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J. G. van der Beek · G. Pet · P. Lindhout

# Resistance to powdery mildew (*Oidium lycopersicum*) in *Lycopersicon hirsutum* is controlled by an incompletely-dominant gene *Ol-1* on chromosome 6

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**Abstract** The inheritance of resistance to powdery mildew (*Oidium lycopersicum*) in *Lycopersicon hirsutum* was investigated by disease tests in segregating populations obtained by hybridising tomato (*L. esculentum*) cv Moneymaker with the wild relative *L. hirsutum* G1.1560. One incompletely-dominant gene *Ol-1* was found to largely control resistance to the disease. To map *Ol-1*, DNA pools from seven resistant and ten susceptible $F_2$ plants were analyzed for random amplified polymorphic DNA (RAPD). With 32 primers tested, one RAPD, primed with the sequence 5′-GACGTGGTGA-3′, was observed between the susceptible and the resistant bulks, which cosegregated with resistance in the $F_2$ population of *L. esculentum* × *L. hirsutum* G1.1560. This RAPD was mapped on chromosome 6 by using an $F_2$ (*L. esculentum* × *L. pennellii*) already mapped for 49 RFLPs. RFLP analysis of the $F_2$ from *L. esculentum* cv Moneymaker × *L. hirsutum* G1.1560 demonstrated that *Ol-1* maps near the *Aps-1* region on chromosome 6, in the vicinity of the resistance genes to *Meloidogyne spp.* (*Mi*) and to *Cladosporium fulvum* (*Cf-2/Cf-5*).

**Key words** *Lycopersicon hirsutum* · Resistance gene mapping · *Oidium lycopersicum* · Gene clustering Bulked segregant analysis · Powdery mildew · Tomato RAPD · *Ol-1*

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J. G. van der Beek[1] · G. Pet · P. Lindhout (✉)[1,2]
Department of Vegetable and Fruit Crops,
Centre for Plant Breeding and Reproduction Research (CPRO-DLO),
P.O. Box 16, NL-6700 AA Wageningen,
The Netherlands

*Present addresses:*
[1] Institute for Plant Protection Research (IPO-DLO), P.O. Box 9060, NL-6700 GW Wageningen, The Netherlands
[2] Department of Plant Breeding, Wageningen Agricultural University, P.O. Box 386, NL-6700 AJ Wageningen, The Netherlands

## Introduction

The cultivated tomato (*Lycopersicon esculentum*) is highly susceptible to *Oidium lycopersicum*, a fungus belonging to the Erysiphales (powdery mildews), which has occurred in Western Europe since 1986. Although chemical control is effective against this pathogen, breeding resistant varieties is a more appropriate solution for environmentally-safe production. Searching the genus *Lycopersicon* for the occurrence of resistance genes, Lindhout et al. (1993) reported four accessions of *L. hirsutum*, one of *L. parviflorum*, and one of *L. peruvianum*, to exhibit high levels of resistance to *O. lycopersicum*. These accessions are particularly useful as sources of resistance as they are cross compatible with *L. esculentum* (Stevens and Rick 1986; Lindhout and Purimahua 1988). The first part of this paper is aimed at studying the inheritance of the resistance to *O. lycopersicum* originating from *L. hirsutum* G1.1560. By evaluation of $F_2$ and $BC_1$ progenies, obtained by hybridising the susceptible *L. esculentum* cv Moneymaker with the resistant *L. hirsutum* G1.1560, for segregation of resistance to *O. lycopersicum*, it was shown that one incompletely-dominant resistance gene (*Ol-1*) largely controls the resistance to *O. lycopersicum*.

