respectively, whereas "rn3" and "slv" were separated by 5 and 11 map units in coupling and repulsion phase, respectively, on two different linkage groups (Davis 1991). None of these leaf morphology-related genes were genetically mapped or tagged using molecular markers. However, Banerjee et al. (2001) found an association of another leaf morphology-related trait (leaf size) with RAPD marker OPA12c (600), OPD15b (1260), OPK17a (936), and OPA4 (800) on LG1.

Flower and Seed Color

Chickpea flowers are solitary, the calyx united with the white, pink, or blue corolla (Duke 1981). Atanasova and Mihov (2006) reported that flower color in chickpea is controlled by a single gene. The violet flowers are dominant over the white flowers. Chickpea flower and seed color were used as morphological markers and genetically mapped in several intra- and inter-specific maps. A single major gene controlling pigmentation in chickpea flowers was mapped by Simon and Muehlbauer (1997). Kazan et al. (1993) mapped the loci determining pigmentation of flower (P) and seed coat color (T3) along with epicotyl color (Gst) and seed surface (Rs) on same linkage group (LG 8) along with the isozyme locus encoding glucose-l-phosphate transferase (Gpt2). Similar observation of association of these morphological traits was also reported by Pawar and Patil (1983). Based on these findings, it was hypothesized that among the three linked genes (P, T3, and Gst), one could be involved in anthocyanin production in corolla, epicotyl, and seed coat, and remaining two genes modify the expression of the anthocyanin production. Morphological marker for anthocyanin pigmentation was mapped as locus P in an interspecific map (Santra et al. 2000). A major gene (C) controlling pigmentation of flower, stems, and seeds was mapped to linkage group 8 with loose linkage with SSR marker TR33 (Cho et al. 2002). Cobos et al. (2005) mapped flower color (B/b) on LG 1 linked with SSR marker GAA47. The SSR marker GAA47 also linked with anthocyanin pigmentation locus P reported by Santra et al. (2000). Tar'an et al. (2007) mapped flower color (fc) gene on LG 4 linked with SSR marker GAA47. The presence of GAA47 indicates that LG 4 of Tar'an et al. (2007) corresponds to LG 1 of Cobos et al. (2005) and suggests that the fc could be the same as earlier reported P and B gene for flower color in chickpea.

Seed Shape and Size

The chickpea pods normally contain one to two seeds. The seed coat may be smooth, rugose, or granulate (Ladizinsky and Alder 1976). The chickpea seed is characterized with beaked, often angular and wrinkled. The length and width of chickpea seeds vary from 4–12 and 3–8 mm,

respectively. Inheritance study of chickpea seed shape indicated that one to three genes interacting in dominance epistasis type control the seed shape. The pea-type seed shape is dominant in both desi and kabuli, and the desi type is dominant to kabuli-type seed shape (D'Cruz and Tendulkar 1970; Kumar and Singh 1995). Knights et al. (2010) also reported that seed shape in desi is controlled by a major gene, and the rounded shape was dominant over the angular shape. Most of the earlier reported analyses were based on the classification of seed shape into three distinct classes as desi, kabuli, or pea shape based on visual observations. Hossain et al. (2010a) proposed a novel method for seed size classification denoted as roundness index (RI). Based on segregation pattern of "RI" in RIL population, two gene controls of seed shape were observed and identified a QTL located on LG2 between H1AO6 and TA110 associated with the angular seed shape.

Variable reports of seed size regulation from Monogenic (Argikar 1956), oligogenic (Patil and D'Cruz 1964) to polygenic (Kumar and Singh 1995; Malhotra et al. 1997) were observed in chickpea. Two additive genes 'Bsd' and 'Smsd' controlling seed size have been reported in chickpea (Ghatge 1993). Both dominant gene control (Kumar and Singh 1995; Upadhyaya et al. 2006) and recessive (Malhotra et al. 1997) gene control for the small and large seed size have been observed in chickpea. In most of the initial reports, inheritance of seed size was evaluated as a qualitative trait by converting seed weight data into normal, small, and medium groups and analyzed for the Mendelian ratios. However, these studies were mainly conducted to analyze the gene effect and heritability of seed size trait (Kumar and Singh 1995; Malhotra et al. 1997).

Seed size represented by 100-seed weight has been efficiently used in QTL analysis. Cho et al. (2002) using 100-seed weight as a representative of seed size identified a major QTL on LG4 explaining 52% variation for seed size in an intraspecific cross. This locus was also associated with the number of seeds per plant. Using an interspecific cross between Hadas (*C. arietinum*)

with Cr 205 (*C. reticulatum*), Abbo et al. (2005) also identified a significant QTL on the same LG4. Major QTLs for seed size on LG 4 were also identified using different intra- and interspecific crosses (Cobos et al. 2005, 2007, 2009; Radhika et al. 2007; Hossain et al. 2010b; Jamalabadi et al. 2013; Karami et al. 2015). Additionally, minor QTLs on LG1 (Radhika et al. 2007; Hossain et al. 2010b), LG2 (Cobos 2009), and LG 8 (Cobos et al. 2007) were also reported (see Chap. 6-Table 3). A major QTL on LG1 representing 48% variation for seed size was identified using QTL-seq techniques. This QTL region corresponds to 35-Kb genomic region on chromosome 1 and comprising of six genes (Das et al. 2015). Differential gene expression analysis of these genes identified COP9 signalosome complex subunit 8 (CSN8) as a potential candidate gene associated with seed weight in chickpea.

Double Podding

A single recessive gene "s" or "slf" governing double podding in chickpea has been reported in different studies using different genetic materials (Khan and Akhtar 1934; Ahmad 1964; D'Cruz and Tendulkar 1970; Singh and van Rheenen 1994; Kumar et al. 2000). Cho et al. (2002) mapped the gene for double podding (s) to LG5 (which corresponds to LG 6 of most updated genetic map) flanked by SSR markers TR44 and TR35 using an intraspecific cross between ICCV2 X JG 62. By using near-isogenic lines (NILs) for double podding gene "s", developed from a cross between JG 62 as a source of double podding and CA2156 single podded line, TA80 a closest linked (4.8 cM) molecular marker to the double podding 's' gene was identified (Rajesh et al. 2002). Another two major genes related to a number of flowers/pods per axis, i.e., "trf" gene responsible for triple-flower/pods per peduncle (Singh and Chaturvedi 1998) and "cym" gene producing 3–9 flowers/pods per peduncle in cymose inflorescence type (Gaur and Gour 2002) have been reported in chickpea. Allelism test between "sfl" and "cym" showed that the double

and multi-flower trait is controlled by different genes (Gaur and Gour 2002; Srinivasan et al. 2008). However, the allelic relation between sfl and "trf" showed that the genes for double- and triple-flower are allelic and suggested gene symbol "sfld" for "allele" for double flower and "sflt" for the allele for the triple-flower trait (Srinivasan et al. 2008). In addition to TA80 as the closest linked SSR marker to "sfl" gene, new and more tightly linked SSR markers NCPGR33 and NCPGR128 on LG6 were identified (Radhika et al. 2007; Gaur et al. 2011). More recently, the "sfl" locus has been fine mapped and narrowed down to a 92.6-Kb region (Ali et al. 2015). The "sfl" gene genetically mapped to LG6 in four different RILs with JG 62 and ICCV 96029 as the source of double podding and CA2156, ILC 72 and ILC 3579 as a source of single podding. Using five pairs of NILs and additional SNP and SSR markers, the "Sfl" gene was further finely located between SNP marker SDSNP1 and CaGM2777. This region corresponds to 92.6-Kb region of chromosome 6 of CDC Frontier genome assembly which contains seven annotated genes. A RAX2 gene encoding R2R3 MYB transcription factor that involved in accessory side shoot formation during inflorescence development has been predicted as a potential candidate gene for double podding in chickpea.

Fusarium Wilt Resistance

Chickpea plants are affected by various biotic and abiotic stresses. Fungal diseases, especially Fusarium wilt and Ascochyta blight are among the most important biotic factors for major yield loss of this crop worldwide. The causative agent of Fusarium wilt, *Fusarium oxysporum* f.sp. *ciceris,* is a soilborne, facultative, vascular wilt fungus that provokes economically important losses in chickpea. Yield losses due to fusarium wilt can be minimized through the use of resistant cultivars. Understanding the genetics of the resistance is critical the effort to effectively develop resistant cultivars.

Genes for resistance to fusarium wilt race 1, 2, 3, 4, and 5 have been identified and mapped to different chickpea chromosomes (Sharma and Muehlbauer 2007). The H1 (*foc-1*) gene involved in resistance to race 1 was the first fusarium resistance gene tagged by a genetic marker (Mayer et al. 1997). The gene was linked to RAPD markers CS-27700 and UBC-170550. Both markers were linked with resistance locus on the same side with a genetic distance of 9 map unit. These two markers were also found to be associated with resistance to race 4 (Tullu et al. 1999). Ratnaparkhe et al. (1998) tagged the resistance for race 4 with ISSR marker UBC-855500 which is also co-segregates with CS-27700. Subsequently, with the availability of SSR markers and saturated genetic maps, several other closely linked markers to resistance to different wilt races were reported. TR59, TA59, and OPJ20600 closely linked to the *foc-0* (Rubio et al. 2003; Cobos et al. 2005, Halila et al. 2009), TA110 and H3A12 linked to *foc-1* (Sharma et al. 2004, 2005; Sabbavarapu et al. 2013), H3A12 and TA96 linked to *foc-2* (Sharma et al. 2005), TA96 and TA194 linked to *foc-3* (Sharma et al. 2004; Sharma et al. 2005; Gowda et al. 2009), TA96 and CS27 linked to *foc-4* (Ratnaparkhe et al. 1998; Tullu et al. 1998, 1999; Winter et al. 2000; Tekeoglu et al. 2000; Benko-Iseppon et al. 2003; Sharma et al. 2004, 2005) and TA59 and TA96 linked to *foc-5* (Ratnaparkhe et al. 1998; Tekeoglu et al. 2000; Winter et al. 2000; Benko-Iseppon et al. 2003; Sharma et al. 2005) were identified. Resistance gene associated with race 6 has not yet tagged or mapped in chickpea.

Genetic analysis of the all reported resistant genes associated with resistance to different fusarium races indicated that the four race-specific genes (*foc-1, foc-2, foc-3, foc-4,* and *foc-5*) were located on LG 2 (Winter et al. 2000; Sharma et al. 2005; Millan et al. 2006). Based on genetic distance among these genes, two sub-clusters of (*foc-4, foc-2,* and *foc-3*) and (*foc-5* and *foc-1*) were identified. However, this order of wilt resistant genes in the cluster identified in interspecific mapping populations was inconsistent with the order of wilt resistant genes found

in intraspecific population (Sharma et al. 2005; Sharma and Muehlbauer 2007).

Apart from the other fusarium wilt resistance genes, the *foc-01* gene was found linked with RAPD marker OPJ20600 and SSR marker TR59 located on LG3 (Rubio et al. 2003; Cobos et al. 2005), which corresponds to LG5 of a most recent genetic map. Halila et al. (2009) also found a strong association of TR59 with wilt resistance gene *foc-01*. Second resistance gene for race 0 (*foc-02*) was found on LG2 closely linked with foc5 (Huettel et al. 2002; Flandez-Galvez et al. 2003; Cobos et al. 2009; Palomino et al. 2009). Map location of all the reported genes indicated that two major chromosomal loci on LG2 and LG5 govern resistance to the all reported races of fusarium wilt (see Chap. 6 Table 1). The availability of DNA-based genetic markers linked to fusarium wilt resistance would allow breeders to use them in marker-assisted selection to directly infer the genotype of very young plants and avoid time delays caused by phenotype testing, and simultaneously avoid confounding effects of genotype × environment interactions.

Herbicide Resistance

Genetic mapping of herbicide resistance in chickpea was first reported by Thompson and Tar'an et al. (2014). A mutant copy of a chickpea AHAS gene encoding acetohydroxyacid synthase was identified as involved in resistance to imidazolinone (IMI) herbicide and genetically mapped onto LG 5. An intraspecific F2 mapping population derived from a cross between CDC Leader (IMI susceptible) and ICCX860047-9 (IMI resistant) lines was used for analyzing the IMI resistance in chickpea. The resistance to IMI segregated as a single gene and found linked to two SNP markers namely Cav1sc310.1p304295 and Cav1sc1.1p4940145 located to LG5. An allele-specific SNP marker targeting the point mutation in the AHAS1 gene was also mapped to the same locus on LG5 as the IMI resistance confirming the involvement of AHAS-1 in IMI herbicide resistance in chickpea.

Non-nodulation

Six recessive mutant genes rn1 to rn6 and one dominant mutant gene Rn7 involved in non-nodulation phenotype have been identified in chickpea (Davis et al. 1985, 1986; Davis 1988; Singh et al. 1992). Except "rn1", none of these non-nodulation genes were genetically mapped in chickpea. Recently, Ali et al. (2014) identified and fine mapped rn1 gene using a combination of F3 mapping population and NILs developed for non-nodulation trait from a cross between non-nodulation mutant line PM233 (source of rn1) and CA2139 nodulating kabuli-type landrace. The non-nodulation gene (Rn1/rn1 locus) was mapped on chickpea LG5. Medicago transcription factor NSP2 was identified as candidate genes associated with nodulations from the corresponding syntenic with Medicago chromosome 3 (Millan et al. 2010; Nayak et al. 2010). The Medicago SNF2 gene corresponds to chickpea Ca_26279 gene at the position 1,280,031-1,281,533 bp on chromosome 5. This gene was denoted as CaNSP2 as a chickpea candidate gene involved in nodulation signaling transduction pathway. The CaNSP2 gene and non-nodulating (Rn1/rn1) locus were co-segregated and mapped to the same position on LG5, concluding that CaNSP2 can use as a candidate to identify "rn1" gene and suggest a possible role of CaNSP2 in nodulation signaling pathway.

7.3 Conclusion

A combination of linkage mapping and a candidate gene approach has been the most successful method of identifying the causal genes for the trait of interest in chickpea. The candidate gene approach would be useful for quickly determining the association of genetic variants with the expressed phenotype for identifying the gene of modest effect. This approach may complement the traditional linkage mapping efforts in identifying the underlying genes for many important traits in chickpea especially those that are controlled by single or major genes and have a simple mode of inheritance as listed in this chapter. As more

resequencing efforts are being completed in chickpea and, thus, more SNPs are identified throughout the genome, some of those SNPs will also be located within the genes, thereby, allowing researchers to use the candidate gene approach on a genome-wide scale.

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Genetic Mapping and Quantitative Trait Loci

8

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Abstract

Breeding for quantitative traits has become a reality in chickpea due to the huge development of DNA molecular markers which have given rise to detailed genetic maps and fruitfully implementation of QTLs analysis. Also, it has been crucial that the availability of the whole-genome sequences in kabuli and desi chickpea types greatly assists to survey or re-localize QTLs. In this chapter, plant material more frequently employed for QTL analysis was described. Besides, the importance of an accurate phenotypic evaluation is stated. Strategies previously used to get a rapid and efficient screening for abiotic and biotic stresses as well as adaptative traits were commented. It is also provided a summary of QTLs associated with fungal diseases, drought and salt stresses, flowering, growth habit, yield- and quality-related components. Candidate genes suggested by different authors, some of them already used in marker-assisted selection, mark the beginning of new possibilities for chickpea breeders that will be able to choose the best allelic combination for agronomic traits.

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8.1 Introduction

Classical plant breeding is based on the phenotypic selection of traits of interest with the ultimate goal of assembling desirable combinations of genes in new varieties. These practices have been very effective in improving crop productivity during the past decades. However, conventional methods often encounter difficulties that can reduce the effectiveness of phenotypic selection and complicate the identification of superior genotypes (Torres 2009). In recent years, different kinds of molecular markers and highly saturated genetic maps (see Chap. 3) have been developed. These tools have made it possible to associate different genes or genomic regions with the phenotypic variation of qualitative and quantitative traits.

Quantitative traits (also known as "polygenic", "multifactorial", or "complex" traits) are usually controlled by different genes with small effects. Those effects are more strongly influenced by environmental conditions than qualitative traits, exhibiting continuous variation. Epistatic and genotype–environment interactions can also occur. Therefore, breeding for quantitative traits is not an easy task due to their complexity. The development of genetic maps allows to be focused on few or single regions that explain a considerable percentage of the total phenotypic variation of a particular trait. Hence, they simplify the selection of quantitative traits. Regions controlling quantitative traits are known as quantitative trait loci (QTL). That term was proposed by Geldermann (1975). The first example of this simplification was published at the beginning of the last century. Sax (1923) found a significant association between seed size (quantitative) and seed colour (qualitative) in bean and proposed a method to locate quantitative trait loci on a linkage map. In those days, the number of genetic markers available was minimum. However, today, the huge development of DNA molecular markers allows us to obtain saturated genetic maps and successfully implement QTLs analysis.

Molecular markers tightly linked ("in the peak") to QTLs facilitate marker-assisted selection (MAS). The MAS approach is not only a tool for speeding up the process of gene transfer, but also allows pyramiding of desirable QTLs from different genetic backgrounds. In addition, fine mapping of QTL regions facilitates a deeper understanding of plant genomics through candidate-gene analysis as well as germplasm characterization that allows its efficient utilization (Asíns 2002).

