BARD Final Scientific Report

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Date of Submission of the report: March 15, 2010

BARD Project Number: US-3870-06C

Project Title: Integrative Physical and Genetic Mapping of the Chickpea Genome for Fine Mapping and Analysis of Agronomic Traits

Investigators

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Keywords:

Bacterial artificial chromosome libraries, physical map, genetic map, days to first flower, ascochyta blight

Abbreviations:

BAC, bacterial artificial chromosome; BIBAC, plant-transformation-competent binary BAC; QTL, quantitative trait locus; ABR, Ascochyta blight resistance; DTF, days to first flower; SSR, simple sequence repeat

Budget: IS: \$70,000

US: \$230,000

Total: \$300,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1			1
Submitted, in review, in preparation		1		1
Invited review papers				
Book chapters		1		1
Books				
Master theses			1	1
Ph.D. theses				
Abstracts	2	2		4
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Dr. Xiaojun Zhang, Texas A&M University

Cooperation Summary (numbers):

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		3	6	9
Longer Visits (Sabbaticals)				

Description Cooperation:

The research accomplishments resulted from the close collaboration among PIs' labs of both countries. The American labs developed the new BAC and BIBAC libraries for chickpea, fingerprinted the proposed BACs and BIBACs, developed the proposed BAC/BIBAC physical map, conducted ABR fine mapping and prepared the DNA pools and high-density clone membranes from the map source BAC and BIBAC libraries for the project. The Israeli labs conducted the integration of the physical map with the chickpea genetic map, identified the BAC/BIBAC contigs containing or closely linked to the ABR and DTF QTL and analyzed the genetic basis of resistance to pod abortion caused by heat stress. This close collaboration ensured that all research objectives have been well done or exceeded, thus ensuring the success of the project.

Patent Summary (numbers):

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				0
Issued (allowed)				0
Licensed				0

Abstract

Chickpea is the third most important pulse crop in the world and ranks first in the Middle East; however, it has been subjected to only limited research in modern genomics. In the first period of this project (US-3034-98R) we constructed two large-insert BAC and BIBAC libraries, developed 325 SSR markers and mapped QTLs controlling ascochyta blight resistance (ABR) and days to first flower (DTF). Nevertheless, the utilities of these tools and results in gene discovery and marker-assisted breeding are limited due to the absence of an essential platform.

The goals of this period of the project were to use the resources and tools developed in the first period of the project to develop a BAC/BIBAC physical map for chickpea and using it to identify BAC/BIBAC contigs containing agronomic genes of interest, with an emphasis on ABR and DTF, and develop DNA markers suitable for marker-assisted breeding. Toward these goals, we proposed: 1) Fingerprint ~50,000 (10x) BACs from the BAC and BIBAC libraries, assemble the clones into a genome-wide BAC/BIBAC physical map, and integrate the BAC/BIBAC map with the existing chickpea genetic maps (Zhang, USA); 2) fine-map ABR and DTF QTLs and enhance molecular tools for chickpea genetics and breeding (Shahal, Sherman and Dani Shtienberg, Israel; Chen and Muehlbauer; USA); and 3) integrate the BAC/BIBAC map with the existing chickpea genetic maps (Sherman, Israel; Zhang and Chen, USA). For these objectives, a total of \$460,000 was requested originally, but a total of \$300,000 was awarded to the project.

