Review

Genomics-assisted breeding for drought tolerance in chickpea

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Abstract. Terminal drought is one of the major constraints in chickpea (*Cicer arietinum* L.), causing more than 50% production losses. With the objective of accelerating genetic understanding and crop improvement through genomics-assisted breeding, a draft genome sequence has been assembled for the CDC Frontier variety. In this context, 544.73 Mb of sequence data were assembled, capturing of 73.8% of the genome in scaffolds. In addition, large-scale genomic resources including several thousand simple sequence repeats and several million single nucleotide polymorphisms, high-density diversity array technology (15 360 clones) and Illumina GoldenGate assay genotyping platforms, high-density genetic maps and transcriptome assemblies have been developed. In parallel, by using linkage mapping approach, one genomic region harbouring quantitative trait loci for several drought tolerance traits has been identified and successfully introgressed in three leading chickpea varieties (e.g. JG 11, Chefe, KAK 2) by using a marker-assisted backcrossing approach. A multilocation evaluation of these marker-assisted backcrossing lines provided several lines with 10–24% higher yield than the respective recurrent parents. Modern breeding approaches like marker-assisted recurrent selection and genomic selection are being deployed for enhancing drought tolerance in chickpea. Some novel mapping populations such as multiparent advanced generation intercross and nested association mapping populations are also being developed for trait mapping at higher resolution, as well as for enhancing the genetic base of chickpea. Such advances in genomics and genomics-assisted breeding will accelerate precision and efficiency in breeding for stress tolerance in chickpea.

Additional keywords: backcrossing, *Cicer arietinum*, genome sequence, quantitative trait loci, yield.

Introduction

Despite continuous efforts to enhance the productivity of agricultural crops, the improvements in production and productivity tend to be marginal, largely due to the tremendous influence of climate change during the past two decades. The impact of climate change on crop production and productivity in sub-Saharan Africa and South Asia, the two major food-insecure regions, has been extensively reviewed, highlighting important differences in impact between individual crops, continental regions and geographical subregions (Knox *et al.* 2012). Global warming has led to increase in the average global temperatures by 1.2°C over the past century and a further raise of 3°C is estimated to occur by 2100 (Schneider *et al.* 2007). These increased temperatures lead to higher evapotranspiration rates

and thereby decrease soil moisture and the physiological processes of crop plants. As a result, drought has become a global phenomenon and continues to have significant impacts on agricultural production in both developing and developed counties. However, the frequency, severity and duration of drought and its socioeconomic impacts may vary. The impact of drought will be high on crops that are grown after the rainy season, especially cool-season legumes like chickpea (*Cicer arietinum* L.), which is predominantly cultivated on residual soil moisture in the arid and semi-arid regions of the world. During the past three decades there has been a shift in the cultivation of chickpea from cooler to warmer regions (Krishnamurthy *et al.* 2013b). Chickpea is also gaining importance in drier and warmer regions of semi-arid tropics of

East Africa as a major protein source for the poor in arid and semi-arid lands (Kimurto *et al.* 2013).

Chickpea is the second most important food legume cultivated by resource-poor farmers in the arid and semi-arid regions of the world. It is a self-pollinated crop with a basic chromosome number eight and a 739-Mb genome size (see Varshney *et al.* 2013*b*). Globally, chickpea is cultivated on 12.14 million ha with 11.3 million tonnes being produced (FAOSTAT 2012). India ranks first in terms of production and productivity, followed by Pakistan, Turkey, Iran, Myanmar, Ethiopia, Mexico, Australia, Mexico, Canada and the United States. Chickpeas are grouped into two distinct types: the small-seeded 'desi' with a brown-coloured seed coat and the large-seeded cream or beige-coloured 'kabuli'. Desi chickpeas are predominantly cultivated in India, Pakistan, Myanmar, Australia and Bangladesh, whereas kabuli chickpeas are cultivated mostly in Turkey, Ethiopia, Syria, Spain, Canada, the United States, Mexico and Portugal. Chickpea seeds are highly nutritious, comprising ~18–24% protein, 4–10% fat, 52–71% carbohydrate, and 10–23% fibre, minerals and vitamins (Jukanti *et al.* 2012). Furthermore, the seed protein contains essential amino acids like lysine, methionine, threonine, valine, isolucine and leucine. Besides providing the essential components of human dietary and health requirements, they fix atmospheric nitrogen and enrich the soil fertility.

In spite of its economic importance and its role in human health, over the last five decades, neither the area under cultivation nor productivity has increased to meet the current demands (Fig. 1). This slow pace of production trend is due to several abiotic and biotic constraints that have been challenging the crops. The ever-increasing population further aggravates growing demands for food grains, and conventional breeding efforts need to be supplemented by genomics-assisted breeding (GAB) (Varshney *et al.* 2005, 2007). Until only a few years ago, chickpea was considered to be an orphan legume due to meagre genomic resources for implementing GAB. Nevertheless, the availability of chickpea genome sequence information (Varshney *et al.* 2013*b*) and large-scale genomic resources (see Varshney *et al.* 2010*a*, 2010*b*, 2012*a*) have turned the crop into a resource-rich crop like any other major crop species. As a result, GAB activities, including trait mapping and molecular breeding such

as marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS) and advanced backcross quantitative trait loci (AB-QTL) analysis, which are routine in breeding programs for major crops, are also being practiced in chickpea.

In the present review, in addition to summarising efforts being made at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and its partners in India and Sub-Saharan Africa to enhance drought tolerance in chickpea and its research impacts, we have made an attempt to provide a comprehensive overview of research efforts to combat drought across the globe that employ genomics tools for chickpea improvement.

