BARD

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**Genetics and Comparative Molecular Mapping of
Biochemical and Morphological Fruit Characters
in *Capsicum***

I. Paran, M. Jahn

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Project Title: Genetics and comparative molecular mapping of biochemical and morphological fruit characters in *Capsicum*

Investigators

Principal Investigator (PI): Ilan Paran
Organization

Institutions

Agricultural Research

Co-Principal Investigator (Co-PI): Molly Jahn

Cornell University

Collaborating Investigators:

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Abbreviations commonly used: quantitative trait locus (QTL), simple sequence repeat (SSR)

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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	4	1	6	11
Submitted, in review, in preparation			1	1
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Master theses			3	3
Ph.D. theses	1		1	2
Abstracts	1		3	4
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Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

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

Description of Cooperation:

Close cooperation between the PIs has resulted in joint authorships in four manuscripts and a book chapter.

The cooperation was built on the strengths and capabilities of each PI. Field trials and QTL analyses were performed mostly in Israel, while SSR development and mapping was performed in the USA. _____

Patent Summary (numbers)

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

Original objectives: The overall goal of our work was to gain information regarding the genetic and molecular control of pathways leading to the production of secondary metabolites determining major fruit quality traits in pepper and to develop tools based on this information to assist in crop improvement. The specific objectives were to: (1) Generate a molecular map of pepper based on simple sequence repeat (SSR) markers. (2) Map QTL for capsaicinoid (pungency) content (3) Determine possible association between capsaicinoid and carotenoid content and structural genes for capsaicinoid and carotenoid biosynthesis. (4) Map QTL for quantitative traits controlling additional fruit traits. (5) Map fruit-specific ESTs and determine possible association with fruit QTL (6) Map the *C* locus that determines the presence and absence of capsaicinoid in pepper fruit and identify candidate genes for *C*.

Background: Pungency, color, fruit shape and fruit size are among the most important fruit quality characteristics of pepper. Despite the importance of the pepper crop both in the USA and Israel, the genetic basis of these traits was poorly understood prior to the studies conducted in the present proposal. In addition, molecular tools for use in pepper improvement were lacking.

Major conclusions and achievements: Our studies enabled the development of a saturated genetic map of pepper that includes numerous SSR markers. This map has been integrated with a number of other independent maps resulting in the publication of a single resource map consisting of more than 2000 markers. Unlike previous maps based primarily on tomato-originated RFLP markers, the new maps are based on PCR markers that originate in *Capsicum* providing a comprehensive and versatile resource for marker-assisted selection in pepper. We determined the genetic and molecular bases of qualitative and quantitative variation of pungency, a character unique to pepper fruit. We mapped and subsequently cloned the *Pun1* gene that serves as a master regulator for capsaicinoid accumulation and showed that it is an acyltransferase. By sequencing the *Pun1* gene in pungent and non-pungent cultivars we identified a deletion that abolishes the expression of the gene in the latter cultivars. We also identified QTL that control capsaicinoid content and therefore pungency level. These genes will allow pepper breeders to manipulate the level of pungency for specific agricultural and industrial purposes. In addition to pungency we identified genes and QTL that control other key developmental processes of fruit development such as color, texture and fruit shape. The *A* gene controlling anthocyanin accumulation in the immature fruit was found as the ortholog of the petunia transcription factor *Anthocyanin2*. The *S* gene required for the soft flesh and deciduous fruit nature typical of wild peppers was identified as the ortholog of tomato polygalacturonase. We identified two major QTL controlling fruit shape, *fs3.1* and *fs10.1*, that differentiate elongated and blocky and round fruit shapes, respectively.

Scientific and agricultural implications: Our studies allowed significant advances in our understanding of important processes of pepper fruit development including the isolation and characterization of several well known genes. These results also provided the basis for the development of molecular tools that can be implemented for pepper improvement. A total of eleven refereed publications have resulted from this work, and several more are in preparation.