When the map position of a gene is known, its expression and function can be studied in more detail in relation to the genetic background or to environmental conditions. In addition, for breeding purposes neighbouring molecular markers are useful for indirect selection. Restriction fragment length polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD) markers have proven to be powerful tools in mapping studies (Tanksley et al. 1989; (insert:) Welsh and McClelland, 1990; Williams et al. 1990; Gebhardt and Salamini, 1992; Van der Beek et al. 1992). Detailed RFLP linkage maps have been generated, based predominantly on segregating $F_2$ populations (Tanksley et al. 1992). Genes have been mapped by using RFLP and RAPD markers and genotypes which differ only for a target gene(s), for example, near-isogenic lines (NILs; Young et al. 1988; Van der Beek et al. 1992), cultivars

(Young et al. 1988; Van der Beek et al. 1992) and recombinant inbred lines (Burr et al. 1988; Knapp 1991), or by analysing DNA polymorphisms between bulked samples of segregating populations (bulked segregant analysis, BSA; Michelmore et al. 1991). By using different approaches, resistance genes to *Alternaria alternata* (Witsenboer et al. 1989), to *Cladosporium fulvum* (Van der Beek et al. 1992; Jones et al. 1993), to *Fusarium oxysporum* (Sarfatti et al. 1991), to *Stemphylium* (Behare et al. 1991), to *Pseudomonas syringae* (Martin et al. 1991), and to tobacco mosaic virus (Young and Tanksley 1989), have been mapped on the tomato genome by using molecular markers.

The second part of the present study is aimed at mapping the gene in *L. hirsutum* for resistance to *O. lycopersicum* in three steps. Firstly, the identification of RAPD markers that are linked to resistance to *O. lycopersicum* by applying BSA on DNA pools of susceptible and resistant plants from an $F_2$ (*L. esculentum* × *L. hirsutum*) segregating for resistance. Secondly, the mapping of such linked RAPD markers, by using an existing RFLP linkage map from an $F_2$ of *L. esculentum* × *L. pennellii* (Odinot et al. 1992). Thirdly, verification of the map position of the *O. lycopersicum* resistance gene by RFLP analysis of individual plants of the $F_2$ (*L. esculentum* × *L. hirsutum*) with RFLP markers neighbouring the linked RAPD marker. This approach combines the ease and speed of finding a linked RAPD marker with the linkage information of an RFLP map.

## Materials and methods

### Plant and fungal material

The highly-resistant accession *L. hirsutum* G1.1560 (Lindhout et al. 1993) and cv Moneymaker were maintained at the Centre for Genetic Resources (CGN) belonging to CPRO-DLO, Wageningen, The Netherlands. The $F_2$ and the two backcross ($BC_1$) populations to both parents were generated from a limited number of $F_1$ (*L. esculentum* cv Moneymaker × *L. hirsutum* G1.1560) plants. $F_3$ lines were produced by self-pollinating resistant $F_2$ plants.

DNA of the cultivar Motelle and the NILs 83M-R and N118 (Ho et al. 1992) was kindly supplied by Dr. P. Zabel, Agricultural University, Wageningen, The Netherlands.

The stock of *O. lycopersicum*, which originated from diseased tomato plants at CPRO-DLO, was maintained on cv Moneymaker in a heated greenhouse. The inoculum was prepared from freshly-sporulating leaves of heavily-diseased tomato plants (Lindhout et al. 1993).

### Experimental conditions

Two disease tests were conducted during 1989 and 1990. For the first test, seeds of the two parents, the $F_1$, $F_2$ and the two $BC_1$ populations were sown on October 11, 1989. The plants were potted 16 days after sowing and transplanted to a temperature-controlled greenhouse. The temperature was set at 22/17 °C, day/night, and after October 31 at 19/16 °C, day/night, and additional light was supplied by SON lamps. The experimental setup was according to a completely randomized block design, with 17 blocks and per block one plant of each parent, two $F_1$ plants, seven $F_2$ plants and three plants of each $BC_1$ population. The plants were inoculated 28 days after sowing, by spraying with a suspension of $3 \times 10^4$ conidia $ml^{-1}$ (Lindhout et al. 1993). During the first 5 days after inoculation, the plants were kept under a plastic cover to maintain a relative humidity of 100%. After removal of the cover, the relative humidity fluctuated between 30% and 70%.

