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Review

Deciphering Resistance to Root-Knot Nematodes in *Prunus* for Rootstock Breeding: Sources, Genetics and Characterization of the *Ma* Locus

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Abstract: Root-knot nematode (RKN) species are predominant pests of crops, attacking stone fruit crops *Prunus* spp. under Mediterranean climate conditions worldwide. Natural resistance for rootstock breeding is a control method that is gaining interest as an alternative to the highly toxic nematicides. This review first reports an outline of the root-knot nematodes parasitizing stone fruit crops and the *Prunus* species and rootstocks. It then describes the main sources of resistance detected among the *Prunus* germplasm and focuses on the major resistance genes identified and their characteristics (spectrum, durability, histological mechanism, effect of temperature, interaction with other pests and diseases, etc.). In peach, besides the *RMia* reference gene, the new genes *PkMi* and *Mf*, also located on chromosome 2, need to be characterized regarding their spectrum and relationship. The two other *Prunus* reference genes, *Ma* from plum (complete spectrum) and *RMja* from almond (more restricted spectrum), are orthologs that belong to a *TIR-NB-LRR (TNL)* cluster on chromosome 7. The review finally summarizes the positional cloning of the *Ma* gene and the characterization of its unique *TNL* structure, encompassing a five-times repeated post-LRR domain. Deciphering how this structure is functionally involved in *Ma*'s remarkable biological properties is a real challenge for the future.

Keywords: functional validation; gene cloning; natural resistance; *Meloidogyne* spp.; *Prunus* spp.; resistance durability; resistance gene; resistance spectrum; rootstock

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1. Introduction

In many perennials, rootstocks contribute to the control of various abiotic constraints such as drought, salinity and limestone, together with diverse biotic constraints due to soil-borne pests and diseases. Amongst soil pests, the root-knot nematode (RKN) species *Meloidogyne* spp. are a major problem in stone fruit crops *Prunus* spp., and breeding programs throughout the world target their control as one of the major traits to improve [1–4]. These programs may exploit the high variability of the interaction between the pest and the plant species and in particular the natural resistance to the RKN species existing in the *Prunus* germplasm. RKN resistance is an environmentally friendly alternative to the highly toxic chemical nematicides and is thus of an outstanding interest in rootstock breeding.

2. Root-Knot Nematodes

Root-knot nematodes (RKNs) (*Meloidogyne* spp.) are found in Mediterranean, tropical and equatorial areas and to a lesser extent in temperate regions. They have been long considered as the most damaging among nematodes worldwide [5]. The *Meloidogyne* genus includes the nematode species with the largest agricultural impact, causing losses estimated at tens of billions of euros per year [6] when all crops are confounded. RKNs have long reduced fruit production in several economically important *Prunus* species. Peach, almond and plum species grown under Mediterranean climate conditions are those most affected [1,7]. Infections by root-knot nematodes induce typical below-ground root galls and lead to the above-ground stunted growth of young trees. Early root infections

result in extensive tree death in an orchard. Tree defoliation occurs that ultimately results in yield losses. Sandy soils are the most favorable to RKN attacks and gall development.

2.1. Biology and Life Cycle

RKNs are sedentary endoparasitic species and obligate biotrophs that penetrate plant roots and hijack the nutritional resources of the host for their benefit. They are extremely polyphagous and reproduce on thousands of cultivated and wild plants [8]. All *Meloidogyne* spp. have a similar life cycle. Eggs are deposited in gelatinous masses and survive in the soil or in plant residues. The mobile second-stage juveniles (J2s) hatch from the eggs and move towards the root tips. During compatible interaction between susceptible plants, J2s penetrate the root and migrate downwards, between the cells of the cortex, to the apical meristem region. Then, they move upwards towards the vascular cylinder and fix. They become sedentary and develop into swollen third- and fourth-stage juveniles and finally into adult females. While settling, they induce specialized hypertrophied and multinucleate cells (designated giant cells) that form a feeding site. Females may lay more than 1000 eggs in an egg mass, which is often located on the outer surface of the root. The giant feeding cells and the divisions of the surrounding vascular parenchyma cells give rise to the typical root galls [9].

2.2. The RKNs Affecting Prunus Species

The three predominant RKNs affecting the *Prunus* species are *M. arenaria*, *M. incognita* and *M. javanica*, which are all extremely polyphagous, have a worldwide tropical and Mediterranean distribution and reproduce asexually via mitotic parthenogenesis. Another widely distributed species is the northern RKN *M. hapla*, present under temperate climates, which reproduces by meiotic parthenogenesis or amphimixis but develops poorly on *Prunus* spp. [10]. Additionally, several species with a more restricted distribution have been reported. Among them, one species, reported on peach in Florida in the 1960s as overcoming the resistance of 'Nemaguard' and 'Okinawa' rootstocks [11], has been more recently described as the peach RKN *M. floridensis* [12,13]. This new species from the USA has been detected in Florida, neighboring southeastern states [14–18] and California [19]. Towards the RKNs *M. arenaria, M. incognita, M. javanica* and *M. floridensis*, the behavior of predominant rootstocks belonging to peach, almond–peach or plum has been reported [3].