We first developed two new BAC and BIBAC libraries, Chickpea-CME and Chickpea-CHV. The chickpea-CME BAC library contains 22,272 clones, with an average insert size of 130 kb and equivalent to 4.0 fold of the chickpea genome. The chickpea-CHV BIBAC library contains 38,400 clones, with an average insert size of 140 kb and equivalent to 7.5 fold of the chickpea genome. The two new libraries (11.5 x), along with the two BAC (Chickpea-CHI) and BIBAC (Chickpea-CBV) libraries (7.1 x) constructed in the first period of the project, provide libraries essential for chickpea genome physical mapping and many other genomics researches. Using these four libraries we then developed the proposed BAC/BIBAC physical map of chickpea. A total of 67,584 clones were fingerprinted, and 64,211 (~11.6 x) of the fingerprints validated and used in the physical map assembly. The physical map consists of 1,945 BAC/BIBAC contigs, with each containing an average of 39.2 clones and having an average physical length of 559 kb. The contigs collectively span ~1,088 Mb, being 1.49 fold of the 740-Mb chickpea genome. Third, we integrated the physical map with the two existing chickpea genetic maps using a total of 172 (124 + 48) SSR markers. Fourth, we identified tightly linked markers for ABR-QTL1, increased marker density at ABR-QTL2 and studied the genetic basis of resistance to pod abortion, a major problem in the east Mediterranean, caused by heat stress. Finally, we, using the integrated map, isolated the BAC/BIBAC contigs containing or closely linked to QTL4.1, QTL4.2 and QTL8 for ABR and QTL8 for DTF.

The integrated BAC/BIBAC map resulted from the project will provide a powerful platform and tools essential for many aspects of advanced genomics and genetics research of this crop and related species. These includes, but are not limited to, targeted development of SNP, InDel and SSR markers, high-resolution mapping of the chickpea genome and its agronomic genes and QTLs, sequencing and decoding of all genes of the genome using the next-generation sequencing technology, and comparative genome analysis of chickpea versus other legumes. The DNA markers and BAC/BIBAC contigs containing or closely linked to ABR and DTF provide essential tools to develop SSR and SNP markers well-suited for marker-assisted breeding of the traits and clone their corresponding genes. The development of the tools and knowledge will thus promote enhanced and substantial genetic improvement of the crop and related legumes.

Achievements

A. Significance of main scientific achievements

The following results have been achieved from this research project:

1. *Two new large-insert chickpea BAC and BIBAC libraries*: Studies in model and other species have shown that the source BAC and BIBAC libraries constructed with multiple restriction enzymes are crucial to development of whole-genome quality physical maps. Therefore, we constructed one new BAC library (Chickpea-CME) and one new BIBAC library (Chickpea-CHV) for chickpea. These two new libraries (11.5 x), along with the two BAC (Chickpea-CHI) and BIBAC (Chickpea-CBV) libraries (7.1 x) constructed previously, provide comprehensive source libraries for construction of the proposed chickpea physical map and many other genomic and genetic studies of chickpea and related species.
2. *A whole-genome integrated physical map of chickpea*: Integrative physical mapping is the centerpiece of many advanced studies in genomics, genetics and breeding. These include, but are not limited to, marker-assisted breeding, high-resolution mapping of genes and QTL, and positional cloning of genes/QTL. Simultaneously, physical maps also provide desirable platforms for genome sequencing and comparative genomics. Nevertheless, no genome-wide physical map was available for chickpea. Lack of such genomic tools and infrastructure for the species has not only limited the deeper analysis of its agronomic genes and QTLs, but also prevented genomic information flow to chickpea from the model or related legumes, such as *Medicago*, *Lotus* and soybean, whose genomes have been sequenced. Through this project, we developed an integrated BAC/BIBAC physical map of chickpea from the above four chickpea BAC and BIBAC libraries and integrated with two chickpea genetic maps using 172 SSR markers. The availability of the integrated physical map will significantly promote all areas of the above research. Moreover, since the physical map was constructed from both BACs and *Agrobacterium*-mediated plant transformation-ready BIBACs, the BIBACs will further facilitate cloning and applications of the QTL and genes of agronomic importance, such as ABR and DTF, and promote functional analysis of the chickpea genome by genetic transformation.
3. *ABR fine mapping*: ABR and DTF are two major constraints to chickpea production in the semi-arid dryland farming systems in both USA and Israel. However, it is difficult to effectively manipulate these traits in conventional chickpea breeding programs, without assistance of modern molecular tools. In the first period of the project, we genetically mapped the QTLs

controlling the traits using SSR markers; unfortunately, the markers are not sufficiently close to the loci for their effective use in marker-assisted selection for the traits. In this project, we identified additional DNA markers for ABR-QTL1 and ABR-QTL2 and saturated their genomic regions with the markers. These markers provide tools essential for marker-assisted selection and cloning of the genes controlling the traits.