Key target traits: breeding efforts to address drought stress

Morphophysiological traits related to drought are generally categorised either as constitutive traits (affecting yield at low and intermediate drought conditions) or drought-responsive traits (affecting yield only under severe drought conditions) (see Blum 2006; Tuberosa 2012). However, progress achieved thus far in breeding for drought tolerance has mostly been attributed to changes in constitutive traits that affect dehydration avoidance rather than drought-responsive traits (Blum 2005, 2006, 2011). Based on drought tolerance research for several decades, the community has reached a consensus that the target drought tolerance traits for improving yield under drought stress should have high heritability, a strong correlation with yield (Monneveux and Ribaut 2006), good genetic variability and a lack of yield penalties under favourable conditions. In addition, the measurement of the target trait should be nondestructive and noninvasive, high-throughput, precise and accurate, and should use a small number of plants without lengthy procedures and relate to higher levels of functional organisation (Tuberosa 2012).

In chickpea, a range of drought tolerance traits have been targeted for phenotyping, genetic dissection and molecular breeding. These traits include early maturity (drought escape), root traits (drought avoidance), carbon isotope discrimination, rate of partitioning, shoot biomass and grain yield under drought conditions. Several of these traits have been used to screen germplasm collections of chickpea (Upadhyaya *et al.* 2012). For instance, the use of polyvinyl chloride pipe-based high-throughput drought phenotyping for screening large numbers of chickpea germplasm lines and advanced breeding lines including mapping populations have already been successfully documented in several studies (Kashiwagi *et al.* 2005, 2006; Krishnamurthy *et al.* 2010; Upadhyaya *et al.* 2012). By using such phenotyping protocols, the ICC 4958 genotype was identified as a drought-tolerant genotype. This genotype has been used as a drought-resistant donor parent that produces high yield in low productivity, short duration and terminal drought-prone environments in peninsular India.

Differences in crop duration and yield potential (Saxena 2003) are known to contribute to seed yield under drought stress. The removal of these effects from seed yield under stress provides a reliable index or drought tolerance index (Bidinger *et al.* 1987; Saxena 2003). This is, in part, explained by the fact that if the testing site is a short-duration environment for chickpea and
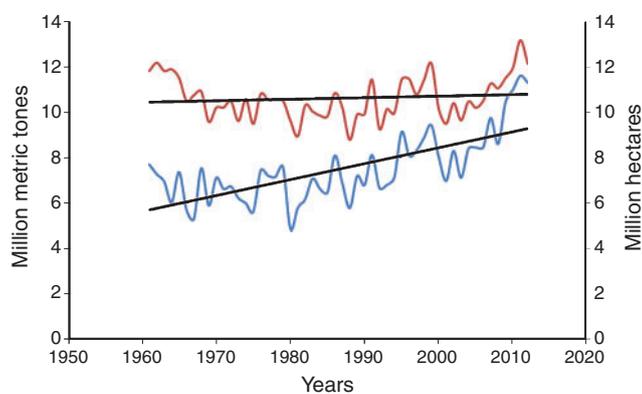
Fig. 1. Production trends and area under cultivation of chickpea across the world during last the six decades.

does not favour long-duration genotypes, these genotypes have to fill their seeds under increasing temperatures and terminal drought (Saxena 2003). For instance, ILC 1799 was identified as being early maturing with a large seed size and higher yield, and is drought-tolerant with wider adaptability (Sabaghpour *et al.* 2006). Previous work has shown that the residual effects after the removal of the effects of drought escape (early flowering) and yield potential (optimally irrigated yield) of a genotype gave a good indication of the true drought tolerance of that genotype (Saxena 2003; Krishnamurthy *et al.* 2010). Employing this method, Krishnamurthy *et al.* (2010) identified the five most drought-tolerant and 20 highly drought-sensitive accessions out of the minicore chickpea germplasm.

Carbon isotope discrimination (Δ^{13}C), an integrator of plant behaviour influencing transpiration efficiency, is an important component of yield under drought. The variation in Δ^{13}C and its association with yield was assessed in the reference collection of chickpea germplasm. The existence of a large variation in Δ^{13}C has been demonstrated (Krishnamurthy *et al.* 2013*a*) and Δ^{13}C also was shown not to contribute to grain yield directly but to have this effect through the harvest index. The partitioning coefficient, an integrator of the grain yield formation process influencing partitioning efficiency, is yet another important component of grain yield under drought (Krishnamurthy *et al.* 1999). Variation in the partitioning coefficient and its association with yield was assessed in the reference collection of chickpea germplasm. This trait showing the greatest association with grain yield had a large range of useful variation across the reference set of chickpea strains (Krishnamurthy *et al.* 2013*b*).

The need for precise drought phenotyping

Recent advances in the area of computational biology, bioinformatics and genomics have helped us to meet the demands set by the genomics revolution to some extent. However, with the deluge of molecular genotypic data generated during last few years, the valid and practical results reported so far have not yielded the expected results (Xu and Crouch 2008; Passioura 2010). This has been partly attributed to slow progress in the area of phenomics, which involves several tools for recording precise and high-throughput phenotyping data. Obtaining a clean set of reproducible and precise phenotypic data on complex quantitative traits like drought tolerance within a larger germplasm collection remains an open challenge even in the era of phenomics-driven technology (Mir *et al.* 2012).