Other RKNs attacking *Prunus* include the polyphagous species of tropical origin, *M. enterolobii* (formerly *M. mayaguensis*), which is uncontrolled by the *Mi-1* gene from tomato and highly aggressive towards many plants [13,20–22], together with *M. morocciensis* [23] and *M. hispanica* [24]. Another RKN species, *M. ethiopica*, is currently limited to South America, where it is destructive when present [25]. Because RKN species cannot be reliably distinguished by symptoms or morphological and morphometrical characters, biochemical (mainly esterases) [26] and molecular (SCARs) [27] tools have been developed.

3. Diversity of *Prunus* Species and Rootstocks

The genus *Prunus*, containing the stone fruit crops, is divided into five main subgenera that comprise more than 400 species distributed worldwide [28]. Among them, a majority of fruit-producing and rootstock species belong to two subgenera:

1. The subgenus Amydalus includes diploid species (2n = 2x = 16) such as the cultivated peach (*Prunus persica*), wild peaches *P. davidiana* and *P. kansuensis*, the cultivated almond (*P. dulcis*) and the wild almond *P. webbii*.
2. The subgenus Prunophora or Prunus *senso stricto* includes hexaploid (*P. domestica*, *P. insititia*; 2n = 6x = 48), tetraploid (*P. spinosa*; 2n = 4x = 32) and diploid (*P. cerasifera*, *P. salicina;* 2n = 2x = 16) plums as well as the apricots (*P. armeniaca, P. mume, P. brigantina*; 2n = 2x = 16).

The genus *Prunus* might have appeared 61 My ago (Eocene) in Oriental Asia. Eurasia harbors the highest diversity, in agreement with its putative native area [29]. Dispersion and speciation events would explain the current distribution from this area. Amygdalus

species moved from oriental to occidental Asia 48 My ago, preceding a diversification and an evolution in which the Himalayan chain separated the almonds (Middle East) and the peaches (Oriental Asia). European plums (*P. cerasifera*, *P. spinosa* and *P. domestica*) migrated from Oriental Asia more recently (31 My) [29]. Nowadays, the distribution area of Myrobalan plum (*P. cerasifera*) ranges from Central Asia to Southern Europe with the highest diversity being found around the Caspian Sea [30].

Stone fruit crops are grown on fruit cultivars carrying selected nutritional and gustative characteristics grafted onto rootstocks providing vigor and adaptation to soil abiotic and biotic conditions. Fruit breeders have to face autosterility within the allogamous species, almond, plums or cherries, and cope with the low genetic diversity of the mainly autogamous species, peaches and apricots. As members of the *Prunus* species can be crossed with each other, rootstock breeders can exploit the high interspecific variability of the different parental species to select and combine their desirable features in hybrid material. In order to obtain polyvalent rootstocks, for example, the Myrobalan plum can be easily crossed with peach, almond or apricot [31].

4. Control of RKNs in *Prunus*: The Resistance Strategy

Interaction between *Prunus* and RKN species is either compatible or incompatible. Compatibility involves a nematode behavior that is similar, at the cellular level as well as in the expression of symptoms, in perennial crops such as *Prunus* and in annual crops such as tomato [32,33]. In *Prunus*, galls may reach sizes that are impressively large despite the lignification of the root tissues.

Incompatibility is a natural resistance phenomenon. Breeding naturally resistant rootstocks is the most economical and environmentally sound method for managing root-knot nematodes in the *Prunus* species and for avoiding their dissemination. Rootstock resistance has been investigated and exploited in several countries. For example, the resistant rootstock 'Nemaguard' has been widely used in California because of the high RKN frequency in almond orchards [3].

4.1. Resistance Sources

Resistance sources have been investigated and characterized mainly in peaches, almonds and plums.

Peach is self-fertile and thus has a low level of genetic variability [34]. Wild relatives, including *P. davidiana* and *P. kansuensis* from China [35,36], are used as sources of resistance to pests and diseases for fruit varieties and rootstocks. Peach was the first *Prunus* species for which RKN resistance (R) factors were reported [37]. The first RKN-resistant peaches, Shalil, Bokhara and Yunnan, originated from northwest India, the Central Asian republics and China, respectively [11], and were resistant to both *M. arenaria* and *M. incognita*. New peach varieties, such as Nemaguard, Nemared and Okinawa, were then selected in the USA, from the 1960s onwards, for their higher resistance towards *M. javanica* [35]. Since the 1990s, several rootstocks for peach have been derived from these accessions, including the complex almond–peach hybrid cv. Flordaguard, the almond–peach cv. Garnem and the peach × *P. davidiana* cv. Cadaman [3].

The almond originates from Central Asia and has been cultivated in the Middle East and Mediterranean areas since Antiquity for its sweet seeds, with bitter-seeded varieties being used as rootstocks. Resistance to *M. javanica* has been detected in bitter seeds of Alnem seedlings in Israel, and these seedlings were later shown to resist *M. arenaria*, *M. enterolobii* and *M. ethiopica*, but they did not show resistance to either *M. incognita* or *M. floridensis* [38–40].

