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Chromosomal localization and molecular-marker tagging of the powdery mildew resistance gene (*Lv*) in tomato

Abstract We report the tagging of a powdery mildew [*Leveillula taurica* (Lév.) Arnaud.] resistance gene (*Lv*) in tomato using RAPD and RFLP markers. DNA from a resistant (cv Laurica) and a susceptible cultivar were screened with 300 random primers that were used to amplify DNA of resistant and susceptible plants. Four primers yielded fragments that were unique to the resistant line and linked to the resistance gene in an F_2 population. One of these amplified fragments, OP248, with a molecular weight of 0.7 kb, was subsequently mapped to chromosome 12, 1 cM away from CT134. Using RFLP markers located on chromosome 12, it was shown that approximately one half of chromosome 12 (about 42 cM), in the resistant variety is comprised of foreign DNA, presumably introgressed with the resistance gene from the wild species *L. chilense*. Further analysis of a backcross population revealed that the *Lv* gene lies in the 5.5-cM interval between RFLP markers, CT211 and CT219. As a prelude to map-based cloning of the *Lv* gene, we are currently enriching the density of markers in this region by a combination of RAPD primers and other techniques.

Key words Powdery mildew (*Leveillula taurica*)
Tomato · RAPD · RFLP · *Lv*

Introduction

Powdery mildew in tomato [*Leveillula taurica* (Lév.) Arnaud.] has become a serious problem to tomato growers and breeders around the world. This air-borne fungus

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causes yellow necrotic lesions and the death of both young and old leaves, resulting in a loss of yield and the quality of produce (Jones and Thompson 1987; Correll et al. 1988). The powdery mildew fungus has a wide host range including crops such as potato, pepper, onion, and artichoke as well as some weed species (Palti 1971, 1974; Correll 1986). Damage caused by this fungus is serious in many of the major tomato-growing regions of the world, especially in hot and dry areas (Kontaxis and Van Maren 1978; Correll et al. 1987). Moreover, the fungicide triadimefon (Bayleton), which is the best means of controlling this disease, has been banned in recent years, increasing the need for resistant varieties (Anonymous 1992).

A group of researchers from Bulgaria introgressed single gene resistance to powdery mildew from the wild tomato, *L. chilense*, (Accession No. LA1969) into the cultivated tomato. This resistance gene, *Lv*, acts as a single dominant gene (Yordanov et al. 1975; Stamova and Yordanov 1987; Hernandes and Stamova 1990; Stamova and Yordanov 1990). Laurica, an introgression line containing the resistance for powdery mildew, is currently the sole source of resistance to this pathogen.

One of the disadvantages of using wild germplasm in breeding programs is linkage drag. Problems of poor horticultural quality, probably due to linkage drag, have been encountered in attempts to use Laurica in breeding programs. Even after many generations of backcross and selection, acceptable lines are still difficult to recover; therefore, currently, no commercial variety exists with this resistance.

In this paper we report the chromosomal localization and molecular tagging of the powdery mildew resistance gene (*Lv*) using random amplified polymorphic DNA (RAPD) primers and RFLP markers. We have also determined that the resistance gene is contained in a large region of foreign DNA (half of chromosome 12) which may account for difficulties in using this resistance in breeding programs. Use of the molecular marker information should allow the linkage drag associated with *Lv* to be broken and may ultimately lead to the isolation of this gene via map-based cloning.