4. *Isolation of BAC/BIBAC contigs containing or closely linked to the QTL of ABR and DTF:* Development of the ABR and DTF closely linked markers and the BAC/BIBAC physical map provide us with advanced tools for the trait studies. Therefore, in this project we isolated the BAC/BIBAC contigs containing or closely linked to QTL4.1, QTL4.2 and QTL8 for ABR and QTL8 for DTF using the physical map and markers. These contigs provide essential tools to develop SSR and SNP markers closer linked and better-suited for marker-assisted selection of ABR and DTF and to clone their corresponding genes.

B. Agricultural and/or economic impacts of the research findings:

Chickpea is the third most important pulse crop in the world, ranks first in the Middle East and a major dietary protein source of vegetarians. This research has made significant progress toward better understanding of the chickpea genome, sequencing of the genome using the next-generation sequencing technology and decoding of its genes, and development of modern genomics tools for enhanced genetic improvement of chickpea germplasm and development of new cultivars adapted to semi-arid dryland farming systems. The research findings will produce at least following immediate impacts on agriculture:

Large-insert BAC and BIBAC libraries have been the choice of resources and tools for many aspects of modern genomics, genetics and breeding research. This research developed two new large-insert chickpea BAC and BIBAC libraries. These new libraries have not only facilitated the proposed BAC/BIBAC physical map development, but also, along with the two BAC and BIBAC libraries developed previously, provided resources and tools essential for comprehensive research of modern genomics and genetics of chickpea and related legume species. Moreover, it has been demonstrated that BIBACs competent for genetic transformation, such as those developed in this project, are powerful tools for large-scale or group of genes transformation, genome functional analysis and molecular breeding. Therefore, the BIBAC libraries developed in this project will promote such research in chickpea and related species.

Whole-genome BAC/BIBAC physical maps have been proven to be the centerpiece of modern genomics, genetics and breeding research. The integrated physical map of chickpea developed in this project provide powerful and essential resources and tools for modern genetic and genomic analyses of the chickpea genome, such as whole genome sequencing and fine mapping, cloning and characterization of genes and QTLs important to chickpea and other legume crops. The cloned genes and QTLs and genomic tools to be developed from such research will in turn greatly promote enhanced chickpea genetic improvement, new variety development and enhanced production in both countries. Importantly, since the recent development of the next-generation sequencing technology has reduced the cost and increased the throughput of DNA sequencing by thousands fold, thus making it feasible and affordable to sequence a genome and develop super-density SNP-based maps, the integrated physical map of chickpea developed in this project provides a framework to sequence the chickpea genome, decode its genes, develop a super-density SNP-based map of the species among its core breeding lines, and establish comparative genomics with related species such as *Medicago*, *Lotus* and soybean whose genomes have been already sequenced.

Development of the chickpea integrated physical map and closely linked DNA markers to the traits of importance has allowed rapid identification of BAC/BIBAC contigs containing or closely linked to ABR and DTF in this project. These contigs provide tools and platforms to develop DNA markers more closely linked to and better-suited for marker-assisted breeding of ABR and DTF, fine map corresponding genes and finally, clone the genes controlling the traits. It has been proven to be difficult to develop varieties of high yield, high quality and early flowering combined with high ABR by conventional breeding. This is because it is difficult to select for plants that are high in yield and quality in the ascochyta-infected condition that is required for selection for ABR. The DNA markers to be developed for ABR and the ABR genes to be cloned by using the BAC/BIBAC contigs will allow breeders to conduct selection for plants of high yield and high quality under the normal (non-infected) breeding conditions, while ABR is selected for by using the DNA markers for the trait. The BIBACs containing ABR or DTF could be directly used in chickpea molecular breeding by BIBAC genetic transformation. Therefore, chickpea breeding will be greatly promoted with the modern genomic tools.