Achievements

The genetic and molecular basis of pungency

A major focus of the proposal was pungency in pepper fruit, a major quality characteristic of this crop that results from the accumulation of capsaicinoids in the placenta of the fruit. We initially mapped the *C* locus required for pungency to chromosome 2 and developed linked molecular markers useful for marker assisted selection (Blum et al. 2002). We then studied the genetic basis of quantitative variation of capsaicinoids content in two mapping populations. The first population was a F₂ population derived from a cross between Maor, a sweet-fruited *Capsicum annuum* cultivar and BG 2816, a wild pungent *C. frutescens* accession. We identified a single major QTL for capsaicinoid content in chromosome 7, termed *cap*, which explained 34-38% of the phenotypic variation for this trait in two growing environments (Blum et al. 2003). We also mapped genes from the capsaicinoids biosynthesis pathway, but neither of them coincided with capsaicinoids content QTL.

The second population was an inter-specific *C. annuum*, NuMex RNaky (mild fruit) x *C. frutescens*, BG 2814-6 (hot fruit) F₃ mapping population (coded FA03). The population was grown and evaluated three times (twice in Israel and once in USA) in field trials for measurement of capsaicinoid content by means of HPLC. DNA that was extracted from the progenitor F₂ population was used for mapping of molecular markers and QTL identification for capsaicinoid content. A total of 17 QTLs and three digenic interactions were detected for different capsaicinoids components in at least two environments (Appendix A), of which one that involved loci at chromosomes 2 and 7 had the greatest effect on capsaicinoids content.

A putative acyltransferase gene was isolated from placental tissue of pungent fruit and was identified as the *C* (*pun1*) gene (Stewart et al. 2005). A placental-specific partial cDNA clone, AT3, encoding an acyltransferase had been identified from a subtraction library and been considered as one of most possible candidate genes for the *pun1* gene. The full-length cDNA was cloned and used as probe for Northern and Southern blot analysis. The expression profile of AT3 through fruit development coincided with the production of capsaicinoids in pungent peppers. Expression of AT3 could not be detected in non-pungent cultivars. Southern analysis indicated that pungent and non-pungent cultivars are distinguished by a deletion within the AT3 locus. Segregation analysis in a *Pun1* mapping population indicated a

complete co-segregation of AT3 and *Pun1*. Virus-induced gene silencing of AT3 resulted in a decrease in the accumulation of capsaicinoids, a phenotype consistent with *pun1*. These results, collectively, indicate that the *Pun1* locus in pepper encodes a putative acyltransferase.

Development of pepper maps

The FA03 population (100 progenies) was used to generate the largest SSR map reported to date in *Capsicum* (Appendix B). The map consists of over 700 molecular markers, of which 195 are AFLP, 35 RFLP and 489 SSR. Most SSR markers are proprietary and were contributed by the companies Syngenta and DNA Landmark. Primer sequences are currently held by the owners of the markers although at least one company is likely to make a significant donation of a marker set to the public domain. Additional 138 SSR markers are from public sources (Dr. Nagy, Agricultural Biotechnology Center Hungary; Dr. B. D. Kim, Seoul National University, South Korea; Dr. U. Reddy Alcom State University, USA) for which no restriction exists. The total map length is 1317 cM and it is distributed along 12 major linkage groups and 4 small linkage groups that can be assigned to pepper chromosomes (Appendix B).

In addition to the SSR-based map, an integrated genetic map of pepper was constructed by merging segregation data from six mapping populations (Paran et al. 2004; www.plbr.cornell.edu/psi). The integrated map included a total of 2262 loci covering 1832 cM distributed in 13 linkage groups. Both pepper maps allow the improved availability of molecular markers for applications in breeding and genome analysis because of better genome coverage and the use of PCR as the marker technology platform that is suited for high-throughput applications.

Mapping and cloning genes that control additional fruit characteristics

We conducted QTL studies to identify the major chromosomal regions that control fruit shape in pepper. These studies allowed the identification and verification of the two most important loci that control fruit shape in chromosomes 3 and 10 in pepper, (Ben Chaim et al. 2003a; Ben Chaim et al. 2003b). In addition we conducted comparative QTL mapping studies for fruit size and fruit shape QTL in chromosomes

2 and 4 in pepper and tomato (Zygier et al. manuscript submitted) that allowed the determination of orthologous QTL in these species.

