

Genomic resources in chickpea

by Teresa MILLÁN^{1*}, Eva MADRID² and Rajeev K. VARSHNEY³

Abstract: Chickpea has considerably increased the genomic resources in recent years providing highly saturated genetic maps including anonymous or gene-specific markers targeting some agronomic traits of interest. In addition, the publication of the two draft genome sequences of Kabuli and Desi chickpea types opens a new era in genomic tools. Furthering in our understanding of the association between phenotypic traits (Quantitative Trait loci-QTL-or genes) with the transcriptome and gene annotation provided by genome sequencing data will be the future challenge to be able to exploit with success marker-assisted Selection (MAS).

Key words: chickpea, genomics, marker-assisted selection

Introduction

DNA marker technology made possible the generation of genetic maps ensuring the use of MAS and positional cloning of genes of interest. Chickpea genetic maps using biparental populations from narrow and wide crosses were initiated in the nineties and had a great step forward with the incorporation of STMS/SSR (Sequence Tagged Microsatellite Sites/Simple Sequence Repeat)

and ESTs (Expressed Sequence Tags) based markers. Those locus specific markers provided the possibility to compare maps in different populations, to unify nomenclature for linkage groups, establish reference maps and provide anchor points for comparing the genomes of the model species *Medicago truncatula* and chickpea (7, 9, 13). In recent times, Next Generation Sequencing (NGS) technologies have been effectively generated in chickpea large-scale transcriptome data together with genomic markers based on Single Nucleotide Polymorphisms (SNPs) facilitating the development of highly saturated second generation genetics maps (5). Those maps have been developed in Recombinant Inbred Line (RIL) populations including markers from Simple sequence repeat (SSR), Expressed Sequence Tag (EST), Intron Spanning Region (CISR), Genic Molecular Markers (GMMs), BAC-end derived SSR (BES-SSR), Diversity Arrays Technology (Dart) or Tentative Orthologous Genes (TOGs) (4, 11) (Table 1).

Marker-assisted breeding in progress

First chickpea genetic maps were mainly focussed in the location of genomic areas controlling disease resistances, some agronomic traits and few quality components (Table 2). Successful results in marker-assisted backcrossing (MABC) for drought tolerance and fusarium wilt have been achieved mainly using STMS makers. STMS have been widely used in chickpea because their extensive probability of finding polymorphism however the prediction of favourable alleles is less accurate than using gene-specific markers. Examples of allele-specific markers were obtained for genomic areas related to ascochyta blight resistance: CaETR for QTL_{AR1} and SCY17 for QTL_{AR2} proved to be successful in predicting resistant accessions (6).

An approach to progress in the detection of candidate genes has been the development of Near Isogenic Lines (NILs) (1). Phenotypic variation observed between pairs of NILs can be assigned directly to the restricted target region of genome that differs between them.

Broaden genomic resources: sequencing projects

Very recently the first draft of the chickpea genome sequence was published. This project was undertaken by the International Chickpea Genome Sequencing Consortium (ICGSC) led by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in collaboration with University of California in Davis, BGI-Shenzhen, University of Cordoba and several other organizations (12). The consortium featured the reference genome of the kabuli type CDC Frontier chickpea variety and re-sequenced the genomes of 90 cultivated and wild genotypes from 10 different countries. This publication reported the draft genome sequence of ~ 738-Mb which contains an estimation of 28,269 genes. Examination of synteny with other legumes revealed extended (> 10 kb) conserved syntenic blocks with *M. truncatula*. The draft sequence of a desi genotype has also become available now (520 Mb assembly covering 70% of the predicted 740 Mb genome length and more than 80% of the gene space) (8).

Comparison of phenotypic traits located in genetic maps, expression studies and the complete genome sequence will be a very powerful tool in the future, facilitating genetic enhancement and breeding to develop improved chickpea varieties.

