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High-resolution genetic map of the *Lv* resistance locus in tomato

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Abstract Bulked segregant analysis and high-resolution mapping were used to pinpoint the position of the *Lv* gene for resistance to *Leveillula taurica* in tomato. Mapping in an F_2, corresponding to more than 3800 gametes, indicates that *Lv* is positioned within the 0.84-cM interval defined by the RFLP markers CT121 and CT129, with the closest marker, CT121, being only 0.16 cM from the gene. The tight linkage of these markers demonstrates their usefulness in marker-assisted breeding for *Lv*, and the high-resolution map provides a starting a starting point for positional cloning of this resistance gene.

Key words Powdery mildew (*Leveillula taurica*) · Tomato-RAPD-RFLP · Marker-assisted breeding

Introduction

Powdery mildew [*Leveillula taurica* (Lév.) Arnaud.] in tomato is a serious problem in many growing regions world wide, but especially in hot, dry regions (Kontaxis and Van Maren 1978; Correll et al. 1987). The fungus typically attacks late in the season causing yellow necrotic lesions on both young and old leaves. The resulting loss of leaf cover results not only in reduced yield but also causes a loss in fruit quality due to sunscald (Jones and Thompson 1987; Correll et al. 1988).

A single dominant gene, *Lv*, conferring resistance to *L. taurica* Arnaud has been introgressed from the wild tomato relative, *Lycopersicon chilense*, into the cultivated tomato via backcross breeding (Yordanov et al. 1975; Stamova and Yordanov 1987, 1990; Hernandes and Stamova 1990). Chungwongse et al. (1994) reported that *Lv* is located near the centromere of chromosome 12 and is positioned in the 5-cM interval between the RFLP markers CT121 and CT129. Moreover, the introgression of *Lv* into the cultivated tomato was accompanied by a transfer of almost an entire arm of chromosome 12 from the *L. chilense* donor line (Chungwongse et al. 1994).

A high-resolution genetic linkage map is a prerequiste for both the application of marker-assisted selection and map-based gene cloning. To safely use a molecular marker for the indirect selection of a linked target gene, it is necessary to first establish that those loci are very tightly linked (preferably < 1 cM apart). Otherwise, recombination between the marker and target gene will lead to frequent misclassification of the target genotype. For map-based cloning the molecular marker and target gene must be tightly linked both genetically and physically (Tanksley et al. 1995).

The goals of the current study were to: (1) identify more molecular markers in the region of chromosome 12 containing *Lv*, and (2) orient *Lv* precisely with respect to these new markers as well as to previously published RFLP markers. Information from this study will be useful for establishing a reliable molecular-marker screen for *Lv* and for determining the feasibility of applying map-based cloning strategies to the isolation of this gene.

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Materials and methods

Development of nearly isogenic lines for *Lv* and the generation of pools for bulked segregant analysis

L. esculentum cv Laurica, which contains the dominant resistance allele at the *Lv* locus, was hybridized and backcrossed six times to

the susceptible line *L. esculentum* cv E6203. Selection in each generation was made for the resistance genotype at RFLP markers CT79 and CT219 that were known to flank *Lv* (Chunwongse et al. 1994). A single heterozygous BC_6 plant was self-pollinated to generate an F_2 population from which six homozygous-resistant genotypes and 13 homozygous-susceptible genotypes were isolated. DNA was extracted from each homozygous individual using the methods described by Bernatzky and Tanksley (1986). Equal amounts of DNA from each genotype were combined to make resistant and susceptible pools of DNA for bulked segregant analysis (Giovanonni et al. 1991; Michelmore et al. 1991).

Decanucleotide primers were obtained from Operon Technologies Inc., Calif., and used to amplify DNA from resistant and susceptible pools using the polymerase chain reaction (PCR) conditions described in Martin et al. (1991). Amplified products were resolved by electrophoresis on 2% agarose electrophoresis gels for 15 h (1.2 V/cm). Primers revealing polymorphism between the pools were re-tested on the same DNA to verify the polymorphism. Primers giving reproducible polymorphisms were applied to selected recombinants from the mapping population in order to determine their map positions relative to *Lv* (see next section).

