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Inheritance of resistance to the root-knot nematode *Meloidogyne arenaria* in Myrobalan plum

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Abstract The inheritance of resistance of the self-incompatible Myrobalan plum *Prunus cerasifera* to the root-knot nematode *Meloidogyne arenaria* was studied using first a diallel cross between five parents of variable host suitability (including two highly resistant clones P.1079 and P.2175, a moderate host P.2032, a good host P.2646 and an excellent host P.16.5), followed by the G2 crosses P.16.5 × (P.2646 × P.1079) and P.2646 × (P.16.5 × P.1079). A total of 355 G1 and 72 G2 clones obtained from hardwood cuttings sampled from trees in the field experimental design, then rooted in the nursery and inoculated individually in containers (5–10 replicates per clone) under greenhouse conditions, were evaluated for their host suitability based on a 0–5 gall-index rating under a high and durable inoculum pressure of the nematode. In the crosses involving the resistant P.1079 and P.2175 and the hosts P.2646 and P.16.5: (1) all of the G1 crosses of P.1079 were resistant while the G2 crosses segregated 1 resistant to 1 host, (2) the G1 crosses between P.2175 and either P.2646 or P.16.5 segregated 1 resistant to 1 host, and (3) all of the G1 progeny between P.2646 and P.16.5 were host. These results indicate that resistance is conferred by a single major dominant resistance gene (homozygous) in P.1079, and the same, or an allelic or a different, major dominant gene (heterozygous) in P.2175, and that P.2646 and P.16.5 are recessive for this (these) major resistance gene(s). As expected according to the hypothesis of a recessive genotype for P.2032, all of its hybrids with P.1079 were resistant, all of its hybrids with P.2646 and P.16.5 were host, and its hybrids with P.2175 segregated for resistance. Nevertheless, the 3:2 segregation ratio of these latter hybrids suggests that clones bearing the P.2175 gene would have a selective advantage. Both resistance genes are completely dominant and confer a non-host behaviour that totally prevents the multiplication of the nematode. This is the first reported evidence of major nematode resistance genes towards *M. arenaria* in a species of the subgenus *Prunophora* in the genus *Prunus*. The symbols *Ma1* for the P.2175 gene and *Ma2* for the P.1079 gene are proposed.

Key words Diallel · Complete dominance · *Prunus cerasifera* · Oligogenic resistance · Nematode resistance

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Introduction

Root-knot nematodes (RKN) (*Meloidogyne* spp.) are obligate plant endoparasites and represent important pests of many crops (Sasser 1977; Lamberti 1979) all over the world. Almond (*Prunus amygdalus* Batsch.) and peach [*P. persica* (L.) Batsch] are the most severely damaged *Prunus* crops in temperate and Mediterranean areas (Minz and Cohn 1962; Kochba and Spiegel-Roy 1976; Kester and Grasselly 1987; Layne 1987; Scotto La Massèse et al. 1990; Nyczepir 1991; Fernandez at al. 1994 a), particularly in light soils with irrigation. The use of resistant rootstocks was proposed as early as 1929 by Tuft and is today the best control alternative to nematicides against these pests. The three most widely distributed species are *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. *M. arenaria* is the most common species in French orchards (Scotto La Massese et al. 1984).

In *Prunus* spp., the first sources used in rootstock hybridization, such as the peaches Shalil (India) or Yunan (China), proved susceptible to *M. javanica* populations (Day and Tuft 1939; Havis et al. 1950; Chitwood et al. 1952; Burdett et al. 1963; Esmenjaud et al. 1994). The newer resistance sources, mainly Nemaguard and Okinawa, were also resistant to *M. javanica* (Sharpe 1957; Burdett et al. 1963; Sharpe et al. 1969; Sherman et al. 1981;