¹University of Córdoba, Department of Genetics, Córdoba, Spain (teresa.millan@uco.es)

²CSIC, Institute for Sustainable Agriculture, Córdoba, Spain

³International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India

Table 1. Second generation genetic maps developed in chickpea based on RIL populations

Newly developed markers	N° of loci	Coverage (cM)	Average inter marker distance (cM)	Reference*
SSR, GMMs, CISR	300	766.56	2.55	(4) ^a
BES-SSR, DarT	1291	845.56	0.65	(11) ^a
EST-SSR, ITPs, SNPs	406	1497.7	3.68	(2) ^a
SNPs, SSR	368	1808.7	1.7	(3) ^b
CKAMs, TOGs-SNPs	1328	788.6	0.59	(5) ^a

*a: map in population derived from *C. arietinum* ICC 4958 x *C. reticulatum* PI 489777;
b: map in population derived from ICCV2 x JG62

Final remarks

The current focus in applied breeding is leveraging biotechnological tools to develop more and better markers to allow marker assisted selection with the hope that this will speed up the delivery of improved cultivars to the farmer. To date, progress in marker development and delivery of useful markers has been increasingly fast in chickpea. Nowadays, markers currently targeting resistance genes or QTL are in majority microsatellite type but high-throughput SNP genotyping platforms are overtaking SSR as the choice of markers type to be used in the screening of germplasm collections (5). Besides, the development of transcript maps and information of the genome sequence will increase marker density in the genomic regions controlling traits of interest. Available tools facilitate the identification of gene families involved in resistance mechanism as NBS-LRR genes, or the analysis of orthologous genes related with agronomics traits (i.e. flowering time, growth habit, double podding etc.) present in other legumes. Similarly, recent advances in genomic technology will assist the exploiting of natural diversity by association mapping conducted on germplasm collections. ■

Table 2. Traits and locus-specific markers localized in different linkage groups of the chickpea genetic map (10)

Linkage group (LG)	Traits	Gene/QTL	Indicative Markers*
LGI	β-carotene	QTL	GA11, TA122
	Seed weight	QTL	GA11
	Days to first flower	QTL	H1F022, GAA40
LGII	Fusarium wilt race 0	<i>Foc-0₂/foc-0₂</i>	TA59, TS47
		<i>foc-1</i>	H3A12, TA110
		<i>foc-2</i>	TA96, H3A12
		<i>foc-3</i>	H1B06y, TA194
		<i>foc-4</i>	TA96, CS27
		<i>foc-5</i>	TA27, TA59, TA96
	Ascochyta blight	<i>ar1</i> , QTL _{AR1}	GA16, TA194, TR
	Seed weight	QTL	TA110-TAA60
	Days to first flower	QTL	H4B09, H1B06
LGIII	Growth habit	<i>Hg</i>	Pgd-c
	β-carotene	QTL 2	TA64, STMS28
	Days to flower	QTL	TS57, TA127, TA142
	Ascochyta blight	QTL	STMS28, TS12, TA64
LGIV	Seed testa color	<i>T3</i>	<i>P</i>
	Flower color	<i>P, B/b</i>	TA61
	Seed coat thickness	<i>Tt/tt</i> , QTL	<i>B/b</i>
	Seed number	QTL	TA130
	Seed weight	QTL	GA24, STMS11, GA2
	Days to flower	QTL	GAA47
	Ascochyta blight	QTL _{AR1} , QTL _{AR2}	CaETR, SCY17 ₅₉₀
LGV	Fusarium wilt race 0	<i>Foc-0₁/foc-0₁</i>	OPJ20 ₆₀₀ , TR59
LGVII	Single/Double pod	<i>s</i>	TR44, TA80
	Seed weight	QTL	TA120, TR40
	Days to flower	QTL	TS57, TA127
	Ascochyta blight	QTL	TA176
	Botrytis grey mould	QTL	SA14-TS71rts36r
LGVIII	Rust	<i>Uca1/uca1</i>	TA18, TA180
	Lutein concentration	QTL	TA25
	Seed weight	QTL	OPE09 ₁₅₉₄ -MER05 ₁₆₄₅
	Ascochyta blight	QTL	TA3, TS46, TS45, H3C11a
	Botrytis grey mould	QTL	TA25, TA144, TA159, TA118

