Resistance Response of the Ma genes from ‘Myrobalan’ Plum to Meloidogyne hapla and M. mayaguensis

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Abstract. In ‘Myrobalan’ plum (Prunus cerasifera Ehr.), Ma1 and Ma2 are single major dominant genes that control the resistance to the predominant root-knot nematode (RKN) species Meloidogyne arenaria (Neal) Chitwood, M. incognita (Kofold & White) Chitwood, and M. javanica (Treub). These genes were evaluated for activity to the northern RKN M. hapla Chitwood and the tropical RKN M. mayaguensis Rammah & Hirschmann, neither of which is controlled by the Mi gene from tomato. This study was conducted under greenhouse conditions using a resistance screening based on high and durable inoculum pressure by the nematodes. Tests were conducted simultaneously for: greenhouse conditions using a resistance screening based on high and durable inoculum (heterozygous for either reference for the M. arenaria inoculum pressure, no effect of the parental clones and progenies were completely resistant to indicating that the significant economic damage to javanica subgenus used (Kester and Grasselly, 1987). Among the environment.

Root-knot nematodes (RKN) cause significant economic damage to Prunus crops in many countries (Kochba and Spiegel-Roy, 1976; Pincot et al., 1989; Scotto La Massèse et al., 1984). The three most widely distributed species in the Mediterranean region are Meloidogyne arenaria, M. incognita, and M. javanica. Almond P. amygdalus Batsch] and peach [P. persica (L.) Batsch] on susceptible rootstocks can be heavily damaged by Meloidogyne sp. in Mediterranean areas (Kester and Grasselly, 1987; Lambert, 1979; Nyczepir, 1991; Pincot et al., 1990). Resistant rootstocks are the best alternative to chemical control by using preplant fumigation, which is costly, short-lived, and may pollute the environment.

Diverse sources of resistance have been used (Kester and Grasselly, 1987). Among the subgenus Amygdalus (including the peach and almond species), the peach ‘Nemarguad’ and the related ‘Nemared’ rootstocks express a near-complete resistance spectrum (Eszeniaud et al., 1997). Their resistance was overcome by one population detected in Florida, a new species thus designated M. sp. Florida (Eszeniaud et al., 1997; Rubio-Cabetas et al., 1999). Another source of RKN resistance is ‘Myrobalan’ plum, P. cerasifera (Scotto La Massèse et al., 1990), which belongs to the subgenus Prunophora (plum and apricot species), and no RKN population has yet overcome this resistance. Resistance to major RKN species in ‘Myrobalan’ plum is controlled by single dominant genes, termed the Ma genes. These genes confer a high-level and wide-spectrum resistance to the major RKN species M. arenaria, M. incognita, and M. javanica in the related ‘Nemared’ rootstocks express a near-complete resistance spectrum (Eszeniaud et al., 1997). Their resistance was overcome by one population detected in Florida, a new species thus designated M. sp. Florida (Eszeniaud et al., 1997; Rubio-Cabetas et al., 1999). Another source of RKN resistance is ‘Myrobalan’ plum, P. cerasifera (Scotto La Massèse et al., 1990), which belongs to the subgenus Prunophora (plum and apricot species), and no RKN population has yet overcome this resistance. Resistance to major RKN species in ‘Myrobalan’ plum is controlled by single dominant genes, termed the Ma genes. These genes confer a high-level and wide-spectrum resistance to the major RKN species M. arenaria, M. incognita, and M. javanica and to the undescribed population M. sp. Florida (Eszeniaud et al., 1996; Lecouls et al., 1997). Consequently, they differ from the putative genes involved in the resistance of the Amygdalus subgenus and are of particular interest in breeding programs for new Prunus rootstocks. Marker-assisted selection is in progress and one sequence characterized amplified region (SCAR) marker has recently been described (Lecouls et al., 1999).

Nevertheless, no study has been conducted on the response of these genes to the northern RKN, M. hapla, and to the tropical RKN, M. mayaguensis (Rammah and Hirschmann, 1988), both of which reproduce on tomatoes (Lycoptersicon esculentum Mill.) carrying the Mi gene (Fargette et al., 1996; Hadisoeganda and Sasser, 1982). The species M. hapla, although it is of a temperate origin, can develop on various legume and horticultural crops in Mediterranean climates where it has been widely introduced. The species M. mayaguensis, distributed at least in Africa (Fargette et al., 1996) and in Central America (Rammah and Hirschmann, 1988), is highly aggressive and is a potential pest in southern Mediterranean regions in Prunus orchards planted for early fruit production. Our purpose was to determine the response of the Ma1 and Ma2 genes to M. hapla and M. mayaguensis both for practical reasons and also to obtain basic knowledge of the resistance spectrum of both genes that might be introduced in a wider range of plant species using biotechnology.

