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The *Am* Gene Controlling Resistance to *Alfalfa mosaic virus* in Tomato Is Located in the Cluster of Dominant Resistance Genes on Chromosome 6

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ABSTRACT

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The dominant gene *Am* from *Lycopersicon hirsutum* f. sp. *glabratum* PI134417 confers resistance to most strains of *Alfalfa mosaic virus*, including the recently identified necrotic strains. The phenotypic response includes a lack of symptom development following mechanical inoculation of leaves. To study the resistance mechanism controlled by *Am*, biological (back-inoculation to susceptible hosts), serological (double-

antibody sandwich, enzyme-linked immunosorbent assay), and molecular (reverse transcription-polymerase chain reaction and hybridization with specific riboprobes) methods of virus detection have been conducted on mechanically inoculated PI134417 leaves. The virus was never recovered, indicating that *Am* acts by an inhibition of viral accumulation during the early events of the virus life cycle. *Am* has been mapped genetically to the short arm of tomato chromosome 6 in the resistance hotspot, which includes the R-genes *Mi* and *Cf-2/Cf-5* and the quantitative resistance factors *Ty-1*, *Ol-1*, and *Bw-5*.

Additional keywords: disease resistance, gene clustering, gene mapping, introgression lines, resistance mechanism.

Alfalfa mosaic virus (AMV; genus *Alfamovirus*; family *Bromoviridae*) is a widely distributed pathogen infecting more than 400 plant species, including several vegetable and woody crops (28, 36). Control of AMV is especially difficult due to its very wide host range, with many weeds that constitute important virus reservoirs and transmission in a nonpersistent manner by at least 22 distinct aphid species. Moreover, cases of seed transmission have been reported in some alfalfa cultivars and solanaceous crop species (8).

In tomato crops, AMV infections are generally sporadic, and consequently, the disease is not considered of major importance. Natural infections have been reported in several countries and were connected, in most cases, to the presence of alfalfa crops nearby (42). However, in the last few years, important and repeated outbreaks of AMV in tomato crops have occurred due to the emergence of necrotic strains (25). AMV symptoms in tomato range from bright yellow mosaic on the leaves caused by non-necrotic strains, sometimes with necrotic patches and deformation of the fruits, to severe necrosis of the leaves and fruits, in cases of necrotic strains. Infection of seedlings with necrotic strains can result in plant death. The incidence of these necrotic strains is probably underestimated in tomato fields because of the frequent occurrence of mixed infections and the confusion of AMV-induced symptoms with those of *Cucumber mosaic virus* (CMV) and *Tomato spotted wilt virus* (TSWV) infection. Phylogenetic analysis conducted on the coat protein sequences of several AMV strains, belonging to the nonnecrotic and necrotic type, showed

that they clustered in two monophyletic groups, subgroup I and subgroup II. The existence of two groups was not related with precise biological characteristics, such as symptoms or host range, but reflect mainly a geographic structure (25).

Sources of resistance to AMV have been identified in three accessions of the wild tomato *Lycopersicon hirsutum* Mill.: PI134417 (PI13), LA1777, and Bruinsma (26). Resistance of *L. hirsutum* PI13 against the necrotic AMV strain LYH1 has been studied most extensively and was shown to be inherited as a single dominant gene, named *Am* (27).

Elucidation of the molecular structure of dominant plant disease resistance genes (R-genes) operating on the basis of the gene-for-gene hypothesis (12,18) revealed strong sequence similarities both within a given host for different pathogens and between host species (7). Sequence similarities include well-defined motifs such as nucleotide-binding sites (NBS) and leucine-rich repeats (LRR). Among plant-virus interactions, two distinct but related responses both mediated by NBS-LRR genes can be observed: the hypersensitive response (HR), characterized by rapid cell death at the primary infection site and represented by the *N* gene controlling resistance against *Tobacco mosaic virus* (TMV) in tobacco (6,39), and the extreme resistance (ER), characterized by normal-appearing tissue following virus inoculation and represented by the *Rx* gene controlling resistance against *Potato virus X* (PVX) in potato (1-3). Another feature is that the ER type of viral resistance mechanism is active in protoplasts (19), whereas the HR type is not expressed in isolated protoplasts, indicating that HR requires cell-to-cell contact and is a tissue-related response. It has been shown recently that HR and ER are components of the same defense response that is activated following recognition between the product of the avirulence gene (*Avr*, the elicitor) and the product of the R-gene (the receptor). Current

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data suggest that most R-Avr protein interactions are not direct but instead involve perception of pathogen-derived proteins within a complex (34). According to these data, the occurrence of HR or ER would depend on the affinity between the components of the complex (which includes the elicitor and receptor), on their concentration, and on their rate of production.

