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BARD

FINAL REPORT

PROJECT NO. US-3034-98

**Genomics Tools for Legume Agronomic Gene
Mapping and Cloning, and Genome Analysis:
Chickpea as a Model**

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Project Title: Genomics Tools for Legume Agronomic Gene Mapping and Cloning, and
Genome Analysis: Chickpea as a Model

Investigators

Principal Investigator (PI):
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Collaborating Investigators:
None

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Keywords:

Bacterial artificial chromosome libraries, simple sequence repeat markers, genetic map, days to flower, grain weight and Ascochyta blight

Abbreviations:

BAC, bacterial artificial chromosome; BIBAC, plant-transformation-competent binary BAC; QTL, quantitative trait locus; RIL, recombinant inbred line; SSR, simple sequence repeat.

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution
Charlene Miller
Assistant Vice President

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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		2	3
Submitted, in review, in preparation	2		1	3
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses			1	1
Abstracts	1			1
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.

None.

Cooperation Summary (numbers):

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		1		1
Longer Visits (Sabbaticals)		1	1	2

Description Cooperation:

The research accomplishments resulted from the close collaboration among three PIs' labs of both countries. The American lab developed the proposed large-insert chickpea BAC library, generated SSR markers for map construction and trait mapping, and trained the Israeli scientist in SSR marker genetic mapping. The Israeli labs developed and characterized the mapping populations, constructed the chickpea genetic map and mapped the target traits of agronomic importance to chickpea production.

Patent Summary (numbers):

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

Abstract

The goals of this project were to develop essential genomic tools for modern chickpea genetics and genomics research, map the genes and quantitative traits of importance to chickpea production and generate DNA markers that are well-suited for enhanced chickpea germplasm analysis and breeding. To achieve these research goals, we proposed the following research objectives in this period of the project: 1) Develop an ordered BAC library with an average insert size of 150 - 200 kb (USA); 2) Develop 300 simple sequence repeat (SSR) markers with an aid of the BAC library (USA); 3) Develop SSR marker tags for *Ascochyta* response, flowering date and grain weight (USA); 4) Develop a molecular genetic map consisting of at least 200 SSR markers (Israel and USA); 5) Map genes and QTLs most important to chickpea production in the U.S. and Israel: *Ascochyta* response, flowering and seed set date, grain weight, and grain yield under extreme dryland conditions (Israel); and 6) Determine the genetic correlation between the above four traits (Israel).

Chickpea is the third most important pulse crop in the world and ranks the first in the Middle East. Chickpea seeds are a good source of plant protein (12.4-31.5%) and carbohydrates (52.4-70.9%). Although it has been demonstrated in other major crops that the modern genetics and genomics research is essential to enhance our capacity for crop genetic improvement and breeding, little work was pursued in these research areas for chickpea. It was absent in resources, tools and infrastructure that are essential for chickpea genomics and modern genetics research. For instance, there were no large-insert BAC and BIBAC libraries, no sufficient and user-friendly DNA markers, and no intraspecific genetic map. Grain sizes, flowering time and *Ascochyta* response are three main constraints to chickpea production in drylands. Combination of large seeds, early flowering time and *Ascochyta* blight resistance is desirable and of significance for further genetic improvement of chickpea. However, it was unknown how many genes and/or loci contribute to each of the traits and what correlations occur among them, making breeders difficult to combine these desirable traits.

