Euphytica (2006) 149: 309–316
DOI: 10.1007/s10681-005-9079-y


# Inheritance of resistance against *Phytophthora infestans* in *Lycopersicon pimpenellifolium* L3707

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Received 28 June 2005; accepted 14 December 2005

*Key words:* genetics, late blight, tomato, *Solanaceae*

**Summary**

*Lycopersicon pimpenellifolium* L3707, resistant to the late blight oomycete *Phytophthora infestans* was crossed with the susceptible *Lycopersicon pimpenellifolium* 14377 or the susceptible *Lycopersicon esculentum* ZH. Progeny F1 and F2 generations were scored at the 5-leaf stage for resistance against 175 field and recombinant isolates of the pathogen. F1 plants exhibited various levels of moderate resistance and F2 plants segregated 3:6:7 resistant/moderately resistant/susceptible. The data support the hypothesis that race-non-specific resistance in L3707 is controlled by two independent genes: a partially-dominant gene and a dominant epistatic gene.

## Introduction

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is a most devastating disease of tomato and potato world-wide. This oomycete pathogen attacks leaves, stems, fruits and seeds of tomato (Rubin et al., 2001; Rubin & Cohen, 2004a). Disease may be initiated by air borne sporangia or by oospores harboring the soil or the seeds (Rubin & Cohen, 2004a). Within-season spread occurs via sporangia. Aggressive genotypes to tomato appeared due to the recombination of the A1 and A2 mating types of *P. infestans* in nature (Gavino et al., 2000).

In Israel, the late blight resistant tomato varieties carrying the genes *Ph*-1 (e.g. New Yorker) or *Ph*-2 (e.g. Pieraline, Macline, Piline) for resistance provide inadequate resistance against the local population of the pathogen (Cohen, 2002) and farmers rely on frequent fungicide application for disease control. However, resistance of the pathogen to fungicides (e.g. metalaxyl) often hampers disease control (Gisi & Cohen, 1996).

A new gene for resistance *Ph*-3 was reported by AVRDC (Asian Vegetable Research and Development Center) in accession L3708 of *Lycopersicon pimpenellifolium* (Jusl.) Mill. (Black et al., 1996a; Black et al., 1996b)

Whereas *Ph*-1 and *Ph*-2 were mapped to chromosomes 7 and chromosome 10, respectively (Moreau et al., 1998, and literature cited therein), *Ph*-3 was mapped to chromosome 9 (Chunwongse et al., 2002). The gene *Rpi-moc1* for late blight resistance in *Solanum mochiquense* resides on chromosome 9 linked to the marker TG328 in the neighborhood of *Ph*-3 (Smilde et al., 2005).

All three genes condition race specific resistance against *P. infestans* in tomato: *Ph*-1 is a single dominant allele effective against race T0; *Ph*-2 is a partially dominant allele highly effective against race T0 and partially effective against race T1; and, *Ph*-3 is a single partially dominant allele effective against isolate Pi-16 from Taiwan that overcomes *Ph*-1 and *Ph*-2 (Chunwongse et al., 2002). Later studies showed that L3708 became infected in two locations in Taiwan. Indeed, four isolates out of 59 Taiwanese field isolates of *P. infestans* were virulent on L3708 (Chunwongse et al., 2002).

Kim and Mutschler (2003) incorporated the resistance to late blight from L3708 into their tomato lines. They revealed that the resistance in their bred fixed

lines is controlled by more than one gene. One of these genes was missing from the AVRDC- breeding lines carrying the resistance from L3708.

Studies conducted in North Carolina by R. Gardner (personal communication) showed that the local population of *P. infestans* has overcome all three resistance genes against late blight under field conditions.

L3707 is another accession of *L. pimpenellifolium* from AVRDC resistant to late blight (Black et al., 1996a; Black et al., 1996b). Data supplied by Agrogene Ltd in Israel (Ben-Daniel et al., 2004) suggested that L3707 performed resistant under late blight epiphytotic conditions in nature.

The purpose of this study was to produce inbred lines of L3707 with race-non-specific resistance against *P. infestans* and to elucidate the mode of inheritance of this resistance in crosses between the selected resistant inbred lines and susceptible lines of *L. pimpenellifolium* or *L. esculentum*. Preliminary results were published earlier (Ben-Daniel et al., 2004; Irzhansky & Cohen, 2004).

