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* (Kofoid & 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: *M. arenaria* (as a reference for the *Ma* genes); *M. hapla* and *M. mayaguensis* from crosses segregating for either *Ma2* alone or *Ma2* and *Ma1* and involving the resistant parental clones P.2175 (heterozygous for *Ma1*) and P.1079 (homozygous for *Ma2*); and the host parental clone P.2646 (recessive for both *Ma* genes). Each parental clone and each individual of the segregating progenies reacted in a similar way to *M. arenaria* and *M. mayaguensis*, indicating that the *Ma* genes also control resistance to *M. mayaguensis*. By contrast, all parental clones and progenies were completely resistant to *M. hapla*, and, despite high inoculum pressure, no effect of the *Ma* genes on this species could be established.

Root-knot nematodes (RKN) cause significant economic damage to *Prunus* crops in many countries (Kochba and Spiegel-Roy, 1976; Pinochet 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; Lamberti, 1979; Nyczepir, 1991; Pinochet 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 ‘Nemaguard’ and

the related ‘Nemared’ rootstocks express a near-complete resistance spectrum (Esmenjaud et al., 1997). Their resistance was overcome by one population detected in Florida, a new species thus designated *M. sp. Florida* (Esmenjaud 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 also to the undescribed population *M. sp. Florida* (Esmenjaud 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 (*Lycopersicon 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)3 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)3 (segregates for *Ma2*) were evaluated (Table 1). The designations (P.2646 x P.1079)9 and (P.2646 x P.1079)3 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*

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

Clone	Phenotype ^z	Genotype
P.2175	R	<i>Ma1 ma1, ma2 ma2</i>
P.1079	R	<i>ma1 ma1, Ma2 Ma2</i>
P.2646	H	<i>ma1 ma1, ma2 ma2</i>
(P.2646 x P.1079) 3	R	<i>ma1 ma1, Ma2 ma2</i>
(P.2646 x P.1079) 9	R	<i>ma1 ma1, Ma2 ma2</i>

^zR = resistant; H = host.

genes (Esmenjaud et al., 1996), was also included. All three cultures were isolates obtained from one single egg mass and maintained on tomato cv. Piersol, which has the *Mi* resistance gene (Roberts et al., 1990). The identity of the three RKN species was confirmed by electrophoresis patterns (Janati et al., 1982).

Evaluation of plant material. Parental and hybrid genotypes were propagated at the Institut National de la Recherche Agronomique (INRA), Villenave d'Ornon, France, from softwood cuttings collected from juvenile or adult trees. Cuttings (25-cm length, 5-mm diameter) were harvested either in May or in June. The cuttings were rooted individually in plates in the nursery up to late autumn to allow development of rooted plants, and shipped in December to the Laboratoire de Biologie des Invertébrés at INRA, Antibes, France, for resistance evaluations during the following year. Cuttings were then repotted by pairs during winter into 5-L containers filled with a sandy substrate. All containers were then placed on iron benches in a greenhouse, and grown at a mean temperature of 25 °C (extremes 22 to 28 °C) until rating. In mid-March, tomato plantlets were planted into a sandy substrate in 250-mL plastic containers in the same greenhouse. Juveniles, 24 to 72 h old, of the RKN species to be tested, were deposited, at the rate of 500/pot, into two holes 2 cm deep and 2 cm from the plant stem. The level of inoculum was chosen based on a previous methodological study with *M. arenaria* 'Monteux' (Esmenjaud et al., 1992). Juveniles were obtained in a mist chamber from tomato roots previously inoculated with the same RKN species.

In mid-May, 2 months after inoculation, the tops of the tomato plants were removed and one entire tomato root system with the surrounding soil was transplanted into each *Prunus* container. Containers infested with the same *Meloidogyne* species were arranged in a completely randomized block design on a greenhouse bench. Groups of containers corresponding to different species were separated from each other with transparent splash screens. Each plant clone-RKN species combination was replicated six times (three containers with two cuttings each).

The 'Myrobalan' plants were harvested 4 months after inoculation. The roots of each plant were washed and given a root gall index (GI) rating according to a 0–5 scale (0 = no gall; 1 = 1% to 10% of root system galled; 2 = 11% to 30%; 3 = 31% to 70%; 4 = 71% to 90%; 5 >90%), using 0.5 steps when galling was estimated to be intermediate between two categories. Extraction and counting of nematodes (Jenkins, 1964) were performed only on 12 *Ma* segregating individuals. Since six replicates of each tested clone-RKN species combination were available, mean GI ratings were analyzed separately for each RKN species and each progeny (completed with the corresponding parental clones) using a one-way analysis of variance (ANOVA) (Noe, 1985). Then the clones were compared by the Newman-Keuls multiple range test at $P \leq 0.01$ for distribution into different classes.

Results and Discussion

Meloidogyne arenaria. For the reference species *M. arenaria*, parental clones P.2175, P.1079, (P.2646 x P.1079)3, and (P.2646 x P.1079)9 were quite free of galls and confirmed their high level of resistance (Esmenjaud et al., 1996). They were highly significantly separated ($P \leq 0.01$) 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 *Ma* recessive 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*

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.

Clone	No. hybrid genotypes	Gall index				χ^2	P-value
		Observed		Expected ratio ²			
		R ^y (≤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

^aBased on the hypothesis presented in Table 1.

^bR = resistant; H = host; R and H classes were determined by ANOVA ($P \leq 0.01$).

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.

Clones	Genes involved	No. and distribution of clones evaluated for:		
		MA	MM	MH
<u>Parents</u>		Resistance		
P.2175		R ^z	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
<u>Tested crosses</u>		No. of clones		
P.2646 x (P.2646 x P.1079) 3	<i>Ma2</i>			
R		4	4	10
H		6	6	0
P.2175 x (P.2646 x P.1079) 9	<i>Mal</i> and <i>Ma2</i>			
R		40	40	60
H		20	20	0
Total				
R		44	44	70
H		26	26	0

^aR = resistant; H = host; R and H classes were determined by ANOVA ($P \leq 0.01$).

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 *Mi*-resistant 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 (*Mi* 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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