Chickpea (*Cicer arietinum* L.) genetic maps were initiated in the early 1990 of the last century with the aim of detecting markers associated with simple phenotypic traits isozymes and interspecific crosses between *C. arietinum* and each of *C. reticulatum* (wild ancestor) and *C. echinospermum* (Gaur and Slinkard 1990; Kazan et al. 1993). Later, Simon and Muehlbauer (1997) included RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers in the chickpea map establishing synthenic relationships with other legumes for the first time. The STMS (sequence tagged microsatellite site) markers developed by Hüttel et al. (1999) and Winter et al. (1999) initiated a new era in chickpea genetic maps. The first reference map was constructed by Winter et al. (2000). Also, STMS enabled the transfer of linkage information among populations and provided anchor points for establishing comparison with the model species *Medicago truncatula* genetic map (Millan et al. 2010; Nayak et al. 2010). More recently, next-generation sequencing (NGS) technologies generated in chickpea large-scale transcriptome data together with genomic markers based on single-nucleotide polymorphisms (SNPs) have facilitated the development of highly saturated second-generation genetics maps (Gujaria et al. 2011; Thudi et al. 2011; Gaur et al. 2012; Hiremath et al. 2012).

An accurate identification of QTLs on the genetic maps requires appropriate plant material and a thorough phenotypic evaluation to be able to identify candidate genes and tightly linked markers useful in MAS as it will be described in following sections.

8.2 Plant Material for QTL Analysis

In chickpea, an autogamous species, most QTLs have been detected using recombinant inbred line (RIL) populations. RILs are derived from the cross of two inbred lines and successive advance of generations by the single-seed descent method (Johnson and Bernard 1962). This type of segregating populations that can be considered as a series of homozygous lines can be multiplied without genetic change occurring. This is a great advantage because it allows to perform phenotypic evaluations in different environments and make it possible the analysis of genotype × environmental (G × E) interactions and the detection of epistatic interactions. The main disadvantage of RILs is that they are derived from two founder genotypes, and it is necessary to invest several years to reach an advanced generation (Doerge et al. 1997).

Examples of QTL analysis using F_2 populations have been also described in chickpea. F_2 are easy to construct and require only a short time to be produced compare to RILs. However, in order to include repetitions in phenotypic evaluations of quantitative traits, it is necessary either to evaluate $F_{2:3}$ progenies or to use techniques to multiply individual F_2 plants. Several authors have developed a methodology to multiply individual F_2 by stem cuttings in chickpea (Tar'an et al. 2007; Anbessa et al. 2009; Kottapalli et al. 2009).

Another issue to consider in the accuracy of QTL analysis is the presence of regions of distorted segregation in the genetic maps. In chickpea F_2 and RILs, it is possible to detect deviations of observed marker genotypic frequencies from the expected Mendelian ratio (Winter et al. 2000; Flandez-Galvez et al. 2003; Cobos et al. 2009; Castro et al. 2011). Segregation distortion can affect genes controlling the desired trait either negatively or positively. Misinterpretation of results may influence the success of breeding programmes, especially if genes or QTL for agronomically important traits is located in regions affected by distorted segregation. If the markers presenting distorted segregation are discarded and they are not included in the genetic maps, QTL linked to these markers may be missed and not considered in MAS. Castro et al. (2011) reported an example of a genomic region presenting segregation distortion where QTLs (QTL_{AR1} and QTL_{AR2}) for one of the most important diseases in chickpea (Ascochyta blight) had been located. This region, which deviated towards the susceptible parent, was confirmed in a F_2 population from the same cross. A cluster of distorted markers on LG4 was also described by Abbo et al. (2005) in a different F_2 population supporting previously mentioned results. Those deviations from expected Mendelian ratios could be caused by gametophytic factors that selectively eliminate or reduce the competition of the male or the female gamete during the reproductive development Castro et al. (2011). This fact should be taken into account by the breeders in the designing of the crossing schemes because some parental lines may have alleles linked to gametophyte loci favouring particular gametes affecting the frequency at which a particular allele is transmitted to the progeny.

Near isogenic lines (NILs) have been also used to produce fine maps of genomic regions related to genes or QTLs for agronomic traits. The advantage of NILs is that only a small target region of the genome is segregating; consequently, the genetic background noise can be eliminated. They are particularly interesting to validate specific QTL. An advantage of NILs over RILs is that they often allow the detection of minor QTLs avoiding complicated segregation of multiple loci (Keurentjes et al. 2007). In chickpea, NILs for qualitative traits have been mostly used (Rajesh et al. 2002; Castro et al. 2010; Ali et al. 2014). Recently, NILs for growth habit were a useful tool to identify a new QTL (QTL_{Hg2}) in the chickpea genetic map (Ali et al. 2015).

In order to increase possibilities of genetic variation, second-generation mapping resources have been proposed (Rakshit et al. 2012). Thus, nested association mapping (NAM) populations consist in a central parent crossed with other diverse parents in star design (Huang et al. 2011). It has been established in maize (Yu et al. 2008;

Ogut et al. 2015), showing the higher prediction abilities for joint-family QTL analysis compared to single-family QTL analysis. In chickpea, there have not been designed NAM populations on purpose to date, but combination of results from RIL populations with a parent in common was helpful to carry out a fine mapping for simple/double-pod trait (Ali et al. 2016). Furthermore, multiparent advanced generation intercrosses (MAGIC) populations have been suggested as ideal resource to generate high-density maps using germplasm of direct relevance to the breeders. To create MAGIC, *n* founder lines are taken. These lines need to be intercrossed for *n/2* generations till all the founders are combined with equal proportions. Once the intercrossing is over, RILs may be derived from them upon selfing. The use of eight founders and a fixed population of 1000 individuals is considered most appropriate for practical purposes (Cavanagh et al. 2008; Rakshit et al. 2012). The disadvantage of these types of populations is that they require long time and hard work to be generated. In addition, the statistical analysis complexity is higher than in biparental crosses. A chickpea MAGIC population has been obtained at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). It consists of 1200 F_6 lines derived from eight parents including cultivars and elite breeding lines from India, Kenya and Ethiopia. These lines provide a large number of accumulated recombination events (Gaur et al. 2012).

Apart from the plant material previously mentioned, germplasm collections have been employed to identify QTLs. Association mapping, also known as linkage disequilibrium (LD) mapping, has emerged as a tool to resolve complex trait variation down to the sequence level by exploiting historical and evolutionary recombination events at the population level. Two broad categories can be differentiated: (i) candidate-gene association mapping, which relates polymorphisms in selected candidate genes controlling phenotypic variation for specific traits; and (ii) genome-wide association (GWAS) mapping, which surveys genetic variation in whole genomes (Zhu et al. 2008). In

chickpea, candidate gene-based association analysis revealed strong genetic association of TFFDM (transcription factor functional domain-associated microsatellite) markers with three major seed and pod traits (Kujur et al. 2013). This study was performed in 96 genotypes (representing 85% diversity of 417 global core germplasm collections), which were selected based on their phenotypic and genotypic diversity estimate measures. GWAS also made it possible to detect markers associated with quality components in chickpea crop. Thus, using 94 diverse accessions of chickpea, it was possible to identify one SNP located on chromosome 1 (chr1) associated with both iron and zinc concentrations. Besides, three SNPs associated with zinc concentration and two SNPs for iron concentration were detected on chr4 (Diapari et al. 2014). Similarly, it has been possible to identify QTLs related to protein content using a set of 187 genotypes comprising both international and exotic collections (Jadhav et al. 2015).

8.3 Phenotyping

In order to locate consistent and stable QTLs in genetic maps, it is necessary to carry out an accurate phenotypic evaluation using well-designed experimental trials with the aim of getting a good correlation between phenotype and genotype. Therefore, experiments with repetitions or without repetitions but using control lines should be performed to quantify experimental errors (environmental variation). Results will depend on the heritability of the studied trait, the population size and an adequate measuring method.

Phenotypic Evaluation for Biotic Stresses

The most important biotic factors limiting chickpea production are fungal diseases such as Ascochyta blight and Fusarium wilt. Other fungal diseases such as botrytis grey mould or, in a minor extent, rust have increased in importance

in the last decade. Evaluations for resistance reaction have been done in field and under controlled conditions. It is required to include in the experiments resistant and susceptible check genotypes as well as differential lines in the case that they have been described. For aerial fungus in field experiments, highly susceptible lines should be used as disease spreaders to get homogenous infections.

A large proportion of QTL analysis for disease resistance has been focused on Ascochyta blight (AB), caused by the fungus *Ascochyta rabiei* (*Pass.*) *Labr*, that affects all aerial parts of the plant. The pathogen is favoured by cool (15–25 °C) and moist conditions (Bayaa and Chen 2011). Evaluation of AB resistance depends on several crucial factors including the choice of appropriate isolates for screening, methodology and the sources of resistance. The attempts to classify virulence in *A. rabiei* have been inconclusive because of the variable number of categories proposed by different studies, the lack of reproducibility of disease phenotypes among laboratories and the lack of comparable standard check cultivars or pathotypes (Peever et al. 2007). Field screening for AB usually consists of planting a susceptible genotype every two or four tested entries, which serves as an indicator/spreader line, scattering infected plant debris collected in the previous season and maintaining high humidity through sprinkler irrigation. If needed, entries could be sprayed with a spore suspension in the evening of a cloudy day (Pande et al. 2005). It could be interesting to include in the trial reported differential lines for different pathotypes. Usually, nonparametric methods are employed for quantifying host response and the phenotype is grouped into discrete classes. The scale developed by Singh et al. (1981) is one of the most widely employed. It is based on the severity of the infection on leaves, stems and pods, and host response is classified as: 1 = no infection, 2 = highly resistant (1–5%), 3 = resistant (6–10%), 4 = moderately resistant (11–15%), 5 = intermediate (16–40%), 6 = moderately susceptible (51–75%) and 9 = plants dead. Area under the disease progress curves (AUDPC) of each line (Campbell and Madden 1990) is frequently calculated in order to study the progress of the disease in each line. Genotypes can be scored weekly from the week when the susceptible check genotypes show the first disease symptoms to the week the check genotypes reach a score of 9.

Methods for screening AB under controlled environment require high level of relative humidity during the first 24-h post-inoculation (Udupa and Baum 2003; Chen et al. 2005). Also, spore concentration in the inoculum is a significant factor. Conidia of individual isolates are harvested from two-week-old cultures, and spore concentration is adjusted to the lowest that causes sufficient disease in a majority of host genotypes (proposed 2×105 pycnidiospores mL^{-1}). Two-week-old plants are sprayed with this conidial suspension to run-off and immediately incubated 24 h at 20 °C and 100% relative humidity (RH) to facilitate infection. There are several methods for scoring AB phenotype (Chen et al. 2004). The nonparametric 1–9 rating scale of Reddy and Singh (1984) is the method most commonly used for controlled condition evaluations. The lesions are scored as follows: 1 = healthy plant—no disease, 2 = lesions present but small and inconspicuous, 3 = lesions easily seen but plant is mostly green, 4 = severe lesions clearly visible, 5 = lesions girdle stems, most leaves show lesions, 6 = plant collapsing, tips die back, 7 = plant dying but at least three green leaves present, 8 = nearly dead plant (virtually no green leaves left) but still with a green stem and 9 = dead plant (almost no green parts visible).

Fusarium wilt (FW) caused by *Fusarium oxysporum* f. sp. *ciceris* is a soil fungus and chickpea plants may be infected at any growth stage. Different evaluation methods have been developed for screening under controlled and field conditions (Sharma and Muehlbauer 2007; Li et al. 2015). The level of resistance and susceptibility of each line is usually calculated as percentage of wilt incidence (number of wilted plants/total number of plants $\times$ 100). Based on wilt incidence, the data can be converted into categorical records. Sharma et al. (2005)

proposed the following scores range: 0–10% wilting = resistance, 11–89% wilting = intermediate, 90% wilting = susceptible. In order to search for Fusarium wilt resistance genes, most studies have treated the character as a qualitative trait (see Chap. 3), and only few considered the disease as a quantitative trait (Table 8.1).

Botrytis grey mould (BGM) caused by *Botrytis cinerea* Pers.ex.Fr. is widely distributed and is the second most important disease in

tropical areas (Pande et al. 2006; Davidson et al. 2007). Different screening techniques have been used for germplasm under controlled and field conditions (Pande et al. 2006). Here, we will describe scale employed by Anuradha et al. (2011) to phenotype a RIL population for BGM. The authors determined QTLs associated with BGM. The severity of the disease was recorded following a 1–9 rating scale, where 1 = no infection on any part of the plant; 2 = minute

Table 8.1 QTLs linked to biotic stresses in different genetic backgrounds

Trait	LG[a]	QTL	R^2 (%)[b]	Tightly linked markers	Source of resistance[c]	References
Ascochyta blight (AB)						
	LG2	QTL_{AR3}	44.3	GA16, TA194, TR19, Ein3	ILC3279, ILC72	Udupa and Baum (2003), Cobos et al. (2006), Iruela et al. (2007), Anbessa et al. (2009), Madrid et al. (2014)
	LG3	QTL_{AR4}	18	STMS28, TS12, TA64, TR58	ICC1, CDC Frontier, Amit	Flandez-Galvez et al. (2003), Ta'ran et al. (2007), Anbessa et al. (2009), Kottapalli et al. (2009)
	LG4	QTL_{AR1}	16.9	GAA47, GA24, H3C041, CaETR	FLIP84-92C, Hadas, ILC3279	Santra et al. (2000), Tekeoglu et al. (2002), Lichtenzveig et al. (2006), Madrid et al. (2013)
		QTL_{AR2}	29.4	SCAR733b, TA2, TA146, TA72, TA130, TA132, TS54, H1G20, SCY17	FLIP84-92C, ILC3279, Hadas, CDC Frontier	Santra et al. (2000), Udupa and Baum (2003), Cho et al. (2004), Iruela et al. (2007), Lichtenzveig et al. (2006), Ta'ran et al. (2007), Anbessa et al. (2009), Kottapalli et al. (2009)
	LG6	QTL_{AR5}	11.6	TA176, TA80, TA22, TA40	CDC Frontier	Ta'ran et al. (2007), Anbessa et al. (2009)
	LG8	QTL_{AR6}	16	TA3, H3C11a, TS46, TS45	CDC Corinne	Flandez-Galvez et al. (2003), Lichtenzveig et al. (2006), Anbessa et al. (2009)
Fusarium (Foc)						
	LG5	QTL_{Foc01}	37.8	TR59, TS35	JG62	Cobos et al. (2005)
	LG2	$QTL_{Foc02/Foc5}$	46.5	TA59	ICCL81001	Cobos et al. (2009)
	LG6	FW-Q-APR-6-1 (race1)	16.4	CaM1402, CaM1101	WR315	Sabbavarapu et al. (2013)
Botrytis grey mould (BGM)						
	LG6	QTL1	12.8	SA-14, TS71	ICCV2	Anuradha et al. (2011)
	LG8	QTL2/QTL3	9.5–48	TA25, TA144 TA159, TA118		
Rust						
	LG7	QTL_{Uca}	73.7	TA18, TA180	Cr5-10	Madrid et al. (2008)

Only QTLs with LOD ≥ 3 were considered
[a]Linkage groups; [b]Maximum value published; [c]*Cr Cicer reticulatum*

water-soaked lesions on emerging tender leaves, usually not seen; 3 = minute water-soaked lesions on 1–5% emerging and upper-most tender leaves, usually seen after careful examination; 4 = water-soaked lesions on 6–10% upper-most tender leaves and tender shoots; 5 = water-soaked lesions, soft rotting of 11–25% of tender leaves and shoots; 6 = water-soaked lesions and soft rotting of 26–40% of top leaves and shoots; 7 = soft rotting and fungal growth on 41–55% of the leaves and branches; 8 = soft rotting, fungal growth on 56–70% of the leaves, branches and stems; 9 = extensive soft rotting, fungal growth on above 70% of the leaves, branches and stems (Pande et al. 2006; Anuradha et al. 2011).

Rust caused by the fungus *Uromyces ciceris-arietini* has been reported to be affecting chickpea production in the Mediterranean region, South Africa, Mexico, Australia, Italy and the USA (Li et al. 2015). Till now, only one QTL related to rust resistance has been published (Madrid et al. 2008; Table 8.2). The evaluation of plant material was carried out under field and controlled conditions. Field evaluations were performed inoculating the plants by spraying with a spore suspension (200 mg spores/l). Disease severity (DS) was rated as a percentage of the host tissue covered by pustules at weekly intervals following (Sillero et al. 2012). Inoculations in growth chamber were carried out spraying the seedlings at the fourth leaf stage with an aqueous suspension of urediospores (2 mg per plant) diluted in pure talcum powder (1:10). Infection type (IT) under controlled conditions was rated using the scale proposed by Stakman et al. (1962) where 0 = no symptoms, ";" = necrotic flecks, 1 = minute pustules barely sporulating, 2 = necrotic halo surrounding small pustules, 3 = chlorotic halo, 4 = well-formed pustules with no associated chlorosis or necrosis.

Phenotypic Evaluation for Abiotic Stresses

There are several kinds of abiotic stresses (salinity, heat, drought) affecting the chickpea production. Among all of them, drought stress is a major constraint to chickpea production and affects yield stability in arid and semi-arid regions of the world, especially when it happens at the end of the growing season. Drought is a quantitative complex trait, and, therefore, experiments must be carefully designed. Molecular breeding seems to be the best strategy to face this trait. It can be deployed targeting drought-tolerant component traits aided by molecular markers. Varshney et al. (2014a) reported a "*QTL-hotspot*" in the chickpea genome for several drought tolerance-related traits (Table 8.2).