Details of cooperation:

This project is a synergistic collaborative project among two American (Zhang and Muehauer/Chen) and three Israeli (Abbo, Sherman and Shtienberg) laboratories. The cooperation among the American and Israeli labs significantly promoted project objectives, and the linkage and exchange in biotechnology and knowledge between scientists of two countries. Without the close collaboration, the project would need much more efforts to approach the research goals of the project. The project combined the leading BAC, BIBAC and genome physical mapping technologies (Zhang) and the advanced chickpea genetic mapping work (Muehauer/Chen) in American labs with advanced field evaluation of chickpea, mapping populations and marker technology in the Israeli labs. The labs and expertise of the PIs/collaborators of the project are well-complemented to each other, and formed an excellent team for the project.

The cooperation among the researchers' laboratories on this project was extremely successful. The American labs focused their research activities on the new BAC and BIBAC library construction, BAC/BIBAC fingerprinting, physical map development and ABR mapping, whereas the Israeli labs focused on DTF analysis and physical/genetic map integration by BAC/BIBAC library screening with mapped SSR markers. Zhang Lab prepared high-density clone filters from the source BAC and BIBAC libraries and distributed to both the USA and Israeli laboratories for library screening with SSRs and the DNA markers closely linked to our targeted QTL: ABR and DTF QTL. Furthermore, Zhang lab also constructed 3-D library pools of the physical map source BIBACs, isolated DNA from each pool and optimized the library screening protocol using the library pools. The DNA of the pools was mailed to the other American and Israeli labs. Moreover, the project research results were shared and discussed regularly among all investigators. Meetings were held in January of 2007, 2008 and 2009 among the USA and Israeli investigators by taking advantage of the Annual International Plant & Animal Genome Conference held in California, USA to discuss the project progress and subsequent research plans. These activities not only allowed transfer of the research materials and technologies from the American labs to the Israeli labs and enhanced chickpea genome research, importantly, they have also promoted deeper collaboration and linkage between American and Israeli scientists in agricultural science and technology research, especially in chickpea.

List of publications resulting from the BARD project:

- Zhang X, Scheuring CF, Dong JJ, Zhang Y, Huang JJ, Lee M-K, Abbo S, Sherman A, Shtienberg D, Chen W, Muehlbauer F, Zhang H-B. 2010. A BAC/BIBAC-based physical map of chickpea, *Cicer arietinum* L. BMC Genomics (accepted with revision)
- Zhang MP, Li Y, Zhang H-B. 2008. Isolation of megabase-sized DNA fragments from plants. In: *Handbook of Nucleic Acid Purification*. D. Liu (ed.). Taylor & Francis Group, LLC, Florida, USA, pp. 513-524.
- Rajesh PN, O'Bleness M, Till B, Cook D, Henikoff S, Roe B, Chen W, Muehlbauer F. 2009. Understanding Ascochyta blight resistance in chickpea using molecular genetics and genomic approaches. Abstract presented at the Second International Ascochyta Workshop, Pullman, WA, USA. June 28 - July 2, 2009.
- Zhang X, Scheuring C, Huang JJ, Lee M-K, Zhang Y, Abbo S, Muehlbauer F, Sherman A, Shtienberg D, Chen W, Zhang H-B. 2009. A first generation BAC/BIBAC-based integrated physical and genetic map of chickpea (*Cicer arietinum* L.). Presented at the Cool Season Legumes Workshop, Plant & Animal Genome XVII Conference.
- Zhang X, Scheuring, Huang J, Lee M-K, Abbo S, Muehlbauer, Sherman A, Shtienberg D, Chen W, Zhang H-B. 2008. Toward a BAC/BIBAC-based integrated physical and genetic map of chickpea. Plant & Animal Genome XVI Conference. P387
- Rajesh PN, Avcioglu B, Nayak S, Winter P, Varshney R, McPhee K, Zhang H-B, Muehlbauer F, Chen W. 2008. Integration of additional molecular markers and genetic analysis of *Ascochyta* blight resistance in chickpea. Plant & Animal Genome XVI Conference. P386.