We identified two genes that control very well known dominant mutations in pepper, the *A* mutation required for anthocyanin accumulation in different plant tissues and the *S* mutation causing a soft flesh and deciduous fruit nature typical to wild fruits. The *A* gene was identified as a homolog of Petunia *Anthocyanin2*, a MYB-domain transcription factor that controls the expression of genes in the anthocyanin biosynthesis pathway (Borovsky et al. 2004). The *S* gene was identified as a fruit polygalacturonase known to control fruit texture in many plants (Rao and Paran, 2003). Finally, the *chlorophyll retainer (cl)* mutation causing inhibition of chlorophyll degradation during fruit ripening and used to breed fruits with mature brown color was mapped to chromosome 1 (Efrati et al. 2005). This opens the way to develop markers for *cl* for use in marker assisted selection and eventually to clone the gene.

Agricultural and/or economical impacts of the research findings

The studies conducted provided major advances in the understanding of key processes involving pepper fruit development such as pungency, fruit shape and size, fruit color and texture. The practical outcomes are the development of breeding tools enabling more efficient selection protocols for pepper improvement. A notable example is the cloning of *pun1* that allows the development of markers for the detection of pungency, a trait that is of prime importance to pepper breeders, yet one of the most difficult ones to select for or against. The mapping of numerous SSR markers throughout the genome will provide additional molecular tools for diverse targets in the genome.

Details of cooperation

Close cooperation between the PIs has resulted in joint authorships in four manuscripts and a book chapter that summarizes genome mapping in *Capsicum*. The cooperation was built on the strengths and capabilities of each PI. Field trials, traits measurements and QTL analyses were performed mostly in Israel, while SSR development and mapping and isolation of the *pun1* locus was performed in the USA.

List of publications

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- Ben Chaim, A., Borovsky, Y., De Jong, W. Paran, I. 2003b. Linkage of the *A* locus for the presence of anthocyanin and *fs10.1*, a major fruit-shape QTL in pepper. *Theor. Appl. Genet.* 106: 889-894.
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- Rao, G. U. Paran, I. 2003. Polygalacturonase: a candidate gene for the soft flesh and deciduous fruit mutation in *Capsicum*. *Plant Molecular Biology* 51: 135-141.
- Stewart, C.S., B.C. Kang, K. Liu, M. Mazourek, E.Y. Yoo, S.L. Moore, B.D. Kim, I.

Paran and M.M. Jahn. 2005. The *Pun1* gene in pepper encodes a putative acyltransferase. Plant J. accepted.

Appendix

Table of content:

Appendix A

Table 1. QTLs detected for capsaicinoids content in the FA03 population

Table 2. Digenic interactions detected in the FA03 population.

Figure 1. The genetic model for the control of capsaicinoids content in the FA03 population.

Appendix B

Table 1. Mapping summary of the FA03 population.

Table 2. Markers information in the FA03 population.

Manuscripts:

Efrati, A., Eyal, Y. and Paran, I. 2005. Molecular mapping of the *chlorophyll retainer* (*cl*) mutation in pepper (*Capsicum* spp.) and screening for candidate genes using tomato ESTs homologous to structural genes of the chlorophyll catabolism pathway. Genome In Press

Stewart, C.S., B.C. Kang, K. Liu, M. Mazourek, E.Y. Yoo, S.L. Moore, B.D. Kim, I. Paran and M.M. Jahn. 2005. The *Pun1* gene in pepper encodes a putative acyltransferase. Plant J. accepted.

Paran, I., Ben Chaim, A., Kang, BC., Jahn, M. (2005). The Genomes. A Series on Genome Mapping, Molecular Breeding & Genomics of Economic Species. C. Kole (Ed), Vol 5. *Capsicum*. In Press

S. Zygier, A. Ben Chaim, A. Efrati, G. Kaluzky, Y. Borovsky and I. Paran. QTL mapping of fruit size and shape in chromosomes 2 and 4 in pepper and comparison of gene function with tomato. Submitted to TAG