Mapping population

The high-resolution mapping population consisted of 1906 F_2 plants derived from the cross *L. esculentum* cv Laurica (R/R) × *L. esculentum* cv UC82L (S/S). DNA was isolated from seedlings using the microprep procedure (Fulton et al. 1995). These individuals were assayed with CT79 and CT219 which were previously shown to delineate a 5-cM interval containing *Lv* (Chungwongse et al. 1994). One hundred and forty six plants were identified as being recombinant in this interval and were subsequently transferred to the greenhouse to obtain F_3 progeny. The same plants were also assayed for RFLP markers CT121 and CD129 which had previously been mapped to the CT79-CT219 interval but whose orientation relative to each other was unknown (Chungwongse et al. 1994; Chungwongse 1995). From 72 of these recombinants, homozygous lines were isolated from F_3 progeny by selection with CT79, CT121, CD129 and CT219. F_4 seeds from these lines were then subjected to disease screening with the *L. taurica* (Lév.) Arnaud pathogen as described in Correll (1986).

Results and discussion

Identification of RAPD markers in the *Lv* region

An average of 4.3 genomic fragments were amplified by each of the 633 RAPD primers that were used to screen the *Lv* susceptible and resistant DNA pools. The screen therefore represented a sampling of approximately 2700 loci. Eight primers revealed polymorphism between the susceptible and resistant pools. These primers could thus be attributed to loci within the introgressed region of chromosome 12 that contains *Lv* and which is defined by the interval between RFLP markers CT79 and CT100 (Fig. 1). Table 1 lists the sequences of these eight primers and the molecular weights of the polymorphic bands. The exact position of these RAPD loci was determined by amplifications of DNA from individuals recombinant within this region (see below).

Table 1 RAPD primers amplifying loci in the *Lv* region of chromosome 12, NB = no band

Primer designation	Primer sequence	MW polymorphic fragment	
		Resistant	Susceptible
OPAM-01	TCACGTACGG	1700?	NB
OPD-02	GGACCCAACC	900	NB
OPAJ-17	ACCCCCTATG	NB	550
OPB-13	TTCCCCCGCT	NB	800
OPAG-14	CTCTCGGCGA	NB	550
OPO-11	GACAGGAGGT	NB	550
OPB-06	TGCTCTGCCC	NB	2000?
OPD-13	GGGGTGACGA	400	NB

High-resolution mapping of the *Lv* locus

Of 1906 F_2 individuals screened, 146 were determined to be recombinant for markers CT79 and CT219. When these recombinant individuals were screened with CT121 and CT129 the precise order of these four markers could be determined (Fig. 1). The entire interval encompasses 3.9 eM. In the previously published high-density molecular map of tomato, this same interval contained approximately 10 cM (Tanksley et al. 1992). The reduced recombination observed in the current study may be due to the fact that the region in question was heterozygous for DNA from *L. chilense* (the wild species from whence *Lv* was introgressed) and *L. esculentum* (cultivated tomato). Previous studies have shown that, when such foreign DNA is introduced into the cultivated tomato genome, recombination typically diminishes in the introgressed segment (Rick 1969; Paterson et al. 1990).

Homozygous lines were derived from 72 of the 146 recombinants described above and 9–22 progeny from each line were subjected to inoculation with *L. taurica* (Lév) Arnaud. The results were clear cut in the majority of cases (i.e., all progeny were resistant or all progeny were susceptible). The few ambiguous cases were eliminated from further analysis. Based on this pathological screen the *Lv* locus could be placed in the 0.84-cM interval between CT121 and CT129. Placement of *Lv* in this interval was based on the 16 plants (out of 72) that had a single crossover in this interval. No double crossovers were observed within the interval. *Lv* was three crossovers away from CT121 (0.16 cM) and 13 crossovers away from CT129 (0.68 cM) (Fig. 1). These results suggest that either CT121 or CT129 could be used safely as co-dominant markers for the indirect selection of the *Lv* resistance gene in breeding programs.

A subset of 16 homozygous recombinant F_3 lines was selected for placement of the eight RAPD loci in the *Lv* region. These lines were choosen to represent at least three independent crossovers in each of the intervals defined by CT79, CT121, CT129 and CT100. The goal was to determine whether any of the RAPD