For the second test, seeds of the two parents and the seven $F_3$ lines were sown on September 28, 1990. The experimental setup was a completely randomized block design with nine blocks, each block consisting of 10–30 plants per $F_3$ line. Experimental conditions and the inoculation procedure were as described above.

### Disease evaluation

The disease symptoms were evaluated three times, at 12, 19 and 30 days after inoculation. The evaluation was performed per plant according to the following index: 0; healthy (no visible symptoms); 1; one or two powdery mildew spots visible on inoculated leaves; 2; three to five powdery mildew spots; 3; more than five powdery mildew spots, but less than 10% foliar area affected; 4; between 10 and 50% foliar area affected; and 5; more than 50% foliar leaf area affected.

### Bulked segregant analysis

Total DNA was extracted from frozen young leaves according to the method described by Van der Beek et al. (1992). DNA of seven healthy (resistant) and ten very diseased (susceptible) $F_2$ plants was extracted, pooled, and analyzed for RAPD according to the bulked segregant analysis as proposed by Michelmore et al. (1991). The seven $F_2$ plants for the resistant bulk were selected on the basis of the nearly complete absence of symptoms in their respective $F_3$ progenies. Polymerase chain reactions (PCRs) were performed in 40 µl mixtures according to Klein Lankhorst et al. (1991a) with some minor modifications. Amplification was carried out in a DNA Thermal Cycler (Perkin Elmer Cetrus) which was programmed for 45 cycles of 1 min at 94 °C, 1 min at 34 °C and 2 min at 72 °C. Twenty microliters of the reaction mixture were loaded on 2% agarose gels containing ethidium bromide. Amplified DNA fragments were separated by electrophoresis and visualised by UV light.

### RFLP analysis

Plant DNA (5 µg) was digested with the restriction enzymes *Eco*RI and *Xba*I (Bethesda Research Laboratories). RFLP analysis was performed as described by Van der Beek et al. (1992). The TG markers used in this study were provided by S. D. Tanksley, Cornell University, New York, USA, and GP79 was kindly supplied by C. Gebhardt, Max Planck Institut für Züchtungsforschung, Köln, Germany.

### Linkage studies

Map distances between RAPD and RFLP markers and *Ol-1* were estimated using the linkage program JoinMap as described by Stam (1993), based on the classification of $F_2$ plants as resistant or susceptible. To circumvent misinterpretation of the disease evaluation data, the rank sum test of Kruskal-Wallis was applied on every marker separately. The frequency distribution of the disease index for each genotype class of a marker was calculated and the mean rank per genotype class was determined (Van Ooijen et al. 1993). The Kruskal-Wallis test statistic (K*) gives the probability that differences between the mean ranks of the genotype classes of a marker are significant. A significant difference indicates an association between the marker and disease resistance (Van Ooijen et al. 1993).

## Results

### Inheritance of resistance to *O. lycopersicum*

The inheritance of resistance to *O. lycopersicum* was studied by disease tests of segregating progenies of *L. esculentum* cv Moneymaker × *L. hirsutum* G1.1560. Most plants could be classified unambiguously based on the