Invasive or destructive methods of plant phenotyping are now being replaced by high-throughput precise and nondestructive imaging techniques. Several phenomics platforms are now available worldwide that provide good phenotyping facilities. These facilities include: (i) infrared cameras to scan temperature profiles and transpiration, (ii) fluorescent microscopy and spectroscopy to assess photosynthesis and photosynthetic rates, (iii) three-dimensional cameras to record minute changes in growth responses, (iv) lidars (light detection and ranging) to measure growth rates, and (v) magnetic resonance imaging to examine root or leaf physiology (Finkel 2009; Gupta *et al.* 2012). Digital imaging allows us to monitor, measure and track many aspects of plant development, function and health that were

unimaginable using conventional measurement techniques. Several software programs have been developed for extracting data from digital images taken from roots, shoots, leaves, seeds and grains (Sozzani and Benfey 2011; Cobb *et al.* 2013). These phenomics tools and those to be developed in future will allow the scanning of thousands of plants in a working day, similar to high-throughput DNA sequencing in the field of genomics (Finkel 2009). The precise and accurate data generated from these facilities is very important and useful for meaningful genetic dissection and GAB applications for crops, including chickpea improvement. These phenomics tools will be used in the near future in chickpea to get valid results out of large number of genomics resources, including the whole genome sequence, which is now available.

Genetic and genomic resources

Over 97 400 chickpea germplasm accessions are conserved in gene banks globally; ICRISAT's gene bank alone conserves 20 267 accessions. Details on the germplasm conserved and the genetic resources available in the case of chickpea have been extensively reviewed in recent publications (Upadhyaya *et al.* 2011; Gaur *et al.* 2013). For efficient and effective germplasm management and conservation, the concept of core and minicore collections have been advocated (Upadhyaya and Ortiz 2001), and trait-specific germplasm has been identified to aid breeding and genomics-assisted selection (Upadhyaya *et al.* 2012). Further attempts were also made to characterise the chickpea germplasm at the molecular level in several studies (Iruela *et al.* 2002; Croser *et al.* 2003; Nguyen *et al.* 2004; Rao *et al.* 2007; Upadhyaya *et al.* 2008; Sefera *et al.* 2011; Choudhary *et al.* 2012) separately from phenotypic characterisation (Upadhyaya *et al.* 2012; Krishnamurthy *et al.* 2013*a*, 2013*b*). Nevertheless, for understanding the genetics of complex traits like drought tolerance, trait mapping is essential for identifying the genes underlying drought tolerance. Based on the evaluation of the minicore collection for terminal drought tolerance, germplasm lines with prolific root systems were identified and three recombinant inbred line mapping populations (Annigeri × ICC 4958, ICC 4958 × ICC 1882 and ICC 283 × ICC 8261) were developed at ICRISAT (see Gaur *et al.* 2008). Similarly, several other mapping populations were also developed for gaining insights into most prevalent biotic and abiotic stresses (see Gaur *et al.* 2013). Furthermore, for creating novel alleles and for functional validation of candidate drought-responsive genes, a 'target-induced local lesions in genome' (TILLING) population, comprising 3072 M2 chickpea lines, was also developed at ICRISAT. A next-generation sequence-based TILLING approach is being adopted to mine novel and potential alleles for some genes associated with terminal drought tolerance (ICRISAT, unpubl. data).

Until 2005, chickpea was considered as an 'orphan legume' in the context of genomic resources; however, recent decoding of the kabuli chickpea genome sequence, the resequencing of 90 genomes of chickpea by Varshney *et al.* (2013*b*), Ruperao *et al.* (2014), desi chickpea genome sequencing (Jain *et al.* 2013) and large-scale development of genomic resources (see Varshney *et al.* 2010*a*, 2010*b*, 2012*a*) have turned chickpea into a 'genomic resource-rich' crop. More than 3000 simple sequence repeat

(SSR) markers have been developed over the last 8 years (Lichtenzveig *et al.* 2005; Sethy *et al.* 2006; Nayak *et al.* 2010; Gujaria *et al.* 2011; Thudi *et al.* 2011; Agarwal *et al.* 2012). In addition, a huge number of SSRs have become available from genome analysis (Varshney *et al.* 2013*b*), which have further enriched the available marker repertoire for chickpea. In order to help geneticists and breeders, a database named the 'Chickpea Microsatellite Database' (http:// cicarmisatdb.icrisat.org, accessed 6 June 2014), an easy-to-use web interface, was developed recently. Recently, Kujur *et al.* (2013) reported development of 1108 transcription factor gene-derived microsatellites and 161 transcription factor functional domain-associated microsatellite markers from 707 transcription factors of chickpea. Recently, a genome-wide physical map spanning ~981 Mb (574 Mb of which was assembled in 1174 contigs, and 3256 singletons represent 407 Mb of the genome) has also been developed (Varshney *et al.* 2012*b*, 2014*a*). This physical map has also been used to link the genetic and genomic maps.

Next-generation mapping populations

Mapping populations involving multiple parents are termed next-generation mapping populations. For instance, multiparent advanced generation inter-cross (MAGIC) population development involves crossing 4–20 parental lines and leads to an increase in genetic variability. In addition, the incorporation of multiple parents ensures the population can be segregated for multiple QTLs for multiple traits, and cytoplasm effects can be modelled. The utility of MAGIC populations has been demonstrated in model species like *Arabidopsis thaliana* (L.) Heynh. (Kover *et al.* 2009), wheat (*Triticum aestivum* L.) (Huang *et al.* 2012) and rice (*Oryza sativa* L.) (Bandillo *et al.* 2013) for fine mapping of several known QTLs and the identification of novel QTLs. At ICRISAT, a set of eight well adapted and drought-tolerant lines (ICC 4958, ICCV 10, JAKI 9218, JG 11, JG 130, JG 16, ICCV 97105 and ICCV 00108), originating from Ethiopia, Kenya and India, have been selected for developing MAGIC populations. Currently 1200 F_6 MAGIC lines are being grown in the field. Furthermore, MAGIC populations provide a platform for a community-based approach for gene discovery, characterisation and deployment for understanding complex traits (Glaszmann *et al.* 2010). In addition, nested association mapping populations, multiline cross inbred lines and recombinant inbred advanced intercross lines are other next-generation multiparental populations that can be developed in chickpea.