To perform a rapid and efficient screening for drought resistance in chickpea, ICARDA (International Center for Agricultural Research in the Dry Areas) proposed a 1–9 scale. This scale has been extended by adding in heat effects because heat stress is usually accompanied by drought stress. The drought and heat resistance (DHR) score was: 1 = very high resistance (free from drought and high-temperature effects, early flowering, very good plant vigour and 100% pod set), 2 = high resistance (early flowering, good plant vigour and 96–99% pod set), 3 = resistant (early flowering, good plant vigour and 86–95% pod set), 4 = moderately resistant (early flowering, moderate plant vigour and 76–85% pod set), 5 = tolerant (medium flowering, poor plant vigour and 51–75% pod set), 6 = moderately susceptible (medium flowering, lack of plant vigour and 26–50% pod set), 7 = susceptible (late flowering, lack of plant vigour and 11–25% pod set), 8 = highly susceptible (late flowering, lack of plant vigour and 1–10% pod set) and 9 = very highly susceptible (no flowering, no pod set and 100% plants died) (Canci and Toker 2009). Several studies have focused on the identification of morphological and physiological traits associated with drought tolerance. Roots traits, such as root depth, root biomass and root length density, have been identified as the most promising plant trait in chickpea for terminal drought tolerance, as these greatly help extract the available soil moisture (Varshney et al. 2014a). Other traits as stomata conductance, characters related to leaf (area, morphology) and

Table 8.2 QTLs for main abiotic stresses detected in chickpea. Only QTLs with LOD $\geq$ 3 were considered

Trait	LG	QTL	Population[a]	QTL number (related traits)[b]	R² (%) Min–Max	Linked markers	References
Drought							
	LGV1	Cluster 1	ICCRIL 03	2 (Y)	3.60–10.67	NCPGR 184, ICCM 0009b	Varshney et al. (2014a)
		Cluster 2	ICCRIL 03	1 (Y)	14.36	cpPb 679915, CaM 0393	Varshney et al. (2014a)
			ICCRIL 04	3 (D, Ph)	6.08–15.57	TA 103II, TA 122, TA 113	Varshney et al. (2014a)
		Cluster 3	ICCRIL 03	2 (D, Y)	5.16–13.98	NCPGR 136, CaM 0046	Varshney et al. (2014a)
			ICCRIL 04	1 (D)	12.12	NCPGR 136, TA 203	Varshney et al. (2014a)
		Q1-1	ILC 588 (T) × ILC 3279 (S)	8 (CTD, D, M, Ph, Y)	6–24	H5A 08, TA 8	Rehman et al. (2011)
	LG2	QR4yld02	ICCRIL 04	1 (Y)	18.55	TA 59, TA 200	Varshney et al. (2014a, b)
			ILC 588 × ILC 3279	1 (M)	8.5	TA 96	Hamwieh et al. (2013)
	LG3	Cluster 4	ICCRIL 03	1 (M)	10	TA 34, NCPGR 49	Varshney et al. (2014a)
			ICCRIL 04	2 (Ph, Y)	13.79–18.97	CaM 1753, cpPb 677529	Varshney et al. (2014a)
		Clusters	ILC 588 × ILC 3279	17 (D, M, Ph, Y)	8.4–24.2	H4G-07, H6C-07, H3G 09, NCPGR-50, TA 179	Hamwieh et al. (2013)
		Q3-1	ILC 588 × ILC 3279	6 (CTD, D, Ph, Y)	9–33	TA 125, TA 6, NCPGR 12, NCPGR 10	Rehman et al. (2011)
	LG4	Cluster 5 (*Hotspot*)	ICCRIL 03	12 (M, R, Y)	10.90–58.20	NCPGR 127, NCPGR 21, TAA 170, ICCM 0249, TR 11, GA 24, STMS 11, ICCM 0065	Varshney et al. (2014a), Jaganathan et al. (2015), Kale et al. (2015)
		QR4100 sdw02	ICCRIL 04	1 (Y)		CaM 2093, ICCM 0249, TA 130, CaM 1214, NCPGR 142, TAA 170	Varshney et al. (2014a)
		2 Clusters[d]	ILC 588 × ILC 3279	10 (D, Ph, Y)	5.1–11.8	H1H-15, H1B-17, H5G 01	Hamwieh et al. (2013)
		Q4-4	ILC 588 × ILC 3279	1 (CTD)	13	GA 137, TA 46	Rehman et al. (2011)
	LG5	Cluster 6	ICCRIL 04	2 (M, Ph)	14.04–16.79	CaM 029, TA 11	Varshney et al. (2014a)
	LG6	Cluster 7	ICCRIL 04	3 (Ph, Y)	2.95–12.06	CaM 1257, ICCeM 15, NCPGR 200, CaM 0317	Varshney et al. (2014a)
		Cluster 8	ICCRIL 03	3 (M, Ph, R)	10.26–12.13	TA 106, H1I 16, CaM 1760, CaM 399	Varshney et al. (2014a)
		Q6-3	ILC 588 × ILC 3279	1 (CTD)	13	GA 21, CaSTMS 2	Rehman et al. (2011)
	LG7	Q7-1	ILC 588 × ILC 3279	2 (Ph)	5–6	TA 28, CaSTMS 25	Rehman et al. (2011)
	LG8	Cluster 9	ICCRIL 03	4 (M, Ph, Y)	9.11–26.87	NCPGR 164, CaM 1918, CaM 2187	Varshney et al. (2014a)
			ICCRIL 04	6 (M, Ph, Y)	8.77–31.32	CaM 0772, TA 118, TS 45, NCPGR 138, GA 6	Varshney et al. (2014a)
		Q8-2	ILC 588 × ILC 3279	1 (Ph)	8	TA 159, GA 6	Rehman et al. (2011)

(continued)

Table 8.2 (continued)

Trait	LG	QTL	Population[a]	QTL number (related traits)[b]	R^2 (%) Min–Max	Linked markers	References
Salinity[c]							
	LG1		ICCV 2 (S) × JG 11 (T)	1 (Ph)	66.75	CaM 1301, CKAM 1971	Pushpavalli et al. (2015)
		QTL-STI/QTL-Sw	ICC 6263 (S) × ICC 1431 (T)	3 (Y)	8.3–9.6	TR 31, H1B 04, TA 47, H2I 01	Saminemi (2010)
	LG2	QTL-YD	ICC 6263 × ICC 1431	2 (Y)	7.5	TR 37, TR 14	Saminemi (2010)
	LG3		ICCV 2(S) × JG 62 (T)	1 (Y, late genotypes)	19.2	TA 106-Podnode	Vadez et al. (2012)
		QTL-SSI	ICC 6263 × ICC 1431	1 (Y)	7.7	TR 24	Saminemi (2010)
	LG4		ICCV 2 × JG 11	2 (Ph, Y)	49.13–59.95	CKAM 0003, CKAM 1003	Pushpavalli et al. (2015)
			ICCV 2 × JG 62	2 (Ph, late genotypes)	18.5–24.5	TA 144, NO_Y_13, TA 186, TA 46	Vadez et al. (2012)
	LG5		ICCV 2 × JG 11	4 (Ph, Y)	24.98–40.69	CaM 0463, ICCM 272	Pushpavalli et al. (2015)
			ICCV 2 × JG 62	3 (Ph, Y)	11.5–19.5	TA 114, NO_X_1, TA 78	Vadez et al. (2012)
	LG6	Cluster	ICCV 2 × JG 62	4 (Y)	18.2–43.2	TR 20s, TA 46; TA 186; GA 137, GA 25	Vadez et al. (2012)
	LG7	QTL-BM	ICC 6263 × ICC 1431	2 (Y)	7–8.1	CaM 0604, CaM 1432	Saminemi (2010)
			ICCV 2 × JG 62	(Y, early genotypes)	27.6	TA 11, TA 42	Vadez et al. (2012)
			ICCV 2 × JG 11	1 (Ph)	7.95	CaM 2031, CKAM 0165	Pushpavalli et al. (2015)
	LG8		ICCV 2 × JG 11	3 (Ph, Y)	37.75–56.87	CKAM 1903, CKAM 0343	Pushpavalli et al. (2015)

[a]ICCRIL 03 derived from ICC 4958 (T) × ICC 1882 (S)/ICCRIL 04 derived from ICC 283(S) × ICC 8261(T), S (sensitive), T (tolerant)

[b]*D* drought indices; *M* morphological traits; *Ph* phenological traits; *R* root traits; *Y* yield-related traits, *CTD* canopy temperature differential

[c]QTLs for salinity have been considered only in the experiments under salinity conditions

[d]QTLs with LOD < 3 identified by Hamwieh et al. (2013) on LG4 have been considered due to the importance of this region in drought tolerance

osmotic adjustment would be necessary for a fine evaluation.

Salt stress is one of the major abiotic stresses ranking only second to drought. Germination, flower, pod production and gamete development of chickpea are adversely affected by salinity, leading to severe yield loss. Salinity reduces plant height, leaf number, leaf, stem and root dry weights and seed emergence (Toker et al. 2007). Although screening methods in the field have been reported for selection of salt-tolerant (Dua 1992; Saxena et al. 1993; Vadez et al. 2007), its routine use in breeding programmes seems to be very limited due to the complex nature of salinity. Moreover, field screening for salinity tolerance requires considerable time, labour and other resources and it is difficult to separate environmental effects from genetic variation. A number of other non-field-screening methods are available for selection of salt-tolerant chickpea (Epitalawage et al. 2003). Several criteria have been used to asses salinity tolerance including cell survival, germination, dry matter accumulation, leaf death and senescence, ion concentrations (ratio Na^+/K^+ or K^+/Na^+), leaf necrosis, osmoregulation and yield. No single selection criterion has been found for salinity tolerance (Toker et al. 2007); however, the lines ICCV2 and JG62 were reported as sensitive and tolerant, respectively (Vadez et al. 2007). Later, these lines were employed to evaluate seed yield and seed number under saline conditions considering two different phenology groups (early and late flowering), in a QTL study for these traits (Vadez et al. 2012; Table 8.2).

Phenotyping for Adaptive and Yield Traits

Growth habit is one of the morphological traits that plays a role in the adaptability to different environments in legumes, affecting yield and yield stability (Rubio et al. 2004; Gaur et al. 2007). This trait is not only related to plant height but also includes differences related to plant structure affecting production. It is affected by the number of branches and, consequently, the number of sites for pod setting. In chickpea, growth habit was initially classified as: (i) prostrate, when primary branches form a right angle with a vertical axis, (ii) semi-erect (bushy), when form a less acute angle with a vertical axis and (iii) erect, when form an acute angle with a vertical axis (Cubero 1987). Erect and semi-erect (bushy) types are present in cultivated chickpea, while prostrate is usually referred to the growth habit that is only present in annual wild *Cicer* species (Muehlbauer and Singh 1987). As we will see in Sect. 6.4., growth habit was recently studied in a QTLs analysis using a RIL population segregating for the three different phenotypes, indicating that, probably, there was more than one gene present in the RIL (Ali et al. 2015).

Days to flowering is another major component of crop adaptation. This trait is evaluated as the number of days from sowing to onset of flowering when at least 50% of the plants from the experimental unit (plot, row, etc.) had set the first flower. The number of genes controlling the inheritance of this trait in chickpea differs depending on the genotypes used as early and late parents and the environment in which the evaluations were performed. Chickpea is a long-day photoperiod species, but when it was introduced at low latitude like India and East Africa, new mutations insensitive to photoperiod arose. Some authors reported the presence of a major gene for flowering time (Gumber and Singh 1996; Or et al. 1999; Kumar and van Rheenen 2000). However, quantitative inheritance has been also reported in chickpea populations where several genes are segregating for flowering time (Cho et al. 2002; Lichtenzveig et al. 2006; Cobos et al. 2007).

Yield is a complex trait resulting from complete development of the plant. In chickpea, correlations between seed yield and its components (seeds per pod, pods per plant, seeds per plant, yield per plant, seed size, etc.) have been reported (Muehlbauer and Singh 1987; Maynez et al. 1993; Talebi and Karami 2011). To perform an accurate evaluation of yield, if it is possible, it would be necessary to design experiments with repetitions in different locations and adequate unit plot size. QTLs associated with these traits have been identified (Table 8.3).

Table 8.3 QTLs for adaptative and other traits detected in chickpea

Trait	LG	QTL	Population[b]	R^2 (%)[c]	Tightly linked markers	References
Days to flowering	LG1	QTL1, *CaqDF*1.1[a]	Hadas × ICC 5810, ICC 4958 × ICC 17160 ILC 3279 × ILC 588	23.6	H1F 022, GAA 40, CID_C_477164	Lichtenzveig et al. (2006), Das et al. (2015a)
	LG2	QTL2 *CaqDF*2.1[a]	Hadas × ICC 5810, ICC 4958 × ICC 17160 ILC 3279 × ILC 588	20.4	H4B 09, H1B 06 CID_C_4642382, CID_S_5036	Lichtenzveig et al. (2006), Das et al. (2015a)
	LG3	QTL$_{DF3}$ *CaqDF*3.1[a]	ICC 81001 × *Cr* 5 ICC 4958 × ICC 17160, ILC 3279 × ILC 588	27.5	TS 57, TA 127, TA 142 CID_C_4431329, CID_S_93427	Cho et al. (2002), Cobos et al. (2009), Das et al. (2015a)
	LG4	QTL$_{DF1}$	CA 2156 × JG 62	23	GAA 47	Cobos et al. (2007)
Growth habit	LG1	QTL1	IL C72 × *Cr* 5	15.4	SCA 09	Ali et al. (2015)
Seed number	LG4	QTL	ICCV 2 × JG 62	31	TA 130	Cho et al. (2002)
Seed size/weight	LG1	QTL2	Hadas × *Cr* 205 ICC 3996 × S 95362, S 95362 × cv. Howzat, ICC7 184 × ICC 15061 Collection of 96 genotypes	47.6	GA 11 CaSNP 8, CASNP 10 TFFDMS 16; TFFDMS 18; TFFDMS 21	Abbo et al. (2005), Hossain et al. (2010), Das et al. (2015b), Kujur et al. (2013)
	LG2	QTL$_{SW3}$	CA 2156 × JG 62 ICC 81001 × *Cr* 5	14	TA 110-TAA 60	Cobos et al. (2007, 2009)
	LG4	QTL$_{SW1}$	ICCV 2 × JG 62; Hadas × *Cr* 205 CA 2156 × JG 62; ICC 81001 × *Cr* 5 ICC 3996 × S 95362; S 95362 × Howzat	52	TA 130, GA 24, STMS 11, GA 2	Cho et al. (2002), Abbo et al. (2005), Cobos et al. (2007, 2009), Hossain et al. (2010)
	LG8	QTLSW2	CA 2156 × JG 62	10.1	TA 03	Cobos et al. (2007)
β-carotene	LG3	QTL2, QTL3	Hadas × *Cr* 205	–	TA 64, STMS 28, TS 19	Abbo et al. (2005)
Protein	LG3		Collection of 187 genotypes	16.8	TR 26	Jadhav et al. (2015)
	LG5			12.9	CaM 1068	

Only QTLs with LOD ≥ 3 were considered
[a]Coincident with days to maturity QTL in Das et al. (2015a)
[b]*Cr Cicer reticulatum*
[c]Maximum value of percentage of phenotypic variation explained considering different publications

Phenotyping for Quality Traits

Breeding for improving protein content and other nutritional components using molecular markers has been rarely performed in chickpea. Recently, Jadhav et al. (2015) identified two QTLs for protein content by association mapping (AM) analysis. They estimated the crude protein content using NIR (near infrared spectroscopy) SpectraAlyzer calibrated by Kjeldahl method using 30 genotypes representing both desi and kabuli types. NIR has the big advantage that it is a nondestructive methodology. The quality traits beta-carotene and lutein concentration have been studied in chickpea $F_{2:3}$ families from a wide cross between *C. arietinum* (Hadas) and *C.*

in the genetic maps (Cobos et al. 2005; Gowda et al. 2009; Halila et al. 2009) (see Chap. 3). However, some studies considered the resistance as a quantitative trait (Table 8.1). QTL analysis in the population CA2139 × JG62, where two resistance genes were segregating, allowed to confirm the presence of genomic regions associated with resistance to *foc*-0 on LG2 and LG5 (Cobos et al. 2005; Halila et al. 2009). Cobos et al. (2009) reported that a high percentage of the phenotypic variation for the resistance to *foc*-5 and *foc*-0 (46.5 and 73%, respectively) was explained by a QTL on LG2 in a RIL derived from an interspecific cross (Table 8.1). Another QTL on LG6 was associated with the resistance to *foc*-1 (Sabbavarapu et al. 2013). In spite of all these studies, only microsatellites markers have been located in the genomic regions where QTLs for *foc* are located. There are very little reports available on the genetics of BGM and rust. Three genomic areas controlling resistance to BGM were identified by Anuradha et al. (2011): QTL1 on LG6 explained 12.8% of the total phenotypic variation, while QTL2 and QTL3 on LG8 explained 9.5% and 48%, respectively. On the other hand, a QTL explaining around 80% for rust resistance was located on LG7 by Madrid et al. (2008).