In this study, we investigated the nature of *Am*-mediated resistance in PI13 using biological, serological, and molecular approaches. We demonstrate that *Am*-mediated resistance belongs to the ER type, with a lack of visible symptoms and virus accumulation in the inoculated leaves. In a second step, bulked segregant analysis (BSA) (23) with amplified fragment length polymorphism (AFLP) markers was applied to tag the *Am* gene. The *L. hirsutum* introgression lines (ILs) were used as a bridge to place *Am* on the tomato genetic linkage map. *Am* is located in the resistance hotspot of tomato chromosome 6.

MATERIALS AND METHODS

Plant material, virus strains, and inoculation. The AMV-resistant *L. hirsutum* f. sp. *glabratum* PI134417 (herein abbreviated PI13) accession and the AMV-susceptible *L. hirsutum* f. sp. *glabratum* PI247087 (PI24) accession were used in this study. PI13 and PI24 were crossed and F₂ and reciprocal BC₁ populations (named BC₁-R and BC₁-S) were generated. PI13 and PI24 plants used to determine the nature of *Am*-mediated resistance were grown in a growth chamber with a photoperiod of 16 h and a temperature of 24°C-day and 16°C-night. The parental lines, the F₁ hybrid, and 120 plants of the BC₁-S progeny were maintained in an insect-proof greenhouse for disease resistance evaluation, DNA extractions, and AFLP tagging experiments. Additional resistance tests were performed on the F₂ (PI24 × PI13) and BC₁-R progenies to confirm inheritance of the resistance. To determine the relationship between AMV resistance in PI13 and *L. hirsutum* f. sp. *typicum* LA1777, PI13 and LA1777 were intercrossed and F₂ and BC₁ seeds were harvested from individual F₁ plants. A total of 204 (PI13 × LA1777) F₂ plants, 169 PI13 × (PI13 × LA1777) BC₁ plants, and 159 LA1777 × (PI13 × LA1777) BC₁ plants were screened with AMV to determine whether any segregation for susceptibility occurred. To place the *Am* resistance gene on the tomato genome map, a set of 53 ILs of LA1777 in cultivated tomato *L. esculentum* cv. E6203 (E62) was used (24). ILs were maintained in an insect-proof greenhouse for seed production and DNA extractions.

AMV strains used in this study are described in Table 1. Genetic analyses were conducted using strains LYE80 (necrotic in to-

mato) and LYH1 (necrotic in tomato). The strains were propagated in *Nicotiana tabacum* L. cv. Xanthi for 15 to 20 days before inoculation to obtain high titer inoculum for tests. Inoculum and mechanical inoculation procedures were as described previously (26). *N. tabacum* cv. Xanthi and *Vigna unguiculata* L. cv. Blackeyes were used as susceptible hosts for back-inoculation experiments.

Double-antibody sandwich, enzyme-linked immunosorbent assay. Presence and absence of the viral capsid protein (CP) was assessed by the double-antibody sandwich, enzyme-linked immunosorbent assay (DAS-ELISA) (5). Antiserum against lettuce AMV strain LAS1 was supplied by H. Lot (INRA, Plant Pathology Station, Montfavet, France). Absorbance values at 405 nm were measured with a spectrophotometer (Titertek Multiscan Plus; Titertek Instruments, Huntsville, AL) after 2 h of incubation. Samples were considered positive when the absorbance was at least three times greater than the average value of the healthy controls (noninoculated plants).

Reverse transcription-polymerase chain reaction. Total RNA from whole inoculated leaves or cotyledons was isolated as described by White and Kaper (38). First-strand cDNA synthesis and polymerase chain reaction (PCR) were performed with specific primers defined on the AMV CP gene (CPAMV1, upstream primer complementary to position 1187 to 1205 of AMV-425M RNA3 [EMBL Accession No. K02703] and CPAMV2, downstream primer complementary to position 1920 to 1938 of AMV-425M RNA3) as described by Finetti-Sialer et al. (11). The entire amount of the total RNA extracted was subjected to first-strand cDNA synthesis and then amplified by PCR.