References

- (1) Castro P, Piston F, Madrid E, Millan T, Gil J, Rubio J (2010) Development of chickpea near-isogenic lines for Fusarium wilt. *Theor Appl Genet* 121:1519-1526
- (2) Choudhary P, Khanna SM, Jain PK, Bharadwaj C, Kumar J, Lakhera PC, Srinivasan R (2012) Genetic structure and diversity analysis of the primary gene pool of chickpea using SSR markers. *Genet Mol Res* 11:891-905
- (3) Gaur R, Azam S, Jeena G, Khan AW, Choudhary S, Jain M, Yadav G, Tyagi AK, Chattopadhyay D, Bhatia S (2012) High-throughput SNP discovery and genotyping for constructing a saturated linkage map of chickpea (*Cicer arietinum* L.). *DNA Res* 19:357-373
- (4) Gujaria N, Kumar A, Dauthal P, Dubey A, Hiremath P, Prakash AB, Farmer A, Bhide M, Shah T, Gaur PM, Upadhyaya HD, Bhatia S, Cook DR, May GD, Varshney RK (2011) Development and use of genic molecular markers (GMMs) for construction of a transcript map of chickpea (*Cicer arietinum* L.). *Theor Appl Genet* 122:1577-1589
- (5) Hiremath PJ, Kumar A, Penmetsa RV, Farmer A, Schlueter JA, Chamarthi SK, Whaley AM, Carrasquilla-Garcia N, Gaur PM, Upadhyaya HD, Kavi Kishor PB, Shah TM, Cook DR, Varshney RK (2012) Large-scale development of cost-effective SNP marker assays for diversity assessment and genetic mapping in chickpea and comparative mapping in legumes. *Plant Biotechnol J* 10:716-732
- (6) Madrid E, Chen W, Rajesh PN, Castro P, Millán T, Gil J (2012) Allele-specific amplification for the detection of ascochyta blight resistance in chickpea. *Euphytica* 189:183-190
- (7) Millan T, Winter P, Jüngling R, Gil J, Rubio J, Cho S, Cobos MJ, Iruela M, Rajesh PN, Tekeoglu M, Kahl G, Muehlbauer FJ (2010) A consensus genetic map of chickpea (*Cicer arietinum* L.) based on 10 mapping populations. *Euphytica* 175:175-189
- (8) Jain M, Gopal M, Patel RK, Priya P, Jhanwar S, Khan AW, Shah N, Singh VK, Garg R, Jeena G, Yadav M, Kant C, Sharma P, Yadav G, Bhatia S, Tyagi AK, Chattopadhyay D (2013) A draft genome sequence of the pulse crop chickpea (*Cicer arietinum* L.). *Plant J* 74:715-729
- (9) Nayak SN, Zhu H, Varghese N, Datta S, Choi H-K, Horres R, Jüngling R, Singh J, Kishor PBK, Sivaramakrishnan S, Hoisington DA, Kahl G, Winter P, Cook DR, Varshney RK (2010) Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. *Theor Appl Genet* 120:1415-1441
- (10) Rubio J, Gil J, Cobos MJ, Millán T (2012) Chickpea. In: Pérez de la Vega M, Torres AM, Cubero JI, Kole C (eds) *Genetics, Genomics and Breeding of Cool Season Grain Legumes*. Science Publishers, Boca Raton, USA, 205-233
- (11) Thudi M, Bohra A, Nayak SN, Varghese N, Shah TM, Penmetsa RV, Thirunavukkarasu N, Gudipati S, Gaur PM, Kulwa PL, Upadhyaya HD, KaviKishor PB, Winter P, Kah G, Town CD, Kilian A, Cook DR, Varshney RK (2011) Novel SSR markers from BAC-end sequences, DArT arrays and a comprehensive genetic map with 1,291 marker loci for chickpea (*Cicer arietinum* L.). *PLoS ONE* 6: e27275
- (12) Varshney RK, Song C, Saxena RK, Azam S, Yu S, Sharpe AG, Cannon S, Baek J, Rosen BD, Tar'an B, Millan T, Zhang X, Ramsay LD, Iwata A, Wang Y, Nelson W, Farmer AD, Gaur PM, Soderlund C, Penmetsa RV, Xu C, Bharti AK, He W, Winter P, Zhao S, Hane JK, Carrasquilla-Garcia N, Condie JA, Upadhyaya HD, Luo M-C, Thudi M, Gowda CLL, Singh NP, Lichtenzweig J, Gali KK, Rubio J, Nadarajan N, Dolezel J, Bansal KC, Xu X, Edwards D, Zhang G, Kahl G, Gil J, Singh KB, Datta SK, Jackson SA, Wang J, Cook DR (2013) Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nat Biotechnol* 31:240-248
- (13) Winter P, Benko-Iseppon A-M, Hüttel B, Ratnaparkhe M, Tullu A, Sonnante G, Pfaff T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G, Muehlbauer FJ (2000) A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* x *C. reticulatum* cross: Localization of resistance genes for fusarium wilt races 4 and 5. *Theor Appl Genet* 101:1155-1163