QTLs for Abiotic Stresses

Drought and salt stresses are two of the major abiotic stresses in chickpea affecting crop productivity in many parts of the world. Chickpea is known for its superior drought tolerance compared to most other cool-season legumes. However, drought reduces chickpea yields and can even lead to total crop failure. Since it is a complex trait and the screening methods are laborious, the use of molecular markers is a powerful approach for dissecting the genetic control of drought. QTLs for drought-related traits have been identified in several studies (Rehman et al. 2011; Hamwieh et al. 2013), though their validation has not been reported yet. Rehman et al. (2011) detected QTLs associated with different traits affecting drought tolerance in

the RIL population ILC588 (drought tolerant) × ILC3279 (sensitive). The QTLs located on LG1 (Q1-1) and LG3 (Q3-1) showed effects on many traits related to drought (yield components and phenological traits, plant height, drought tolerance score and canopy temperature differential) (Table 8.2).

The same RIL population was used by Hamwieh et al. (2013). They detected 93 QTLs (LOD ≥ 2.0), although only 19 of them showed LOD values ≥3 for 12 drought-related traits studied across the genome in different environments. Eight QTLs were observed in more than one environment. Two interesting regions were located on LG3 and LG4 and showed pleiotropic effect for several traits (Table 8.2). The first region on LG3 comprised indicative markers (H4G-07 and H6C-07) associated with days to flowering and maturity, drought resistance indices (D) and yield-related traits (Y). The second region on LG3 (indicative markers H3G09, NCPGR-50 and TA179) was associated with phenological (Ph) and yield-related traits (Y) (Table 8.2). Similarly, two common QTLs for different traits were observed on LG4. The first region comprised two markers (H1G-20 and H5G-01) associated with drought resistance (D), phenological (Ph) and yield-related traits (Y). The second region on LG4 comprised the markers H1H-15 and H1B-17, indicative of a common significant QTL for phenological (Ph) and seed number (Y).

In a recent study, Varshney et al. (2014a) reported 45 robust main-effect QTLs (M-QTLs) and 973 epistatic QTLs (E-QTLs) that explained up to 58.20 and 92.19% of the phenotypic variation for several targeted traits, respectively. The authors used two RIL mapping populations [ICC 4958 (drought tolerant) × ICC 1882 (sensitive) and ICC 283 (sensitive) × ICC 8261 (tolerant)]. This study provides nine QTL clusters associated with roots, morphological, phenological, yield-related traits and drought indices (Table 8.2). Among these QTL clusters, the QTL Cluster 5 on CaLG04, referred as *"QTL-hotspot"* (∼29 cM and 7.74 Mb), harboured stable and consistent QTLs for several drought-tolerant traits. This cluster containing seven markers (ICCM0249, NCPGR127, TAA170, NCPGR21,

TR11, GA24 and STMS11) is the most significant region and is being employed in molecular breeding for improving yield under terminal drought conditions (see Sect. 6.6). This region has been refined by Jaganathan et al. (2015) to 14 cM on a genetic map corresponding to ~4 Mb on the physical map. The current analysis integrated 49 new SNP markers in the "QTL-hotspot" region, thereby enriching the same region from 7 to 55 markers. Also, Kale et al. (2015) employing SkimGBS approach refined the "QTL-hotspot" region of ~3 Mb size into two smaller regions, "QTL-hotspot_a and b". A cluster of QTLs (Cluster 9) on CaLG08 also seems to be an interesting genomic region for targeting for drought-tolerant molecular breeding (Table 8.2).

Salinity is another complex abiotic stress, and only few studies have reported the presence of QTLs for salinity tolerance in chickpea (Table 8.2). Saminemi (2010) identified QTLs associated with yield-related traits (seed yield, 100-seed weight and shoot biomass) on LG1, LG2, LG3 and LG7 under salinity conditions using the RIL population from ICC6263 (salt sensitive) × ICC1431 (tolerant). The genomic regions on LG1 and LG7 were found to be important, although these QTLs have not been validated. Later, Vadez et al. (2012) identified QTLs on LG3 and LG6 for seed yield and components under salinity conditions using the RIL population ICCV2 (salt sensitive) × JG62 (tolerant) (Table 8.2). The population segregated also for flowering time, and the lines were separated by "early and late" phenology. Several QTLs were identified for seed yield and its components under saline conditions within each phenology group with limited overlap. Nevertheless, no major QTL was identified when the analysis was carried out on the entire set of the RIL population, highlighting the importance of phenology in the genotypic response to salt stress.

Recently, Pushpavalli et al. (2015) located a total of 46 QTLs that included 19 QTLs for phenological traits and 27 QTLs for yield-related traits across years and treatment (control and salinity) in a RIL population derived from ICCV 2 (salt sensitive) × JG 11 (tolerant). QTLs were clustered on different genomic regions of LG5, LG7 and LG8. The genomic regions on CaLG05 (28.6 cM) and CaLG07 (19.4 cM) were the most interesting because they hold QTLs for traits that were significantly related to yield under salinity explaining 12 and 17% of the phenotypic variation, respectively (Table 8.2).

QTLs for Adaptative and Yield Traits

One of the most important characteristics for crop adaptation to a particular environment is days to flowering. The use of early flowering and, consequently, early maturity in chickpea can be very interesting in semi-arid and Mediterranean environments because it allows the escape from terminal drought and high temperature at the end of the growing season causing a positive effect on chickpea yield (Rubio et al. 2004; Gaur et al. 2007). Even though flowering time can be seen as a relatively simple trait, it participates in a complex network of interactions with other developmental processes being affected by several loci related to light perception, circadian clock, photoperiod response, etc (Weller and Ortega 2015).

Classical genetic studies under both long- and short-day conditions determined that chickpea flowering time could be controlled by one or two genes (Gumber and Singh 1996; Or et al. 1999; Kumar and van Rheenen 2000; Anbessa et al. 2006). However, other authors considered this trait as quantitative. First QTLs detected were associated with markers today situated on LG8 (Cho et al. 2002; Lichtenzveig et al. 2006). They were later validated by Vadez et al. (2012). Other QTLs on LG1, LG2, LG3, LG4 and LG5 have been also published (Table 8.3). It should be pointed out the QTL on the central region of LG3, which has been validated in different genetic backgrounds and is synthenic with QTLs for flowering time detected in *Medicago*, *Vicia faba* and *Lupinus*. This genomic area in chickpea is harbouring a cluster of *FT* family genes as well as *CYCLING DOF FACTOR (CDF)* homologs, a LUX like gene and an ortholog of the *CO*-like gene *COLh* (Weller and Ortega 2015).

Differences in growth habit are not only related to plant height but also include plant structure affecting yield and yield stability (Rubio et al. 2004). The important adaptative trait growth habit was considered to be controlled by a single gene (Hg) in interspecific crosses segregating for prostrate versus semi-erect or erect and was mapped on LG3 of the chickpea genetic map (Kazan et al 1993; Cobos et al. 2009; Aryamanesh et al. 2010). However, phenotypic evaluations considering intermediate values (0 = prostrate, 1 = semi-erect and 2 = erect) revealed a genomic area on LG1 (QTL_{Hg2}) involved in the control of this trait and coincided with the location of a second gene (Hg2) responsible for semi-erect versus erect phenotypes (Ali et al. 2016; Table 8.3; see Chap. 3).

QTLs for yield-related components have been also analysed in several studies (Table 8.3). Thus, regions associated with seed number of plants were detected on LG1 and LG4 in a population derived from an intraspecific cross (Cho et al. 2002). A QTL for seed weight co-located on the same area of LG4 (indicative marker TA130). These two traits were negatively correlated ($r = -0.476$), and, therefore, the co-location of QTLs for both traits could indicate pleiotropic action of a single QTL (Cho et al. 2002). The QTL for seed weight on LG4 has been widely validated using wide and narrow crosses. As it has been above mentioned, yield and yield components have been also analysed in drought and salinity tolerance studies.

QTLs Associated with Quality Traits

Chickpea is mainly used for food, and its nutritional value is well recognized (Asif et al. 2013; Sánchez-Chino et al. 2015). However, only few QTLs associated with quality components in chickpea seeds have been published. A recent study detected two QTLs for protein content using a set of 187 genotypes comprising both international and exotic collections with protein content ranging from 13.25 to 26.77%. Those QTLs were situated on LG3 and LG5 (Table 8.3), but a larger numbers of markers and

genotypes should be used to confirm these associations (Jadhav et al. 2015). Abbo et al. (2005) explored beta-carotene and lutein concentration variation in an F_2 population derived from an interspecific cross (Hadas × C. reticulatum). Two QTLs with LOD values >3 were identified in two regions of LG3 linked to markers TA64, STMS28 and TS19 (Table 8.3).

8.5   Fine Mapping and Candidate Genes

The recent development of high-density genetic maps and the public release of the whole chickpea genome sequence provide a framework to narrow down the targeted genomic regions and identify candidate genes. The accurate location of QTL in the genetic maps may be limited by the size of the populations, but this may be overcome combining data from different populations (Anbessa et al. 2009; Hossain et al. 2010; Ali et al. 2015). Another strategy is the use of NILs, sharing the same background but differing in the target trait (Ali et al 2016). Additionally, setting up the correlation between positions in genetic and physical maps of key markers makes it possible to search for candidate genes in genomic areas where QTL has been reported.

Fine mapping of QTLs governing AB resistance is one of the challenges in order to develop molecular markers reliable for MAS. The establishment of the relationship between marker positions in genetic and physical maps allowed the location of one resistant candidate gene (Ein3) in QTL_{AR3} (LG2) (Madrid et al. 2014). This gene (Ein3), together with one previously identified on LG4 (CaETR1) (Madrid et al. 2012), is the only gene directly tagging AB-resistant genomic regions.

Two different approaches were carried out to refine the position of the "QTL-hotspot" region on LG4 harbouring several QTLs for drought tolerance-related traits (Table 8.2). (Jaganathan et al. 2015), using genotyping-by-sequencing (GBS), developed a high-density intraspecific genetic map of chickpea comprising 1007 markers spanning 727.29 cM. The map included 49 new

SNP markers in the "*QTL-hotspot*" region reducing its area from 29 to 14 cM that corresponds to 3 Mb on the physical map. The authors developed inexpensive and easy-to-use markers that are ready to be employed in MAS for introgression of this "*QTL-hotspot*" region into elite cultivars. Also, this study proposed several candidate genes retrieved from the chickpea draft genome sequence (Varshney et al. 2013) and the blasted against NCBI-nr protein database. One of those genes was dehydration-responsive element-binding protein (DREB), which is a well-known transcription factor involved in abiotic stress including drought tolerance (Liu et al. 1998; Lata and Prasad 2011). Likewise, thiamine thiazole synthase, involved in stress-related mechanisms (Rapala-Kozik et al. 2012), and some trait specific genes like E3 ubiquitin–protein ligase and TIME FOR COFFE (TIC) were also identified in the "*QTL-hotspot*" region (Jaganathan et al. 2015).

Kale et al. (2015) performed fine mapping of the same "*QTL-hotspot*" region using a skim sequencing approach (Golicz et al. 2015) to construct a high-density bin mapping. They identified 53,223 SNPs segregating in the 222 RILs from the cross ICC 4958 × ICC 1882, providing a total of 1610 bins. Firstly, bins were used as molecular markers for developing a genetic map and the order of bins on the genetic map was compared with their physical position on the chickpea genome sequence, showing an excellent concordance. Secondly, QTL analysis using the bin map revealed that "*QTL-hotspot*" region of ∼3 Mb size was divided into two smaller regions: "*QTL-hotspot_a*" (bin_4_13239546–bin_4_13378761, equivalent to 139.22 Kb) and "*QTL-hotspot_b*" (bin_4_13393647–bin_4_13547009, equivalent to 153.36 Kb). Further, bin mapping and gene enrichment analysis identified a set of 12 candidate genes. Some of the identified genes such as E3 ubiquitin ligase, serine threonine protein kinases and homocysteine S-methyltransferase were annotated as candidate drought-tolerant genes. The expression profile of these genes suggested that they are involved in drought tolerance in chickpea Kale et al. (2015).

Fine mapping for QTLs related to salinity tolerance has not been performed as extensively as in QTLs for drought. Two key genomic regions comprising QTLs linked to salinity tolerance-related traits have been described on CaLG05 and CaLG07 (Table 8.2). Based on gene ontology (GO) annotation, 31 putative candidate genes were found on CaLG05 and 17 genes on CaLG07 in a distance of 11.1 and 8.2 Mb on the chickpea reference genome. Across CaLG05 and CaLG07, ten candidate genes were found to have a vital role in ABA (abscisic acid) biosynthesis, metabolism and ABA-dependent signalling pathways (Pushpavalli et al. 2015).

A high-resolution genetic linkage map harbouring robust QTLs governing flowering and maturity time has been recently obtained by the identification of large-scale genome-wide InDel markers (Das et al. 2015a). The authors searched for InDel markers comparing *in silico* high-throughput genotyping data available for different chickpea genotypes (ICC 4958, ICC 4951, ICC 12968 and ICC 17160). InDel markers were validated, and the ones that were polymorphic between ICC 4958 and ICC 17160 were genotyped in 190 individuals of an F_5 mapping population segregating for flowering and maturity time. The integration of genetic and physical maps delineated five InDel markers at three major genomic regions linked to three robust QTLs in three different chromosomes, coinciding with flowering time and days to maturity (Table 8.3). QTL intervals were demarcated in less than 200 kb. Interestingly, five InDel marker-containing genes among were MADS [MCM (minichromosome maintenance) AGAMOUS DEFICIENS SRF (human serum response factor)], TCP [teosinte branched 1 (tb1), cycloidea, proliferating cell factor (PCF)] and DOF (DNA-binding one zinc finger) transcription factors. All of them are known to be involved in transcriptional regulation of diverse known flowering time pathways in crop plants, including legumes (Weller and Ortega 2015).

Another strategy for fine mapping a region linked to seed weight on LG1 has been recently published combining QTL-seq and classical QTL mapping (Das et al. 2015b) (Table 8.3). Two DNA bulks from a segregating mapping

population with extreme values were re-sequenced using NGS technology. An initial region of 1.37 Mb containing 177 genes was reduced to a 35-kb physical interval carrying six genes. Differential expression studies indicated that the SNP locus (CaSNP8: G/A) in the coding region of constitutive photomorphogenic 9 (COP9) signalosome complex subunit 8 (CSN8) gene was one of the potential candidates regulating seed weight in chickpea. The seed-specific expression, as well as differential up-/down-regulation of this candidate gene in high and low seed weight parental genotypes and homozygous individuals, was relevant. However, the authors proposed that functional validation and a detailed molecular characterization of this gene should be performed to understand its role in seed weight regulation in chickpea (Das et al. 2015b).

8.6   MAS for Complex Traits

It is well known that MAS helps breeders to increase efficiency, precision, selection intensity and selection of favourable combinations of genes in early generation, resulting in increased genetic gain (Kumar et al. 2011). In chickpea, as in other crops, MAS has been more effective for relatively simple traits than for complex ones (Bouchez et al. 2002; Lecomte et al. 2004, among others). However, some examples of successful use of MAS for quantitative inherited traits can be mentioned. As seen throughout this chapter, QTLs for resistance to Ascochyta blight have been located and validated on LG4 (QTL_{AR1} and QTL_{AR2}), LG2 (QTL_{AR3}) and LG3 (QTL_{AR4}) of the chickpea map using different mapping populations (Table 8.1). Due to the complex inheritance of this disease, the incorporation of MAS for AB resistance into breeding programmes would greatly accelerate the development of new chickpea cultivars.

Castro et al. (2015) studied phenotypic classical selection and MAS to select blight-resistant genotypes and compared the effectiveness of both methods. The phenotypic evaluation showed that the resistance to AB could be recessive in the material employed. Phenotypic selection in $F_{2:4}$ and $F_{2:5}$ generations leads to an increase in the frequency of the allele associated with the resistance of the markers CaETR and GAA47 (linked to QTL_{AR1}), indicating the usefulness of these markers for MAS. The markers TA72 and SCY17 (linked to QTL_{AR2}) could be also useful for MAS, but the high distorted segregation towards the susceptible parent in the region where these markers were located could explain their low effectiveness. The costs associated with phenotypic selection and MAS for AB resistance during three cycles of selection showed that MAS was more expensive than phenotypic selection. Nevertheless, the use of markers reduced the length of time necessary to select resistant lines. The authors concluded that it is recommended to employ MAS in early generations of chickpea breeding programmes because it makes it possible to develop populations with a high frequency of favourable alleles conferring resistance to blight. Thus, the use of MAS in combination with phenotypic selection was more fruitful for a complex trait such as AB because genotypic selection could help to retain other QTL with differential expression and to select loci involved in epistatic interactions.

Two co-dominant markers associated with AB resistance were used by Bouhadida et al. (2013) to explore their usefulness in discriminating between resistant and susceptible chickpea genotypes. CaETR and $SCARY17_{590}$ markers developed by Madrid et al. (2012) and Iruela et al. (2006), respectively, were employed to genotype a set of 23 chickpea accessions to select advanced lines and varieties from the Tunisian chickpea breeding programme. As a result, the advanced line V10 was selected and presented the resistance allele for CaETR but was heterozygous for the $SCARY17_{590}$. This line was characterized as resistant to moderately resistant in field studies and under controlled conditions. The authors concluded that V10 could be very useful for developing a new variety carrying both resistance alleles and expressing good levels of resistance to AB in different chickpea cropping environments. Both markers contributed efficiently in the selection of new chickpea varieties

with better combinations of alleles to ensure durable resistance to AB. These markers were previously used by Madrid et al. (2013) detecting AB-resistant alleles in 90% of the resistant accessions in a collection of landraces, advances breeding lines and cultivars.