Riboprobe production and dot blot hybridization. The pCPLYH plasmid containing the CP open reading frame of AMV strain LYH1 (25; EMBL Accession No. AJ130709) was used to produce plus-strand and minus-strand riboprobes against RNA3 of AMV, thereafter named (+)RNA3 and (−)RNA3. An RNA labeling and detection kit (Boehringer Mannheim, Germany) was used to label riboprobes according to the manufacturer's instructions. For hybridization, total RNA and reverse transcription (RT)-PCR products (described previously) were denatured in 50 mM NaOH and 5 mM EDTA and blotted overnight on Hybond N⁺ nylon membrane (Boehringer Mannheim). Before blotting, PCR reactions were purified (Microcon Centrifugal Filter Units; Millipore, Bedford, MA). Membranes were air dried and the nucleic acids were cross-linked to the membrane by exposure to UV irradiation for 3 min. Membranes were hybridized overnight at 50°C in 4 ml of Dig Easy Granules buffer (Boehringer Mannheim) containing 70 ng ml^{−1} of labeled riboprobe. Membranes were washed at 65°C in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% sodium dodecyl sulfate. Detection was performed with an anti-digoxigenin antibody linked to alkaline phosphatase and the chemiluminescent phosphatase substrate CDP Star (Boehringer Mannheim). ChemiDoc System apparatus and Quantity One software (Bio-Rad Laboratories, Hercules, CA) were used to detect chemiluminescent signals.

Molecular markers analysis. Total DNA was extracted from approximately 8 to 20 mg of fresh young leaves as described by Caranta et al. (4). DNA samples from 10 BC₁-S plants resistant to AMV and 10 BC₁-S plants susceptible to AMV were pooled for BSA. The different steps used to generate AFLP markers were essentially as described by Vos et al. (37). Two hundred fifty nanograms of genomic DNA was digested with 2.5 units of the restriction enzymes *Hind*III (rare cutter) and *Mse*I (frequent cutter). The first amplification was done with a single selective nucleotide primer combination (primer *Hind*III, 5'-AGACTGCG-TACCAGCTTA-3' and primer *Mse*I, 5'-GATGAGTCCTGAGTA-AC-3') and the second one with primer combinations with three selective nucleotides (described below). Primers were provided by Keygene N.V. (Wageningen, The Netherlands). A preliminary search for polymorphism using AFLP markers was performed on the two DNA pools and parental lines PI13, PI24, E62,

TABLE 1. *Alfalfa mosaic virus* (AMV) strains used to evaluate PI13 resistance

AMV strain	Subgroup ^a	Source	Phenotype on tomato ^b
126A	I	<i>Portulaca oleracea</i>	Mosaic
195AN	I	<i>Lycopersicon esculentum</i>	Mosaic
CAA1	II	<i>Capsicum annuum</i>	Necrotic
CAA65	nd	<i>Capsicum annuum</i>	Necrotic
CAA133	nd	<i>Capsicum annuum</i>	Necrotic
CAA296	nd	<i>Capsicum annuum</i>	Necrotic
CI1637	nd	<i>Cicoria</i> sp.	Necrotic
Da	I	<i>Lycopersicon esculentum</i>	Necrotic
DAC16	II	<i>Daucus carota</i>	Necrotic
F430	I	<i>Phaseolus vulgaris</i>	Necrotic
LAO1	nd	<i>Lavandula officinalis</i>	Necrotic
LAS1	II	<i>Lactuca sativa</i>	Mosaic
LIP1	nd	<i>Lippia citrodora</i>	Necrotic
LYE1	nd	<i>Lycopersicon esculentum</i>	Necrotic
LYE80	II	<i>Lycopersicon esculentum</i>	Mosaic
LYH1	II	<i>Lycopersicon hirsutum</i>	Necrotic
SOT1	nd	<i>Solanum tuberosum</i>	Necrotic

^a According to Parrella et al. (25).

^b Symptoms observed on apical, noninoculated leaves.

and LA1777. Primer combinations generating polymorphism between the resistant and the susceptible DNA pools were subsequently mapped on the complete BC₁-S progeny and on the 53 ILs when the same polymorphism (AFLP fragments with the same molecular weight) was observed between PI13/PI24 and E62/LA1777.

The restriction fragment length polymorphism (RFLP) procedure was essentially the same as described by Saliba-Colombani et al. (29). Screening for RFLP between the PI13 and PI24 was performed with *Eco*RI, *Hind*III, and *Xba*I restriction enzymes and with probes previously mapped on the short arm of tomato chromosome 6 (CT and CD, tomato cDNA derived from mRNA from tomato epidermal tissue; and TG, tomato genomic DNA clones) (31).

