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**Tagging Plant Genes with Tightly-Linked
RELP Markers**

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Tagging Plant Genes with Tightly-Linked RFLP Markers

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Abstract

Many tomato genes of economic importance have been placed on the genetic map and being introgressed into modern cultivars. The objective of this project was to identify RFLP markers which are tightly linked to disease resistance genes. Such markers could be used to enhance the rate of progress in breeding programs. RFLP markers tightly linked to the following resistance genes were identified: Tm1 (resistance to tobacco mosaic virus); Tm2 (resistance to tobacco mosaic virus); Ve (resistance to *Verticillium*); I (Resistance to *Fusarium* race 1); I1 (Resistance to *Fusarium* race 1); I2 (Resistance to *Fusarium* race 2); I3 (Resistance to *Fusarium* race 3); Mi (Resistance to root knot nematodes); Sm (Resistance to *Stemphylium*) and Pto (Resistance to *Pseudomonas syringae*).

RFLP markers tightly linked to disease resistance genes could be used as starting points in a chromosome walk aimed at cloning those genes. To achieve this objective we proposed to produce high resolution map for the target genes of interest. This has been accomplished for the genes Mi, Tm2 and I2 and projects were initiated aimed at cloning these genes.

Other studies conducted within the framework of the BARD project contributed to our understanding of the evolution, genome organization and the pattern of inheritance of quantitative traits in tomato.

Objectives

The objective of this project was to develop RFLP (restriction fragment length polymorphism) tags for a number of disease resistance genes in tomato. The targetted genes were *Tm1* (resistance to tobacco mosaic virus); *Tm2* = (resistance to tobacco mosaic virus), *Ve* (resistance to *Verticillium albo-atrum*), *I* (resistance to *Fusarium oxysporium*, race 1), *Mi* (resistance to root knot nematodes). In addition to these originally-targetted genes, we were also able to develop RFLP tags for additional resistance genes: *Sm* (resistance to *Stemphylium*), *I2* (resistance to *Fusarium oxysporium*, race 2), *I3* (resistance to *Fusarium oxysporium*, race 2), and *Pto* (resistance to *Pseudomonas syringae*). Secondary objectives were to develop high resolution maps for some of these genes as a prelude to chromosome walking to isolate the genes. This has been accomplished for *Mi*, *Tm2* and *I2*. Currently work is underway to chromosome walk to these genes using a recently developed YAC (yeast artificial chromosome) library for tomato.

Linked RFLP markers for the above resistance genes were to be used to in a breeding program to begin developing multiple resistant tomato stocks for hybrid production. This work is currently underway using the tagged markers developed during this project.

Research Accomplishments

I. Identification of RFLP markers tightly linked to disease resistance genes in tomato.

A primary objective of this project has been to "tag" disease resistance genes in tomato with RFLP markers. Tightly-linked RFLP markers can provide a tool for selection for plant breeders and may ultimately lead to the cloning of these genes via chromosome walking.

A. Fusarium resistance -- I1 gene. A backcross mapping population of 140 individuals has been established from a cross of an F1 hybrid between *L. pennellii* which segregates for the I1 gene and which is being used to locate the exact position of the I1 gene. In addition Cornell and Rehovot have jointly developed a recombinant inbred population between *L. esculentum* cv. VFNT cherry and *L. cheesmanii* which segregates for I1 as well as several other disease resistance genes (*Ve*, *Mi* and *Tm2a*) and which will be used for mapping of the I1 gene.

B. Fusarium resistance -- I2 gene. Using chromosome 11 specific clones provided by Cornell, Zamir's lab in Rehovot has identified RFLP markers tightly linked to the I2 gene for resistance to *Fusarium oxysporum* race 2 (Sarfatti et al. 1989). Presently work is underway to walk to the I2 gene using a Cosmid library developed in Israel and a YAC library developed in the U.S.

C. Fusarium resistance -- I3 gene. I3 is a new gene recently introgressed from *L. pennellii* and which confers to race 3 of *Fusarium*. Screening of breeding lines with RFLP markers has allowed placement of the I3 gene on the RFLP map and to determine the size of introgressed segments containing this gene (Tanksley and Costello 1991). Currently more than 10 RFLP/RAPD markers have been identified within 5 cM of the gene and a high resolution map is underway. A PCR-based screen for the gene is also being developed based on the most tightly linked RFLP marker.