Transcriptomic resources

Recent advances in next generation sequencing technologies have greatly facilitated the ability to sequence the genome and transcriptomes of several plant species (Varshney *et al.* 2009*b*; Thudi *et al.* 2012). Novel transcriptomic approaches such as RNA sequencing-based gene expression profiling can also be used for identifying and isolating drought-responsive genes. The identified candidate drought stress associated genes should provide insights into the molecular mechanisms of stress tolerance and ultimately help to develop improved drought-tolerant chickpea varieties.

Transcriptome sequencing in chickpea

A major aim of genomic studies in plants is the identification of genes and pathways that affect crop production. EST databases provide basic sequence information and facilitate the identification of candidate genes for agronomic traits. EST collections through Sanger sequencing have proven extremely useful in many plant species (Sreenivasulu *et al.* 2002). Large-scale transcriptome sequencing in chickpea using Sanger sequencing and next-generation sequencing approaches have provided ample information about the gene content in chickpea. For instance, extensive early effort was made and an abundance of ESTs from a range of tissues, including developmental and stress-challenged tissue, were generated in chickpea. Complementary DNA libraries have been generated and 20 162 ESTs have been generated from plants grown under drought and salt stress (Varshney *et al.* 2009*a*). Subsequently, a comprehensive transcriptome assembly comprising 103 215 tentative unique sequences was developed based on 435 018 FLX/454 reads and 21 491 Sanger ESTs (Hiremath *et al.* 2011). Using the Illumina sequencing platform, another 53 409 contigs representing ~28 Mb of unique transcriptome sequence were assembled (Garg *et al.* 2011*a*). The same group, by using FLX/454 and Illumina sequencing technologies, defined another set of 34 760 contigs representing ~4.8% (35.5 Mb) of the chickpea genome (Garg *et al.* 2011*b*). By combining these different datasets, a hybrid assembly with 46 369 transcript assembly contigs has been developed from RNA samples from >22 tissues representing a range of developmental stages and eight tissues challenged by different stresses collected from 17 different chickpea genotypes (Kudapa *et al.* 2014).

Many studies reported several genes or ESTs to be involved in various stress responses based on transcriptomic and proteomic studies (Pandey *et al.* 2006, 2008; Mantri *et al.* 2007; Molina *et al.* 2008, 2011; Varshney *et al.* 2009*a*). However, gene discovery has been very limited in chickpea. Hence, only a few candidate genes have been cloned and functionally validated (Kaur *et al.* 2008; Shukla *et al.* 2009; Tripathi *et al.* 2009; Peng *et al.* 2010; Kudapa *et al.* 2013). By studying two chickpea varieties (PUSABGD 72 and ICCV 2) for differences in transcript profiling during drought stress treatment by withdrawal of irrigation at different time points, Jain and Chattopadhyay (2010) reported that most of the highly expressed ESTs in the tolerant cultivar predicted that most of them encoded proteins involved in cellular organisation, protein metabolism, signal transduction and transcription. Deokar *et al.* (2011), in addition to studying the genes that are up- and downregulated in a drought-tolerant genotype (ICC 4958) under terminal drought stress and in a drought-susceptible genotype (ICC 1882), also studied gene expression between the bulk of the selected recombinant inbred lines exhibiting extreme phenotypes. Of 3062 unigenes identified, 51.4% were novel and 2185 genes had significant similarity to those in the National Center for Biotechnology Information (NCBI) nonredundant database. Furthermore, the expression status of 830 unigenes in response to terminal drought was evaluated and the expression of 10 genes was also validated through quantitative real-time PCR (Deokar *et al.* 2011).

In terms of differential expression studies, Sanger ESTs generated from drought-challenged tissues of drought-tolerant (ICC 4958) and drought-sensitive (ICC 1882) were used for *in silico* expression studies (Varshney *et al*. 2009*a*). However, in another comprehensive study, after aligning 37 million Illumina short sequence tags generated from drought-challenged root tissues of the same genotypes as used in the transcriptome assembly, Hiremath *et al*. (2011) identified 2974 TUSs with significant expression changes, 2823 of which could be associated with gene ontology annotations. Furthermore, the expression patterns of many genes suggested that their role in various pathways of secondary metabolism. In a different study, a wide range of expression levels were observed by mapping all reads onto a nonredundant set of chickpea transcripts, where the number of reads corresponding to each transcript ranged from 14 (0.16 reads per million) to 270 894 (3137 reads per million), with an average of 1617 (18.7 reads per million) (Garg *et al*. 2011*a*). This report identified 250 transcripts with root-specific expression and 217 transcripts with shoot- specific expression.

Recently, several functional genomics studies have been performed in chickpea to identify abiotic stress-responsive transcripts using approaches such as suppression subtractive hybridisation, super serial analysis of gene expression (SuperSAGE), microarray and EST sequencing (Buhariwalla *et al*. 2005; Matsumura *et al*. 2005; Molina *et al*. 2008). Sequencing-based expression profiling using serial analysis of gene expression and SuperSAGE allow us to quantify global gene expression. If serial analysis of gene expression is combined with one of the next-generation sequencing platforms, it is more precisely called deepSuperSAGE. By using SuperSAGE, Kahl *et al*. (2007) investigated drought- and salt-stress transcriptomes of chickpea by analysing 360 000 transcripts representing 40 000 unique mRNAs, and identified 3000 transcripts responding to these stresses. In another deepSuperSAGE application, 80 238 tags representing 17 493 unique transcripts from drought-stressed

Table 1. Summary of main-effect, stable and consistent quantitative trait loci (QTLs) for drought tolerance related traits in chickpea