Segregation analysis for molecular markers (AFLP and RFLP) and resistance data was performed with the Mapmaker/Exp version 3.0 software (21) with a minimum LOD score of 4.0 and a maximum recombination fraction of 0.3. The Kosambi's mapping function was used to convert the recombination fractions into centimorgans (cM) (20).

RESULTS AND DISCUSSION

***Am*-mediated resistance belongs to the ER type.** All PI13 plants mechanically inoculated on the cotyledons with AMV-LYE80 infectious sap 11 days after sowing were uniformly resistant without symptoms of any type, either on the inoculated organs or on apical noninoculated leaves (thereafter named apical leaves). No HR-like lesions were observed on the inoculated organs at the macroscopic level (data not shown). Moreover, the virus was not detected by DAS-ELISA from the inoculated and noninoculated tissues at 15 days postinoculation (dpi). As expected, PI24 plants inoculated at the same stage presented typical mosaic symptoms on the apical leaves and high DAS-ELISA values (at least 10-fold the value of the mock-inoculated controls). To further characterize the resistance phenotype of PI13, back-inoculation to susceptible hosts and DAS-ELISA tests were performed on inoculated leaves each day during 7 dpi. PI13 appeared unable to accumulate AMV-LYE80 in the inoculated leaves because the virus was never recovered using back-inoculation to tobacco and cowpea, nor was it detected using DAS-ELISA (DAS-ELISA values were similar to those from mock-inoculated leaves). The virus was recovered from PI24 inoculated leaves by back-inoculation at 1 dpi and by DAS-ELISA at 2 dpi.

To test whether PI13 resistance also is effective against other AMV strains, 16 AMV strains differing in their geographic origins, subgroups, and pathogenic phenotypes on tomato (Table 1) were manually inoculated to PI13 and PI24 plants (10 plants per genotype per strain). The different strains were not detected at 15 dpi by DAS-ELISA from both inoculated and apical PI13 leaves, whereas high DAS-ELISA values were detected from both PI24 inoculated and apical leaves (at least 10-fold the value of the mock-inoculated controls), showing that *Am*-mediated resistance is effective against a wide range of strains.

Finally, RT-PCR and dot blot hybridization were conducted on inoculated leaves to detect viral RNAs. Amplification of the CP region of AMV RNA3 was not observed from total RNA extracted from PI13 inoculated leaves at 1, 2, 3, and 4 dpi, whereas an amplified product was obtained from total RNA of PI24 inoculated leaves at 1 dpi (Fig. 1). To detect amplified products not visible by ethidium bromide staining, RT-PCR products were transferred to a nylon membrane and hybridized with the (+)RNA3 AMV riboprobe. In this experiment, viral RNA was detected from PI24 inoculated leaves at 1 dpi but no signal was observed from PI13 inoculated leaves at 1, 2, 3, and 4 dpi (Fig. 1). Dot blot hybridization with specific (+)RNA3 and (−)RNA3 riboprobes confirmed that no virus could be detected in inoculated leaves of PI13 challenged with AMV-LYE80 (Fig. 2).

Although a very low accumulation of viral RNA cannot be excluded due to the detection limits of the methods used, our results clearly indicate that virus accumulation is strongly inhibited in PI13 inoculated leaves. At present, direct evidence for AMV-LYE80 replication in PI13 leaves, such as the presence of (−)RNA, is lacking. Thus, the *Am* gene acts by an inhibition of viral accumulation during the early events of the virus life cycle, i.e., translation and/or replication in the inoculated cells. This, together with the fact that PI13 leaves containing *Am* remain symptomless after mechanical inoculation suggest that the *Am*-mediated resistance belongs to the ER type of virus resistance like *Rx*-mediated resistance (1) and *Rsv1*-mediated resistance against *Soybean mosaic virus* in soybean (14).

Another feature of the ER type of plant virus R-genes is that they have the potential to initiate an HR-like mechanism in graft-inoculated plants (14). This remains to be determined for the *Am* gene. The *Rx*-PVX interaction also suppresses accumulation of viruses that are taxonomically unrelated to PVX (1,19), but this feature has never been reported for other plant virus R-genes. Preliminary data of co-inoculation experiments indicate that *Am* is not able to reduce accumulation of viruses unrelated to AMV. In these experiments, CMV, TSWV, TMV, or *Potato virus Y* were co-inoculated to PI13 plants with AMV strain LYE80 (data not shown).