## Materials and methods

### *Plant material*

*Lycopersicon pimpenellifolium* (Jusl.) Mill. accessions L3707 (PI365951, Peru) was obtained in 1996 from L.L. Black, AVRDC, Taiwan. Plants were grown under late blight epiphytotic conditions in two locations in Israel by Agrogene Ltd. Resistant individuals were self-pollinated for 10 generations (two generations/year) and five progeny inbred lines were used.

*Lycopersicon pimpenellifolium* (Jusl.) Mill. accessions 14277, 14322, 14377, 14341, and 14345 were obtained in 2000 from F. Nuez, Universidad Politecnica de Valencia, Spain. Screening for resistance to late blight in intact plants in growth chambers revealed that all five accessions were susceptible to late blight.

*Lycopersicon esculentum* Mill. breeding line ZH, highly susceptible to late blight (Cohen, 2002), is from own breeding collection.

Plants were grown in the glasshouse (22–34 °C) in 128-cell trays, 1 plant/cell, filled with a mixture of peat and perlite (1:1. v/v) and fertilized once a week with 0.5% solution of N:P:K (20:20:20). When plants developed 5 fully expanded leaves they were taken for inoculation.

Resistant plants were transplanted to the field and grown in shade houses covered with 50 mesh insect-proof white plastic nets.

Plants were self-pollinated or crossed with susceptible partners (see below) and progeny offspring plants were grown as above before inoculated.

### *Crosses*

Inbred lines of the resistant *Lycopersicon pimpenellifolium* L3707 were crossed with either the susceptible *Lycopersicon pimpenellifolium* 14377 or the susceptible *Lycopersicon esculentum* breeding line ZH. The F1 and F2 progeny plants were inoculated with mixed isolates of *P. infestans* in order to elucidate the mode of inheritance of race-non-specific resistance against the blight.

### *Pathogen isolates*

A total of 175 isolates of *Phytophthora infestans* (Mont.) de Bary were used for inoculations. Three isolates were obtained from Switzerland, two from Japan, two from Turkey, four from the USA (belonging to genotypes US-1, US-7 and US-8) and 164 isolates were from Israel. Of the Israeli isolates, 74 were collected from potato and tomato fields during the period 1983–2004 (Cohen, 2002) and 90 isolates were F1 hybrids created in our laboratory by crossing either one of four A1 isolates with one A2 field isolate (Rubin & Cohen, 2004b).

Isolates were each inoculated onto detached leaflets of the standard 11 Black potato differentials (Malcolmson & Black, 1966) as well as tomato lines carrying the genes *Ph*-1 and *Ph*-2. Inoculated leaves were incubated at 20 °C (12 h light/day) for 7 days. Sporulation of *P. infestans* was assessed and the race structure (virulence profile) of each isolate was determined. An isolate was considered virulent to a cultivar if sporulation was apparent. When no symptom was seen or HR (hypersensitive response) developed, the isolate was classified as avirulent.

Isolates were maintained by repeated inoculations of detached tomato leaflets of inbred ZH in growth chambers at 13–14 °C (12 h light/day).

### *Inoculation*

Two types of assays were performed in order to evaluate the level of resistance against late blight. The first was done with intact 5-leaf plants growing in 128-cell trays and the second, with detached leaved taken from shade-house-grown plants. Both types of assays were done with sporangial suspension of mixed-isolates.

Sporangia were collected from ZH infected leaflets into cold distilled water, mixed together, adjusted to about 5000 sporangia/ml and sprayed onto intact plants or the lower leaf surfaces of detached leaflets (3–5 leaflets/genotype) placed on wet filter paper in plastic trays. Inoculated plants or leaves were kept in the dark at 18 °C, 100% relative humidity, for 20 h to ensure infection and thereafter transferred to 20 °C growth chambers for 7 days to allow for symptoms development. Detached leaves were constantly maintained under moist conditions in sealed transparent plastic trays so that the intensity of sporulation of the pathogen could be quantified.