QTL explaining >10% of phenotypic variation are referred to as main-effect QTLs; QTL for a given trait appearing in more than one location are referred to as 'stable' QTL; QTL appearing in more than 1 year or season are considered to be 'consistent' QTL (Varshney *et al*. 2014*b*). R : T ratio, ratio of root dry weight to stem dry weight

Traits	Linkage group	Position of QTL (cM)	Phenotypic variation explained (%)	Marker interval
Root traits				
Root length density	CaLG04	62.56–73.06	10.90	NCPGR127–NCPGR21[A]
Root surface area	CaLG06	91.97–105.84	10.26	TA106–H1I16
R : T ratio	CaLG04	68.09–73.06	16.67	TAA170–NCPGR21[A]
Morphological traits				
Shoot dry weight	CaLG04	68.09–73.06	17.59	TAA170–NCPGR21[A]
Plant height	CaLG03	12.71–13.67	10.00	TA34–NCPGR49
	CaLG04	62.56–73.06	30.20	NCPGR127–NCPGR21[A]
	CaLG06	80.68–123.07	13.12	CaM1760–CaM399
	CaLG08	0.0–9.65	14.73	NCPGR164–CaM2187
Phenological traits				
Days to 50% flowering	CaLG04	62.56–68.09	24.49	NCPGR127–TAA170[A]
	CaLG08	0.0–9.65	26.87	NCPGR164–CaM1918
Days to maturity	CaLG06	91.97–123.07	12.13	TA106–CaM0399
	CaLG08	0.0–22.86	18.83	NCPGR164–CaM1918
	CaLG04	62.56–68.09	19.71	NCPGR127–TAA170[A]
Yield-related traits				
Pods per plant	CaLG04	62.56–73.06	23.18	NCPGR127–NCPGR21
Seeds per pod	CaLG04	68.09–73.06	42.07	TAA170–NCPGR21[A]
100-seed weight	CaLG01	0.0–16.65	10.31	NCPGR184–ICCM0009b
	CaLG04	62.56–73.06	58.20	NCPGR127–NCPGR21[A]
Biomass	CaLG04	62.56–73.06	21.32	NCPGR127–NCPGR21[B]
	CaLG08	0.0–22.86	10.95	NCPGR164–CaM1918
Harvest index	CaLG04	68.09–73.06	11.69	TAA170–NCPGR21[B]
	CaLG01	41.48–61.71	14.36	cpPb-679915–CaM0393
	CaLG01	0.0–16.65	10.67	NCPGR184–ICCM0009b
Yield	CaLG01	68.47–70.38	13.98	NCPGR136–CaM0046
	CaLG04	68.09–73.06	15.72	TAA170–NCPGR21[A]
Drought indices				
Drought tolerance index	CaLG01	41.48–70.38	11.23	cpPb-679915–CaM0046

[A]These markers are already being deployed in several marker-assisted backcrossing programs, as listed in Table 2.

and nonstressed control roots in chickpea have been identified (Molina *et al.* 2008, 2011).

Gene cloning is an approach for isolating candidate genes that are functionally related to the trait of interest. A forward genetics approach for the identification of genes controlling a trait is positional cloning. The published genome sequence assemblies for crop legumes such as soybean (*Glycine max* (L.) Merr.), chickpea, pigeonpea (*Cajanus cajan* (L.) Millsp.) and common bean (*Phaseolus vulgaris* L.) will eventually make map-based cloning easy. Efforts are under way to clone genes from within a drought tolerance QTL region in chickpea (Varshney, unpubl. data). After discovering trait-associated genes by any of the abovementioned approaches, the next step is their functional validation. Several approaches such as overexpression, RNAi, virus-induced gene silencing and TILLING have been applied for this purpose. Optimised

protocols with higher efficiency are already available in chickpea (Acharjee *et al.* 2010). Validation of genes through genetic transformation, RNAi or virus-induced gene silencing is a time-consuming process in legumes, mainly due to the lack of efficient transformation systems in legumes. This situation has promoted the application of TILLING to study gene function in legumes.

Trait mapping

Both linkage analysis and association mapping are currently being employed for genetic dissection of complex traits in chickpea. To date, several trait mapping studies have been conducted in the case of chickpea. However, most of these studies focussed on mapping biotic stresses like *Fusarium* wilt (Benko-Iseppon *et al.* 2003; Cobos *et al.* 2005; Gowda *et al.* 2009;

Table 2. Summary of parental polymorphism assessment between donor and recurrent parental genotypes of chickpea from India, Kenya and Ethiopia

Crosses highlighted in bold are ongoing marker-assisted backcrossing programs. P, polymorphic; M, monomorphic; NA, no amplification