In this period of the project, we developed the resources, tools and infrastructure that are essential for chickpea genomics and modern genetics research. In particular, we constructed the proposed large-insert BAC library and an additional plant-transformation-competent BIBAC library from an Israeli advanced chickpea cultivar, Hadas. The BAC library contains 30,720 clones and has an average insert size of 151 kb, equivalent to 6.3 x chickpea haploid genomes. The BIBAC library contains 18,432 clones and has an average insert size of 135 kb, equivalent to 3.4 x chickpea haploid genomes. The combined libraries contain 49,152 clones, equivalent to 10.7 x chickpea haploid genomes. We identified all SSR loci-containing clones from the chickpea BAC library, generated sequences for 536 SSR loci from a part of the SSR-containing BACs and developed 310 new SSR markers. From the new SSR markers and selected existing SSR markers, we developed a SSR marker-based molecular genetic map of the chickpea genome. The BAC and BIBAC libraries, SSR markers and the molecular genetic map have provided essential resources and tools for modern genetic and genomic analyses of the chickpea genome. Using the SSR markers and genetic map, we mapped the genes and loci for flowering time and *Ascochyta* responses; one major QTL and a few minor QTLs have been identified for *Ascochyta* response and one major QTL has been identified for flowering time. The genetic correlations between flowering time, grain weight and *Ascochyta* response have been established. These results have provided essential tools and knowledge for effective manipulation and enhanced breeding of the traits in chickpea.

Achievements

Significance of main scientific achievements: From this project, the following results have been achieved. These results have provided essential resources and tools for modern genetic and genomic analyses of the chickpea genome, and for enhanced chickpea genetic improvement and new variety development in both countries.

1. Two large-insert chickpea BAC and BIBAC libraries – Studies have documented that large-insert BAC libraries are essential resources for modern genetics and genomics research (e.g., <http://hbz.tamu.edu>). The chickpea BAC and BIBAC libraries developed in this project have not only provided a unique source for development of user-friendly DNA markers for marker-assisted breeding, as demonstrated in this project, but also are essential for subsequent chickpea integrative physical and genetic mapping, and gene and QTL cloning and characterization.
2. SSR markers and the SSR-based genetic map – SSR markers have been proven well-suited for marker-assisted breeding in crop plants. The SSR markers developed in this project for chickpea have provided essential tools not only for chickpea molecular genetic map construction and gene and QTL mapping, as demonstrated in this project, but also desirable markers to analyze the chickpea germplasm and decipher their relationships and pedigrees, thus providing a reference to breeders in variety ownership and purity protection, and parent selection in breeding programs. The SSR genetic map of chickpea developed has laid a foundation for high-resolution genetic map construction of the chickpea genome, mapping and cloning of genes and QTLs important to chickpea production and comparative analysis between the legume genomes.
3. SSR markers for Ascochyta response, flowering date and grain weight - Ascochyta response, flowering date and grain weight are three major constraints to chickpea production in the semi arid dryland farming systems. However, it is difficult to effectively manipulate these traits in conventional chickpea breeding programs. The SSR markers developed have provided new tools to manipulate and select for the traits in chickpea germplasm evaluation and breeding programs.
4. Correlations between flowering time, Ascochyta response and grain weight – It has been recognized that combining early pod set and Ascochyta blight resistance in chickpea is difficult. It was unclear whether the constraint on combining the above two traits is due to linkage or other reasons. In this project we determined, for the first time, the genetic correlations between the two traits and identified differential pod response to Ascochyta infection. This information is most valuable for chickpea breeders and will assist in combining early flowering into modern high yielding materials without compromising Ascochyta blight resistance or grain weight.

Agricultural and economic impacts of the research findings: Chickpea is the third most important pulse crop in the world, ranks the first in the Middle East and is a major dietary protein source for vegetarians. This research has made significant progress toward development of modern molecular 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:

The knowledge of the correlations among flowering time, Ascochyta response and grain weight, and their SSR markers generated in this project have provided a guide and new tools for germplasm evaluation and breeding of chickpea for the traits. Without such knowledge and tools, it is difficult to develop varieties of high yield, high quality and early flowering combined with Ascochyta blight resistance. 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 Ascochyta blight resistance. The SSR markers developed for the Ascochyta blight resistance will allow breeders to conduct selection for plants of high yield and high quality under the normal (non-infected) breeding conditions, while the Ascochyta blight resistance is selected by using its SSR markers.

This research has generated three RIL ($F_5 - F_7$) populations from three chickpea breeding crosses. These populations are not only essential for high-density molecular genetic map construction of chickpea and precise mapping of QTLs in multiple years x locations, but also have provided several elite germplasm lines for chickpea breeding and elite strains that can potentially be made of direct use in chickpea production.