Resistance to AMV-LYE80 and allelism relationship between PI13 and LA1777. Among the 120 BC₁-S plants, 54 were resistant and 66 were susceptible to AMV-LYE80. The F₂ progeny segregated 76 resistant and 24 susceptible plants and no susceptible plant was observed among the 100 BC₁-R plants challenged with AMV-LYE80. A perfect correspondence was observed on the susceptible plants between the symptoms and the presence of viral CP antigen detected by DAS-ELISA at 30 dpi. On the contrary, the virus was never detected using DAS-ELISA on inoculated and apical leaves of plants without symptoms and con-

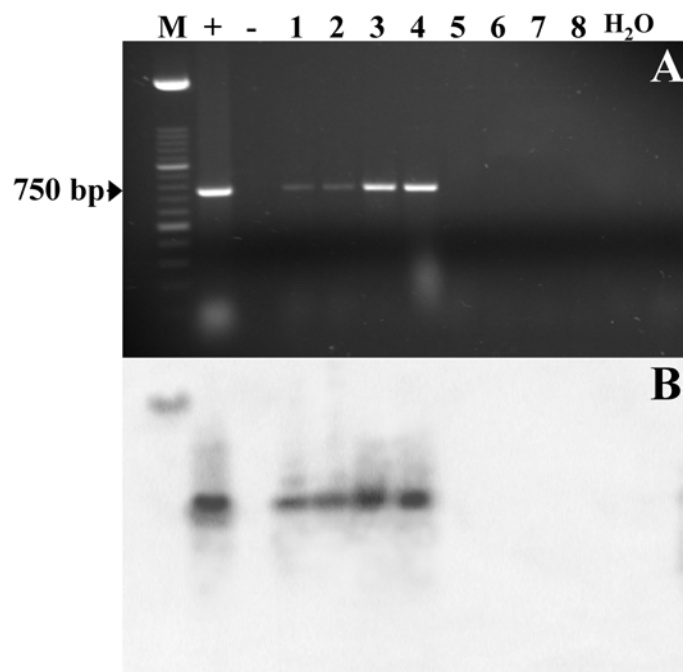


Fig. 1. A, Reverse transcription-polymerase chain reaction and **B,** Southern blot analyses of the amplified products obtained with primers CPAMV1 and CPAMV2 (*Alfalfa mosaic virus* [AMV] capsid protein [CP] gene) from total RNA extracted at intervals of 24 h during 4 days postinoculation (dpi) from inoculated leaves of PI24 (lanes 1, 2, 3, and 4, RNA extracted 1, 2, 3, and 4 dpi, respectively) and PI13 (lanes 5, 6, 7, and 8, RNA extracted 1, 2, 3, and 4 dpi, respectively). The Southern blot hybridized a specific riboprobe against (+)RNA3 of AMV-LYH1. M, molecular size marker; +, positive control (RNA extracted from AMV-infected tomato); −, negative control (RNA extracted from healthy tomato); and H₂O, RNA-free sample.

sidered resistant. Segregation frequencies confirmed that a single dominant gene is involved in the resistance to nonnecrotic AMV strain LYE80 (expected ratio of BC₁-S (1S:1R), $\chi^2 = 1.2$, $P = 27.3\%$; expected ratio F₂ (3R:1S), $\chi^2 = 0.053$, $P = 81.7\%$; and

expected ratio BC₁-R (0S:1R)). These results are in complete agreement with a previous study of inheritance of resistance to the necrotic AMV strain LYH1 (27) and confirm the efficiency of *Am*-mediated resistance against different AMV isolates.