Ramming and Tanner 1983). Nemaguard was widely used in California and South-Eastern United States for many years (Nyczepir 1991) and is an example of the long-term efficiency of a resistance strategy against *Meloidogyne* spp. (Cook and Evans 1987; Scorza and Okie 1990; Roberts 1992; Nyczepir and Halbrendt 1993). Nevertheless this rootstock was attacked in Florida by an agressive population identified as *M. incognita* race 3 (Sharpe and Perry 1967; Sharpe et al. 1969; Sherman et al. 1981; Sherman and Lyrene 1983). Consequently, the selection of a resistant rootstock towards this population was performed from crosses and open-pollinated seedlings of Chico 11, Okinawa and *P. davidiana* (Kester and Asay 1986; Sherman et al. 1981, 1991). All these already mentioned selections belong to the subgenus *Amygdalus*. By contrast, in France, new resistance sources were selected in the subgenus *Prunophora* (plum, prune and apricot), in which two species, *P. cerasifera* Ehr. (Myrobalan plum) and *P. insititia*, are known as particularly well adapted to the heavy and calcareous soils often found in new fruit tree areas (Bernhard 1962; Bernhard et al. 1979; Renaud et al. 1988; Salesses et al. 1992, 1994).

Myrobalan clones range from highly resistant to susceptible towards *M. arenaria* (Scotto La Massèse et al. 1990; Esmenjaud et al. 1992, 1993). The two clones P.1079 and P.2175 proved highly resistant to 22 root-knot nematode populations from different geographical origins belonging to *M. arenaria*, *M. incognita*, *M. javanica*, *M. hispanica*, *M. hapla* and an unclassified root-knot species (Esmenjaud et al. 1994). These clones were also resistant to five other mixed *M. incognita* and five other mixed *M. javanica* populations (Fernandez et al. 1994 a). Moreover, the resistance of these clones was not affected by high temperature and high inoculum pressure whereas the resistance obtained from representatives of the subgenus *Amygdalus*, such as Nemaguard or derived clones like hybrids G.×N. (Fernandez et al.1994 b; Esmenjaud et al. 1996), was affected, though only lightly, by these factors. Consequently, these Myrobalan clones appear particularly useful for the creation of new *Meloidogyne* resistant rootstocks by intra- and inter-specific hybridization.

The genetic control of resistance in *Prunus* has been poorly investigated mainly because studies on these perennial plants are time and space consuming in contrast with annual crops such as tomato (Sidhu and Webster 1973, 1975; Cap et al. 1993), common bean (Omwega et al. 1990) or maize (Williams and Windham 1990). In the Myrobalan plum, early tests using young softwood cuttings are not satisfactory and resistance evaluation has to be based on older softwood cuttings, or better still on hardwood cuttings, because a reliable response of *Prunus* plants to RKN needs root-tissue maturation (Canals et al. 1992; Esmenjaud et al. 1995). Inheritance studies on resistance were only carried out under field conditions on some sources of the subgenus *Amygdalus*, the peaches Okinawa to *M. incognita*, Okinawa and *P. davidiana* to *M. javanica* (Sharpe et al. 1969), and the almond Alnem to *M. javanica* (Kochba and Spiegel-Roy 1975). No investigation has so far been carried out on any species of the subgenus *Prunophora*.

The objective of the present study is to establish the genetic determinism of Myrobalan-plum resistance to *M. arenaria*. Considering that *P. cerasifera* is self-incompatible, this study is based on the analysis of the behaviour of G1 hybrids from a diallel cross involving clones ranging from highly resistant to excellent host and completed with appropriate G2 crosses. From a preliminary study (Scotto La Massèse et al. 1990), the presence of (a) major gene(s) involved in the resistance of the highly resistant clones was hypothesized. We report here the results and conclusions of our complete study in which the evaluation of clones was performed with a previously described method (Esmenjaud et al. 1992) providing a high and durable inoculum pressure of the nematode.

Materials and methods

Plant material

Five clones of *P. cerasifera* introduced in the years 1960–1970 from various geographical origins were used to establish a diallel cross at INRA Villenave d'Ornon (France). Their host suitability is defined on a resistant/host terminology. The parental clones and their main characteristics are as follows: P.1079 (south western France; red leaf) and P.2175 (Bucarest, Rumania), highly resistant to the Monteux isolate of *M. arenaria* (Scotto La Massèse et al. 1990); P.16.5 (northern Alps , France; red leaf) and P.2646 (Balsgard, Sweden), the best hosts towards this same isolate; and P.2032 (rootstock registered as 'Myrabi'; south-eastern France) considered as a moderate host.