*Disease rating*

In intact plants the proportion of leaf and stem area showing blight symptoms was visually estimated by using a 0–4 rating index as follows:

0 = no visible symptoms apparent.
0.1 = a few minute lesions (about 1 mm in diameter) on one or two bottom leaves.
0.2, 0.3, 0.4 = increasing number of minute lesions on the 2 bottom leaves.
0.5 = about 10% of the total leaf area is blighted. Symptoms usually confined to the 2 bottom leaves. No symptoms on the stem.
1 = about 25% of the total leaf area is blighted. No symptoms on the stem.
2 = about 50% of the total leaf area, and about 10–25% of the stem are blighted.
3 = about 75% of the total leaf area, and about 30–50% of the stem are blighted.
4 = leaves and stem are fully blighted (dead plants).

In detached leaves, the leaf area occupied by blight lesions was visually estimated by using 0–4 rating index, similar to the above rating. In some assays individual leaflets were placed in 10 ml of 50% ethanol and the number of sporangia produced was counted with the aid of a haemocytometer.

## Results

*Virulence profile of isolates*

Inoculations of detached leaves with each of the 175 isolates showed that the isolates used in this study could break through all *Solanum demissum* resistances present in the standard 11 Black potato differentials

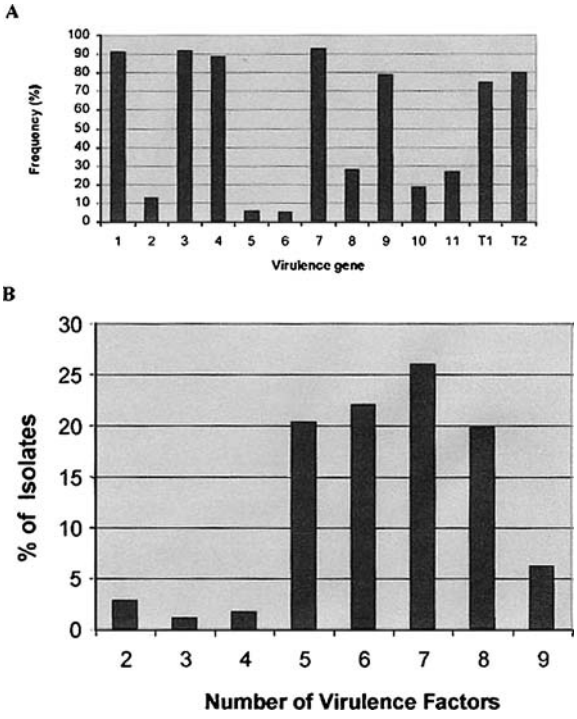


*Figure 1.* Virulence race structure of *Phytophthora infestans* isolates used for inoculations of tomato plants in this study. A total of 175 isolates were used, of which 90 isolates were F1 hybrids. A. The frequency of virulence factors in isolates population. Virulence factors 1–11 can overcome the resistances conferred by potato R genes 1–11; T1 and T2 can overcome tomato resistances conferred by the genes *Ph*-1 and *Ph*-2. B. Percentage of isolates that carry 2 to 9 virulence factors.

(Malcolmson & Black, 1966) as well as the resistances performed by the tomato genes *Ph*-1 and *Ph*-2 (Figure 1A and B).Virulence factors 1,3,4,7,9,T1 and T2 were most frequent whereas 5 and 6 were least frequent (Figure 1A). Most isolates were complex races. About 21, 22, 26 and 20% of the isolates carried 5, 6, 7 and 8 virulence factors, respectively and about 6% carried 9 virulence factors Figure 1B).

*Evaluation of race-non-specific resistance in* Lycopersicon pimpenellifolium

Whole plants (5-leaf stage) were inoculated in growth chambers with a mixture of all Israeli field isolates.

All five inbred lines of L3707 were highly resistant to late blight, producing a mean DI (Disease Index rating) of 0.5 or less whereas accessions 14341, 14345, 14322, 14277, and 14377 were fully susceptible, scored DI = 4.