Materials and methods

Three hundred decanucleotide primers were obtained from Operon Technologies Inc., Calif., and used to amplify DNA from Laurica (R) and JH195(S). The reactions were prepared as described by Martin et al. (1991) and were amplified with either a Perkin-Elmer/Cetus DNA thermal cycler or an MJ Research PTC100 Programmable Thermal Controller. Amplified products were resolved on 2% Nusieve GTG agarose (FMC) and 1% agarose electrophoresis gels for 15 h (1.2 V/cm). The four primers described in this paper have the following nucleotide sequences: OP 57, 5′ d[GACGTGGTGA]3′, OP58, 5′ d[GTGGAGTCAG]3′, OP217, 5′ d[AGCGGAAGTG]3′ and OP248, 5′ d[AAGCCCCCCA]3′. Selected amplified products were excised from the gel and purified by a glass-milk-based method (Geneclean, BIO/101, Calif.). Purified products were then P^{32}-labeled using the random hexamer method (Sambrook et al. 1989) and used to probe Hybond-N^+ filters (Amersham) carrying tomato DNA digested with five different enzymes: EcoRI, EcoRV, DraI, HindIII, and HaeIII. Southern hybridization and washing conditions were as described by Bernatzky and Tanksley (1986).

Three populations were used to determine the map position of Lv relative to RAPD and RFLP markers. The first population (referred to as LXE) consisted of 161 plants from an F_2 population generated from a cross between Laurica (a line containing the introgressed Lv gene) and a powdery-mildew-susceptible commercial cultivar, JH195. The resulting F_2 progeny were screened for both response to the powdery mildew pathogen and the segregation of selected molecular markers. Seedlings (or, in some instances, cuttings) were inoculated with an isolate of L. taurica collected from an infested tomato field in California as described by Correll (1986). Disease severity was scored 2–3 weeks after the initial inoculation. DNA for RAPD analysis was isolated from the source using the method of Bernatzky and Tanksley (1986). Association between the RAPD markers and the disease phenotype was determined by a χ^2 test for independence using Statview 512+ for MacIntosh.

A second population (EXP) was used in this laboratory to create a high-density molecular linkage map and consists of 67 F_2 plants from a cross between L. esculentum and L. pennellii (Tanksley et al. 1992). This population was used to locate the precise map position of the DNA fragments obtained from RAPD isoline analysis and shown from the previous population to be linked to Lv. RAPD markers were located on the tomato linkage map using the computer program MAPMAKER (Lander et al. 1987).

To identify the position of the introgression piece from L. chilense, isoline filters were prepared by digesting DNA of Laurica and three tomato cultivars: San Marseno, Moneymaker, and E6203, with four different enzymes: EcoRI, EcoRV, XbaI and BstNI. These filters were hybridized as described above with selected RFLP markers from chromosome 12 of tomato in order to determine the size of the introgressed piece from L. chilense.

Finally a population of 167-BC_1 plants from a cross of Laurica and the susceptible line E6203 (LXE) was used to fine map Lv relative to RFLP markers on chromosome 12.

Results and discussion

RAPD analysis and chromosome localization of Lv

Of the 300 random primers used to amplify DNA from Laurica (R) and JH195 (S), four, OP 57, OP58, OP 217 and OP248, revealed polymorphisms. One primer, OP57, amplified codominant bands. The other three primers yielded presence/absence polymorphisms. All polymorphic bands were shown to be linked to resistance in the LXE population ($P < 0.001$, Fig. 1). The polymorphic DNA fragments amplified by each primer were purified, radiolabeled and

hybridized to tomato genomic DNA. PCR products from OP57, OP58 and OP217 were found to contain repeated DNA sequences. However OP248 yielded a 700-bp single-copy fragment which mapped to chromosome 12 approximately 1 cM away from RFLP marker CT134 in the EXP population (Fig. 2).

Recombination suppression and linkage drag

To determine the size of the introgressed chromosomal segment from L. chilense in Laurica, DNA from Laurica and three susceptible cultivated tomato varieties were digested with four restriction enzymes and probed with selected RFLP markers from chromosome 12. Laurica was found to contain alleles not normally present in L. esculentum within the region of chromosome 12 that spans TG180 to CT134, a distance of at least 42 cM (Fig. 2).