Production of G1 and G2 generations

The parental clones used for hybridization were trees grown in the field or in greenhouse containers to prevent climatic problems for flowering and pollination. Field trees were isolated in cages from parasite pollination by wind or insects. In both field or greenhouse conditions, flowers were emasculated at the 'white button' stage and pollination was performed after 24 to 48 h with a paint brush using pollen previously collected and eventually stored at 4°C. Hybrid seeds were germinated in vitro by embryo culture on a gelified hormone-free medium containing half-strength Knop macronutrients, Heller micronutrients and 20 g/l of sucrose (Gautheret 1959). Seedlings were first grown in the greenhouse and tranferred into the 'hybrid' experimental field. At the beginning of the resistance evaluations, the parents and 355 hybrid clones were available as adult trees growing in the field at INRA Villenave d'Ornon (France) and the production of G2 progenies was in progress.

Nematode isolate

The isolate "Monteux" of *M. arenaria* obtained from Monteux, Vaucluse (France) was maintained in the greenhouse on the tomato var. St Pierre (*Lycopersicon esculentum* Mill.). As this population reproduces by mitotic parthenogenesis (Triantaphyllou 1985), an isolate was reared from a single egg mass and this isolate was also maintained on tomato var. St Pierre. The identity of the isolate was verified each year before inoculation via its isoesterase phenotype (Janati et al. 1982).

Evaluation of plant material

For each clone, the assessing material was propagated at INRA Villenave d'Ornon from hardwood cuttings sampled on adult trees for G1 clones and on 2–4 year-old recently obtained trees for G2 clones.

Table 1 Gall index[a] and resistance classification of the diallel parents to *M. arenaria* (ten replicates)

Parent	P.1079	P.2175	P.2032	P.2646	P.16.5
Gall index (GI)	0.0 a[b]	0.0 a	2.6 b	3.3 bc	4.2 c
Classification	Highly resistant	Highly resistant	Moderate host	Good host	Excellent host
Resistance level	HR $0 \leq GI < 1$	HR $0 \leq GI < 1$	MH $2 \leq GI < 3$	GH $3 \leq GI < 4$	EH $4 \leq GI < 5$

[a] 0 is no gall; 5 is >90% of root system galled
[b] Gall index ratings followed by the same letter do not differ according to Newman-Keuls multiple range test at $P \leq 0.01$

Homogeneous cuttings (50 cm long, 1 cm diameter) were harvested in November or December, dipped into 1500 ppm of indolebutyric acid aqueous solution and placed in polyethylene bags in a dark room kept at 18–20°C until callus formed at their basal section. Then, cuttings were planted in the nursery up to next late autumn to allow for the development of rooted plants, which were then harvested and kept in sand until the following March. Hardwood cuttings were supplied as bare roots to the Laboratoire de Biologie des Invertébrés at INRA Antibes (France) for resistance evaluation. Each cutting was individually planted at mid-March under greenhouse conditions and in a 5-l container filled with a sandy substrate. Containers were placed on iron benches and irrigated individually every 2 days with a 5 N-11.5 P_2O_5–7.5 K_2O nutrient solution at 3 g/l containing trace elements (Algoflash: Algochimie, Tours, France) and grown between April and July at a mean temperature of 25°C (extremes 22–28°C). On the same date in March, tomato plantlets grown in a greenhouse maintained at 25°C minimum in 250-ml plastic containers were inoculated with 500 24–72-h old juveniles of the Monteux isolate deposited into two holes, 2 cm deep and 2 cm from the stem. Juveniles were obtained in a mist chamber from tomato roots previously inoculated with the same isolate. The level of inoculum chosen was based on a previous methodological study on six resistant and five host Myrobalan clones (Esmenjaud et al. 1992).

At mid-May, 2 months after inoculation, the top parts of the tomato plants were cut and removed and one whole soil and root system content was transferred into each *Prunus* container. Four months after inoculation, *Prunus* plants were harvested. Each plant was carefully washed individually over a bucket and given a root-gall index rating according to a 0–5 scale (Barker 1985) (0=no gall; 1=1–10% of root system galled; 2=11–30%; 3=31–70%; 4=71–90%; 5>90%) and completed with 0.5 steps when galling was estimated to be at the limit between two groups. After rating the root systems were individually frozen at –20°C until nematodes were extracted. Frozen root systems were transferred to a refrigerator (5°C) to be thawed progressively. Fine roots (diameter≤1 mm) were separated, weighed and 20 g, sampled randomly, were ground with an ultra grinder (20 000 rpm) for 2 s. The freed nematode stages were collected into a beaker through a 250-µm pore sieve. Non-ground roots and rootlets were recovered from the sieve and were ground two more times. Then the content of the beaker was centrifuged twice (Jenkins 1964). Females, males, J3-J4, J2 and eggs were counted under a binocular.