Appendix:

1. Published papers:

- Zhang MP, Li Y, Zhang H-B. 2008. Isolation of megabase-sized DNA fragments from plants. In: *Handbook of Nucleic Acid Purification*. D. Liu (ed.). Taylor & Francis Group, LLC, Florida, USA, pp. 513-524 (attached).

2. Papers ‘in press’, ‘accepted’ or ‘submitted’:

Zhang X, Scheuring CF, Dong JJ, Zhang Y, Huang JJ, Lee M-K, Abbo S, Sherman A, Shtienberg D, Chen W, Muehlbauer F, Zhang H-B. 2010. A BAC/BIBAC-based physical map of chickpea, *Cicer arietinum* L. BMC Genomics (accepted with revision) (attached).

3. Summaries of unpublished data:

ABR fine mapping and physical/genetic integration (Chen and Muehlbauer): We constructed a robust linkage map of 338 DNA markers with 8 linkage groups covering 486 cM (Figure 2). Out of the 338 DNA markers, 124 were derived from the chickpea BACs and BIBACs used in the chickpea physical map, thus this genetic map partially integrated with the physical map. We identified tightly linked markers for ABR-QTL1 and increased marker density at ABR-QTL2 with field data (Figures 3B and 3C). With greenhouse data, one major and two minor QTL for less virulent pathotype I (*Ar*19) and no major QTL for high virulent pathotype II (*Ar*628) were identified. Of these three QTLs for pathotype I, two minor QTL (Figures 3A and 3D) were the same as ABR-QTL1 and 2, and the third was a new major QTL (34%; LOD 13) (Figure 3E).

This interspecific cross (CRIL7) is a reference population; therefore, this genetic map will be useful for the research community. ABR-QTL1 and 2 are mapped to linkage group IV (LGIV). This LG splits into two different groups at LOD 7 and so are the two QTLs. In conclusion, we have increased the marker density at ABR-QTL regions, which are essential for finally cloning the genes of ABR by positional cloning.

Genetic basis of resistance to pod abortion caused by heat stress (Abbo and Sherman): Additionally, we studied the underlying genetic basis of resistance to chickpea pod abortion. The rationale underlying the decision to study this phenotype is that yield losses due to hot spells are a major problem in the east Mediterranean. The severity of the problem is expected to increase if the predicted pattern of extreme weather events associated with the global changes will persist. The specific objectives for the 3 seasons were as follows: [1] To screen hybrid progeny (F_2 , through F_5 populations) from a cross between cv. Hadas (susceptible to heat stress) and ICC14088 (resistant to heat stress). [2] To identify resistant progeny with Kabuli seed type (large beige). [3] To estimate the heritability of the pod abortion (caused by heat stress) trait. [4] To develop DNA marker(s) linked to promoting alleles for marker assisted selection.

The hybrid progeny of the different generations were evaluated in replicated experiments during the 3 field seasons (see Figure 1) within commercial chickpea fields alongside the parental lines. However, the timing and intensity of the extreme hot weather events varied from year to year and interfered with the precision of our phenotypic scoring. In all of the experiments, highly significant

Figure 1 Experimental design and procedures

differences were observed between the two parental lines (Table 1). Despite relatively high

heritability values ($h^2=0.5-0.6$) obtained in certain experiments, no consistent year to year performance was observed among the progeny of resistant or susceptible selections (data not shown). Having screened the hybrid progeny from F3 through the F5 generation, we failed to produce high- and low-abortion lines. Our inability to identify the desired genotypes was also reflected in our inability to identify a linked DNA marker either using our SSR set or by DArT reaction. Nevertheless, the selected parental lines and populations developed in the study have provided materials essential for molecular analysis of the important trait under a controlled condition.

Table 1 The flowering and podding data of the two parental lines tested in kibbutz Gat during the 2008 growing season.

Cultivar	Days to flower	Aborted pods
Hadas	110.4±0.5 A	2.20±0.08 A
Susceptible lines		0.94±0.80 B
Resistant lines		1.03±0.80 B
ICC14088	102.2 ±0.7 B	0.35±0.09 C