The basis of marker-assisted backcrossing (MAB) strategy is to transfer a specific allele at the target locus from a donor line to a recipient line while selecting against donor introgressions across the rest of the genome. Tar'an et al. (2013) used MAB to introgress resistance to AB and double pod into adapted chickpea cultivars. The authors carried out backcrosses between moderately resistant donors (CDC Frontier and CDC 425-14) and the adapted varieties CDC Xena, CDC Leader and FLIP98-135C. They included two backcrosses and selection for two QTLs for AB resistance (*Abr* QTL3 and *Abr* QTL4 on LG4 and LG8, respectively) and a locus associated with double podding on LG6. An average of three markers per chromosome and the average distance of 25 cM between two markers were used. This procedure allowed selecting plants with 92% of the recipient genome by BC2F1. The selected plants possessed the majority of elite parental type SSR alleles on all fragments analysed except the segments of LG4, LG6 and LG8 that possessed the targeted QTL. Therefore, using MAS and two generations of backcrosses followed by one generation of selfing, Tar'an et al. (2013) obtained an improved version of CDC Xena, CDC Leader and FLIP98-135C. This practical example of marker-assisted selection clearly illustrates the superiority of using MAB compared to conventional backcrossing because obtaining such a small donor region within only a few backcross generations would be impossible using conventional methods. With the availability of large numbers of SSR and SNP markers, high-throughput genotyping and fine mapping of major QTLs, any major QTL can now be virtually introgressed into a variety without changing the desirable agronomic characteristics.

Recently, Varshney et al. (2014b) developed two parallel marker-assisted backcrossing programmes by targeting *foc1* locus and two QTL regions, ABQTL-I (ar1, ar2a and QTL_{AR3}) and ABQTL-II (ar2b, QTL_{AR1} and QTL_{AR2}), to introgress resistance to *Foc* and AB in the genetic background of C214 (an elite chickpea cultivar high yielding but susceptible to both diseases). WR315 was selected as donor parent for introgression of the genomic segment carrying *foc1* and *foc3*. ILC3279 was used as donor for transferring the two QTL clusters associated with AB. In the case of *Foc*, six markers (TR19, TA194, TAA60, GA16, TA110 and TS82) linked to *foc1* were conducted for foreground selection. Eight markers (TA194, TR58, TS82, GA16, SCY17, TA130, TA2 and GAA47) linked to ABQTL-I and ABQTL-II were used for AB. After undertaking three rounds of backcrossing and three rounds of selfing, 22 BC_3F_4 lines were generated from the cross C 214 × WR 315 and 14 MABC lines from C214 × ILC 3279. Phenotyping of these lines identified three lines (with 92.7–95.2% of recurrent parent genome) resistant to *foc1* and seven lines (with 81.7–85.40% of recurrent parent genome) resistant to AB. This study demonstrates the usefulness of MABC to develop superior lines with enhanced resistance to *Foc* race 1 (and possibly race 3) and AB.

Today, many efforts have been focused on developing markers linked to QTL for drought tolerance. MABC breeding approach has been applied with proven success in chickpea for enhancing drought tolerance by introgressing "*QTL-hotspot*" (Table 8.2) into elite cultivars at the ICRISAT and its National Agricultural Research System. The elite Indian chickpea variety JG11, obtained from the donor parent ICC4958 (drought tolerant), was used as the recurrent parent for three generation of backcrossing. True F_1 plants were selected for first backcrossing using JG11 as a female parent. Based on foreground selection with QTL linked markers and background selection with AFLP (amplified fragment length polymorphism) markers, the backcross progenies carrying drought-tolerant alleles for the target "*QTL-hotspot*" and with maximum recurrent parent genome (RPG) recovery were selected for undertaking another round of backcrossing. After undertaking three rounds of backcrossing, selected plants were selfed twice for making

plants homozygous as well as for multiplication of seeds. The SSR markers ICCM0249, TAA170 and STMS11 were used for foreground selection at different backcross generations. A total of 29 introgression lines were developed with $\sim 93\%$ recurrent parent genome recovery. The introgression lines developed from JG11 $\times$ ICC4958 were found to possess higher root length density (average 0.41–0.20 cm^{-3}), root dry weight (average 1.25–0.08 g cyl^{-1}) and rooting depth (average 115.21–2.24 30 cm) than the donor and recipient parents; these are the most important target traits for enhancing drought tolerance in chickpea (Thudi et al. 2014).

Preliminary analysis of phenotypic evaluation of these lines in India, Kenya and Ethiopia indicated that several lines had an increase in yield higher than 10% under rainfed conditions and around 20% under irrigated conditions. Based on the preliminary results, other partners like Indian Institute of Pulses Research (Kanpur) and Indian Agricultural Research Institute (New Delhi) in India, Egerton University (Kenya) and the Ethiopian Institute of Agricultural Research (Ethiopia) in sub-Saharan Africa have initiated introgressing this region into genetic backgrounds of elite cultivars from their regions (Thudi et al. 2014).

At ICRISAT four desi genotypes have been selected based on their performance: ICCV 04112, ICCV 05107, ICCV 93954 (released as JG 11 in India) and ICCV 94954 (released as JG 130 in India). Elite lines were used to make two crosses (JG 11 $\times$ ICCV 04112 and JG 130 $\times$ ICCV 05107). To pyramid the superior alleles of the favourable QTLs identified based on F_3 genotyping data and F_5 phenotyping data (from Ethiopia, Kenya and India), a set of eight lines were selected for each cross using OptiMAS (Valente et al. 2013). These efforts are expected to develop superior lines with enhanced drought tolerance for other regions (Thudi et al. 2014).

8.7   Conclusion

QTLs analysis in chickpea has been fruitful, and important genomic areas in the chickpea genome have been related to important biotic, abiotic and yield-related traits. We have reported through the chapter that areas targeting QTLs associated with disease resistance have been consistently validated by different research groups using different genetic backgrounds. These QTLs have a great advantage over the QTLs for complex traits as drought or salinity because they are based on accurate and well-established phenotyping methods. However, the phenotypic evaluations for drought or salinity require dissecting the physiological responses. Despite these difficulties, it has been possible to detect genomic regions explaining important percentages of the total variation. Moreover, detailed genetic studies for key traits as flowering time have been transferred from model species to chickpea in the last years. Markers linked to some of those QTLs have been successfully applied in breeding programmes originating new varieties with the desired traits. As we described in Sect. 6.6, most of them were based on MAB (Tar'an et al. 2013; Thudi et al. 2014; Varshney 2016). However, still insufficient efforts have been undertaken to target resistances to minor pests and diseases as well as tolerance to abiotic stresses such as chilling and freezing.

The availability of the whole-genome sequences in kabuli and desi chickpea types opens new possibilities to survey or re-localize QTLs. The analysis of either segregating populations or genomic resources using next-generation sequencing (NGS) technologies produces genome-wide high-density single-nucleotide polymorphisms (SNPs) with a relatively low cost, allowing to identify haplotype blocks significantly correlated with quantitative trait variation (Barabaschi et al. 2016). Parallel efforts will be required to large-scale and systematic phenotyping in order to achieve accurate reporting of experimental protocols, data management and integration with modelling (Fiorani and Schurr 2013).

Compiled information applied in multiparent populations such as MAGIC will provide a unique opportunity to explore environmental interactions and better predict allelic effects in diverse backgrounds (Huang et al. 2015). Today, it is also increasing the number of QTL studies related to different "-omics" by profiling and

quantifying transcripts, proteins and/or metabolites, resulting in expression-QTLs (eQTLs), protein-QTLs (pQTLs) and metabolite-QTLs (mQTLs), respectively. The compilation of this huge amount of molecular and phenotypic data through genome-wide meta-analysis will allow breeders to more effectively exploit QTL allelic diversity (Salvi and Tuberosa 2015). In the future, it could be possible that marker-assisted selection (MAS) in chickpea, as in other crops, will consist in choosing the best allelic combination across the entire genome instead of using markers focused in particular QTLs.

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Requirement of Whole-Genome Sequencing and Background History of the National and International Genome Initiatives

Mahendar Thudi and Rajeev K. Varshney

Abstract

Chickpea is the second most important grain legume for food and nutritional security in the arid and semi-arid regions of the world. The genome sequence provides the basis for a wide range of studies, from the important goal of accelerated breeding to identifying the molecular basis of key agronomic traits, in addition to understanding the basic legume biology. The discussions during 5th International Conference on Legume Genetics and Genomics, held during July 8–10, 2010 in Asilomar, USA, provided the platform for the genesis of International Chickpea Genome Sequencing Consortium (ICGSC http://ceg.icrisat.org/gt-bt/ICGGC/ICGSC.htm), and as result of global research partnership co-led by ICRISAT, UC-Davis, and BGI-Shenzhen, involving 49 scientists from 23 organizations in 10 countries the draft genome of kabuli genotype CDC Frontier was published. On the other hand, the Next Generation Challenge Programme on Chickpea Genomics (NGCPCG) initiative unraveled the genome sequence of desi genotype ICC 4958. This chapter summarizes the background history of two independent efforts to generate draft genome sequence of kabuli and desi chickpea genomes. In addition, the chapter also highlights key developments of application of genome sequence for crop improvement.

9.1 Introduction

The genus *Cicer* is a member of the monogeneric tribe *Cicereae* Alef; subfamily *Papilionaceae*, family *Leguminosae*, which includes 9 annual and 34 perennial species (van der Maesen 1987). Chickpea (*C. arietinum* L.) is the only *Cicer* species cultivated on a large scale, self-pollinated diploid ($2n = 2x = 16$) with a genome size

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of $\sim$740 Mbp (Arumuganathan and Earle 1991). It is the second most important pulse crop in the world covering an area of 13.9 mha (FAO 2016). Two distinct chickpea types, different in their morphology and used in different ways of processing have been described: desi and kabuli. Desi-type chickpeas have purple flower and small, dark, and angular seeds; it is largely consumed in India and Pakistan. Kabuli chickpeas have white flower and large, cream-colored seeds; it is preferred in the Mediterranean Basin and Central Asia, mainly consumed as whole seed. The kabuli type constitutes only $\sim$15% of global chickpea production, but good quality large-seeded kabuli chickpea are very much appreciated in the market and fetches three times higher price than desi cultivars. Although India is the largest producer, it imports chickpea from Australia, Canada, Mexico, Turkey, Ethiopia, etc., to cater the need of ever-growing population. Similarly, Spain also needs to import approximately double than the Spanish chickpea production (FAO 2010). This is because of low productivity (<1 tons per hectare) as a result of exposure of the crop to a number of abiotic stresses such as drought, salinity, and biotic stresses (e.g., Fusarium wilt (FW) and Ascochyta blight (AB)).

9.2 Need for Draft Genome Sequence

Increasing and stabilizing the seed yield while minimizing inputs is the major aim of chickpea breeding. This goal can be achieved by developing cultivars better adapted to stresses in local environments. The recent developments in high-throughput or next-generation sequencing (NGS) technologies are opening up a wealth of possibilities for pulse breeding. A reference chickpea genome sequence provides a foundational resource for this important crop which also possesses a relatively modest genome size ($\sim$740 Mb). Availability of genome sequence information will dramatically accelerate complete identification of genomic variations as it is easy to generate re-sequence data from different genotypes which can be aligned with the reference genome and then be linked with phenotypes, to obtain biological insights as well as for breeding applications. In addition, the reference genome will aid in elucidation of complex genetic interactions in chickpea, which in turn facilitates pulse geneticists and breeders to develop a full understanding of the variations found in each genotype. Analyses beyond sequencing include finding candidate gene(s), variation for traits related to nutritional quality, bioactive compounds and bioavailable micronutrients in chickpea, will enable integration of these outputs into the applied pulse breeding activities like (a) selection of parents for crossing, (b) screening the early generations for the desired genotypes that contain all (or the majority of) favorable alleles, and (c) integration of the selected lines into elite cultivar development.

This chapter summarizes the background history of two independent efforts to generate draft genome sequence of kabuli and desi chickpea genomes. International Chickpea Genome Sequencing Consortium (ICGSC) was led by ICRISAT to decode the draft genome of kabuli genotype CDC Frontier, while The Next Generation Challenge Programme on Chickpea Genomics (NGCPCG) unraveled the genome sequence of desi genotype ICC 4958.

9.3 ICGSC Efforts to Unravel Draft Genome Sequence of CDC Frontier Genotype

Discussions initiated during the 5th International Conference on Legume Genetics and Genomics (ICLGG), held during July 8–10, 2010 in Asilomar, USA, led to the development of one consortium named as "International Chickpea Genome Sequencing Consortium (ICGSC)" co-led by ICRISAT, UC-Davis and BGI-Shenzhen, with the main objective of decoding the genome sequence information and making it available to chickpea research community. ICGSC comprised of seven leading research institutes of the world that have extensive expertise in both basic as well as applied genomics of chickpea.

CDC Frontier, a high yielding medium seeded kabuli chickpea variety was selected for developing the genome sequence. This variety was developed at University of Saskatchewan, Canada from the cross FLIP 91-22C × ICC 14912 in 1993. While FLIP91-22C was developed by the International Center for Agricultural Research in the Dry Areas (ICARDA), in Aleppo, Syria, and ICC 14912 was developed by the ICRISAT, India.

9.4 Consortium Partners and Strengths

ICRISAT with a global mandate to improve chickpea crop has lead the efforts of unraveling the draft genome of chickpea. For >40 years, ICRISAT has been engaged in pre-breeding research and has been sharing the breeding lines with national partners for their evaluation and release of the varieties in the targeted zones/countries. ICRISAT, in collaboration with its partners developed significant amount of genetic and genomic resources as given in Tables 9.1 and 9.2. For instance, large-scale SSR markers, SNP markers, DArT markers, several inter- and intra-specific genetic maps and QTL maps have been developed. ICRISAT has developed genome-wide physical map of chickpea in collaboration with UC-Davis and National Institute of Plant Genome Research (NIPGR), India (Varshney et al. 2013). The ICRISAT genebank has the largest collection of 20,267 accessions in genus *Cicer* from 60 distinct countries across five continents (Asia, Africa, Americas, Europe, and Oceania-pacific) including 308 accessions of 18 (eight annual and ten perennial) wild *Cicer* species.

University of California, Davis, USA—The research group led by Douglas Cook, possessed extensive expertise in the areas of comparative and structural genomics of the legume family and transcriptional profiling. They have a special focus to understand the molecular and genetic basis of symbiotic nitrogen fixation and legume–pathogen interactions. Apart from this, UC-Davis in collaboration with ICRISAT under Phase I of Tropical Legumes I, funded by Bill and Melinda Gates Foundation contributed to develop numerous SNPs (based on Sanger, 454 and Solexa re-sequencing, as well as an Illumina SNP GoldenGate platform); large collections (∼2800) of SSRs; bacterial artificial chromosome (BAC) libraries and >30 Mbp of BAC-end sequence information at NCBI; a comprehensive inventory of >400 NBS-LRR disease resistance genes.

BGI-Shenzhen, China is a premier genomics research organization, with a goal for developing projects and platforms that are on the cutting edge of research and technologies. Further, they focus on developing all kinds of applications, including de novo sequencing and assembly of plant and animal genomes, large-scale genome re-sequencing, genetic association studies, gene expression profiling, whole transcriptome assembly, miRNA detection, ChIP-Seq studies, DNA methylation characterization and metagenomics.

University of Saskatchewan, Canada—The Crop Development Centre (CDC) at University of Saskatchewan developed more than 15 kabuli and desi varieties that have been released in Canada. In addition, several cultivars of specific market classes such as green and black desi and green cotyledon kabuli have been released. University of Saskatchewan has a breeding program that focuses on enhancing yield, resistance to AB, earliness, grain visual, and processing qualities. Steady gains in yield potential together with the improvement in resistance to AB have been achieved over the past decade. Many recently released cultivars yield up to 20% or more than those that were released in mid-1990s. Molecular breeding efforts to develop improved genotypes for AB are underway (Tar'an et al. 2007a, b). Seed qualities like seed size, shape, and seed coat color were main focus and have been working in collaboration with ICRISAT, France and Australia for developing inter-specific hybrids in chickpea. In terms of genomics research, identified several SNPs from 454 sequencing of various tissues of CDC Frontier. The CDC chickpea breeding program has developed a number of populations to facilitate studying of AB blight disease resistance and others in chickpea (Table 9.3).