Cross	Polymorphism with markers				Summary of marker polymorphism for each cross
	TAA170	ICCM0249	GA24	STMS11	
India					
JG 11 × ICC 4958	P	P	M	P	Three out of four markers
ICCV 10 × ICC 4958	P	P	M	M	Two out of four markers
Pusa 362 × ICC 4958	M	M	M	M	No polymorphic marker
DCP 92–3 × ICC 4958	P	P	M	M	Two out of four markers
KWR 108 × ICC 4958	P	P	M	M	Two out of four markers
RSG 888 × ICC 4958	P	P	M	M	Two out of four markers
JG 315 × ICC 4958	P	P	M	M	Two out of four markers
BG 256 × ICC 4958	M	P	M	M	One out of four markers
BGD 72 × ICC 4958	M	P	M	M	One out of four markers
C 235 × ICC 4958	P	M	M	M	One out of four markers
JG 16 × ICC 4958	P	NA	M	M	One out of four markers
ICC 8261 × ICC 4958	NA	NA	M	M	No polymorphic marker
Kenya					
ICCV 97105 × ICC 4958	P	P	M	M	Two out of four markers
ICCV 95423 × ICC 4958	P	P	M	M	Two out of four markers
ICCV 96329 × ICC 4958	P	P	M	M	Two out of four markers
ICCV 92311 × ICC 4958	P	P	M	M	Two out of four markers
ICCV 92318 × ICC 4958	P	P	M	M	Two out of four markers
ICCV 97110 × ICC 4958	P	P	M	M	Two out of four markers
ICCV 00108 × ICC 4958	M	P	M	M	One out of four markers
ICCV 97126 × ICC 4958	M	P	M	M	One out of four markers
ICCV 92944 × ICC 4958	M	M	M	M	No polymorphic marker
ICCV 97306 × ICC 4958	NA	M	M	M	No polymorphic marker
Ethiopia					
Ejere × ICC 4958	P	P	P	P	Four out of four markers
Arerti × ICC 4958	P	P	P	P	Four out of four markers
Dubie × ICC 4958	M	P	M	M	One out of four markers
Habru × ICC 4958	P	M	M	P	Two out of four markers
Xariye × ICC 4958	M	P	M	M	One out of four markers
Natoli × ICC 4958	P	P	M	M	Two out of four markers
Shasho × ICC 4958	P	M	M	M	One out of four markers
Teji × ICC 4958	P	P	P	P	Four out of four markers
Worku × ICC 4958	P	P	M	M	Two out of four markers
Akaki × ICC 4958	M	M	M	M	No polymorphic marker

Sabbavarapu *et al*. 2013), *Aschochyta* blight (Udupa and Baum 2003; Iruela *et al*. 2006, 2007; Anbessa *et al*. 2009; Kottapalli *et al*. 2009; Aryamanesh *et al*. 2010) and *Botrytis* grey mould (Anuradha *et al*. 2011), and agronomically important traits (Gowda *et al*. 2011). Trait mapping studies have been discussed in detail in Varshney *et al*. (2012*a*).

In terms of abiotic stresses, Vadez and colleagues (2012) at ICRISAT identified QTLs for salinity tolerance and inferred that tolerance is associated with earliness in chickpea. Although efforts were made to understand drought tolerance (Rehman *et al*. 2011; Hamwieh *et al*. 2013), the studies aimed to understand the performance of agronomic or physiological traits. Recent research endeavours at ICRISAT by Varshney *et al*. (2014*b*) may eventually lead to a comprehensive understanding of drought tolerance in chickpea. For understanding the complex nature of drought tolerance, precise phenotypic data (from 20 drought component traits evaluated in one to seven seasons at one to five locations in India on two intraspecific mapping populations (ICC 4958 × ICC 1882 and ICC 283 × ICC 8261) where analysed, alongside extensive genotyping data. Comprehensive QTL analysis has provided several stable, consistent and robust main-effect QTLs for 13 out of 20 drought tolerance traits explaining 10–58.20% of phenotypic variation (Table 1; Varshney *et al*. 2014*b*). Markers flanking these QTLs can be deployed for enhancing drought tolerance as well as individual trait improvement through MABC breeding. A genomic region referred to as 'QTL-hotspot', spanning ~29cM on *Cicer arietinum* Linkage Group 04 (CaLG04) of an intraspecific genetic map (ICC 4958 × ICC 1882), was found to harbour 12 out of 25 main-effect QTLs for 12 traits explaining ~58.20% of phenotypic variation (Varshney *et al*. 2014*b*; Table 1). Seven SSR markers (ICCM0249, NCPGR127, TAA170, NCPGR21, TR11, GA24 and STMS11) present in *QTL-hotspot* are the most important markers for marker-assisted introgression of this genomic region into elite genetic backgrounds for enhancing drought tolerance through MABC.

Genomics-assisted breeding for enhancing drought tolerance

Until recently, breeding efforts to improve drought tolerance have been hindered due to a quantitative genetic basis and a poor understanding of the physiological basis of yield in water-limited conditions. Although GAB was like a dream until 5 years ago in chickpea, a range of GAB approaches are now being used.

Marker-assisted backcrossing

A MABC breeding approach has been successfully deployed in chickpea for enhancing drought tolerance by introgressing "*QTL-hotspot*" into elite cultivars. Out of seven SSR markers present in the "*QTL-hotspot*" region (ICCM0249, NCPGR127, TAA170, NCPGR21, TR11, GA24 and STMS11), four markers (ICCM0249, TAA170, GA24 and STMS11) available at the initiation of the molecular breeding programs at ICRISAT and its partner institutions (Indian Institute of Pulses Research (IIPR), Kanpur; Indian Agricultural Research Institute (IARI), New Delhi; Egerton University, Kenya and the Ethiopian Institute of Agricultural Research, Ethiopia), were tested for marker

polymorphism on 32 recurrent parents with respect to the donor parent (ICC 4958). In total, 18 out of 32 cross combinations showed polymorphisms with at least two markers; the remaining 43.75% (14) cross combinations had either one or no marker (Table 2). This indicates that saturation of this region with additional markers is essential to enable introgression of "*QTL-hotspot*" into different elite genetic backgrounds.