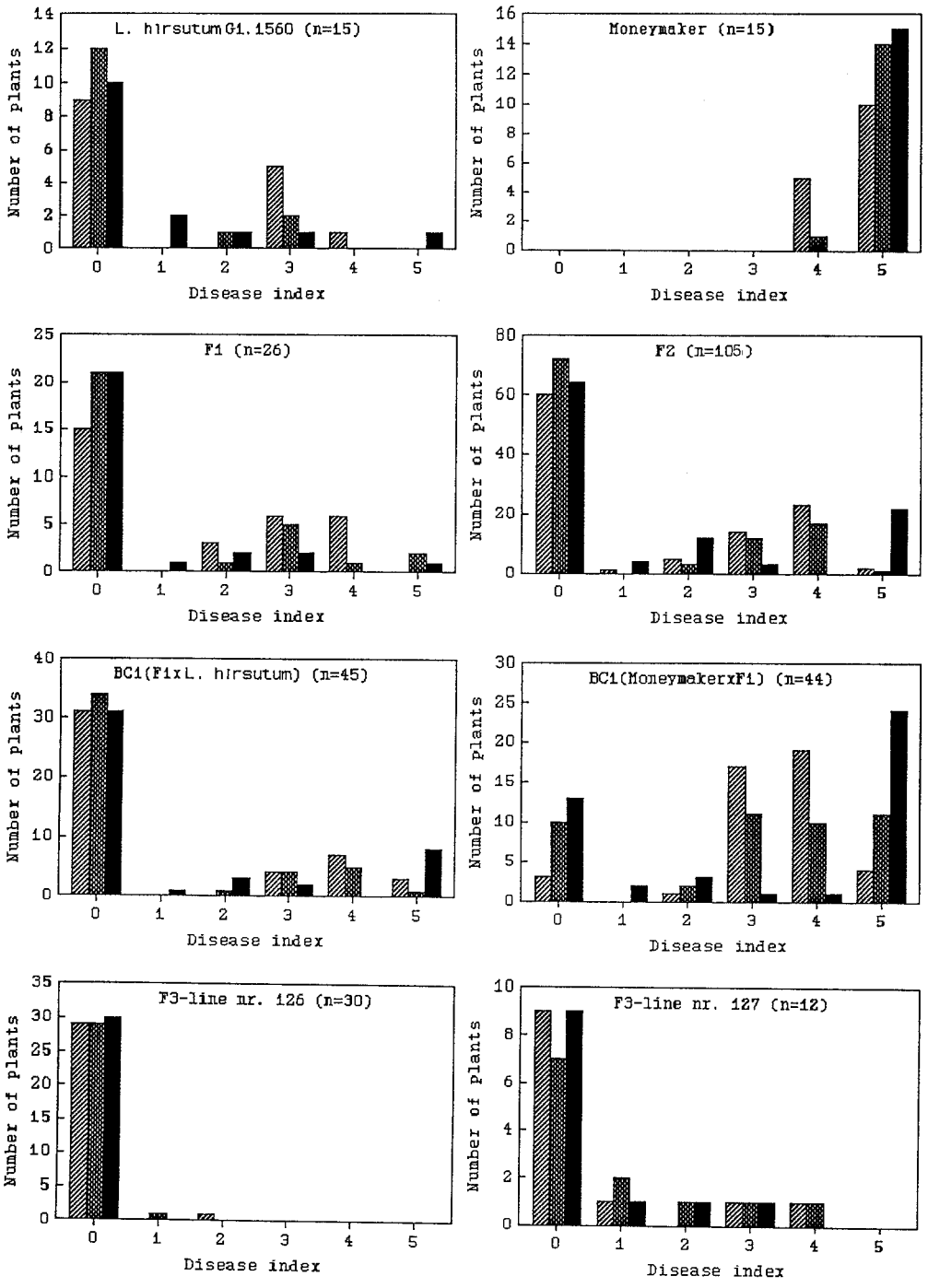



**Fig. 1** Frequency distribution of symptoms for *O. lycopersicum* infection of *L. hirsutum* G1.1560, *L. esculentum* cv Moneymaker, and their progenies, as indicated in the panels. The *number* of plants per population is shown between brackets. The disease index was defined as: *0*, healthy (no visible symptoms); *1*, one or two powdery mildew spots visible on inoculated leaves; *2*, three to five powdery mildew spots; *3*, more than five powdery mildew spots, but less than 10% foliar area affected; *4*, between 10 and 50% foliar area affected; and *5* , more than 50% foliar leaf area affected