Planning of the tests

Trials were performed over 3 years. During the first 2 years, the G1 tests were conducted and ten cuttings per clone were evaluated for resistance following the complete procedure described above, i.e. gall-index rating and nematode extraction. A previous study (Esmenjaud et al. 1992) had established that the gall index was highly significantly correlated with the $\log_{10}(x+1)$ transformed numbers of the different nematode stages in the roots. The best linear correlation was observed with females, followed by eggs and juveniles. For these latter stages, representing the reproductive potential of the nematode in the plant, the gall index proved to be a good criterion for evaluating host suitability in *P. cerasifera*. As this previous study was confirmed by the results of the 2 first years, replicates were reduced to five (except for parents for which ten replicates were kept) in the G2 tests conducted during the third year and a gall-index rating was attributed to each tested plant.

Parental clones were always simultaneously tested with their respective G1 and G2 progenies as references. The highly resistant clones P.1079 and P.2175, the two best host clones P.16.5 and P.2646, and the hybrids between these four parents were evaluated during the first year. Hybrids between P.2032 and the four other diallel parents were tested during the second year. Second-generation progenies were evaluated during the third year.

Statistical analysis

As replicates (ten in years 1 and 2 and five in year 3) of each tested clone were available, data from the same year test were analyzed using a one-way analysis of variance. Nematode densities obtained from each of the 2 first years were $\text{Log}_{10}(x+1)$-transformed for analysis (Noe 1985). Means of transformed nematode densities and gall-index ratings were compared by a Newman-Keuls multiple range test at $P \leq 0.05$ and $P \leq 0.01$. When the tested clones were in excess of 30, chi-square tests were conducted on G1 and G2 data to determine the goodness of fit between observed and expected segregation ratios for resistant and host classes.

Results

Evaluation of the parental clones during the 3 years allowed us to clarify their relative level of resistance. Their gall index (GI), based on results of the second year, are reported in Table 1. Clones P.1079 and P.2175 were quite free of galls and confirmed their high level of resistance [GI=0; highly resistant (HR)]. They were highly significantly separated from host parents P.2032 [GI=2.6; moderate host (MH)], P.2646 [GI=3.3; good host (GH)] and P.16.5 [GI=4.2; excellent host (EH)]. Mean gall-index ratings of the tested clones were arranged into five levels [HR level: $0 \leq GI < 1$; intermediate level (I): $1 \leq GI < 2$; MH level: $2 \leq GI < 3$; GH level $3 \leq GI < 4$; EH level: $4 \leq GI < 5$]. As over 50% of the hybrids of HR parents were also HR, the resistant class was defined as corresponding to HR clones, whereas the host class was defined by the I, MH, GH and EH clones. Data on G1 and G2 progenies are summarized in Tables 2 to 4, in terms of the major gene hypothesis deduced from a preliminary study (Scotto La Massese et al. 1990), as follows: P.1079=RR; P.2175=Rr; other clones=rr where clones RR and Rr are resistant and clones rr are host.

Table 2 Segregation of G1 progenies from resistant (R; P.1079 and P.2175: GI=0) and host (H; P.2646: GI=3.3 and P.16.5: GI=4.2) parents evaluated for *M. arenaria* on a 0–5 gall index (GI) rating