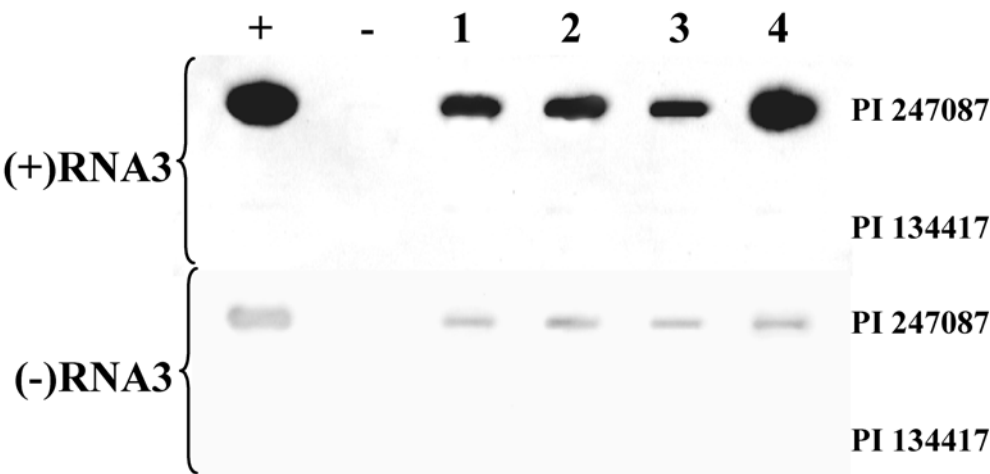


Fig. 2. Dot blot hybridization of total RNA extracted at intervals of 24 h during 4 days postinoculation (dpi) from inoculated leaves of PI247087 and PI134417 (lanes 1, 2, 3, and 4, RNA extracted 1, 2, 3, and 4 dpi, respectively). Membranes were probed with specific riboprobes against (+)RNA3 and (-)RNA3 of *Alfalfa mosaic virus* (AMV)-LYH1. +, positive control (RNA extracted from AMV-infected tomato); and -, negative control (RNA extracted from healthy tomato).