This introgressed region was found to exhibit reduced recombination (as measured in the LXE population) compared to the EXP population which does not contain the Lv gene. The distance from the top to the end of the introgressed piece (from TG180 to CT134) is about 42 cM in the EXP but only 28.5 cM in the LXE population (Fig. 2). The reduction in map distance is pronounced in two regions: between TG180 and TG68, 13.8 cM in EXP but only 4.3 cM in LXE (68% reduction), and between TG68 and CT211, 18.7 cM in the first population but 10.4 cM in the latter (43% reduction). However, the recombination frequency increased toward the middle of the chromosome and appeared to be as high, or higher, in the LXE population than in the mapping population.

Recombination suppression is known to be caused by abnormalities in chromosome structure such as inversion or translocation (Burnham 1991). However, this is not likely to be the cause in this instance because the single-copy markers mapped in this region retained the same order in both populations. Rick (1969) found that the recombination frequency in the interspecific tomato cross was reduced. This suggests that there might be a certain degree of recombination suppression where chromosome segments from remote species pair and that the degree of suppression might vary as seen in the LXE and EXP populations.

Fine mapping of the Lv locus

The LXE population was used to fine map Lv on chromosome 12. DNA from 167-BC_1 plants was extracted, digested with EcoRV, and hybridized with TG180 and CT134, markers that flank the introgressed segment (Fig. 2). Forty recombinants were selected and tested for their disease resistance reaction. In addition, these recombinants were tested with additional RFLP markers located within the introgressed piece. From the disease screening and marker survey results, the Lv gene can be positioned midway between the RFLP markers CT211 and CT219, which are 5.5 cM apart (Fig. 2). The markers that flank the

Fig. 1 RAPD markers that amplified polymorphic fragments between resistant and susceptible lines. *P* and *F2* stand for parents, Laurica (*R*), HJN (*S*), JH150 (*S*), and JH195 (*S*), and the *F2* segregating population. *R* and *S* represent resistance and susceptibility based on disease scoring. *M* is a 1-kb ladder, molecular weight marker
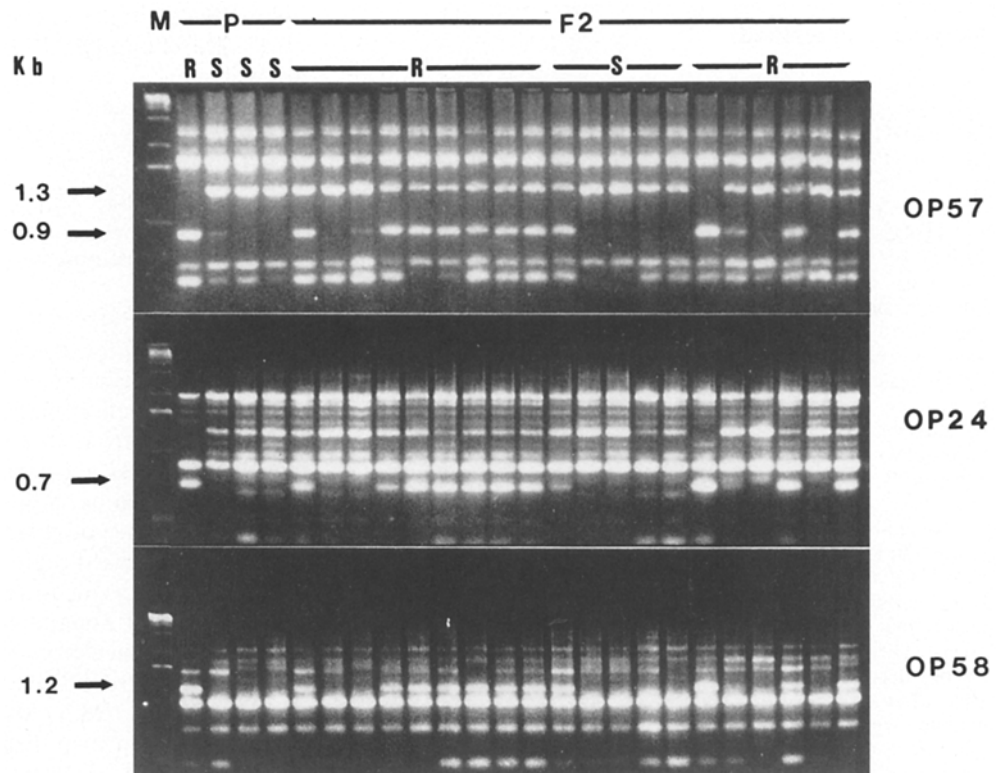
Lv gene, CT211 and CT219, are likely to be physically far apart, as on average 5.5 cM corresponds to 4–5 Mb (Tanksley et al. 1992). Therefore, we are now in the process of focusing on this region to obtain more molecular markers through a combination of RAPD analysis and other techniques.