*Table 1*. Late blight symptoms and sporangial production of *Phytophthora infestans* in detached leaves of resistant and susceptible tomato inbred lines as affected by inoculum density of mixed isolates of *Phytophthora infestans*

| | Inoculum density, sporangia/ml $\times 10^3$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2.5 | | 5 | | 7.5 | | 10 | |
| Tomato line | DI[a] | SI[b] | DI | SI | DI | SI | DI | SI |
| L3707[c] | $0.05 \pm 0.1$ | 0 | $0.2 \pm 0.17$ | 0 | $0.6 \pm 0.2$ | 0 | $0.9 \pm 0.3$ | 0 |
| 14377/5 | 4 | $56 \pm 6$ | 4 | $113 \pm 4$ | 4 | $134 \pm 6$ | 4 | $153 \pm 4$ |
| ZH | 4 | $54 \pm 4$ | 4 | $103 \pm 6$ | 4 | $130 \pm 4$ | 4 | $151 \pm 5$ |

[a]DI = Mean disease index rating (0–4 scale).
[b]SI = Mean number of sporangia $\times 10^3$ per leaflet.
[c]Tests were performed with inbred lines of L3707/1, L3707/4, L3707/5 with 3 leaflets/line.

Table 1 presents mean data for detached leaflets inoculated with a mixture of all field isolates: L3707 was remarkably resistant even at increasing inoculum densities whereas 14377/5 and ZH were highly susceptible. No sporulation was detected in L3707 leaves whereas in the susceptible lines it ranged between 54-153 $\times$ $10^3$ sporangia/leaflet, depending on the inoculum density.

Microscopical examinations of leaf discs inoculated with isolate 367 (see 7) showed profuse sporulation in ZH with no apparent response of the host tissue as against necrotic minute lesions in L3707 with no sporulation. The mean number of sporangia/leaf disc produced in L3707 was 0, whereas in ZH it reached a mean of 11.6 $\times$ $10^3$ sporangia/leaf disc, similar to that in the susceptible 14377/5.

To assess the compatibility of individual *P. infestans* isolates with L3707, detached leaflets of the five resistant inbred lines of L3707 were drop-inoculated (four 10 $\mu$l droplets/leaflet, about 20 sporangia/droplet) with each of the 175 isolates of *P. infestans*. Leaves of ZH served as susceptible controls. At 7 days post inoculation DI was evaluated and the number of sporangia produced on a leaflet was determined with the aid of a haemocytometer.

Mean DI produced by all individual field isolates in L3707 lines ranged between 0.37 $\pm$ 0.56 and 0.58 $\pm$ 0.79, with no significant differences between the lines, whereas the mean DI produced by all hybrid isolates ranged between 0.01 $\pm$ 0.05 and 0.06 $\pm$ 0.02, with no significant differences between the lines. All isolates produced DI of 4 in ZH. No sporangia were detected in leaves of L3707 regardless of the line or isolate inoculated whereas in ZH a mean of 170 $\pm$ 81 $\times$ $10^3$ sporangia/leaflet were counted for the field isolates and a mean of 136 $\pm$ 78 $\times$ $10^3$ sporangia/leaflets for the hybrid isolates. Overall, the hybrid isolates were less aggressive to L3707 and less compatible with ZH than the field isolates. The results indicate on a total inhibition of sporulation of *P. infestans* in late blight lesions produced in L3707.

### *Inheritance of resistance in* L. pimpenellifolium *L3707* $\times$ L. pimpenellifolium *14377*

Four inbred lines of the resistant L3707 were each crossed with the susceptible 14377. L3707 served as the male parent in three crosses whereas 14377 served as a male parent in one cross. Offspring progeny plants at the F1, F2 generations were inoculated at the 5-leaf stage with a mixture of 175 isolates of *P. infestans*. Disease rating was done 7 days post inoculation. Results are presented in Table 2. All 4 inbred lines of L3707 were resistant showing DI of 0–0.9 (Table 2), whereas 14377 was susceptible with DI of 3–4. All F1 plants (except one) and the reciprocal E1 showed various levels of moderate resistance to late blight with DI ranging between 1 and 2.4 (Table 2). The moderate level of resistance in F1 and E1 plants indicate that resistance in L3707 is partially dominant.

Four F2/E2 populations (out of 5 populations) segregated into resistant/moderately resistant/susceptible at a ratio of 3:6:7 (Table 2), supporting the hypothesis that two genes, one partially dominant and one epistatic dominant, confer the resistance against late blight in L3707.