Table 1 Genetic resources developed at ICRISAT

Cross	Generation	No. of RILs	Segregating traits/significance
Mapping populations			
ICC 4958 × ICC 1882	F_{10+}	264	Root traits
ICC 283 × ICC 8261	F_{10+}	281	Root traits
Annigeri × ICC 4958	F_{10+}	257	Root traits
ICCV 2 × JG 11	F_3	290	Salinity tolerance
ICC 6263 × ICC 1431	F_6	286	Salinity tolerance
ICC 506-EB × Vijay	F_9	328	*Helicoverpa* resistance
ICC 3137 × IG 72953	F_5	244	*Helicoverpa* resistance
ICC 3137 × IG 72933			*Helicoverpa* resistance
ICCV 2 × JG 62	F_{10+}	573	Fusarium wilt resistance, Botrytis gray mold resistance, *Helicoverpa* resistance and salinity tolerance
WR 315 × C104	F_{10+}	84	Fusarium wilt resistance
ICCV 2 × ICC 1496	F_8	249	Botrytis gray mold resistance
ICCV 10 × ICC 1496	F_8	250	Botrytis gray mold resistance
Pb 7 × ICCV 04516	F_4	281	Ascochyta blight resistance
ICC 995 × ICC 5912	F_6	246	Protein content
MABC populations			
JG 11 × ICC 4958	BC_3F_2		For enhancing drought tolerance
ICC 92318 × ICC 8261	BC_3F_2		For enhancing drought tolerance
KAK 2 × ICC 8261	BC_3F_2		For enhancing drought tolerance
MARS populations			
JG 11 × ICCV 04112	F_5	188	For accumulation of favorable alleles for drought tolerance
JG 130 × ICCV05107	F_5	188	For accumulation of favorable alleles for drought tolerance

Table 2 Genomic resources developed by ICRISAT and its partners

Marker resources			Transcriptomic resources
SSRs	SNPs	DArT	
311 from SSR-enriched library (in collaboration with University of Frankfurt, Germany); 1344 from BAC-end sequences (in collaboration with UC-Davis, USA)	9,000 identified and 768 on GoldenGate assay (in collaboration with UC-Davis, USA, NCGR, USA)	Ca. 5,000 extended array with 15,360 (in collaboration with DArT Pty Ltd, Australia)	20,665 Sanger ESTs; 435,018 454/FLX reads; 103,215 TUSs; and ~118 million Solexa reads (in collaboration with NCGR, USA and UC-Davis, USA)

Table 3 RIL populations and polymorphic traits available at University of Saskatchewan

Cross	Polymorphic traits/markers[a]	Reference
ILC 72 × Cr 5-10	Blight, *B/b*, *Tt*, *Hg*, Isoenz, cross-genome markers, ISSR, RAPD, STMS	Cobos et al. (2006)
ICCL 81001 × Cr 5-9	*B/b*, *Fs*, FOC5, *Hg*, *Rt*, *Df*, ISSR, RAPD, STMS	Cobos et al. (2009)
ILC 3279 × WR315; WR 315 × ILC 3279	*Blight, B, FOC5, RAPD, SCAR, STMS*	Iruela et al. (2006, 2007)
CA 2139 × JG 62	*B/b, Tt, Sfl, FOC0, ISSR, RAPD, STMS*	Cobos et al. (2005, 2007) Halila et al. (2009)
CA2156 × JG62	*B/b, Tt, Sfl, FOC0, ISSR, RAPD, STMS*	Cobos et al. (2005)

[a]*FOC* Fusarium wilt resistance genes; *B* flower color (*pink/white*); *f* days to flower
g 100 seed weight; *Gh* Growth habit; *LS* length of the seed; *SC* seed color; *Sfl* and *s*: single/double pod; *STC* stem color; *Tt* testa thickness

CSIRO/University of Western Australia/ Curtin University, Australia—Dr. Karam Singh's group had world leading expertise on biotic stresses in legumes and highly relevant expertise on crop and patho-genomics. The Australian group has made excellent use of the model legume *Medicago truncatula* to progress legume disease and pest research. Of high relevance to this effort is their expertise on the major fungal pathogens of chickpea worldwide namely *Ascochyta rabiei* and *Fusarium oxysporum*. In the case of *A. rabiei,* they have generated a genome sequence using NGS technology involving Illumina 75 bp paired-ends reads at ∼23X coverage and have identified ∼12,000 protein encoding genes. In the case of *F. oxysporum*, they have generated the sequence of a medic isolate again using NGS technology. This group also has excellent expertise on another economically important soil-borne fungal pathogen, *Rhizoctonia solani*, which is an important problem for chickpea. They identified key transcriptional regulators in *M. truncatula* that can give high levels of resistance to *R. solani* when overexpressed in the roots of composite plants without any deleterious effects on plant growth (Anderson et al. 2010).

University of Córdoba/IFAPA, Spain—The research group in Córdoba (IFAPA and Univ. of Córdoba, Spain) has been running plant breeding programmes focused in obtaining new cultivars, better adapted to Mediterranean conditions together with the quality required in Spanish market. Integration of marker-assisted selection (MAS) in traditional breeding programs accelerates the achievement of productive cultivars. Involved in chickpea map development (Millán et al. 2010) but still it is necessary to target some agronomic traits or saturate genomic areas in order to have useful makers for MAS. Development of trait-specific germplasm for instance, recent development of near isogenic lines (NILs) differing in resistance for FW could facilitate the identification of different genes (Castro et al. 2010) and race-specific resistance to *F. oxysporum*. The most significant QTLs involved in AB resistance are two genomic regions in LG4 enclose two clearly differentiated QTLs (QTL1 and QTL2) more than 30 cM apart. Efforts to find candidate genes for QTL1 and QTL2 have been attempted (Iruela et al. 2009). Other traits like bushy growth habit and double-podded mutation also have a positive effect on yield and yield stability in chickpea crop under Mediterranean conditions (Rubio et al. 1998, 2004). Both traits are controlled by a single gene: simple/double pod (*S/s* or *Sfl/sfl*) and erect/bushy habit (*Gh/gh*) (Muehlbauer and Singh 1987).

National Centre for Genome Resources (NCGR), Santa Fe, USA has a worldwide

reputation for sequencing and the development of custom bioinformatic resources for research communities. The NCGR Sequencing Center undertakes massively parallel sequencing services using Illumina® (Solexa) Genome Analyzer and ABI SOLiD4 instruments and also provides genotyping, the gold standard for high-throughput SNP screening, and supplies software tools and services for analysis of genome and transcriptome projects worldwide. NCGR contributed to informatics and data analysis of chickpea genome sequences data.

Above-mentioned institutes were the part of ICGSGC (http://ceg.icrisat.org/gt-bt/ICGGC/ICGSC.htm). The key scientists from each institute leveraged resources from various funding organization including the CGIAR Generation Challenge Programme (GCP), US National Science Foundation (NSF), Saskatchewan Pulse Growers (Canada), Grains Research & Development Corporation (Australia), Indo-German Science Technology Corporation (Germany and India), National Institute for Agricultural and Food Research and Technology (Spain), National Research Initiative of US Department of Agriculture's National Institute of Food and Agriculture (USA), Ministry of Education, Youth and Sports of the Czech Republic and the European Regional Development Fund, University of Cordoba, ICAR (India), BGI (China) and ICRISAT for decoding the genome sequence of chickpea.

9.5 Efforts of NGCPCG to Unravel the Genome Sequence of ICC 4958 a *Desi* Genotype

NGCPCG was initiated by a group of nine NIPGR scientists, with three main objectives: (1) Chickpea genome sequence analysis and its alignment to genetic map; (2) Functional genomics of stress tolerance in chickpea; (3) Functional genomics of chickpea seed development and nutrition. The NGCPCG is purely the work of scientists belonging to just one Indian institute, the NIPGR. The NGCPCG, apart from deciphering the genes, had also worked on finding markers distributed all over the genome which could be used by plant breeders for creation of better variety of chickpea. Complexity of the genome is very high, and it reflects on the nature of biological evolution that there has been more than one line of evolution. The chickpea cultivar ICC4958 was used for generating the draft genome.

9.6 Announcement of Chickpea Genome

Chickpea draft genome sequence decoded was published on January 27, 2013, in a high impact factor Journal "Nature Biotechnology." This was the result of global research partnership led by ICRISAT, involving 49 scientists from 23 organizations in 10 countries. This genome sequence breakthrough was announced by Mr. Ashish Bahuguna, the then Secretary, Department of Agriculture & Cooperation, Dr. Swapan Datta, the then Deputy Director General, Crop Science, ICAR, Dr. William Dar, the then Director General, ICRISAT, and Dr. Rajeev K. Varshney, Coordinator, ICGSC on January 28, 2013 in Krishi Bhawan, New Delhi (Fig. 9.1).

During the press conference announcing the decoding of the chickpea genome sequence at Krishi Bhavan, New Delhi, the then Director General Dr. Dar said, "In the face of growing global hunger and poverty amid the threat of climate change, the chickpea genome sequence will facilitate the development of superior varieties that will generate more income and help extricate vulnerable dryland communities out of poverty and hunger for good, particularly those in the drylands of Asia and sub-Africa for whom ICRISAT and our partners are working." In addition Mr. Ashish Bahuguna, the then Secretary, Ministry of Agriculture, Government of India, recognizing the efforts of the global research team, said, "Decoding of the chickpea genome would facilitate the development of

Fig. 1 (Left–Right) Dr. Swapan Datta, the then DDG (Crop Science), ICAR; Mr. Ashish Bahuguna, the then Secretary, Ministry of Agriculture, Government of India; Dr. William Dar, the then Director General, ICRISAT; and Dr. Rajeev K. Varshney, coordinator of ICGSC and Director—Center of Excellence in Genomics, ICRISAT during the press conference announcing the decoding of the chickpea genome sequence at Krishi Bhavan, New Delhi

improved varieties with higher yields and greater tolerance to biotic and abiotic stresses. This would help chickpea farmers to increase productivity, reduce cost of inputs, and realize higher incomes." He added: "This development is of great importance to India, the largest producer and consumer of chickpea." Dr. Swapan Datta, the then Deputy Director General—Crop Science, Indian Council of Agricultural Research (ICAR), highlighted that "The chickpea genome sequence is expected to help in the development of superior varieties with enhanced tolerance to drought and resistance to several biotic stresses. India will benefit most from this genome sequence, our country being the largest producer of chickpea. This, in my opinion, is by far the most significant collaboration between ICAR, ICRISAT, and the global genomics community." While addressing the addressing the media during the press conference Dr. Rajeev K. Varshney, mentioned that

"Genetic diversity, an important prerequisite for crop improvement, is very limited and has been a serious constraint for chickpea improvement. This study will provide not only access to "good genes" to speed up breeding but also to genomic regions that will bring genetic diversity back from landraces or wild species to breeding lines. Currently, it takes 4–8 years to breed a new chickpea variety. This genome sequence could reduce by half the time to breed for a new variety with market-preferred traits." Prof. MS Swaminathan, Member of Indian Parliament and renowned agricultural scientist said, "I would like to compliment the excellent scientific work done by Rajeev K. Varshney of ICRISAT and his colleagues in developing a high-quality genome sequence of chickpea. I am confident that the knowledge provided by this study will help accelerate the improvement of this crop through marker-assisted breeding."

9.7 A Road Map for Chickpea Improvement

Genome sequence will play a crucial role in speeding up the development of improved varieties that will ensure the food and nutritional security and enhance the income for small holder farmers. In addition, genome sequence also provides the basis for a wide range of studies, from the important goal of accelerated breeding to identifying the molecular basis of key agronomic traits, in addition to understanding the basic legume biology. In addition to developing superior varieties tolerant to drought, heat, *Fusarium* wilt and *Ascochyta* blight, genome sequence can also be used to develop early maturing varieties as well as varieties amenable for mechanical harvesting so that chickpea varieties can be introduced to new niches and drudgery of women can also be reduced. This would help chickpea farmers to increase productivity, reduce cost of inputs and realize higher incomes. Based on the discussions with higher officials and extensive consultations with stakeholders, a road map was developed for enhancing chickpea productivity in India.

For utilizing the genome sequence information of chickpea, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India funded a project entitled "Utilizing chickpea genome sequence for crop improvement" to a consortium of leading chickpea breeders and genomics scientists from different institutes like ICRISAT, ICARDA—New Delhi, Indian Institute of Pulses Research (IIPR)—Kanpur, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya (RVSKVV), RAK College of Agriculture (RAKCA), Sehore, MP, India, and Rajasthan Agricultural Research Institute (RARI)—Durgapura, Junagadh Agricultural University (JAU)—Junagadh. This project had a major emphasis on (i) identification of superior lines, (ii) integrate genomic selection (GS) approach in chickpea breeding, (iii) identification of molecular markers associated with trait of interest for chickpea using nested association mapping (NAM) and linkage mapping approach, and (iv) mapping of targeted traits and harnessing the germplasm diversity using genome-wide association study (GWAS) approach. Similarly, Indo-Australian Biotechnology Fund (IABF) and Department of Biotechnology, Government of India jointly funded a project entitled "Improving Chickpea Adaptation to Environmental Challenges in Australia and India." This proposal is a collaboration between ICRISAT, Indian Agricultural Research Institute (IARI), India, South Australian Research and Development Institute, Australia, and The University of Western Australia, Australia. The project has a major focus on identification and delivering genetic improvements in chickpea that will support breeding for enhanced abiotic and biotic stress.

9.8 Conclusion

In the year 2010, ICGSGC came into existence with main objective of decoding the chickpea genome sequence, and as a result of efforts of the consortium, the genome sequence was made public in 2013. Ever since the genome sequence information is available to chickpea research community, there have been efforts to utilize this information for crop improvement. For instance, the funding organizations like Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, Indo-Australian Strategic Research Fund have already encouraged research groups that are making use of chickpea genome sequence and re-sequence information for developing the climate resilient chickpeas.

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Sequencing the Chickpea Genome

10

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Abstract

The importance of chickpea and constraints in chickpea production urged the need of chickpea genome. Varshney and colleagues in 2013 reported the draft genome of chickpea (kabuli). The genome assembly was 532.29 Mb spanning across 7,163 scaffolds and consisted of 28,269 gene models. The estimated size of chickpea genome was 738.09 Mb based on k-mer analysis. The draft genome assembly covered 73.8% of the total estimated genome size for chickpea. Gene annotation was carried for predicted gene models, though the UTRs and promoters have not been yet been predicted. Genome duplication and synteny analysis with other closely related legume crops showed gene conservation and segmental duplications spread across the draft genome assembly. The genome assembly provides resource for targeting genes responsible for disease resistance which are of agronomic importance. The genome assembly has been used for genome-assisted breeding and is further utilized to study the diversity and domestication of chickpea.

10.1 Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated, diploid $(2n = 2x = 16)$ legume crop primarily grown by resource-poor farmers in the semi-arid regions of the world. The nitrogen fixing ability and high protein content of chickpea make it a crop of high economic importance in developing countries. Based on the grain size and seed coat color, two market classes of chickpea, namely desi and kabuli, are cultivated extensively. Advances in genomics technologies facilitated the adoption of genomics

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tools in crop improvement referred as genomics-assisted breeding (Goodwin et al. 2016; Varshney et al. 2014; Koboldt et al. 2013; Metzker 2010). The availability of draft genomes of major cereals including rice (*Oryza sativa*; IRGSP 2002; Goff et al. 2002), sorghum (*Sorghum bicolor*; Paterson et al. 2008), maize (*Zea mays*; Schnable et al. 2009), and legumes such as pigeonpea (*Cajanus cajan*; Varshney et al. 2012) facilitates the deployment of genomic information in crop improvement.

Owing to the economic importance of chickpea and given the usefulness of draft genomes, the International Chickpea Genome Sequencing Consortium (ICGSC) led by ICRISAT decoded the draft genome of kabuli genotype CDC frontier. This chapter mainly summarizes the tools and strategies used for generating the draft genomes and various analyses for understanding the genome architecture of chickpea and synteny with other sequenced legumes. In addition, this chapter also provides a comparative view of both desi and kabuli genomes available.

10.2 Strategies and Tools for Sequencing

The chickpea genome sequencing was carried out using the short reads from Illumina HiSeq 2000 and bacterial artificial chromosome (BAC) end sequencing (Varshney et al. 2013). The Illumina short reads were assembled into contigs which were further used to construct the scaffolds. The BAC end sequencing was used to form the backbone for the scaffolding. Further, the high-density genetic maps were used to anchor the scaffolds on to the pseudomolecules. The unanchored scaffolds and contigs were reported separately along with pseudomolecules, as a part of the final assembly. Paired-end sequencing libraries (11 in total) were formulated with insert sizes of ~170 bp, 500 bp, 800 bp, 2 Kb, 5 Kb, 10 Kb, and 20 Kb. For the development of assembly, scaffolds' construction and gap closure, SOAPdenovo2 (Luo et al. 2012) was used. The genetic marker sequences along

with flanking regions were searched in the assembly using BLASTN (Altschul et al. 1997) and also using e-PCR (Schuler 1997) in case of the presence of only primer sequences, to place these sequences on the scaffolds. The microbial contamination was eliminated from the genome assembly using searches against the bacterial and fungal genomes with the help of Megablast. Further, BLAT (Kent 2002) was used to screen for contamination of organellar DNA, chloroplast genome sequence of chickpea, and Lotus (*L. japonicus*) mitochondrion in the chickpea genome assembly. The completeness of the genome assembly was verified by mapping the transcriptome assembly contigs to the genome assembly using BLAT. The exome coverage prediction was carried out by mapping the core eukaryotic genes, identified by core eukaryotic gene mapping approach CEGMA v.2.3 (Parra et al. 2007), to the genome assembly.