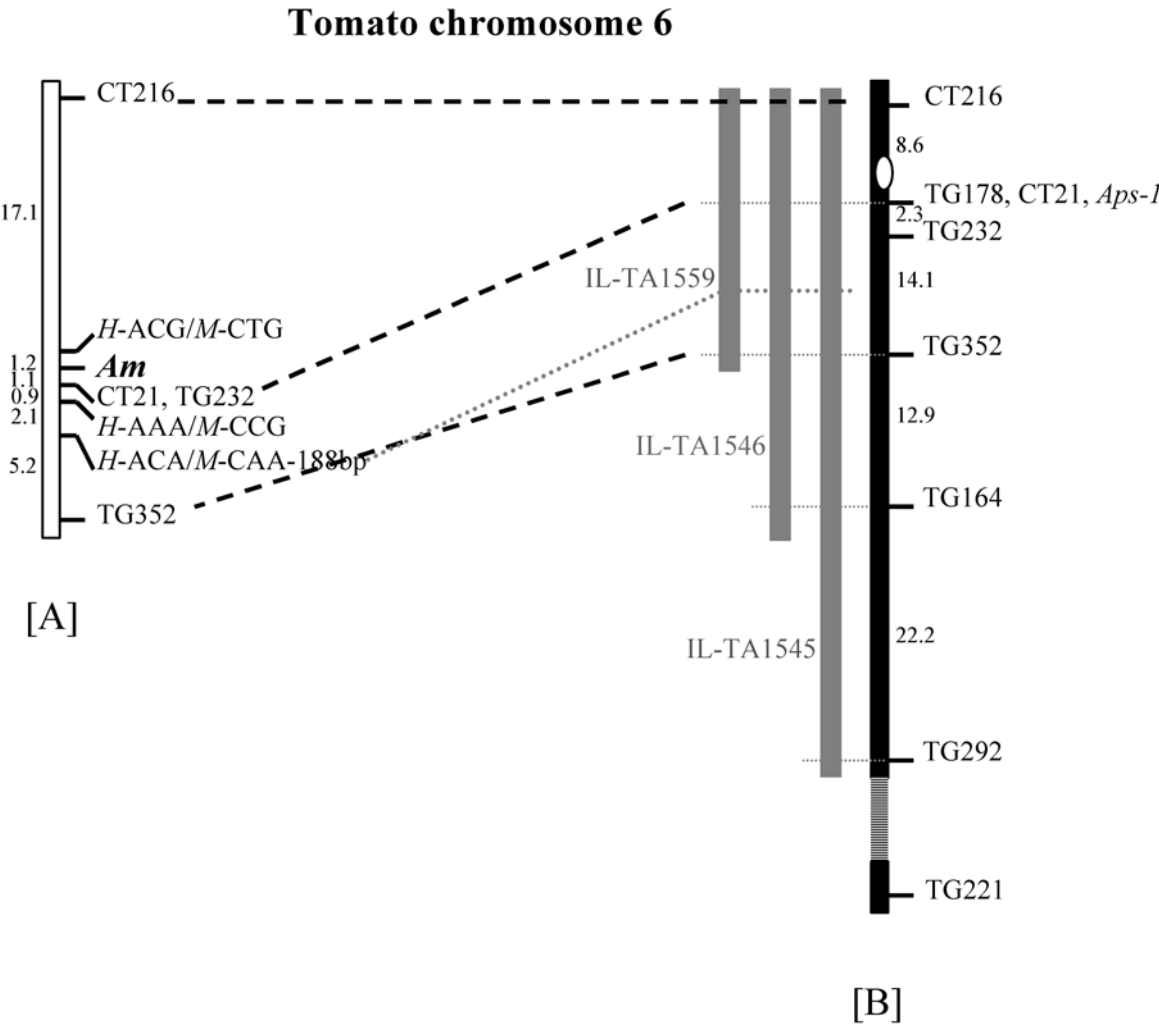


Fig. 3. Map location of the *Am* resistance gene on tomato chromosome 6. **A**, The marker order around the resistance gene *Am* in the BC₁ progeny PI24 × (PI13 × PI24). Distances in centimorgans are indicated on the left of the linkage group. **B**, The marker order on chromosome 6 (in black) as determined by Tanksley et al. (31). The centromere is indicated by a white circle on the chromosome. Introgression fragments IL-TA1559, IL-TA1546, and IL-TA1545 from chromosome 6 of *Lycopersicon hirsutum* (24) are superimposed on the map with restriction fragment length polymorphism markers used to determine the introgression fragments. Distances in centimorgans are indicated on the right of the linkage group.

Among the 204 F₂ and 328 BC₁ plants from the F₁ hybrid between PI13 and LA1777 (another resistant *L. hirsutum* accession) inoculated with AMV, no susceptible segregants were recovered. While definitive proof of allelism is not possible for dominant genes via genetic complementation analysis (i.e., complementation in the F₁ hybrid), the results obtained strongly suggest that both PI13 and LA1777 possess alleles of the same gene. Alternatively, if resistance is due to distinct genes, these loci must be tightly linked.

The *Am* resistance gene maps in tomato chromosome 6. One hundred and nine primer combinations were screened on both the resistant and susceptible DNA pools and the parental lines PI24, PI13, E62, and LA1777. Five primer combinations amplified DNA fragments in the pool only from resistant plants. This observation is in agreement with the fact that BSA on BC₁-S plants segregating for a dominant gene in combination with dominant AFLP markers is expected to detect only markers linked to the resistance allele. These five primer combinations (*Hind*III-AAA/*Mse*I-CCG, *Hind*III-AAA/*Mse*I-CTC, *Hind*III-AAC/*Mse*I-CGA, *Hind*III-ACA/*Mse*I-CAA, and *Hind*III-ACG/*Mse*I-CTG) produced five AFLP markers linked at less than 5 cM from the *Am* resistance gene. The resistance locus was flanked by AFLP markers *Hind*III-ACG/*Mse*I-CTG and *Hind*III-AAA/*Mse*I-CCG at 1.2 and 2 cM, respectively (Fig. 3).

Among the five AFLP markers linked to *Am*, only the 188-bp fragment generated by the primer combination *Hind*III-ACA/*Mse*I-CAA and localized 4.1 cM from *Am* was polymorphic between the parental lines of the *L. hirsutum* ILs, LA1777 and E62 (Fig. 4). The four other *Am*-linked markers were not amplified in the ILs parental lines. To place the *Am* gene on the tomato genetic linkage map, the 53 ILs were screened with this marker. Three *L. hirsutum* ILs (TA1545, TA1546, and TA1559), carrying overlapping fragments of chromosome 6, presented a polymorphism for the 188-bp fragment, suggesting that the *Am* gene mapped on the short arm of tomato chromosome 6 (Figs. 3 and 4). To confirm the map location of *Am*, four RFLP markers previously mapped on tomato chromosome 6 (CT216, CT21, TG232, and TG352) and polymorphic between *L. hirsutum* PI24 and PI13 were used to survey the BC₁-S progeny. RFLP analysis confirmed the preliminary association of *Am* with tomato chromosome 6. *Am* was mapped at 1.1 cM from the RFLP markers CT21 and TG232, very close to the centromeric region (Fig. 3).