Detached leaf assays were performed with another set of F1, E1 and parents. Leaves were taken from middle stem of adult plants growing in a shade- house in the field. Six days after spray-inoculation with a mixture of 175 isolates DI was estimated and the number

*Table 2.* Inheritance of race-non-specific resistance against *Phytophthora infestans* in crosses between the resistant *Lycopersicon pimpinellifolium* L3707 and the susceptible *Lycopersicon pimpinellifolium* 14377/5

| | | Number of plants | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Disease rating index | | | | | | | | Total | | | Expected ratio[b] | | | |
| Pedigree | Generation | 0–0.4 | 0.5–0.9 | 1–1.4 | 1.5–1.9 | 2–2.4 | 2.5–2.9 | 3–4 | R[a] | MR | S | R | MR | S | $\chi^2_{(2)}$ | *P* |
| 14377/5 | $P_1$ | – | – | – | – | – | – | 11 | 0 | 0 | 11 | – | – | – | – | – |
| L3707/1 | $P_2$ | 3 | 13 | – | – | – | – | – | 16 | 0 | 0 | – | – | – | – | – |
| $P_1 \times P_2$ | $F_1$ | – | 1 | 3 | 3 | 2 | – | – | 1 | 8 | 0 | – | – | – | – | – |
| $P_1 \times P_2$ | $F_2$ | 6 | 14 | 4 | 10 | 5 | 6 | 32 | 20 | 25 | 32 | 3 | 6 | 7 | 2.756 | 0.252 |
| 14377/5 | $P_1$ | – | – | – | – | – | – | 12 | 0 | 0 | 12 | – | – | – | – | – |
| L3707/2 | $P_2$ | 5 | 8 | – | – | – | – | – | 13 | 0 | 0 | – | – | – | – | – |
| $P_1 \times P_2$ | $F_1$ | – | – | 2 | 5 | 7 | 1 | – | 0 | 15 | 0 | – | – | – | – | – |
| $P_1 \times P_2$ | $F_2$ | 12 | 8 | 8 | 8 | 11 | 3 | 26 | 20 | 30 | 26 | 3 | 6 | 7 | 4.612 | 0.100 |
| 14377/5 | $P_1$ | – | – | – | – | – | – | 13 | 0 | 0 | 13 | – | – | – | – | – |
| L3707/4 | $P_2$ | – | 15 | – | – | – | – | – | 15 | 0 | 0 | – | – | – | – | – |
| $P_2 \times P_1$ | $E_1$ | – | – | 3 | – | – | – | – | 0 | 3 | 0 | – | – | – | – | – |
| $P_1 \times P_2$ | $F_2$ | 3 | 11 | 8 | 3 | 15 | 1 | 22 | 14 | 27 | 22 | 3 | 6 | 7 | 2.008 | 0.366 |
| $P_2 \times P_1$ | $E_2$ | 11 | 5 | 9 | 1 | 5 | 1 | 23 | 16 | 16 | 23 | 3 | 6 | 7 | 4.227 | 0.121 |
| 14377/5 | $P_1$ | – | – | – | – | – | – | 11 | 0 | 0 | 11 | – | – | – | – | – |
| L3707/8 | $P_2$ | 2 | 11 | – | – | – | – | – | 13 | 0 | 0 | – | – | – | – | – |
| $P_2 \times P_1$ | $E_2$ | 8 | 15 | 12 | 3 | 10 | 0 | 28 | 23 | 25 | 28 | 3 | 6 | 7 | 6.632 | 0.036* |

[a]R = resistant (DI = 0–0.9), MR = moderately – resistant (DI = 1–2.9) and S = susceptible (DI = 3–4).
[b]Based on a model of one partially dominant gene and one epistatic dominant gene. * = model not accepted.

of sporangia produced was counted. Leaflets of 14377 were fully blighted whereas those of L3707 showed no symptoms. Leaflets of F1 and E1 showed necrotic lesions covering about 30–40% of their area. Mean numbers of sporangia/leaflet (in thousands) in 14377, F1, E1, and L3707 were $90 \pm 7$, $0.30 \pm 0.20$, $0.22 \pm 0.20$ and 0, respectively. These results show that inheritance of resistance against lesion production is partially dominant whereas resistance against sporangial production is (almost) dominant.

Kim and Mutschler (2003) reported that detached leaves of homozygous L3708 inbred lines were resistant to all 5 strains of *P. infestans* they have used whereas the response of the heterozygous F1 hybrids varied amongst the strains, being susceptible to US-7.