Material and Methods

Plant material. The three ‘Myrobalan’ plum parental clones, P.1079, P.2175, and P.2646, and appropriate crosses were chosen for this study. P.1079 and P.2175 are highly resistant to RKN and carry, respectively, the genes Ma2 (homozygous) and Ma1 (heterozygous) (Lecouls et al., 1997). The clone P.2646 (recessive for both genes) is a host for all tested RKN.

Intraspecific crosses involving the parents P.2175 and P.2646 and their progeny clones P.2175 x (P.2646 x P.1079)9 and P.2646 x (P.2646 x P.1079)9 were used. All tested crosses segregate for resistance to M. arenaria, M. incognita, and M. javanica. Sixty individuals of P.2175 x (P.2646 x P.1079)9 (segregates for Ma1 and Ma2) and 10 of P.2646 x (P.2646 x P.1079)9 (segregates for Ma2) were evaluated (Table 1). The designations (P.2646 x P.1079)9 and (P.2646 x P.1079)9 are used to identify the individual selections 9 and 3, respectively, from the cross between the female parent P.2646 and the male parent P.1079.

Nematode populations. Two RKN populations, M. hapla ‘Canada’ originally introduced from Canada and M. mayaguensis ‘VSS1’ originally introduced from Senegal, were used. Another population, M. arenaria ‘Monteux’, belonging to the RKN reference species, which was used to identify the Ma

Table 1. Phenotype and genotype of parental clones for Ma genes.

| Clone | Phenotype | Genotype |
|-------|-----------|----------|
| P.2175 | R | Ma1 ma1, ma2 ma2 |
| P.1079 | R | ma1 Ma1, Ma2 Ma2 |
| P.2646 | H | ma1 ma1, ma2 ma2 |
| (P.2646 x P.1079) 3 | R | ma1 Ma1, Ma2 ma2 |
| (P.2646 x P.1079) 9 | R | ma1 ma1, Ma2 ma2 |

R = resistant, H = host.

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Results and Discussion

**Meloidogyne arenaria.** For the reference species *M. arenaria*, parental clones P.2175, P.1079, (P.2646 x P.1079), and (P.2646 x P.1079)9 were quite free of galls and confirmed their high level of resistance (Eschenjaud et al., 1996). They were highly significantly separated (P ≤ 0.01) from the host parent P.2646. Mean GI ratings of the individuals of the segregating progenies, including parents, clearly separated two highly significantly different classes: the resistant (R) class with clones with a GI ≤ 0.2 and the host (H) class grouping the host clones with a GI > 0.9 (Table 2). No intermediate clones were observed. Thus, segregation of both progenies for Ma genes was confirmed. In the cross with the largest numbers, P.2175 x (P.2646 x P.1079)9, which involves the Ma1 and Ma2 genes, the segregation ratio was distorted (2R:1H instead of 3R:1H), but remained at an acceptable probability with the chi square test (0.1 < P < 0.2). In the cross (P.2646 x (P.2646 x P.1079)3, the expected 1R:1H ratio was observed, although the population size was very small.

**Meloidogyne hapla.** All of the parental clones, and particularly the Ma recessive clone P.2646, were highly resistant to *M. hapla* (Table 3). In the progenies tested, no segregation of resistance was observed. To strengthen these results, roots were carefully reexamined to locate eventual minor galls that could have escaped the first rating observation and might indicate initiation of a nematode feeding site (NFS), but no symptoms were observed. In addition, nematode extraction from the roots of 12 individuals segregating for Ma (6 R and 6 H) recovered no second-stage juveniles (data not shown). Additionally, 26 individuals (8 R and 18 H to *M. arenaria*) from another segregating cross (P.16.5 x (P.16.5 x P.1079)29, involving another *Marecessive clone* (P.16.5), were also evaluated for resistance to *M. hapla* (data not shown). This complementary work was justified because clone P.16.5 is a better host for *M. arenaria* and thus may be a better host for *M. hapla*. No symptoms and no NFS were detected on any root system.