Details of cooperation: The cooperation among the three 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 cooperation, it would have been difficult to achieve the research goals of the project. The project combined the leading DNA marker technology available in the American lab with advanced field evaluation of novel chickpea germplasm and mapping populations contributed by the Israeli collaborators. The labs and expertise of the PIs are complementary, forming an excellent team for the project.

The cooperation among the three PIs' laboratories on this project was extremely successful. Zhang's lab in the U.S. focused on the BAC library construction and SSR marker development, whereas the labs of Abbo and Bonfil in Israel focused on the RIL mapping

population development and characterization, field trait phenotyping and analysis, SSR genetic map construction and QTL mapping. The Israeli PI, Abbo, visited the American lab to discuss the cooperation on the genetic map construction, field data collection and QTL mapping. A Ph.D. student of Abbo, J. Lichtenzweig, then visited the American laboratory and was trained in the SSR mapping techniques. Lichtenzweig later worked for half a year in SSR analysis and mapping in the American laboratory. These activities allowed transfer of the SSR technology and related BAC technologies from the American lab to the Israeli lab and promoted the SSR genetic map construction and QTL mapping of the project.

Publications resulting from the BARD project:

1. Kumar J, Abbo S (2001) Genetics of flowering time in chickpea and its bearing on productivity in semi-arid environments. *Adv. Agron.* **72**, 107.
2. Lichtenzweig J, Shtienberg D, Zhang H-B, Bonfil DJ, Abbo S (2002) Biometric analyses of the inheritance of resistance to *Dydimella rabiei* in chickpea. *Phytopathology* **92**, 417.
3. Abbo S, Shtienberg D, Lichtenzweig J, Lev-Yadun S, and Gopher A (2003) Chickpea, summer cropping, and a new model for pulse domestication in the ancient Near-East. *Quarterly Review of Biology* (tentatively accepted for publication-- subject to revision).
4. Abbo S, Berger J, Turner NC (2002) Evolution of cultivated chickpea: four genetic bottlenecks limit diversity and constrain crop adaptation. *Genome* (under review).
5. Scheuring C, Lichtenzweig J, Abbo S, Bonfil DJ, Zhang H-B (2003) Generation of SSR markers from BAC and BIBAC libraries for genetic map construction, gene and QTL mapping and integrated physical mapping of chickpea. *Theor. Appl. Genet.* (in prep.).
6. Lichtenzweig J, Scheuring C, Bonfil DJ, Shtienberg D, Zhang H-B, Abbo S, (2003). A linkage map of the chickpea (*Cicer arietinum* L.) genome and localization of QTLs for ascochyta response, flowering time and grain weight. *Theor. Appl. Genet.* (in prep.).
7. Lichtenzweig J (2003) Epidemiological, and genetic aspects of Ascochyta response in Chickpea (*Cicer arietinum* L.). Ph.D. Thesis, Hebrew University of Jerusalem (in prep.).
8. Scheuring C, Lichtenzweig J, Abbo S, Zhang H-B (2002) Generation of SSR markers from large-insert BACs for genetic map construction, gene and QTL mapping and integrated physical mapping of chickpea. *Plant, Animal & Microbe Genomes X*: P193

Appendix:

1. Published papers:

Kumar J, Abbo S (2001) Genetics of flowering time in chickpea and its bearing on productivity in semi-arid environments. *Adv. Agron.* **72**, 107 - 138.

Lichtenzveig J, Shtienberg D, Zhang H-B, Bonfil DJ, Abbo S (2002) Biometric analyses of the inheritance of resistance to *Dydimella rabiei* in chickpea. *Phytopathology* **92**, 417 - 423.

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

Abbo S, Shtienberg D, Lichtenzveig J, Lev-Yadun S, and Gopher A (2003) Chickpea, summer cropping, and a new model for pulse domestication in the ancient Near-East. Quarterly Review of Biology (tentatively accepted for publication-- subject to revision).