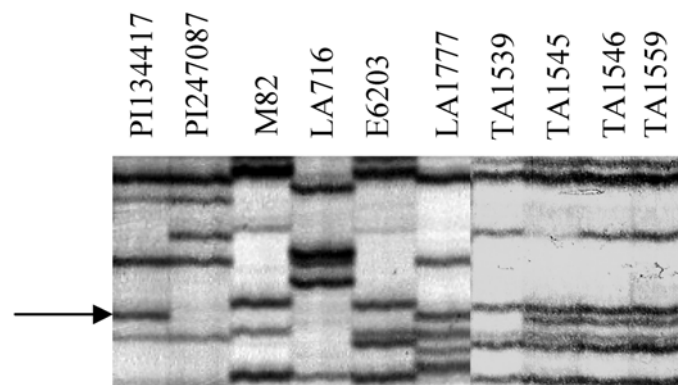


Fig. 4. Polymorphism observed for the 188-bp amplification product from primer combinations *Hind*III-ACA/*Mse*I-CAA. The size of the fragment was determined using the ϕ × 174 DNA *Hinc*II digest ladder from Pharmacia (Uppsala, Sweden). The 188-bp fragment is absent in *Lycopersicon esculentum* E6203, *L. hirsutum* PI247087, and the parental lines (M82 and LA716) of the *L. pennellii* introgression lines (ILs) (9). The 188-bp fragment is present in resistant *L. hirsutum* LA1777 and PI134417. Among the four ILs with overlapping segments of tomato chromosome 6 (TA1539, TA1545, TA1546, and TA1559), ILs TA1545, TA1546, and TA1559 have the 188-bp fragment, indicating that it maps on this chromosome segment.

The efficiency of ILs representing the entire wild genome species for both gene mapping and gene discovery (i.e., exploitation of traits from wild species that are not obvious in the parents) has been described by Eshed and Zamir (9,10). Because the *Am* gene is present in the LA1777 accession (as indicated by the allelism test), the more efficient approach would have been to deduce its location from the direct screening of the set of ILs for resistance to AMV. However, none of the 53 ILs were resistant to AMV (data not shown). This can be explained by the partial coverage of the LA1777 genome and the few number of markers used to define the introgression segments (Fig. 3). Another possibility is that the single haploid *L. hirsutum* LA1777 genome captured in the ILs does not include *Am* (*L. hirsutum* LA1777 is an outcrosser). However, the ILs were useful to map one of the AFLP markers linked to *Am* and consequently to place it on a tomato chromosome. The transfer of AFLP marker from one *L. hirsutum* accession to another was feasible because the same AFLP fragment localized 4.1 cM from *Am* was amplified from both LA1777 and PI13. Further confirmation of the map localization of *Am* using RFLP markers indicated that this AFLP fragment probably corresponds to the same locus in both *L. hirsutum* accessions. The parental lines of the *L. pennellii* ILs (9) also were screened with the five AFLP markers linked to *Am* but AFLP fragments were never amplified in the parental lines of *L. pennellii* ILs (Fig. 4). These data illustrated the difficulty of transferring such markers from one species to another.

Interestingly, *Am* was located in the resistance hotspot of tomato chromosome 6 containing several genes for qualitative and quantitative resistance to other tomato pathogens: *Mi* for resistance to nematodes (40), *Cf-2* and *Cf-5* for resistance to *Cladosporium fulvum* (16) and the major-effect quantitative trait loci *Ol-1* for resistance to *Oidium lycopersicon* (33), *Ty-1* for resistance to *Tomato yellow leaf curl virus* (41), and *Bw-5* for resistance to *Ralstonia solanacearum* (32). Among those, *Mi*, *Cf-2*, and *Cf-5* were isolated by positional cloning and shared sequence similarities. *Mi* is a member of the plant resistance protein family characterized by the presence of NBS and LRR domains (40), and all *Cf* genes encode membrane-anchored glycoproteins that also contain many LRRs (17). The clustering of genes involved in resistance to related and unrelated pathogens is now a well-documented phenomenon (15), including species of *Solanaceae* (13). Genomic clustering of resistance genes is observed either at complex loci consisting of linked resistance gene homologues (30,40) or at larger genomic regions where numerous resistance genes may span a few to 20 cM (13). This organization can be related to their evolutionary processes (15,22). Some of these genes may have evolved from a common ancestor by local gene duplications and subsequent functional diversification. The fact that relatively small changes in resistance gene sequence can lead to resistance against entirely different pathogen species supports this idea (35). Taken together, these data can lead to the hypothesis that occurrence of *Am* in this cluster reflects shared sequence similarities (NBS, LRR, or a combination of both domains) with the dominant R-genes belonging to the resistance hotspot on tomato chromosome 6.

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