Our data showed that F1 and E1 progenies of 14377 × L3707 failed to support any sporulation of also two isolates of US-7 (one from Ithaca, NY and the other from Raleigh, NC) in detached leaves. Leaflets of the susceptible parents 14377/2 and 14377/5 allowed a mean production of 119 and 127 $\times 10^3$ sporangia/leaflets, respectively, whereas no sporangia were produced in leaflets of the resistant parents L3707/1, L3707/2, L3707/5 and L3707/8. These results suggest that resistance in L3707 might be controlled differently from that in L3708.

*Inheritance of resistance in* L. pimpenellifolium *L3707* × L. esculentum *ZH*

Two inbred lines of the resistant L3707 (male parent) were crossed with the susceptible inbred line ZH and the F1 and F2 progeny plants were inoculated at the 5-leaf stage with a mixture of 175 isolates of *P. infestans*. Data presented in Table 3 show that F1 plants were moderately resistant to late blight indicating on a partial dominance of the resistance trait.

Both F2 populations segregated 3:6:7 resistant/moderately resistant/susceptible, supporting the hypothesis that L3707 carries one partially dominant gene for resistance and one dominant gene epistatic to the first.

Detached leaf assays were made with the parents and F1 as described above. ZH showed a mean DI = 4 with a mean of $63 \pm 4 \times 10^3$ sporangia/leaflet, whereas L3707 had a mean DI = $0.05 \pm 0.05$ with no sporangia produced. The F1 exhibited a mean DI = $2.3 \pm 0.6$ with $0.4 \pm 0.4 \times 10^3$ sporangia/leaflet, suggesting again that resistance in the heterozygote L3707 performs in an almost dominant manner against reproduction of the pathogen.

Crosses were also made between *L. esculentum* cv. Pieraline which carries the *Ph*-2 resistance gene

*Table 3.* Inheritance of race-non-specific resistance against *Phytophthora infestans* in crosses between the resistant *Lycopersicon pimpinellifolium* L3707 and the susceptible *Lycopersicon esculentum* inbred ZH

| | | Number of plants | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Disease rating index | | | | | | | Total | | | Expected ratio[b] | | | | |
| Pedigree | Generation | 0–0.4 | 0.5–0.9 | 1–1.4 | 1.5–1.9 | 2–2.4 | 2.5–2.9 | 3–4 | R[a] | MR | S | R | MR | S | $\chi^2_{(2)}$ | *P* |
| ZH | $P_1$ | – | – | – | – | – | – | 17 | 0 | 0 | 17 | – | – | – | – | – |
| L3707/1 | $P_2$ | 22 | 3 | – | – | – | – | – | 25 | 0 | 0 | – | – | – | – | – |
| $P_1 \times P_2$ | $F_1$ | – | – | 5 | 7 | 5 | 5 | – | 0 | 22 | 0 | – | – | – | – | – |
| $P_1 \times P_2$ | $F_2$ | 28 | 41 | 36 | 18 | 33 | 25 | 126 | 69 | 112 | 126 | 3 | 6 | 7 | 2.873 | 0.238 |
| ZH | $P_1$ | – | – | – | – | – | – | 17 | 0 | 0 | 17 | – | – | – | – | – |
| L3707/5 | $P_2$ | 19 | 13 | – | – | – | – | – | 32 | 0 | 0 | – | – | – | – | – |
| $P_1 \times P_2$ | $F_1$ | – | – | 2 | 4 | 1 | 10 | – | 0 | 17 | 0 | – | – | – | – | – |
| $P_1 \times P_2$ | $F_2$ | 20 | 31 | 15 | 17 | 30 | 19 | 111 | 51 | 81 | 111 | 3 | 6 | 7 | 1.983 | 0.371 |

[a]R = resistant (DI = 0–0.9), MR = moderately – resistant (DI = 1–2.9) and S = susceptible (DI = 3–4).
[b]Based on a model of one partially dominant gene and one epistatic dominant gene.

(Moreau et al., 1998) and L3707/2. About 44% of the F2 plant population (253 plants) were susceptible. The proportion of susceptible plants (7/16) suggests that *Ph*-2 is not allelic with the resistance loci of L3707.