In view of the above, "*QTL-hotspot*" has been successfully introgressed into the genetic background of the elite varieties JG11, KAK2 and Chefe. Three SSR markers (TAA170, ICCM0249 and STMS11) were used for foreground selection and 10 amplified fragment length polymorphism (AFLP) primer combinations were used for background selection after

Table 3. Summary of genomics-assisted breeding efforts for chickpea at the International Crop Research Institute for the Semiarid Tropics (ICRISAT) and its National Agricultural Research Systems partners
MABC, marker-assisted backcrossing; EIAR, Ethiopian Institute of Agricultural Research, EU, Egerton University; IIPR, Indian Institute of Pulses Research; IARI, Indian Agricultural Research Institute; NIL, near isogenic line; MARS, marker-assisted recurrent selection; MAGIC, multiparent advanced generation intercross

Institution	Cross or parents	Number of lines and generation
MABC for drought tolerance		
ICRISAT, India	JG 11 × ICC 4958	20 BC_3F_5 lines
ICRISAT, India	Chefe × ICC 8261	8 BC_3F_5 lines
ICRISAT, India	KAK2 × ICC 8261	2 BC_3F_5 lines
EIAR, Ethiopia	Ejere × ICC 4958	384 BC_2F_1
EIAR, Ethiopia	Arerti × ICC 4958	27 BC_3F_4 lines
EU, Kenya	ICCV 97105 × ICC 4958	33 BC_3F_1
EU, Kenya	ICCV 95423 × ICC 4958	10 BC_3F_5 lines
ICRISAT, India	ICCV 10 × ICC 4958	22 BC_3F_5
IIPR, India	DCP92–3 × ICC 4958	60BC_1F_1
IIPR, India	KWR 108 × ICC 4958	7 BC_1F_1
IARI, India	Pusa 362 × ICC 4958	170 BC_2F_1
NIL development		
ICRISAT, India	JG 11 × ICC 4958	25 BC_6F_4
ICRISAT, India	ICC 1882 × ICC 4958	8 BC_6F_2
ICRISAT, India	ICC 1882 × ICC 8261	21 BC_6F_2
ICRISAT, India	ICC 283 × ICC4958	5 BC_6F_2
ICRISAT, India	ICC 283 × ICC 8261	10 BC_6F_2
MARS populations		
ICRISAT, India	JG 11 × ICCV 04112	RC_1F_1 Plants
ICRISAT, India	JG 130 × ICCV 05107	RC_1F_1 Plants
IIPR, India	ICCV10 × DCP92–3	15 F_1 seeds harvested
IARI, India	Pusa372 × JG 130	1000 F_2 seeds harvested
MAGIC population		
ICRISAT, India	ICC 4958, ICCV 10, JAKI 9218, JG 11, JG 130, JG 16, ICCV 97105 and ICCV 00108	1200 F_7 lines

each generation of backcrossing while introgressing "*QTL-hotspot*" into JG 11 genetic background. A total of 29 introgression lines were developed with ~93% recurrent parent genome recovery after three backcross cycles followed by two generations of selfing (Varshney *et al.* 2013*a*). The introgression lines developed from JG11 × ICC 4958, were found to possess higher root length density (average 0.41 ± 0.20 cm cm^{-3}), root dry weight (average 1.25 ± 0.08 g cyl^{-1}) and rooting depth (average 115.21 ± 2.24 30 cm) compared in both the donor and recipient parents; these are the most important target traits for enhancing drought tolerance in chickpea (Varshney *et al.* 2013*a*). Furthermore, preliminary analysis of phenotypic evaluation of these lines in India (Patancheru, Dharwad, Nandyal, Durgapura and Gulbarga), Kenya and Ethiopia indicated that several lines with >10% increase in yield under rainfed conditions and ~20% increase in yield under irrigated conditions were available. Based on the preliminary results, other national partners like Indian Institute of Pulses Research (Kanpur) and Indian Agricultural Research Institute (New Delhi) in India, and Egerton University (Kenya) and the Ethiopian Institute of Agricultural Research (Ethiopia) in sub-Saharan Africa initiated introgressing this region into genetic backgrounds of elite cultivars in their regions (Table 3).

Marker-assisted recurrent selection

Several minor and superior drought-responsive alleles may be present in one or more different genetic backgrounds. In such cases, tapping these alleles for enhancing drought tolerance through MABC will be a daunting task. Henceforth, in addition to introgressing the QTLs or genes for enhancing drought tolerance, efforts are also being made to utilise the genomic resources in enhancing the level of tolerance by accumulating superior alleles through MARS approaches (see Varshney *et al.* 2012*a*). MARS has proven to be successful in private breeding programs in enhancing genetic gains and is effective at improving quantitative traits in maize (*Zea mays* L.), soybean and sunflower (*Helianthus annuus* L.) (Johnson 2004; Eathington *et al.* 2007). In brief, MARS is a modern breeding approach that enables us to increase the frequency of several beneficial alleles with an additive effect and small individual effects in recurrent crosses (Bernardo and Charcosset 2006). Although several multinational companies are using MARS in crops like maize and soybean, only a few public-sector institutes have started to use MARS in crops like wheat (Charmet *et al.* 2001), sorghum (*Sorghum bicolor* (L.) Moench) (Abdallah *et al.* 2009) and rice (Grenier *et al.* 2012).
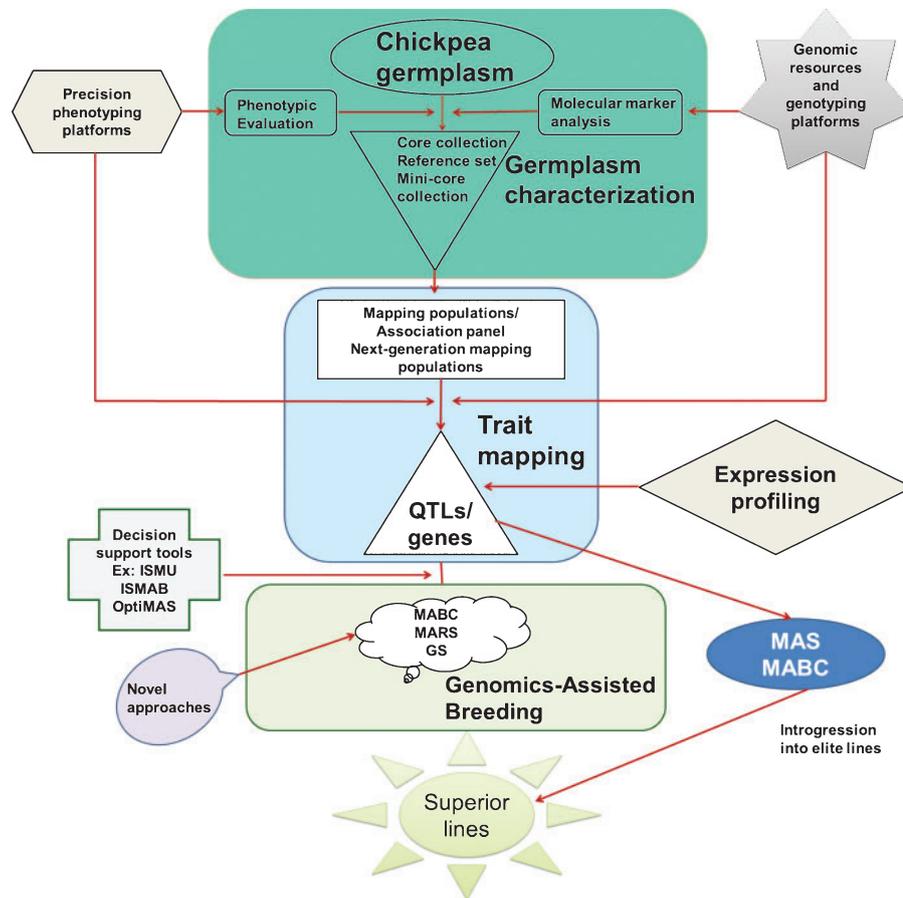
Fig. 2. Integrated genomics approaches for developing superior lines. ISMU, integrated SNP mining and utilisation pipeline; ISMAB, information system for marker-assisted breeding.