clear presence or absence of symptoms, but for some plants the disease evaluations were ambiguous. Consequently, all plants were evaluated for the relative severity of symptom expression on a 0 to 5 scale. The majority of the $F_1$ plants showed no symptoms (Fig. 1), which is in accordance with a dominantly-inherited resistance. However, a small percentage of both the resistant parent and the $F_1$ plants showed some symptoms. Therefore, genotypes were only considered as resistant if they were free from symptoms for at least one of the three evaluation dates. Alternatively, plants were considered as susceptible when they showed symptoms at each evaluation. The severity of the symptoms of plants considered susceptible was usually higher than 3. Under this assumption, the $F_2$ segregated 80 resistant and 25 susceptible plants, which is in accordance with one dominantly-inherited resistance gene. Though other more complex hypotheses could not be excluded, the most simple "one dominant gene hypothesis" was largely confirmed by the segregation ratios in the backcross populations (Fig. 1): the $BC_1$ ($F_1 \times L.$ *hirsutum* G1.1560) was nearly as resistant as *L. hirsutum* and the $F_1$ (*L. esculentum* cv Moneymaker $\times$ *L. hirsutum* G1.1560), and the $BC_1$ (*L.*

*esculentum* cv Moneymaker × $F_1$) showed some over representation of susceptible plants (16 resistant versus 28 susceptible plants).

Most of the susceptible plants of the $F_2$ population were less diseased than the susceptible parent cv Moneymaker. This suggests that, apart from a major resistance gene, one or more additional genes with minor effects influence symptom expression. Seven $F_2$ plants without any symptoms were propagated to $F_3$ lines and these lines were evaluated for disease resistance. These $F_3$ lines showed fewer susceptible plants than the resistant parent (Fig. 1), indicating they were probably derived from homozygous resistant $F_2$ plants.

The symbol *Ol-1* is proposed to designate the incompletely-dominant gene from *L. hirsutum* G1.1560 for resistance to *O. lycopersicum*.

## Mapping the resistance gene *Ol-1*

Though the segregation data favoured the one gene hypothesis, other genetic models could not be excluded. To circumvent promiscuous conclusions about the genetics of resistance, research was carried out to map the proposed gene *Ol-1*. For mapping purposes sufficient DNA polymorphisms should occur between the susceptible and resistant parents. Using the restriction enzymes *Eco*RI, *Xba*I and *Taq*I, 78 of the 94 probes tested showed an RFLP between cv Moneymaker and *L. hirsutum* f. *glabratum* with at least one enzyme (data not shown), indicating a fair chance to also find polymorphisms between *L. esculentum* and *L. hirsutum* G1.1560.

To identify a RAPD marker linked to *Ol-1*, DNA bulks were prepared from seven resistant plants of the $F_2$ of *L. esculentum* × *L. hirsutum*, from which the $F_3$ progenies were completely resistant, and from ten of the most susceptible $F_2$ plants. Thirty-two primers were used for RAPD analyses of these two DNA bulks. With the primer 5′-GACGTGGTGA-3′ a DNA fragment of 0.7 kb, designated RAPD17, was amplified in the susceptible pool but was absent in the resistant pool (Fig. 2). By amplifying DNA of the individual $F_2$ plants from the bulks with this primer, all ten susceptible plants amplified RAPD17 while five of the seven resistant plants did not amplify RAPD17. Based on these cosegregation data it was concluded that RAPD17 was most likely associated with the susceptibility allele cv Moneymaker and, consequently, with the locus *Ol-1*.

For mapping RAPD17 on the genome of tomato an $F_2$ population of *L. esculentum* cv Allround × *L. pennellii* LA716 was used. As RAPD17 was amplified in cv Allround, but not in *L. pennellii,* the segregation of RAPD17 could be evaluated in this $F_2$ population. The segregation of RAPD17 was compared with the segregation of 51 RFLP markers on 75 $F_2$ plants (Odinot et al. 1992). The most-closely-linked marker of RAPD17 was TG178, which is close to the *Aps-1* locus on chromosome 6 (Messeguer et al. 1991). Consequently, the map position of RAPD17 is near the *Aps-1* locus.