10.3 Assembly

A total of 153.01 Gb of sequence data was generated for the development of the first draft genome assembly in chickpea. This resulted in coverage of 207.32X from 11 genomic libraries sequenced using Illumina platform with insert sizes ranging from 180 bp to 20 Kb. The high-quality sequence data of 87.65 Gb after filtering was used to assemble into 544.73 Mb of genome sequence scaffolds. The N50 for these scaffolds was 645.3 Kb, and the maximum size of these scaffolds was found to be 6.17 Mb. The chickpea genome is estimated to be of 738.09 in size which shows that the assembled scaffolds were able to cover 73.8% of the genome. The non-assembled genome is believed to be enriched with repetitive sequences as observed by increased read depth in repeat-containing regions in comparison with non-repeat regions and also by having four-fold lower k-mer diversity in non-assembled fraction as compared to non-repetitive assembled fraction. An improved assembly spanning 532.29 Mb with a N50 of 39.99 Mb having 7,163 scaffolds was generated

with the help of 46,270 repeat masked paired bacterial artificial chromosome (BAC) end sequences. The anchoring of 65.23% of this assembly to eight genetic linkage groups was carried out with the help of 1,292 genetic markers reported in previous studies. This data was used to obtain eight pseudomolecules namely, Ca1-Ca8. The anchoring of 93.4% of these scaffolds was validated using restriction-site-associated DNA (RAD) single nucleotide polymorphism (SNP) markers that were discovered between two segregating recombinant inbred line populations. This approach resulted in the identification of low-proportion chimeric scaffolds, i.e., 1.7% of the total scaffolds which amounted to 4.6 Mb of mis-assembled genomic sequence. These chimeric scaffolds were processed by excluding the erroneous part of the scaffold sequences and removing them from the pseudomolecule models. Another synteny-based approach was used to anchor the scaffolds onto the pseudomolecules. In this approach, regions lacking genetic support but showing conserved synteny with Medicago (*Medicago truncatula*) were anchored to pseudomolecules. The regions supported by synteny are hypothetical placements in the pseudomolecules which will be eventually updated upon availability of improved genetic maps supporting these regions or if there are modifications in the assembly of Medicago. The RAD genotyping data was used to anchor 75% of the scaffolds, while the synteny-based approach by comparing scaffolds with Medicago was used to anchor rest of the 25% scaffolds to the pseudomolecules.

10.4 Repetitive Sequences

Repeat regions in the genome were identified using Tandem Repeat Finder (Benson 1999) which resulted in a total of 127,377 such regions. It was observed that 84.9% repeat regions occurred in span of <1 kb, while in gap-spanning clones repeat regions were present in the tracts of 10–103 kb. Out of the total repeat regions identified, 29,018 regions could not be assembled due to low-sequence complexity and the occurrence

of such repeats was masked by adding Ns within the pseudomolecules. Nearly half of the chickpea genome consists of transposable elements (TEs) and unclassified repeat elements similar to the percentage observed in other legume crops such as Medicago (30.5%), pigeonpea (*Cajanus cajan*; 51.6%), and soybean (*Glycine max*; 59%). The most abundant transposable elements are long terminal repeat (LTR) which covers more than 45% of total nuclear genome. The centromere regions are made up of the microsatellites which are dispersed as tandem repeats. The most found tandem repeats within the genome are 163-bp (18%), 100-bp (30%), and 74-bp (13%) unit repeats and constitute a total of 61% of total tandem repeats identified. The 163-bp and 100-bp units correspond to already identified chickpea microsatellites, *CaSat1* and *CaSat2,* respectively, while 74-bp repeat is similar to dispersed highly repetitive element *CaRep2*. Tandem repeat finder was used to filter for the genomic regions >3 copies and >60 bp consensus length across the genome assembly. The genome assembly was scanned for the presence of transposable elements combining two approaches of de novo and homology-based searches. LTR_Finder v 1.03 (Xu and Wang 2007), PILER-DF v 1.0 (Edgar and Myers 2005), and RepeatScout v 1.05 (Price et al. 2005), all three de novo software, were used to build a chickpea repeat database. Repeat Masker v 3.2.7 (http://repeatmasker.org/, v 3.2.2) was deployed to identify repeats with the help of the constructed chickpea repeat database and Repbase (Jurka 1995). Along with these approaches, Repbase was also used to identify repeat-related proteins in the genome using RepeatProteinMask (http://repeatmasker.org/, v 3.2.2).

10.5 Gene Annotation

Gene prediction was done using combined approaches of ab initio modeling and homology-based searches with gene sets taken from six closely related legume species and CaTA transcript sequences. These approaches resulted in a non-redundant set of 28,269 gene

models where average transcript and coding sequence size were 3,055 bp and 1,166 bp, respectively. Majority of these genes show homology with the gene models present in TrEMBL and Interpro (Zdobnov and Apweiler 2001) databases. The functions were assigned to 89.73% genes, while the rest 2,904 genes remained unannotated. The gene density was observed to be on the rise toward the ends of the pseudomolecules. The nonprotein coding genes resulted in the prediction of 684 tRNA, 478 rRNA, 420 miRNA, and 647 snRNA genes in the genome. The 454/Roche transcriptome data generated for CDC frontier line was mapped to the genome assembly for validation of the gene space capture by the draft genome assembly. The gene coverage is calculated to be $\sim 90.8\%$. More than 98% homologs for core eukaryotic genes were found to be conserved in the draft genome assembly. BLASTP search using the chickpea proteome as query against the proteomes of Medicago, soybean, pigeonpea, and Lotus (*Lotus japonicus*) was carried out to estimate the conservation of chickpea gene models present in mentioned species. Proteome of chickpea was found to be most similar to Medicago (89.7% chickpea proteins correspond to Medicago proteins) and least similar to Arabidopsis (*Arabidopsis thaliana*: 79.2% were found similar to Arabidopsis proteins).

Three approaches homology-based, *de novo*, and transcript sequence-based were used for the gene prediction. The results of these approaches were fed to GLEAN (Elsik et al. 2007), which after multiple filtration resulted in a gene set of 28,256 genes. Further, CEGMA identified 453 core genes which are highly conserved across all eukaryotes. Out of these 453 core genes, 13 genes did not align to any gene with the set defined by GLEAN and rest were found present in the genome and hence were added to a final set resulting in 28,269 genes. BLASTP against SwissProt and TrEMBL databases (Magrane and Consortium 2011) was used to assign functions to the final predicted gene set. The presence of motifs and domains in genes was detected using InterProScan against protein databases which include Pfam (Punta et al. 2011), PROSITE

(Sigrist et al. 2010), SMART (Letunic et al. 2012), PRINTS (Attwood et al. 2003), PANTHER (Thomas et al. 2003), and ProDom (Corpet et al. 2000). Genes were assigned gene ontology IDs, and with the information obtained from KEGG database (Kanehisa and Goto 2000) annotated with their associated pathway. tRNAscan-s.e.m. v1.23 (Lowe and Eddy 1997) was used to scan for tRNA genes, and INFERNAL v0.81 (Nawrocki et al., 2009) was used to predict snRNA and miRNA genes by searches against the Rfam database.

10.6 Genome Duplication

The genome duplication events occur in the genome over the course of evolution for a species. The scanning of the genome sequence for the presence of segmental duplications resulted in 110 syntenic blocks that contained 5 to 62 gene pairs. The divergence time was observed to be 58 million years (Myr) ago based on the rates of synonymous substitution per synonymous site (Ks) for the syntenic blocks. The divergence time is in consistence with genome duplication event that occurred at the base of Papilionoideae. The galegoid (Medicago, Lotus and chickpea) and millettioid (soybean, pigeonpea) clades in this family separated around 54 Myr ago. The chickpea species diverged from Lotus around 20–30 Myr ago and from Medicago around 10–20 Myr ago based on the analysis of four-fold degenerate sites using the calculation of genetic distance–transversion rates.

10.7 Synteny with Allied and Model Genomes

Synteny analysis was carried out for chickpea with 6 other closely related crops, namely Medicago, Lotus, pigeonpea, soybean, Arabidopsis, and grape (*Vitis vinifera*). The synteny analyses revealed extensive conservation between chickpea, and other species shows that high percentage of chickpea assembly has conserved regions matching with one or more species included in

the synteny analysis. The maximum number of conserved syntenic blocks (>10 kb) was seen in Medicago, while it was substantially fragmented with Lotus. When compared with legumes, soybean showed the maximum number of syntenic blocks depicting its recent polyploidy ancestry, while fragmented colinearity with pigeonpea suggests the incompleteness of the pigeonpea genome assembly. The 28,269 gene models of chickpea were compared with 230,161 gene models from four legumes and two non-legumes resulting in 15,441 orthologous groups using reciprocal pairwise approach. Of these, 5,940 orthologous groups were observed having a single chickpea gene indicating simple orthology relationship, while 4,468 chickpea genes were observed in species-specific groups, with no ortholog but having paralogs within the genome. These groups may be attributed to the structural rearrangements that lack simple orthology followed by duplication, as is observed in the case of NBS-LRR disease resistance genes. The percentage of the total predicted gene models which were classified into orthologous groups by OrthoMCL gives insights for the genes which have history of duplication after the divergence of legumes from Arabidopsis and grape. The chickpea genome may be attributed to a series of gene loss and gene duplications as it is the same time interval required for whole-genome duplication event at the base of the Papilionoideae. Several genes from each of the 7 species could

not be placed into orthologous groups which may be because of the heterogeneity in gene prediction for each of these species while it may also be due to lineage-specific evolution events. MUMmer (Delcher et al. 2003) and SyMAP (Soderlund et al. 2011) were used in combination for the synteny analysis. Classification of orthologous genes and gene clusters was carried out using OrthoMCL (Li et al. 2003).

10.8   Comparison of Desi and Kabuli Genomes

There was another effort made towards the genome sequencing of chickpea, by whole genome sequencing of the ICC 4958 genotype which is desi type (Jain et al. 2013). There exist various differences in the final assemblies reported by two efforts mentioned above (Table 10.1). The genome size of the kabuli genome was 532.29 Mb, and in case of desi genome, it was 519.84 Mb. The number of gene models reported for the two genomes was similar: 28,269 in kabuli and 27,571 in desi. The number significantly differs for the number of scaffolds assembled and N50 for the two genome assemblies. The number of scaffolds is comparatively too less in case of kabuli genome, and also, the N50 value is comparatively high which states that the kabuli genome is much better in terms of these assembly parameters. As compared to desi,

Table 1 Comparison of the features of first two draft genome assemblies in chickpea

Feature	Varshney et al. (2013)	Jain et al. (2013)
Chickpea type	Kabuli	Desi
Genotype	CDC frontier	ICC 4958
Assembly size	532.29 Mb	519.84 Mb
No of scaffolds	7,163	181,462
N50	39.99 Mb	0.077 Mb
No of gene models	28,269	27,571
Longest scaffold	59.46 Mb	23.37 Mb
Total size in pseudomolecules	347,247,377 bp	124,385,597 bp
Repeat elements	258,057,703 bp	210,201,779 bp
No of miRNA	420	60
No of tRNA	684	627
No of rRNA	478	249
GC content	30.78%	26.93%

kabuli genome has ∼2.8 times sequence anchored at pseudomolecules, and the longest scaffold in kabuli is more than twice the size of longest scaffold observed in desi genome. The number of miRNA, tRNA, and rRNA fragments is significantly higher in the kabuli genome. The GC content is bit higher in the kabuli genome which may be attributed to only Illumina technology used to develop the assembly. The desi genome is more fragmented in comparison with the kabuli genome, and kabuli genome will serve a better resource for genome-based studies in chickpea.

10.9 Subsequent Validation

Both kabuli and desi assemblies were subsequently assessed using a chromosomal genomics approach to determine whether the differences in the genome assemblies represent real differences in genome structure or are artifacts of assembly of one or both genomes. Isolated chromosomes from each of the varieties were sequenced and the data was mapped to the pseudomolecules (Ruperao et al 2014). This analysis demonstrated that the physical genomes of kabuli and desi chickpea types are very similar and the observed differences in the sequence assemblies are due to major errors in the desi genome assembly, including the misplacement of whole chromosomes, portions of chromosomes, and the inclusion of a large portion of sequence assembly which does not appear to be from the genome of chickpea. In contrast, the kabuli assembly is mostly correct. Based on this analysis, updated versions of both kabuli and desi genome assemblies have been produced (http://doi.org/10.7946/P2G596 and http://doi.org/10.7946/P2KW2Q), with GBrowse access at http://www.cicer.info/.

10.10 Conclusion

The chickpea genome sequencing has provided the much needed thrust to genomics based breeding approaches. Further, the re-sequencing of the germplasm will help in better understanding of the diversity present in *Cicer* species. The resource generated from these sequencing efforts will help in improvement of the genome assembly with enhanced coverage. The improved genome assemblies will help in identification of regions linked to important agronomic traits. These sequencing efforts are expected to enhance the chickpea yield and its resistance to biotic and abiotic stresses.

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Impact of Genomics on Chickpea Breeding

11

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Abstract

Chickpea is an economical source of vegetable protein for the poor living in the semi-arid regions globally. As a consequence of climate change and increasing climate variability, the incidences of drought and heat stresses and severity of some diseases, such as dry root rot and collar rot, have increased in chickpea crop, resulting in poor and unstable yields. By improoving the efficiency of crop breeding programs, climate resilient varieties with traits desired by the farmers, industries and consumers can be developed more rapidly. Excellent progress has been made in the development of genomic resources for chickpea in the recent past. Several national and international chickpea breeding programs have started utilizing these genomic resources and tools for genetic improvement of complex traits. One of such examples includes the introgression of "*QTL-hotspot*" containing quantitative trait loci (QTLs) for several drought tolerance-related traits, including root traits, through marker-assisted backcrossing (MABC) for enhancing drought tolerance in popular cultivars. Several drought-tolerant introgression lines with higher yield as compared to the popular cultivars have been identified. Multi-parent advanced generation intercross (MAGIC) populations developed from using 8 parents created large genetic diversity consequently several promising lines. Marker-assisted recurrent selection (MARS) has also been explored for yield improvement in chickpea. Development of diagnostic markers or the identification of candidate genes for several traits is essential for greater use of genomic resources in chickpea improvement.

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11.1 Introduction

Chickpea (*Cicer arietinum* L.) is a cool-season food legume grown mainly in arid and semi-arid regions of the world. Chickpea is cultivated in 13.9 m ha with a production of 13.6 m ton during 2014 (FAOSTAT 2016). Its cultivation and consumption are mainly concentrated in South Asia region, where India alone accounts for more than 70% of global chickpea area and consumption. Due to its cultivation under challenging soil and environmental conditions, the crop is exposed to several biotic and abiotic stresses. As a consequence of climate change, incidence of new races and also new diseases such as dry root rot and collar rot are posing serious threats to chickpea production. Besides classical breeding methods, genomic approaches are particularly useful in handling complex traits, which are usually controlled by several genes and highly influenced by environment. Genomic tools facilitate in the identification of genomic regions/QTLs and favorable alleles of small effects that generally remain unnoticed and are not included in the gene pool used for breeding (Morgante and Salamini 2003; Vaughan et al. 2007).

Most of the qualitative traits are characterized by high heritability and are easy to select for. However, favorable allelic combinations and genetic recombinations for complex traits are difficult to identify through conventional breeding strategies. Advances in genomic technologies enable to capture genome-wide diversity in natural and artificial populations. In recent years, large-scale genomic resources were developed in case of chickpea (Varshney et al. 2010). The draft genome sequence and resequence information of germplasm lines of chickpea (Varshney et al. 2013) including parental lines of several mapping populations (Thudi et al. 2016), varieties (Thudi et al. 2016) provided several thousands of simple sequence repeat (SSR) markers, millions of single nucleotide polymorphism (SNP) and indels for use in genomics-assisted breeding for chickpea improvement. The present chapter discusses the recent advances in the application and impact of the genomic tools in chickpea improvement.

11.2 Marker-Assisted Selection for Chickpea Improvement

In marker-assisted selection (MAS), the genotypes are selected based on the presence or absence of markers instead of the trait itself. The tight linkage between the markers and the major gene or QTL responsible for the trait is necessary for successful implementation of MAS in breeding programs. With the advances in new genomic tools and availability of a large amount of genomic resources, it is now possible to identify the strong associations between markers and traits.

Routine breeding programs involve the combining of two or more genes or QTLs controlling trait(s) of interest into a common genetic background. In this process, selection of plants having all favorable donor alleles based on phenotype for making backcrosses or generation advancement in each generation will be very difficult. In such circumstances, the application of MAS will be of a great help in the process of identifying plants carrying several targeted alleles whose effect on the phenotype is not recognizable. In chickpea, a large number of genomic resources were deployed for the identification of genes/QTLs controlling several qualitative and quantitative traits. Some of them are early-flowering-time genes (Gaur et al. 2016; Mallikarjuna et al. 2017), pod- and seed-related traits for enhancing heat tolerance (Pronob Paul, personal communication), vernalization response (Samineni et al. 2016), root-related traits for enhancing drought tolerance (Chandra et al. 2004; Varshney et al. 2014a), ascochyta blight resistance (Aryamanesh et al. 2010; Cho et al. 2004; Varshney et al. 2014b), fusarium wilt resistance (Sharma et al. 2004; Sharma et al. 2005; Tekeoglu et al. 2000), botrytis gray mold resistance (Anuradha et al. 2011), seed yield traits under salinity (Pushpavalli et al. 2015) and normal growing conditions (Gowda et al. 2011),

double podding (Rajesh et al. 2002; Cho et al. 2002; Ali et al. 2010), flower color, and several other traits. Among these traits, practical application of QTLs identified for improving drought tolerance, fusarium wilt, and ascochyta blight diseases has been successfully demonstrated using marker-assisted backcrossing (MABC).