*Meloidogyne hapla* does not reproduce on ‘Myrobalan’ plum. Our work on this RKN was aimed at favoring nematode development or gall formation by using a high and durable inoculum pressure and to determine any difference, even minor, between *Prunus* material segregating for Ma. Because plant symptoms that could have indicated the initiation of a NFS were not observed, one cannot draw conclusions on the activity of the Ma genes against *M. hapla*. ‘Myrobalan’ plum may be a non-host plant for *M. hapla*, which means that many unfavorable genetic factors prevent the penetration and the development of this RKN and hide the putative expression of the Ma genes.

| Clone | No. hybrid genotypes | Gall index | Observed | Expected ratio | \( \chi^2 \) | P-value |
|-------|----------------------|------------|----------|----------------|-------|--------|
|       |                       | R(≥0.2)    | H(>0.9) R | H              |       |        |
| P.2175 | x                     |            |          |                |       |        |
| P.1079 | x                     |            |          |                |       |        |
| P.2646 | x                     |            |          |                |       |        |
| (P.2646 x P.1079)9 | x |            |          |                |       |        |
| (P.2646 x P.1079)3 | x |            |          |                |       |        |
| P.2175 x (P.2646 x P.1079)9 | 60 | 40 | 20 | 47.25 | 15.75 | 2.3 | 0.1–0.2 |
| P.2646 x (P.2646 x P.1079)3 | 10 | 4 | 6 | 5 | 5 | 0.4 | 0.5–0.6 |

Based on the hypothesis presented in Table 1.

*R = resistant; H = host; R and H classes were determined by ANOVA (P ≤ 0.01).*

Table 2. Distribution in ‘Myrobalan’ plum of two progenies [P.2175 x (P.2646 x P.1079)9 and P.2646 x (P.2646 x P.1079)3] segregating for the Ma1 and Ma2 genes for resistance to *M. arenaria* on a 0–5 gall index (GI) rating.

Table 3. Resistance of parental clones to *M. arenaria* (MA), *M. mayaguensis* (MM), and *M. hapla* (MH) and distribution of ‘Myrobalan’ plum crosses segregating for Ma1 and Ma2 genes and simultaneously tested for resistance to these three RKN species.

| Genes involved | No. and distribution of clones evaluated for: |
|----------------|----------------------------------|
|                | MA | MM | MH |
| Parents        | R  | R  | R  |
| P.2175         | R  | R  | R  |
| P.1079         | R  | R  | R  |
| P.2646         | H  | H  | R  |
| (P.2646 x P.1079)3 | R | R | R |
| (P.2646 x P.1079)9 | R | R | R |
| Tested crosses |          |       |       |
| P.2646 x (P.2646 x P.1079)3 | Ma2 | 4 | 4 | 10 |
| H              | 6 | 6 | 0 |
| P.2175 x (P.2646 x P.1079)9 | Ma1 and Ma2 | 40 | 40 | 60 |
| R              | 20 | 20 | 0 |
| H              | 26 | 26 | 0 |

\( R = \text{resistant}; H = \text{host}; \text{R and H classes were determined by ANOVA (P ≤ 0.01).} \)
genes. Considering that this non-host status is polygenic, circumventing this status might only be possible by considerable modification of the genetic background of ‘Myrobalan’ plum by repeated backcrosses to a host species. As most Prunus species are either poor or non-hosts, such a procedure appears practically impossible to perform currently.

*Meloidogyne mayaguensis*. In comparing *M. arenaria* vs. *M. mayaguensis*, all the individuals behaved in a similar way (Table 3). Whatever the RKN species to which they were exposed, the 60 individuals from the first progeny and the 10 individuals from the second progeny reacted identically to both species (R or H). In other words, all individuals classified as resistant to *M. arenaria* were also classified as resistant to *M. mayaguensis* and all individuals classified as host to *M. arenaria* were also classified as host to *M. mayaguensis*.

This identical resistance behavior to *M. arenaria* and *M. mayaguensis* indicates that the *Ma* genes also control resistance to both RKN species. Consequently, the spectrum of these genes, which already control resistance to the major species of the polyphagous RKN complex, also extends to this minor species. Nematode introduction from tropical and equatorial countries, and especially from Africa where it develops on *M. resistans* tomatoes and is generally very aggressive (Fargette, 1987; Fargette et al., 1996), into *Prunus* growing regions should not be a concern for growers using *Prunus* rootstock material carrying the *Ma* genes. Our data on this RKN species show that the genetic systems involved in tomato (*Mt* gene) and ‘Myrobalan’ plum (*Ma* genes) are not related. This result is not surprising considering that RKN resistance genes generally have been very specific (Roberts, 1995; Williamson and Hussey, 1996), and that their respective botanical families (*Rosaceae* and *Solanaceae*) are very distant from each other.

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