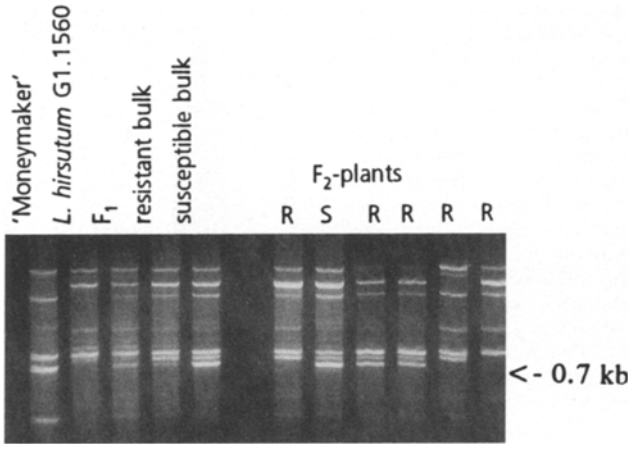



**Fig. 2** RAPD patterns of genotypes with reference to the resistance or susceptibility to *O. lycopersicum*. DNA of the genotypes indicated above the lanes was amplified by a PCR assay primed with 5′-GACGTGGTGA-3′. The $F_2$ plants are classified as *R*, resistant or *S*, susceptible. The absence of the 0.7-kb band, indicated with the *arrow* and designated RAPD17, is associated with resistance. Only 6 of the 46 $F_2$ plants are shown. The 0.7-kb band is present in two of the five resistant plants, indicating either heterozygosity of the *Ol-1* locus or a recombination between *Ol-1* and RAPD17

To obtain an independent verification of the mapping of RAPD17, the cultivars Motelle and Moneymaker and the near-isogenic lines N118 and 83M-R, carrying small introgressed chromosome segments in the *Mi/Aps-1* region on chromosome 6 (Ho et al. 1992), were analyzed for the RAPD17 locus. RAPD17 was amplified in cvs Motelle and Moneymaker but not in the near-isogenic lines 83M-R and N118, demonstrating close linkage of RAPD17 with *Aps-1* (Ho et al. 1993; see Fig. 5).

To confirm the mapping of the *Ol-1* gene on chromosome 6, 61 plants of the $F_2$ of *L. esculentum* cv Moneymaker × *L. hirsutum* G1.1560 were analyzed for segregation of *Ol-1* and the RFLP markers GP79, TG178 and TG153 (Table 1, Fig. 3). Only five recombinants were observed between *Ol-1* and GP79, four between *Ol-1* and TG178 and three between *Ol-1* and TG153, confirming the close linkage of *Ol-1* with these three RFLP loci. All seven resistant $F_2$ plants of the resistant bulk, which were probably homozygous for *Ol-1*, were homozygous for the *L. hirsutum* alleles of TG153, indicating no recombination between *Ol-1* and TG153 in these seven genotypes. This confirms the location of *Ol-1* close to TG153.

This mapping was based on the interpretation of the disease evaluation data in terms of resistance or susceptibility. As stated above, some $F_2$ plants could not be classified unambiguously. Misclassifications will decrease the accuracy of the estimation of genetic distances. To avoid misinterpretation, the original disease evaluation data were analyzed using the Kruskal-Wallis test. This is a nonparametric equivalent of the analysis of variance and is applicable when a nonlinear scale is used, as in the present study (Van Ooijen et al. 1993). This test also showed a very significant linkage ($P < 0.001$) of *Ol-1* with GP79, TG178 and TG153, irrespective of the moment of evaluation (Fig. 4).