D. Nematode resistance -- Mi gene. Five RFLP markers that are tightly linked to the Mi gene, and which are included on the introgressed segment containing this gene, have now been identified at Cornell. More than 1000 F2 plants have been analyzed for Mi as well as segregating RFLP markers in order to develop a high resolution map. The results of the high resolution mapping have been recently published and are being used to guide a chromosome walk to the gene (Messeguer et al. 1991). The linked RFLP markers are also being used to transfer Mi into a series of tomato breeding stocks.

E. Tobacco mosaic resistance -- Tm-2a gene. Fifteen RFLP markers have been isolated which map within 5 map units of the *Tm-2a* gene on chromosome 9. The same F2 population being used to locate the Mi gene is also being used for high resolution mapping of this gene. At least 5 of the 15 markers are apparently less than 1 cM away from the gene. PFGE was also being used to physically map the region of the genome containing this gene (Ganal et al. 1998). Due to apparent suppressed recombination around the Tm2 gene (presumably because of its proximity to the centromere on chromosome 9) we have had to analyze additional large segregating populations and to isolate additional markers (RAPDs) in order to prepare for a chromosome walk. The high resolution map has now been completed and is being used as a guide for a

chromosome walk to the gene (Ganal et al, in preparation). Linked RFLP markers are also being used to select for Tm2 in an associated breeding project.

F. Tobacco mosaic resistance -- Tm-1 gene. The Tm-1 gene has been located near the centromere on chromosome 2. This region is apparently highly suppressed in recombination and as a result, many RFLP markers show tight linkage to this gene (more than 50 markers). Because of the lack of recombination in this region, high resolution has been difficult and this gene is no longer being pursued.

G. Pseudomonas resistance -- Pto gene. Five RFLP markers on chromosome 5 have been shown to be located within the introgressed segment containing the *Pto* gene which confers resistance to *Pseudomonas syringae* (Martin et al. 1991). A population of 500 F2 plants has now been analyzed and a high resolution map prepared for *Pto* (Martin et al, in preparation). Chromosome walking to *Pto* (using a YAC library is currently underway). A PCR based selection screen for *Pto* (based on one of the tightly linked RFLP markers) has now been developed and is being used to breeding for *Pto* resistance.

H. Verticillium resistance -- Ve gene. The map position of *Ve* was previously unknown, but was thought to be on chromosome 12. The gene has now been mapped to chromosome 7 using a recombinant inbred population between *esculentum* and *cheesmanii* (Juvick et al. 1991).

I. Stemphylium resistance -- Sm gene. The gene *Sm* confers resistance to four *Stemphylium* species and was associated with chromosome 11. (Behare et al. 1991).

II. Use of RFLP tags to develop multiply-resistance tomato inbreds

Thus far, tightly linked RFLP markers have been found for all of the originally targetted genes as well as several others that were not originally targetted. We are currently exploiting these linkages (as well as previously established RFLP linkage for soluble solids genes, Paterson et al. 1988) to establish a set of processing and fresh market inbred lines with multiple resistance genes as well as high soluble solids. These lines will be field tested both in the U.S. and Israel in hybrid combination with other previously-established inbreds. By using the RFLP markers, we hope not only to incorporate these useful genes into these inbreds, but to select for minimal 'linkage drag' DNA in order to avoid problems previously encountered with their usage in commercial tomato varieties.

III. Map-based cloning of disease resistance genes.

One of the reasons for seeking RFLP markers tightly linked to disease resistance genes was to provide a starting point for chromosome walking to clone the resistance genes. While chromosome walking was not included in the immediate objectives of this project, we have, nonetheless, been able to use information and markers developed during this project to begin a chromosome walk to four of the resistance genes. These are *I2*, *Pto*, *Mi* and *Tm2a*. RFLP markers have been used to isolate YAC or cosmid clones tightly linked to each of these genes. It is anticipated that chromosome walking will lead to the cloning of one or more of these genes in the relatively near future.

Cooperation

Cooperation on this project has been on several levels. The mapped RFLP clones used both in Israel and the U.S. were provided by the Tanksley lab at Cornell. The *esculentum* x *cheesmanii* recombinant inbred line population was developed jointly with the latter generations (F4 - F7) being produced in Israel. A student from the Zamir lab visited the Tanksley lab in the fall of 1989 to do the RFLP probing on these populations. Materials have been exchanged for development of multiple resistance lines using RFLP-marker selection and the P.I.'s communicated on a regular basis regarding the status of this project. The P.I.'s are currently continuing to collaborate on both chromosome walking and on the development of multiple resistance breeding lines using the markers developed during this project.

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