Abbo S, Berger J, Turner NC (2002) Evolution of cultivated chickpea: four genetic bottlenecks limit diversity and constrain crop adaptation. *Genome* (under review).

3. Summaries of unpublished data:

Scheuring C, Lichtenzveig J, Abbo S, Bonfil DJ, Zhang H-B (2003) Generation of SSR markers from large-insert BAC and BIBAC libraries for genetic map construction, gene and QTL mapping and integrated physical mapping of chickpea. *Theor. Appl. Genet.* (in preparation).

Large-insert BAC and BIBAC libraries are essential resources for many aspects of genomics research. We report large-scale generation of SSR markers from large-insert BAC and BIBAC libraries of chickpea for genetic map construction, and gene and QTL mapping. Small-insert, SSR-enriched DNA libraries have widely been used to develop SSR markers, however, development of SSR markers from large-insert BAC libraries provides several unique advantages. The source BAC of an SSR marker represents a DNA fragment of ca. 150 kb, which provides a desirable bridge for BAC-based integrative physical and genetic mapping, allows development of any type of polymorphic markers for a genomic region that are well-suited for marker-assisted breeding, and streamlines the high-resolution mapping and cloning of genes and QTLs of interest. To develop SSR markers for chickpea genome research, we constructed a large-insert BAC library and a large-insert plant-transformation-competent BIBAC library from

the *Hind* III- and *Bam* HI-partial digests of the nuclear DNA of chickpea, *Cicer arietinum* L. cv. Hadas, respectively. The BAC library contains 30,720 clones and has an average insert size of 151 kb, equivalent to 6.3 x chickpea haploid genomes. The probability of obtaining a clone containing a particular sequence from this library is greater than 99%. The BIBAC library contains 18,432 clones and has an average insert size of 135 kb, equivalent to 3.4 x chickpea haploid genomes. The combined libraries contain 49,152 clones, equivalent to 10.7 x chickpea haploid genomes. The BAC library was screened with the SSR oligos (GA)₁₀, (GAA)₇, (AT)₁₀, (ATT)₇, (TGA)₇, (CA)₁₀, (CAA)₇ and (CCA)₇, and SSR locus-containing BACs were identified. Seven hundred sixty-eight positive BACs were randomly selected and subcloned. Sixty hundred forty-eight SSR-containing subclones were sequenced and 536 of them were confirmed to contain at least one SSR. Sequence analysis showed that 310 SSRs represent new loci in the chickpea genome. Genotypic analysis and mapping of the SSRs indicated that use of a BAC library as a source of SSR markers is a powerful means to large-scale SSR marker generation for molecular genetic map construction and gene and QTL mapping.

Lichtenzweig J, Scheuring C, Bonfil DJ, Zhang H-B, Abbo S, (2003). A linkage map of the chickpea (*Cicer arietinum* L.) genome and localization of QTLs for ascochyta response, flowering time and grain weight Theor. Appl. Genet. (in preparation).

A genetic map was generated with 120 F_{3:6} recombinant inbred lines (RILs) derived from an intraspecific cross between 'Hadas' and ICC5810. A total of 41 SSR markers were assayed for segregation, 24 developed within this project and 17 from published projects. Most markers segregated in a 1:1 Mendelian ratio among the individuals (Prob. of $\chi^2 < 0.05$); five marker showed segregation distortion (GAA40, TA135, H1E12, H1H08, H1I16). About 5% (range of 0 to 13%) of the F_{3:6} lines were heterozygous for most of the markers. Marker order and map distances were calculated with Mapmaker V2.0 (9, 14) at a minimum LOD (log of odds) of 3.0 and a maximum recombination fraction of 0.25. The Kosambi mapping function was employed to compute recombination distances in centiMorgans (cM). The markers cover 78.4 cM in 6 linkage groups; 6 markers remained unlinked. Using the map, one major QTL and a few minor QTLs have been identified for Ascochyta response and one major QTL has been identified for flowering time.