**Table 1** Frequency table for 61 plants of the $F_2$ (*L. esculentum* cv Moneymaker × *L. hirsutum* G1.1560), showing segregation of the resistance gene *Ol-1* and four molecular markers. The symbols refer to: *h*, *L. hirsutum* allele; *e*, *L. esculentum* allele, *Ol-1/.*, homozygous dominant or heterozygous for the *Ol-1* gene; *ol-1/ol-1*, homozygous recessive for the *Ol-1* gene; mv=missing values. The recombinants are underlined

| Genotype | GP79[a] | | | | RAPD17[b] | | | TG178[c] | | | | TG153[d] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *h/h* | *h/e* | *e/e* | mv | *h/h* | *e/.* | mv | *h/h* | *h/e* | *e/e* | mv | *h/h* | *h/e* | *e/e* | mv |
| *ol-1/.* | 17 | 30 | 2 | 1 | 19 | 25 | 6 | 18 | 28 | 2 | 2 | 19 | 19 | 1 | 10 |
| *ol-1/ol-1* | 0 | 3 | 7 | 1 | 0 | 11 | 0 | 0 | 2 | 9 | 0 | 1 | 1 | 8 | 1 |

[a] GP79: *h*, 6.5- and 1.8-kb *Eco*RI fragments and 25-kb *Xba*I fragment; *e*, 4.3-kb *Eco*RI fragment and 13-kb *Xba*I fragment
[b] RAPD17: *h*, no DNA fragment; *e*, 0.7-kb fragment, primed by 5′-GACGTGGTGA-3′
[c] TG178: *h*, 1.6-kb *Eco*RI-fragment; *e*, 2.4-kb *Eco*RI fragment
[d] TG153: *h*, 1.0-kb *Xba*I-fragment; *e*, 2.0-kb *Xba*I fragment

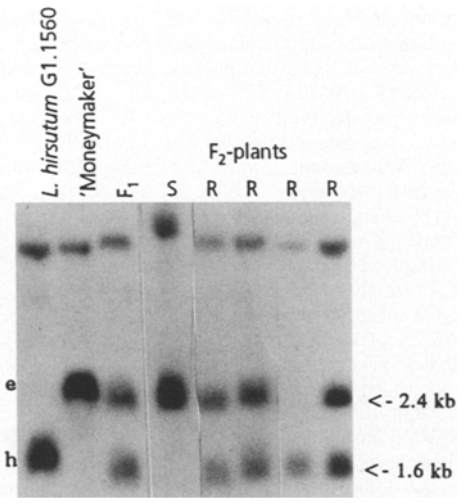
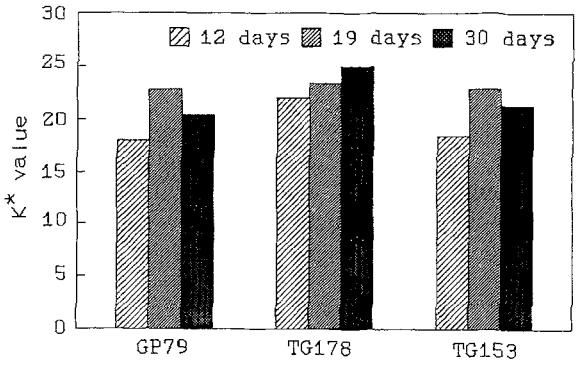



**Fig. 3** Linkage analysis between TG178 and *Ol-1*, the resistance gene to *O. lycopersicum*. Probe TG178 was hybridized onto blots of *Eco*RI-digested DNA from individual plants of the segregating $F_2$ *L. eculentum* cv Moneymaker × *L. hirsutum* G1.1560. The $F_2$ plants are classified as *R*, resistant or *S*, susceptible. The upper band of 2.4 kb is the *L. esculentum* TG178 allele (*e*) and the lower 1.6-kb band is the *L. hirsutum* allele (*h*). Only 5 of the 61 $F_2$ plants are shown