Clone	Nb genotypes	Gall index					R (0–1)	H (1–5)	Expected ratio[a]		χ^2	*P*-value
		0–1 HR[b]	1–2 I	2–3 MH	3–4 GH	4–5 EH			R	H		
P.1079		x					x					
P.2175		x					x					
P.2646					x			x				
P.16.5						x		x				
P.2175 × P.1079[c]	**29**	**29**					**29**	**0**	**29**	**0**		
P.1079 × P.2646	6	6					6	0	6	0		
P.2646 × P.1079	10	10					10	0	10	0		
P.2646 × P.1079	**16**	**16**					**16**	**0**	**16**	**0**		
P.16.5 × P.1079	**15**	**15**					**15**	**0**	**15**	**0**		
P.2175 × P.2646	47	24		14	6	3	24	23	23.5	23.5		
P.2646 × P.2175	3	2			1		2	1	1.5	1.5		
P.2646 × P.2175	**50**	**26**		**14**	**7**	**3**	**26**	**24**	**25**	**25**	**0.04**	**0.8–0.9**
P.2175 × P.16.5	32	18		2	6	6	18	14	16	16		
P.16.5 × P.2175	11	6		2		3	6	5	5.5	5.5		
P.16.5 × P.2175	**43**	**24**		**4**	**6**	**9**	**24**	**19**	**21.5**	**21.5**	**0.58**	**0.4–0.5**
P.16.5 × P.2646	**32**	**0**		**4**	**14**	**14**	**0**	**32**	**0**	**32**		

[a] Expected ratio based on the following hypothesis: P.1079=RR; P.2175=Rr; P.2646 and P.16.5=rr. RR and Rr resistant and rr host
[b] HR = highly resistant; I = intermediate; MH = moderate host; GH = good host; EH = excellent host
[c] The cumulated reciprocal crosses (from the two rows mentioned above when present) are in bold type

Hybrids between P.1079, P.2175, P.2646 and P.16.5 (Table 2)

When reciprocal crosses were available, such as for P.1079 × P.2646 and for P.2175 × P.16.5, the results did not show any maternal effect. Mean gall-index ratings of crosses between the four parents were clearly distributed into two distinct groups (GI<1 and GI≥2) with no representative of the I level. Hybrids involving P.1079 were free of galls and all were in the HR class. Hybrids between P.2175 and P.2646 or P.16.5 segregated into two categories with a ratio of 1 resistant to 1 host ($0.8<P<0.9$ and $0.4<P<0.5$, respectively) as expected from the basic hypothesis of Scotto La Massèse et al. (1990): clones in the HR level were completely free of galls whereas host clones were distributed as three host levels. Gall-index ratings of the whole-HR clones were highly significantly different from those of the whole-host clones (data not shown). Data on nematode numbers confirmed the absence of developing stages of the nematode in the roots of the HR hybrids, and the highly significant difference between clones ranged into the HR and host levels (data not shown). The hybrids between both host clones ranged into the three host levels.

Hybrids involving P.2032 and the other parental clones (Table 3)

All hybrids between P.1079 and P.2032 were highly resistant. When P.2032 was crossed with P.2175, a bimodal distribution was obtained. Sixty per cent of the clones were in the HR level and the remaining 40% were distributed into the four other levels with a maximum in the GH level. Nevertheless, the three clones of the I level (1≤IG<2) were not significantly different from the neighbouring clones of the MH level but were significantly different from those of the HR level. Data on nematode numbers confirmed the absence of developing stages of the nematode in the roots and the significant difference between clones in the HR level and those in the I level (data not shown). Crosses between P.2032 and P.2646, as well as crosses between P.2032 and P.16.5, gave a monomodal distribution with a maximum in the GH and MH-GH levels, respectively, but ranged from intermediate to excellent-host levels.

G2 progenies involving P.1079, P.2646 and P.16.5 (Table 4)

The three tested G2 progenies segregated into two clearly separated resistant and host classes with no intermediate clones. All of the HR G2 clones, as well as their P.1079 and P.1079× host ancestrals, were completely free of gall. In [P.16.5 × (P.2646 × P.1079)9], the most numerous one, and in [P.2646 × (P.16.5 × P.1079)33], clones segregated 1 resistant to 1 host whereas in [P.2646 × (P.16.5 × P.1079)29] a majority of resistant clones (12 out of 19) were obtained. Cumulated numbers fit the 1:1 segregation ($0.6<P<0.7$) expected for a single dominant resistance gene at the homozygous stage in P.1079.