*Resistance of fruit*

Mature green fruits were picked from shade -house-grown plants, placed in moist Petri dishes and spray inoculated with a mixture of 175 isolates of *P. infestans*. Results presented in Table 4 show that all fruits of 14337 were susceptible whereas those of L3707, F1 and F2 (taken from plants resistant in their foliage) segregated resistant/susceptible, indicating that different genes control resistance against late blight in the foliage and the fruits. L3707 should be homozygote for fruit race-non-specific resistance before the mode of inheritance of this trait in fruits could be elucidated.

*Table 4.* Inheritance of race-non-specific resistance against *Phytophthora infestans* in fruits of *Lycopersicon pimpinellifolium*

| | | Number of fruits | | |
|---|---|---|---|---|
| Pedigree/generation | Mean fruit weight, g | Total | Blighted | Healthy |
| 14377[a] | $2.9 \pm 036$[c] | 40 | 40 | 0 |
| L3707[b] | $0.7 \pm 0.09$[a] | 93 | 49 | 44 |
| $F_1$ | $1.56 \pm 0.33$[b] | 28 | 18 | 10 |
| $F_2$[c] | $1.52 \pm 0.3$[b] | 134 | 54 | 70 |

[a]Tweny fruits of each inbred line 14377/2 and 14377/5.
[b]About 18 fruits of each of the five inbred lines.
[c]Plants resistant in their foliage (bulked from 3 crosses).
Fruit weights followed by different letters are significantly different at $p < 0.05$ (Fisher test).

## Discussion

*Phytophthora infestans*, the oomycete causal agent of late blight in potato and tomato is a highly polymorphic organism. Genetic variation has intensified in recent years mainly due the sexual reproduction of this pathogen via mating of A1 and A2 isolates (Cohen, 2002; Gavino et al., 2000; Rubin & Cohen, 2004a; Rubin & Cohen, 2004b). Some recombinant isolates might be more aggressive than their ancestor isolates (Gavino et al, 2000) thus rendering host resistance genes and chemical control (Gisi & Cohen, 1996) inefficient. Searching for durable resistance in tomato against late blight is therefore an important need for the tomato industry.

Three genes for resistance were identified in tomato, *Ph*-1, *Ph*-2 and *Ph*-3. All of them originated from the wild tomato *Lycopersicon pimpinellifolium*. In some growing areas in the world (Chunwongse et al., 2002; Cohen, 2002; Kim & Mutschler, 2003a) these genes currently provide no protection against the local populations of the pathogen.

In this study we provide evidence that L3707, another accession of *L. pimpinellifolium* from Peru, is highly resistant against late blight.

We were interested in selecting a durable, race-non-specific resistance in L3707. Such durability is normally tested under field conditions in various locations overtime. Nevertheless, durable resistance might

also be achieved in one location at a relatively short period of time by inoculating the host plants with a number of pathogen isolates carrying all known virulence factors in simple or complex race structures. The genetic diversity of the pathogen can be further increased by using sexually-produced recombinant isolates, so as to mimic future diversification of the pathogen that might occur in nature.

Our results with detached leaves inoculated with 175 individual isolates showed that L3707 was highly resistant to all 85 field isolates and all 90 F1 hybrid isolates of *P. infestans*. New Yorker carrying the *Ph*-1 gene for resistance was susceptible to 90% of these isolates and Pieraline carrying the *Ph*-2 gene- to 80% of them.

Whole-plant inoculations made with a mixture of these 175 isolates enabled the selection of 5 highly resistant L3707 inbred lines. This series of isolates carried all virulence genes necessary for attacking the potato differential lines R1-R11 as well as the tomato genes *Ph*-1 and *Ph*-2.

Analyses of the response to disease in the F1, E1, F2, and E2 plant populations from the crosses between susceptible *L. esculentum* and L3707, or the susceptible *L. pimpinellifolium* and L3707 indicated that resistance is not maternally-inherited and support the hypothesis that L3707 carries two genes for race-non-specific resistance: a partially-dominant gene R, and an independent, dominant gene, E, epistatic to R, which is necessary for expression of R. The genotypes RREE (as might occur in the parent L3707) and RREe are fully resistant, whereas RrEE and RrEe (as in the F1) are moderately resistant. All other genotypes are susceptible (Table 5). As expected, our F1/E1 plants were moderately resistant whereas 5 out of 6 F2/E2 populations segregated 3:6:7 resistant/moderately resistant/susceptible.