At ICRISAT four superior *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). Two crosses were made by using elite by elite lines (JG 11 × ICCV 04112 and JG 130 × 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 ver. 1.0 (Valente *et al*. 2013). It is anticipated that at the end of the project, RC_3F_4 progenies will be available for evaluation at multiple locations. These efforts will lead to the development of superior lines with more enhanced drought tolerance. Some efforts have been initiated to use MARS in the case of chickpea for assembling favourable alleles for drought tolerance using ICCV 04112 × ICCV 93954 and ICCV 05107 × ICCV 94954 crosses. Nevertheless, IARI and IIPR also have initiated MARS in chickpea by using Pusa 372 × JG 130 and DCP 92–3 × ICCV 10 crosses. These efforts are expected to develop superior lines with enhanced drought tolerance for other ecological regions (Table 3).

Genomic selection

As precise phenotyping is essential and the cost of generating phenotyping data at every generation is very expensive, recent advances in genomics technologies and the availability of a wide range of genotyping platforms have made the cost of genotyping much less expensive compared with phenotyping. Genomic selection is a modern breeding approach that is unlike MABC and MARS; it predicts the breeding values (i.e. the genomics-estimated breeding values) of a line based on historical phenotyping data and the genotyping data. Genomic selection has proven to be successful in several animal breeding programs (Schefers and Weigel 2012; see Eggen 2012) as well as in crop plants like maize (Zhao *et al*. 2012). Efforts to deploy genomic selection in chickpea are underway at ICRISAT. In this regard, a collection of 320 elite breeding lines was selected as the 'training population'. In addition to compiling historical phenotyping data for ~10 years at >10 locations, research has extensively phenotyped the training population was for several traits of agronomic importance at ICRISAT (Patancheru) and IARI (New Delhi) during the cropping season of 2011–12 and 2012–13 under rainfed and irrigated conditions. In parallel, the training population was genotyped using KBioscience Competitve Allele-Specific Polymerase chain reaction (KASPar) assays (651) and diversity array technology arrays (15 360 features). Collected phenotypic data and generated genome-wide marker profiling data (>3000 markers) were used with a range of statistical methods including ridge regression–best linear unbiased prediction, kinship-based ridge regression, BayesCπ, BayesB, Bayesian least absolute shrinkage and selection operator (LASSO) and random forest prediction to predict genomics-estimated breeding values (Roorkiwal *et al*. 2013). Resequencing of the germplasm lines and parents of different mapping populations will enable the identification of genome-wide single nucleotide polymorphism (SNP) markers that can be effectively utilised in genomic selection.

Future perspectives

As drought is a complex phenomenon, no single approach for all locations may be applicable for enhancing drought tolerance. In this context, an integrated effort deploying need-based approaches is essential (Fig. 2). Furthermore, for accelerating the adoption of the molecular breeding for enhanced drought tolerance in chickpea, the development of markers that are easily assayable and technically less demanding, and that do not require high capital equipment for genotyping, termed 'breeder friendly markers', is essential. For instance, conversion of SNPs to Illumina Veracode, cleaved amplified polymorphic sequences or KASPar assays will enable their wider application in breeding programs. In addition, the development of decision support tools is essential for enhancing the precision of selection and to accelerate GAB in crop plants in general. In this area, ICRISAT has developed several important user-friendly decision support tools like the integrated SNP mining and utilisation pipeline, the molecular breeding design tool and the genotyping data management system. Several other tools that aid in genomics-assisted selection have been integrated and made available on an integrated breeding platform (https://www. integratedbreeding.net/molecular-breeding, accessed 6 June 2014). Further well-structured molecular breeding programs are essential for the effective deployment of GAB approaches for crop improvement (Varshney *et al*. 2012c). To achieve this, training in modern plant breeding skills and fostering integrated breeding strategies and sharing of knowledge and expertise among collaborative partners, especially in developing countries with limited infrastructure and human resources, is the need of the hour.

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