Table 3 Distribution of G1 progenies of P.2032 (moderate host; GI=2.6) with resistant (R; P.1079 and P.2175: GI=0) and host (H; P.2646: GI=3.3 and P.16.5: GI=4.2) parents evaluated for *M. arenaria* on a 0–5 gall index (GI) rating

Clone	Nb geno-types	Gall index					R (0–1)	H (1–5)	Expected ratio[a]		χ^2	*P*-value
		0–1 HR[b]	1–2 I	2–3 MH	3–4 GH	4–5 EH			R	H		
P.1079		x					x					
P.2175		x					x					
P.2032				x				x				
P.2646					x			x				
P.16.5						x		x				
P.2032 × P.1079[c]	**15**	**15**					**15**	**0**	**15**	**0**		
P.2175 × P.2032	29	19	1	2	4	3	19	10	14.5	14.5		
P.2032 × P.2175	31	17	2	2	8	2	17	14	15.5	15.5		
P.2032 × P.2175	**60**	**36**	**3**	**4**	**12**	**5**	**36**	**24**	**30**	**30**	**2.4**	**0.1–0.2**
P.2646 × P.2032	31	0	1	6	21	3	0	31	0	31		
P.2032 × P.2646	15	0	3	3	7	2	0	15	0	15		
P.2032 × P.2646	**46**	**0**	**4**	**9**	**28**	**5**	**0**	**46**	**0**	**46**		
P.16.5 × P.2032	15	0	1	8	4	2	0	15	0	15		
P.2032 × P.16.5	34	0	2	9	13	10	0	34	0	34		
P.2032 × P.16.5	**49**	**0**	**3**	**17**	**17**	**12**	**0**	**49**	**0**	**49**		

[a] Expected ratio based on the following hypothesis: P.1079=RR; P.2175=Rr; other parents=rr. RR and Rr resistant and rr host
[b] HR = highly resistant; I = intermediate; MH = moderate host; GH = good host; EH = excellent host
[c] The cumulated reciprocal crosses (from the two rows mentioned above when present) are in bold type

Table 4 Distribution of three G2 progenies of resistant P.1079 (R; GI=0) with host P.2646 (H; GI=3.3) and host P.16.5 (H; GI=4.2) evaluated for *M. arenaria* on a 0–5 gall index (GI) rating

Clone	Nb geno-types	Gall index					R (0–1)	H (1–5)	Expected ratio[a]		χ^2	*P*-value
		0–1 HR[b]	1–2 I	2–3 MH	3–4 GH	4–5 EH			R	H		
P.1079		x					x					
P.2646					x			x				
P.16.5						x		x				
(P.2646 × P.1079)9		x					x					
(P.16.5 × P.1079)29		x					x					
(P.16.5 × P.1079)33		x					x					
(P.16.5 × (P.2646 × P.1079)9)	40	20		1	7	12	20	20	20	20	0	1
(P.2646 × (P.16.5 × P.1079)29)	19	12		2	4	1	12	7	9.5	9.5		
(P.2646 × (P.16.5 × P.1079)33)	13	7			3	3	7	6	6.5	6.5		
Total G2 progenies	72	39		3	14	16	39	33	36	36	0.25	0.6–0.7

[a] Expected ratio based on the following hypothesis: P.1079=RR; P.2646 and P.16.5=rr. RR and Rr resistant and rr host
[b] HR = highly resistant; I = intermediate; MH = moderate host; GH = good host; EH = excellent host

Discussion

From hybrids between both highly resistant and the three host parents, a determism of resistance based on major resistance genes is confirmed. These results establish that resistance in P.2175 is monogenic, completely dominant and heterozygous, whereas P.2032, P.2646 and P.16.5 are recessive. We propose the symbol *Ma1* for this P.2175 major resistance gene. As the entire P.1079 G1 hybrids are resistant and its G2 crosses segregate 1:1, this clone expresses resistance in which a single major gene (homozygous) with complete dominance is also involved. We propose the symbol *Ma2* for this P.1079 major resistance gene. The relationships between *Ma1* and *Ma2* and the corresponding genotypes of the five diallel parents are presented in Table 5.