Applications and future work

The decrease in meiotic recombination in the *L. chilense* segment may have contributed to the retention of the large *L. chilense* chromosome segment during the original introgression of the gene into *L. esculentum*. The large segment of *L. chilense* DNA probably carries many genes in addition to the resistance gene, and these genes might account for the horticultural problems encountered in breeding for powdery mildew resistance. Using conventional breeding methods to significantly reduce the linkage drag associated with *Lv* would require many additional generations. However, the use of linked molecular markers in the region of *Lv* would allow the selection of rare beneficial crossovers and the rapid reduction of linkage drag (Tanksley et al., 1989). Moreover, having the gene tagged with tightly-linked molecular markers, will facilitate selection for resistant genotypes in breeding programs.

Finally, molecular mapping of the *Lv* gene is the first step towards isolation of this gene via map-based cloning. This strategy has proven successful in the cloning of many genes with unknown products, such as genetic diseases in humans (Rommens et al. 1989; Del-Castillo et al. 1993; Gregory et al. 1993) and a disease resistance gene in to-
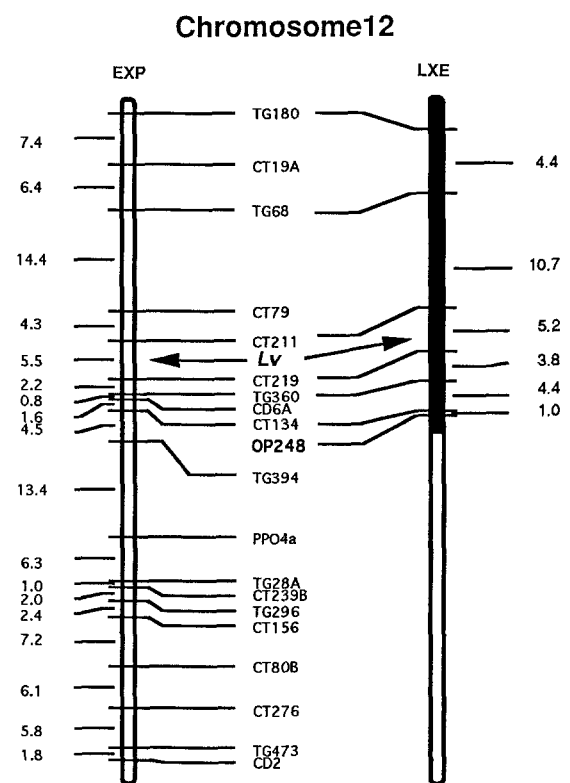

Fig. 2 Reduction in recombination frequency can be observed as depicted in the figure. *EXP* and *LXE* represent chromosome 12 in the *L. esculentum* X *L. pennellii* and the Laurica X *L. esculentum* populations respectively. Distances between markers are displayed in Kosambi cM units. The *solid area* on the short arm of the *LXE* chromosome shows the introgressed region from *L. chilense*

mato (Martin et al. 1993). The *Pto* gene that confers resistance to bacterial speck in tomato was cloned via a map-based strategy and was found to be a member of the serine/threonine protein-kinase family involved in signal transduction. Cloning of the *Lv* gene will be one key step toward elucidating the mechanism of plant defense against fungi which may shed additional light on the molecular mechanism of that defense. Results from this work are likely to have application not only in tomato but also in other crops that host this fungal pathogen.

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