Improvement of Drought Tolerance

In case of drought tolerance, multi-disciplinary activities were converged to improve the response of chickpea to drought stress conditions. The architecture and function of the root system are expected to directly relate to the transpiration efficiency (TE) which in turn is responsible for water balance in the plant during moisture stress conditions. The results indicated that increasing drought tolerance via deep roots along with higher TE was the key trait most likely to give higher grain yield under drought stress conditions (Soltani et al. 2000). Even though roots play a vital role in the extraction of water from the soil layers, very less information about the extent of variation and genetic behavior of these traits was revealed. The reason is obvious that phenotyping of these traits is highly labor-intensive and high influence of growing conditions on the results recorded.

Efforts from physiologists led to the identification of large variation for various root-related traits in the germplasm (Kashiwagi et al. 2005) and RILs (Serraj et al. 2004) that help reduce the negative effects of drought. In this direction, the root length density (RLD) in relatively shallow soil layers and the maximum root depth (RDp) were found to positively influence the seed yield under terminal drought environments (Gaur et al. 2008). One RIL population from Annigeri x ICC 4958 cross was developed and phenotyped for root traits (Serraj et al. 2004). Further, based on the results from screening of chickpea germplasm, two intraspecific mapping populations, namely ICC 4958 × ICC 1882 and ICC 283 × ICC 8261, were developed at ICRISAT (Gaur et al. 2008). These populations were evaluated at multi-locations over multi-seasons in India and genotyped with SSR markers. Results showed that a genomic region referred as "QTL-hotspot" showing 58.20% phenotypic variation for 12 drought tolerance-related traits including root traits was identified on CaLG04 (Varshney et al. 2014a). The "QTL-hotspot," validated in both RIL populations, increased the confidence of chickpea breeders to introgress this genomic region into popular chickpea cultivars for enhancing drought tolerance.

MABC aims to transfer one or more genes/QTLs of interest from one genetic background into popular or elite cultivar to improve the targeted trait. In this direction, chickpea varieties, JG 11, ICCV 10, and KAK 2 from India and Chefe from Ethiopia, were selected to introgress this genomic region from ICC 4958 genotype using MABC scheme. After making three backcrosses and selfing for 4 generations, more than 20 BC_3F_4 introgression lines were developed in each background of JG 11 and ICCV 10. A number of plants selected in each generation for making crosses and the markers used in the foreground and background selection for improving JG 11 were reported in Varshney et al. (2014a). These lines were evaluated at multi-locations for two years during 2011–2014 in India. Several lines giving at least 10% higher seed yield than recurrent parents (JG 11 and ICCV 10) were identified under both rainfed and irrigated conditions. Location-specific genotypes identified and very few genotypes found common in the top yielding lines due to high influence of environment. Interestingly, there was no relationship between yield under rainfed and irrigated conditions. Further, the seed size of most of the lines was increased which is similar to donor parent (ICC 4958). It indicates that the QTL-hotspot region was also influencing/close to genes controlling seed size in ICC 4958.

Heat Stress Tolerance

Increase in temperatures due to global warming reduces accumulation of assimilates, enhances

leaf senescence, disturbs fertilization activities, and thus drastically reduces seed yield of crops, especially in combination with drought stress. As a result, plants show shortened life cycles, less time for photosynthesis (Reynolds et al. 2010), a shorter reproductive phase, and lower yield potential (Ainsworth and Ort 2010).

Being adapted to cool-season environments, cultivation of chickpea under increased day and night temperatures is a big challenge ahead. Recent screening efforts identified several heat-tolerant genotypes in chickpea (Wang et al. 2006; Krishnamurthy et al. 2011; Upadhyaya et al. 2011; Devasirvatham et al. 2012; Gaur et al. 2015). The ability of heat tolerance varies with cultivars and could involve changes in both morphological and physiological traits (Karim et al. 2000; Kumar et al. 2012), and therefore, heat-tolerant genotypes could be of great promise toward achieving stable yields under increasing temperatures. In this direction, a recent study was conducted to identify genomic regions related to heat tolerance in F_{8-9} recombinant inbred line (RIL) population of the cross ICC 4567 (heat sensitive) $\times$ ICC 15614 (heat tolerant). Phenotypic evaluation was done under field conditions with no stress and heat stress treatments. Genotyping-by-sequencing (GBS) approach based on 271 single nucleotide polymorphisms (SNPs) covering the whole genome of chickpea was used for genotyping. The QTL analysis revealed two consistent genomic regions harboring eight QTLs on CaLG05 and CaLG06. Four major QTLs for number of filled pods, number of seeds, grain yield, and % pod setting, located in the CaLG05 genomic region, were found to have cumulative phenotypic variation of above 50%. Moreover, QTL $\times$ environment interaction effects were non-significant except for harvest index and biomass (Pronob Paul, personal communication). Validation of these QTLs in other populations is in progress, and once these are validated, MAS can be effectively implemented in regular breeding programs for enhancing heat tolerance.

Enhancement of Genetic Diversity Through MAGIC (Multi-parent Advanced Generation Intercrossing) Lines

The existence of genetic diversity in the breeding populations is crucial to develop new varieties with resistance to various biotic and abiotic stresses. Using different hybridization techniques, plant breeders create variability for various traits of economic importance, which will expose the rare or important alleles in homozygous condition. In this direction, MAGIC lines were developed in chickpea using eight diverse genotypes selected from South Asia and sub-Saharan Africa. In this development scheme, more number of recombinations and greater genetic diversity in MAGIC lines greatly help in detection of QTLs with high precision. Further, these lines can be used directly in breeding programs for the development of cultivars suitable to diverse agro-ecologies in Asia and sub-Saharan Africa. The incorporation of multiple parents ensures the population segregates for multiple QTLs for multiple traits. Further, MAGIC lines can act as a base for gene discovery, characterization, and deployment of genes for understanding complex traits (Glaszman et al. 2010). The power of such populations has been demonstrated in maize to understand the genetic architecture of several traits (Buckler et al. 2009; Poland et al. 2011).

The parents (ICC 4958, ICCV 10, JAKI 9218, JG 11, JG 130, JG 16, ICCV 97105, and ICCV 00108) of the MAGIC lines from Ethiopia, Kenya, and India were crossed in direct fashion excluding reciprocals. Twenty-eight two-way, 14 four-way, and 7 eight-way crosses were made to develop a MAGIC population. Over 1200 F_2 plants from 7 eight-way crosses were advanced to F_8 using single seed descent (SSD) method and seed was harvested from 1136 $F_{7:8}$ progenies. These progenies were evaluated for two years (2013 and 2014) under field conditions with rainfed and irrigated treatments. Large

variability in phenology (days to flowering: 34–69 d) and yield-related traits (seed yield: 170–4554 kg/ha); 100 seed weight: 10–45 g) was observed under two treatments (Gaur et al. unpublished data). Several promising lines with significantly higher seed yield than the best parents were also identified. On the other hand, eight parents used in the development of MAGIC lines were genotyped using 70 SSR and 747 SNP markers. In addition, the parental lines were resequenced (with 5.79–16.08X coverage) and variable number of SNPs and indels were identified among the parental lines by aligning to CDC Frontier reference genome. The F_8 progenies were also genotyped for identification of diverse MAGIC lines based on haplotype distribution. MAGIC lines will be a valuable source for establishing marker-trait association using genome-wide association study (GWAS) for several complex traits in chickpea.

Marker-Assisted Recurrent Selection (MARS) for Yield Enhancement

Many complex traits are controlled by several minor QTLs. Gene pyramiding becomes very difficult as the number of QTLs increases, and in such cases, MABC has limited application. The more effective strategy would be to deploy MARS to increase the frequency of favorable alleles in the populations. This molecular breeding scheme differs from traditional QTL or MAS studies in that the new mapping study is conducted on each breeding population. This technology was developed first by major commercial maize breeding programs, and it has shown promising in increasing the rate of genetic gain. To evaluate the application of MARS scheme in self-pollinated crops such as chickpea, experiments were conducted with the support from Generation Challenge Program (GCP) during 2010–2014 at ICRISAT, Patancheru, India. Choice of populations was driven by yield performance of genotypes. Two crosses JG 11 × ICCV 04112 and JG 130 × ICCV 05107 were made to combine the favorable alleles of yield QTLs from the respective parents with an assumption that each population had a different

set of QTLs involved. 188 F_3 plants each in two crosses were genotyped using SSR markers. Further, F_3-derived F_5 progenies were evaluated at multi-locations. QTL analysis of phenotyping and genotyping data resulted in identification of few major and several minor QTLs contributing to yield, and yield contributing traits (RK Varshney, personal communication). Based on the QTL(s) information for seed yield, harvest index, biomass, and seed size in different F_5 progenies, 4 lines were selected in JG 11 × ICCV 04112 and 3 lines in JG 130 × ICCV 05107 having different combinations of favorable alleles for recombination cycle. Multiple cycles of MARS increased the frequency of favorable marker alleles associated with agronomic traits. The selected lines were subjected to 1st and 2nd recombination cycles. F_1 plants (27 and 5) having all favorable alleles of QTLs from both parents for seed yield and other yield-related traits were identified in JG 11 × ICCV 04112 and JG 130 × ICCV 05107, respectively. Finally, F_1 plants with all favorable alleles in homozygous condition were grown. Each selected F_1 plant was advanced to F_4 generation separately for field evaluation. On the other hand, for comparing the advantage of MARS over the traditional method of recurrent selection, top 8 high-yielding F_5 progenies were also selected and intercrossing is being completed in each cross. Practical utility of this scheme in regular breeding programs will depend on the genetic gain achieved in terms of selection efficiency, selection accuracy, marker-trait associations, and distribution of favorable alleles between the selected parents used for crossing. Further, marker technology helps in the identification of individual plants having all favorable alleles among large populations when the number of *loci* of interest is higher, and in identifying whether the genotype combining favorable alleles is present in the population (Ishii and Yonezawa 2007). The utility of MARS decreases as the information of the number of small-effect QTLs associated with the trait decreases (Charcosset and Moreau 2004; Bernardo and Charcosset 2006). However, Bernardo and Charcosset (2006) reported that the higher genetic gain was feasible through MARS compared to MABC.

Application of MARS in chickpea
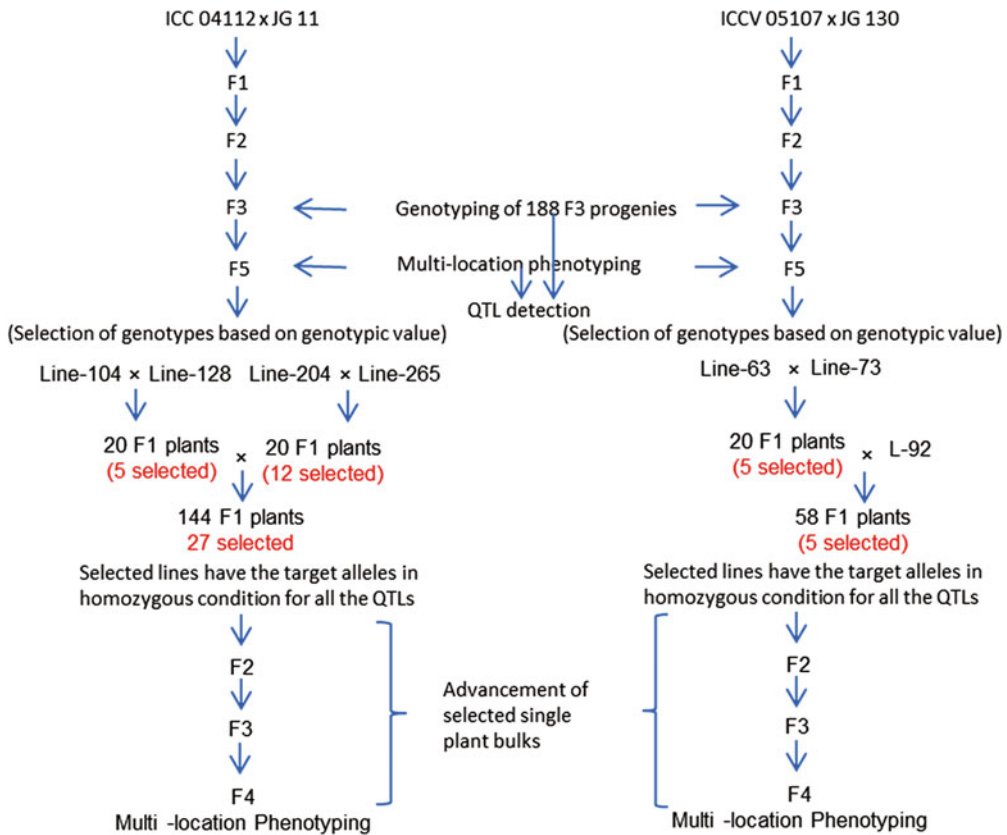
Introgression of Fusarium Wilt and Ascochyta Blight Resistance into Elite Cultivar

In chickpea, genes from different sources which confer resistance against fusarium wilt and ascochyta blight (*Ascochyta rabiei*) diseases have been successfully transferred and genotypes were developed with resistance to fusarium wilt and ascochyta blight diseases. Wilt is the most commonly occurring disease in warm and dry regions. Several stable sources of resistance were identified (Haware and Nene 1982; Nene et al. 1989; Pande et al. 2006) and successfully integrated into the backgrounds of high-yielding lines in the regular breeding programs for enhancing the wilt resistance. Further, the available field and laboratory screening technologies

are cost-effective, yet reliable. However, availability of molecular markers associated with wilt resistance genes could accelerate the selection of resistant genotypes. Using the marker-assisted selection, markers tightly linked to wilt resistance genes can be used to screen a large number of genotypes for the presence of these genes. For example, SSR marker "TA59" has been used to tag genes for wilt resistance in the NIL development (Castro et al. 2010). Several studies suggest the existence of a genomic region harboring several resistance genes in linkage group 2 (LG2), including a cluster of six fusarium wilt resistance genes: foc-0, foc-1, foc-2, foc-3, foc-4, and foc-5 (Tekeoglu et al. 2000; Winter et al. 2000; Cobos et al. 2005; Milla´n et al. 2006; Sharma and Muehlbauer 2007; Halila et al. 2009). Identifying reliable race-specific

diagnostic markers will further enhance the application of molecular markers in regular breeding programs. These markers help in the identification of sources of resistance to different races simultaneously, which has been a difficult task under field screening.

Ascochyta blight (AB) is a major disease of chickpea, especially in areas where cool, and humid weather persists during the crop season. Several sources of resistance to AB were identified (Reddy and Singh 1984; Singh and Kapoor 1985; Singh and Reddy 1990). Breeding efforts at ICRISAT led to the development of varieties with moderate to good level of resistance to AB were released in the names of "Myles" and "Howzat" in USA and Australia, respectively. Genetic studies reported that AB resistance of chickpea is oligogenic in nature. Studies on RILs suggest that several genomic regions (QTLs) were involved in controlling resistance to AB dispersed on different linkage groups (LG2, LG3, LG4, LG6, and LG8) in the genome. LG4 has been reported by several researchers to contain QTLs for AB resistance (Santra et al. 2000; Tekeoglu et al. 2002; Cho et al. 2004; Stephens et al. 2014), while other reports highlight LG2 (Udupa and Baum 2003; Cho et al. 2004), LG3 (Flandez-Galvez et al. 2003b; Anbessa et al. 2009; Kanouni et al. 2009), and LG8 (Lichten-zveig et al. 2006). Markers closely linked to major QTLs have been reported. Two QTLs for pathotype II located on LG4, one is linked to markers CaETR or GAA47 and the other is linked to TA72/ScY17 (Udupa and Baum 2003; Cho et al. 2004). Furthermore, loci TS12b and STMS28 on LG1 and TS45 and TA3b on LG2 were found significantly associated with the disease reaction under controlled environments (Flandez et al. 2003a and b). Similarly, a codominant marker (CaETR) located in the QTLAR1 region of LG4 was also reported (Madrid et al. 2013). However, these markers linked to different AB QTLs need to be validated in diverse populations for their utility in regular breeding programs.

In this direction, an attempt was made to introgress the QTLs controlling FW and AB into a cultivar, C 214 (Varshney et al. 2014). In the foreground selection, six SSR markers (TR19, TA194, TAA60, GA16, TA110, and TS82) linked to foc-1 for FW, and eight markers (TA194, TR58, TS82, GA16, SCY17, TA130, TA2, and GAA47) linked to ABQTL-I and ABQTL-II for AB were used in MABC scheme. After three backcrosses, FW-resistant lines with more than 90% recovery and AB-resistant lines with more than 80% recovery of recurrent parent genome were selected. These lines need to be evaluated under field conditions for disease response and agronomic performance in multi-location trials for possible application of these markers.

11.3 Conclusion

New genomic advances, many of which are already being developed, will make it easier for breeders to obtain new cultivars with improved characteristics, either by facilitating selection or by improving the variation available by using precision breeding approaches. In particular, the present and new genomics tools add great value in the process of genetic dissection and breeding of complex traits. So far, the genomic tools played a key role in QTL identification, and their use in chickpea breeding programs is limited to improving drought tolerance. Identification of reliable diagnostic markers for several other important traits should be given more emphasis for rapid spreading of this technology in NARS breeding programs.

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Future Prospects for Chickpea Research

12

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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 grains at a faster pace.

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

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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 loses. 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 F_2, 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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