Nevertheless, when crosses between P.2175 and the "less favourable" host clone P.2032 are taken into account, resistant and host classes are less clearly separated and a 3:2 ratio indicating a distorsion of segregation is observed. Actually, in the progenies between P.2175 and host clones P.16.5, P.2646 or P.2032, the R:H ranges from 1:1 to 3:2 (the percentages of resistant clones are 52% in P.2646, 55.8% in P.16.5 and 60% in P.2032). The mean percentage of resistant clones in the total accumulated hybrids (153

Table 5 Putative genotypes of the diallel cross parental clones for resistance to *M. arenaria*

Parental clone	Number of genes[a]	
	Two *Ma1* ≠ *Ma2*[b]	One *Ma1* = *Ma2*[c]
P.1079	*ma1 ma1, Ma2 Ma2*	*Ma1 Ma1*
P.2175	*Ma1 ma1, ma2 ma2*	*Ma1 ma1*
P.2032	*ma1 ma1, ma2 ma2*	*ma1 ma1*
P.2646	id.	id.
P.16.5	id.	id.

[a] All genes expressed in a dominant fashion
[b] *Ma1* and *Ma2* linked or independant
[c] *Ma1* same as or allelic to *Ma2*

clones) is 55.5% (Tables 2 and 3). These results suggest that the resistance gene confers a higher survival (which might be linked to a higher vigor) to seedlings that bear it. In G2 data involving P.1079, the R:H ratio also ranges from 1:1 to 3:2 with a mean percentage of resistant clones (54.2% of 72 clones) equivalent to that of previous G1 hybrids involving P.2175, and confirms the distorsion of segregation in favour of resistant clones.

Although our study does not allow us to determine the relationship between *Ma1* and *Ma2* (Table 5), the most probable hypothesis is that they are either the same or else allelic. Nevertheless, [(P.1079×hosts)×P.2175] progenies should be screened to obtain answers to this question. Because resistance in P.2175 and P.1079 is highly efficient against the whole RKN species (Esmenjaud et al. 1994), another step would be to establish if the same or different gene(s) is (are) also involved in their resistance towards the other species.

This is the first reported evidence of major resistance genes in a species of the subgenus *Prunophora*. It is also the first indication of resistance genes in *Prunus* spp. towards *M. arenaria*. In the subgenus *Amygdalus*, Kochba and Spiegel-Roy (1975) identified a major dominant gene towards *M. javanica* in bitter almond, but these selections were galled by *M. incognita* (Scotto La Massèse et al. 1984) showing that this resistance gene does not have a wide range and is relatively specific. In the wild peach *P. davidiana* as in the peach rootstocks Nemaguard and Okinawa, no marked resistance difference between *M. javanica* and the other *Meloidogyne* species was found (Sharpe 1957; Burdett et al. 1963; Malo 1967; Sharpe et al. 1969; Sherman et al. 1981). Considering that, in these later selections, the presence of a single major dominant gene was suggested for the resistance to *M. incognita*, and of at least two other dominant and independant genes for *M. javanica* (Sharpe et al. 1969), it would be interesting to establish the relationships between genes involved in the peach and Myrobalan-plum resistance systems. Testing on the highly resistant *P. cerasifera* clones the Florida population that overcomes the resistance of Nemaguard and Okinawa rootstocks (Sharpe and Perry 1967; Sharpe et al. 1969; Sherman et al. 1981) would provide preliminary data on this point.

The dominant resistance genes *Ma1* and *Ma2* confer a non-host behaviour that completely prevents the multiplication of the nematode. Moreover, this resistance was not overcome by any of the over-30 tested RKN species and isolates (Esmenjaud et al. 1994; Fernandez et al. 1994 a) and was not modified under conditions known to affect plant defense mechanisms to RKN, such as high temperature and high inoculum pressure (Fernandez et al. 1994 b; Esmenjaud et al. 1995). Durable resistance is particularly needed in perennial plants (Johnson 1983) and, in RKN, weeds can maintain high numbers, or at least the continual presence, of the pest and so facilitate selection for virulence (Cook and Evans 1987). According to the classification of fungal resistance genes by Van der Plank (1968), quantitative polygenic resistance should be more durable than monogenic resistance. But current data on plant resistance to RKN do not support such a general argument (Roberts 1992). Consequently, the possibility of introducing major resistance genes to *M. arenaria* and putative major genes to other RKN species from these Myrobalan-plum sources into rootstocks of *Prunus* appears promising. The development of marker-assisted selection is particularly needed for perennial species that require long generation intervals (Chaparro et al. 1994). In our laboratory, the search for RAPD markers of the *Ma1* gene using the BSA method (Michelmore et al. 1991) is in progress.

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