**Fig. 4** Association of three linked RFLP markers on chromosome 6 with disease resistance to *O. lycopersicum*. The Y-axis indicates the Kruskal-Wallis statistic K*, calculated from the mean ranks of the disease evaluations per marker genotype class of 61 plants of an $F_2$ population *L. esculentum* cv Moneymaker × *L. hirsutum* G1.1560




## Discussion

From the segregation data of progenies from the susceptible *L. esculentum* crossed with the resistant *L. hirsutum* preliminary evidence was obtained about the inheritance of resistance to the powdery mildew *O. lycopersicum*, originating from *L. hirsutum* G1.1560. Most of the variation in the $F_2$ could be explained by one major dominant gene, designated *Ol-1*. However, the presence of one or more additional genes with minor effects should be taken into account as the most susceptible $F_2$ and $F_3$ plants were less diseased than the susceptible parent cv Moneymaker (Fig. 1). The effects of these minor genes may be particularly manifest in heterozygous *Ol-1/ol-1* plants. The presence, and possible interaction, of major and minor resistance genes precluded a detailed genetic analysis. However, the mapping of resistance genes provides a powerful tool for the characterization of the effect of individual genes on resistance.

The *Ol-1* gene, particularly when homozygous, will confer a high level of resistance to *O. lycopersicum* and will protect a tomato crop from infection by the fungus. Major gene resistance has been found frequently in plant-pathogen interactions in tomato and the inheritance of resistance to powdery mildew caused by *O. lycopersicum* is a further example.

Despite the ambiguities in the interpretation of the disease evaluation data, the gene mapping approach employed proved to be very efficient. Only 32 primers were required to obtain at least one RAPD that was tightly linked to the *Ol-1* locus. By comparison, Giovanonni et al. (1991), Martin et al. (1991) and Michelmore et al. (1991) found two RAPDs with 200 primers tested, three RAPDs with 100 primers tested and three RAPDs with 144 primers tested, respectively. It has been estimated that 100 primers would yield a marker within an expected distance of 1.9 cM from the target gene (Martin et al. 1991). The number of only 32 tested primers needed to find a RAPD linked to *Ol-1* is in line with this estimate.

RAPD17 was in repulsion phase with the *Ol-1* gene, making the chance to detect recombinants between both genes rather low. Consequently, the estimation of the genetic distance between both genes was inaccurate. For

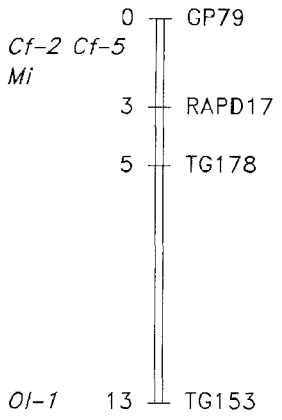



**Fig. 5** Integrated linkage map of chromosome 6 in the region of four disease resistance genes. The genetic distances are calculated from the data of the present study by using JoinMap. The position of the disease resistance genes is from Ho et al. 1993, Dickinson et al. 1993 and from the present study

more reliable mapping the codominant RFLP markers were more useful. The major advantage of the mapping of RAPD17 was the selection of linked RFLP markers. This approach was very efficient as the RAPD analysis quickly identified that part of the genome where *Ol-1* was probably located and only three RFLP markers needed to be screened to confirm the mapping of *Ol-1*.

TG153 is probably the most-closely-linked marker to the *Ol-1* locus. This marker is also linked to *Mi*, *Cf-2* and *Cf-5*, resistance genes to *Meloidogyne spp.* and *Cladosporium fulvum* respectively (Fig. 5; Klein-Lankhorst et al. 1991b; Ho et al. 1991; Dickinson et al. 1993). For plant breeding purposes selection for these disease resistance genes could profitably be employed by selecting indirectly with adjacent molecular markers. This would speed up breeding programmes, as one type of molecular analysis could replace several disease tests. Also, indirect selection with molecular markers is very helpful in elucidating rarely-occurring recombinants between resistance genes, so facilitating the combination of these closely-linked resistance genes into cultivars.

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