Genes for race-non-specific resistance against late blight were reported in potato and tomato. Naess et al. (2000) and Song et al. (2003) demonstrated that *Solanum bulbocastanum* carrying the RB gene is highly resistant to all known races of *P. infestans* and exhibited durable effective resistance in the field (Staples, 2004). van der Vossen et al. (2003) mapped this gene to chromosome 8 and identified a cluster of four resistance gene analogues in this locus. One of these candidate genes provided resistance when transformed to tomato. Smilde et al. (2005) showed that non-specific resistance in *Solanum mochiquese* is located on chromosome 9 linked to the TG328 marker. This marker is in close proximity to the QTL *Ph*-3 of L3708 (Chunwongse et al., 2002). Brouwer et al. (2004) and Brouwer and Clair (Brouwer & St Clair, 2004) identified resistance QTL's on all 12 chromosomes in back crosses of tomato with the wild tomato *Lycopersicon hirsutum*.

*Table 5.* A genetic model for the inheritance of race-non-specific resistance against *Phytophthora infestans* in *Lycopersicon pimpenellifolium* L3707 based on one partially dominant gene R and one dominant gene E epistatic to R

| Genotype | Proportion | Phenotype |
|---|---|---|
| RR EE | 1/16 | Resistant |
| RR Ee | 2/16 | Resistant |
| RR ee | 1/16 | Susceptible |
| Rr EE | 2/16 | Moderately resistant |
| Rr Ee | 4/16 | Moderately resistant |
| Rr ee | 2/16 | Susceptible |
| rr EE | 1/16 | Susceptible |
| rr Ee | 2/16 | Susceptible |
| rr ee | 1/16 | Susceptible |

Segregation ratio in $F_2$ R:MR:S = 3:6:7.

The two genes we have identified in L3707 seemed to be non-allelic with *Ph*-2 of Pieraline (Moreau et al., 1998) because 7/16 of the F2 plant population of the cross Pieraline × L3707 were susceptible.

We do not know if R and/or E of L3707 is/are allelic with *Ph*-3 of L3708 because no crosses were made between these two lines. These two accessions are probably closely related as they have both originated from Peru as L3707 = PI 365951 and L3708 = PI 365957 (L.L. Black, AVRDC, personal communication).

Chunwongse et al. (2002) demonstrated (based on 72, F2 plants) that the AVRDC L3708 carries a single, partially dominant gene for resistance. Black et al. (1996a) indicated earlier on single genes acting additively. Kim and Mutschler (2003) of Cornell tested several lines of L3708 from AVRDC and found that they were fixed for their level of resistance, rather than segregation. Resistance of their own-bred lines derived from the AVRDC L3708 was controlled by more than one gene, at least one of which is missing from the AVRDC lines. Frary el al. (1998) had indication that L3708 contains additional genes for resistance to late blight. They tested the resistance of an F2 population from susceptible tomato × L3708 to California isolates of *P. infestans* under field conditions and found three QTLs associated with this resistance, all located in chromosome 6.

Marker-assisted molecular mapping of the resistance genes of L3707 is required in order to elucidate their relationships with other resistance genes in tomato and potato.

Interestingly, our inbred lines of L3707 segregated in resistance to fruit blight. Segregation was observed in also F2 plants expressing high resistance in their foliage. This suggests that different or additional loci control resistance against late blight in the fruits. Similar phenomenon is known in potato in which cultivars resistant to late blight in the foliage showed susceptibility in tubers (Kadish et al., 1990).

## Acknowledgements

We thank Dr. L.L. Black for providing seeds of L3707; Profs. U. Gisi, W, E. Fry, and J. Ristaino for providing isolates of *P. infestans*; L. Petrov, L. Sheck, and B.H. Ben-Daniel of Agrogene Ltd for performing the screening for disease resistance in shade-houses and providing F10 breeding lines of L3707; E. Brinder of our laboratory for growing the plants; Dr. A. Baider, and D. Gotleib of our laboratory for maintaining the isolates of the pathogen; Dr. E. Rubin for providing with hybrid isolates; and, Dr. Alex Beharav of the Institute of Evolution, University of Haifa for elaborating the genetic model of inheritance.

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