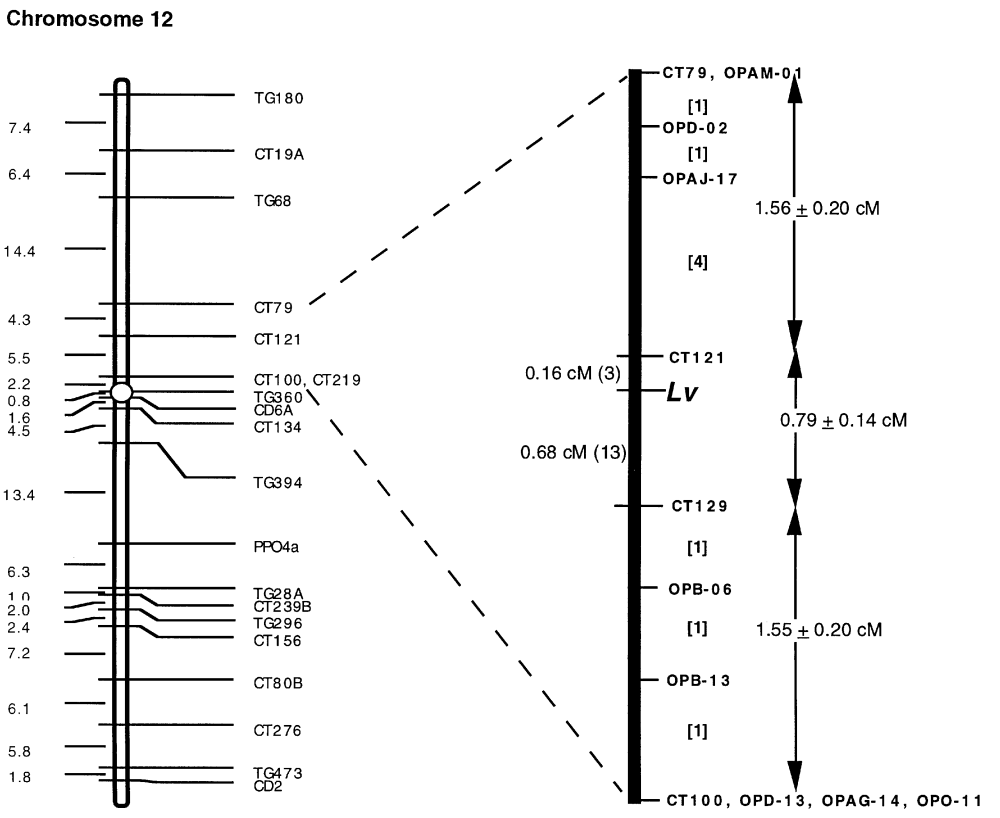

Fig. 1 Left: molecular map of chromosome 12 based on Tanksley et al. (1992). The *open circle* shows the approximate position of the centrometer based on Grandillo and Tanksley (1996). Right: enlargement of the interval CT79–CT100 containing the *Lv* gene. Centimorgan distances on the right are derived from the analysis of 1906 F_2 plants. Distances on left are derived from the disease screening of a subset of 72 individuals determined to be recombinant between CT79 and CT100. *Numbers in parentheses* are the numbers of individuals showing recombination in the corresponding interval. The order of RAPD markers (right side) was determined by the analysis of 16 selected individuals with crossovers in the CT79–CT100 interval. The *numbers* in brackets are the numbers of individuals recombinant in each corresponding interval

markers were closer to *Lv* than CT121 or CT129. Based on an analysis of these recombinants, it was determined that none of the RAPD loci are located in the CT121–CT129 interval which contains *Lv*. One RAPD locus co-segregated with CT79, three co-segregated with CT100, two were localized to the CT79–CT121 interval, and two were localized to the CT129–CT100 interval (Fig. 1). The fact that no RAPDs could be localized between CT121 and CT129 suggests that this interval may represent a relatively small amount of DNA.

Prospects for marker-assisted selection and map-based cloning of *Lv*

Results from this study demonstrate that the RFLP markers CT121 and CT129 define a very small interval approximately (1 cM) containing the *Lv* gene. Because of their tight linkage to *Lv*, either of these markers could be used effectively for marker-assisted selection of this gene in breeding programs. Powdery mildew is a late-onset disease and direct screening with the *L. taurica* pathogen is both time consuming and difficult. Thus, markers for the resistance gene should greatly facilitate its incorporation into new tomato varieties. Also, because *Lv* was introduced from a wild tomato species, and is still surrounded by a large portion of wild species DNA, the linked markers described herein may be useful in selecting for rare crossover events which will reduce linkage drag in further breeding.

Finally, the tight linkage of CT121 and CT129 may provide a starting point for the eventual positional cloning of *Lv*. In tomato, on average, 1 cM corresponds to approximately 950 kb (Ganal et al. 1989). CT121 is estimated to be 0.13 cM from *Lv* which corresponds to an expected physical distance of approximately 100 kb. This distance is small enough that one might be able to use CT121 in a chromosome-landing approach to isolate a single BAC or YAC containing the *Lv* resistance gene (Tanksley et al. 1995).

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