Plum species are diverse and adapted to a broad range of climatic and edaphic conditions. They were domesticated independently in Europe (*P. domestica*, *P. insititia*), China (*P. salicina*) and North America (*P. hortulana*) [41–43]. Most fruit varieties of plums belong to the European species *P. domestica* and *P. insititia*, or to *P. salicina*. Resistance has been identified in a number of plum rootstocks, including the St Julien (*P. insititia*), Damas (*P. spinosa* × *P. domestica*) and Myrobalan plums (*P. cerasifera*) [44]. The Myrobalan plum, a

near-wild allogamous species found in temperate and Mediterranean climates, originated in the Caucasus and Crimean regions [30]. Myrobalan accessions P. 2175, P. 1079 and P. 2980 express complete resistance to more than 30 RKN isolates from *M. arenaria, M. incognita, M. javanica, M. hispanica* and *M. hapla* [10,45]. Recent evaluations showed that this resistance also controls the species *M. ethiopica* and *M. enterolobii* [40].

4.2. Genetics and Mapping of RKN Resistance

A major dominant reference R gene has been identified in each of the plum, almond and peach species (Table 1).

Table 1. Spectrum of resistance conferred by the *R* genes, *Ma, RMia,* and *RMja,* to six root-knot nematodes, *Meloidogyne* spp., attacking *Prunus* crops.

			Root-Knot Nematode Species					
Resistance Gene	**Plant Species**	**Resistance Source**	***M. arenaria***	***M. incognita***	***M. javanica***	***M. ethiopica***	***M. floridensis***	***M. enterolobii***
Ma	Plum—*P. cerasifera*	P.2175 & P.2980	R	R	R	R	R	R
RMia	Peach—*P. persica*	Nemared	R	R	S	R	S	S
RMja	Almond—*P. dulcis*	Alnem 1	R	S	R	R	S	R

R = resistant, S = susceptible.

In plum, the resistance carried out by the Myrobalan plum has been shown to be conferred by a single major dominant gene called *Ma*. The *Ma* gene controls resistance to all tested RKN species and to the predominant RKN species *M. arenaria*, *M. incognita* and *M. javanica* [46,47], in particular. *Ma* also controls the peach RKN *M. floridensis* [47] and the highly aggressive species *M. enterolobii* (=*M. mayaguensis*), which is uncontrolled by the *Mi1-2* R gene from tomato [48]. More recently *Ma* has also been shown to confer resistance to *M. ethiopica* [40] (Table 1), a species widely present in stone fruits and grape in South America [25]. *Ma*, as the RKN reference gene for plum, is located on the linkage group (LG) 7 of the *Prunus* map [49] (chromosome 7 of the peach reference genome [50]). The steps of the genetics and mapping studies in the Myrobalan plum are summarized in the first part of Figure 1.

In almond, bitter cultivars of the 'Alnem' series carry resistance to *M. javanica* conferred by a single gene identified as soon as 1975 [51] and later designated as *RMja* [52]. This almond RKN reference gene also controls *M. arenaria*, *M. ethiopica* and *M. enterolobii* [40] but neither *M. incognita* nor *M. floridensis* (Table 1). The *RMja* gene, first shown as closely linked to the *Ma* gene [52], has recently been characterized as an orthologue of this plum gene [53].

In peach, the 'Nemaguard' and 'Nemared' rootstocks carry the *RMia* gene for resistance to *M. arenaria* and *M. incognita* [49]. This gene, most likely having a population-dependent activity towards *M. javanica,* controls neither *M. enterolobii* nor *M. ethiopica* [40] (Table 1). *RMia,* the RKN reference gene for peach, is independent from *Ma* and *RMja* and is located on LG2 in the *Prunus* map [49] (chromosome 2 of the peach reference genome [50]).

Additionally, a Chinese wild peach (*P. kansuensis*) has been shown to carry an R gene to *M. incognita* named *PkMi* [54,55], which is also located on LG2, but its relationship with *RMia* needs to be clarified. An accession of *P. kansuensis* has also been proposed by other authors as carrying a single-locus dominant (*Mf*)/recessive (*mf*) model for the inheritance of resistance to *M. floridensis* [56], as the cognate *Mf* locus is also located on LG2 [57]. It will be important to determine the spectrum of this resistance towards other RKN species and to shed light on the relationship between the *RMia*, *PkMi* and *Mf* genes from peach resistance sources.

Other R genes have also been detected in other plum species. The *Rjap* gene, identified in the Japanese plum *P. salicina* and located on LG7, carries a wide resistance spectrum that might be the same as that of *Ma* [49]. Even though no fine mapping has been performed for *Rjap*, it is highly plausible that it is an *Ma* orthologous gene. More recently a gene for

resistance to *M. incognita* [58], named *PsoRPM2*, was reported in the wild Chinese plum species *P. sogdiana*, which is close to the Myrobalan plum.

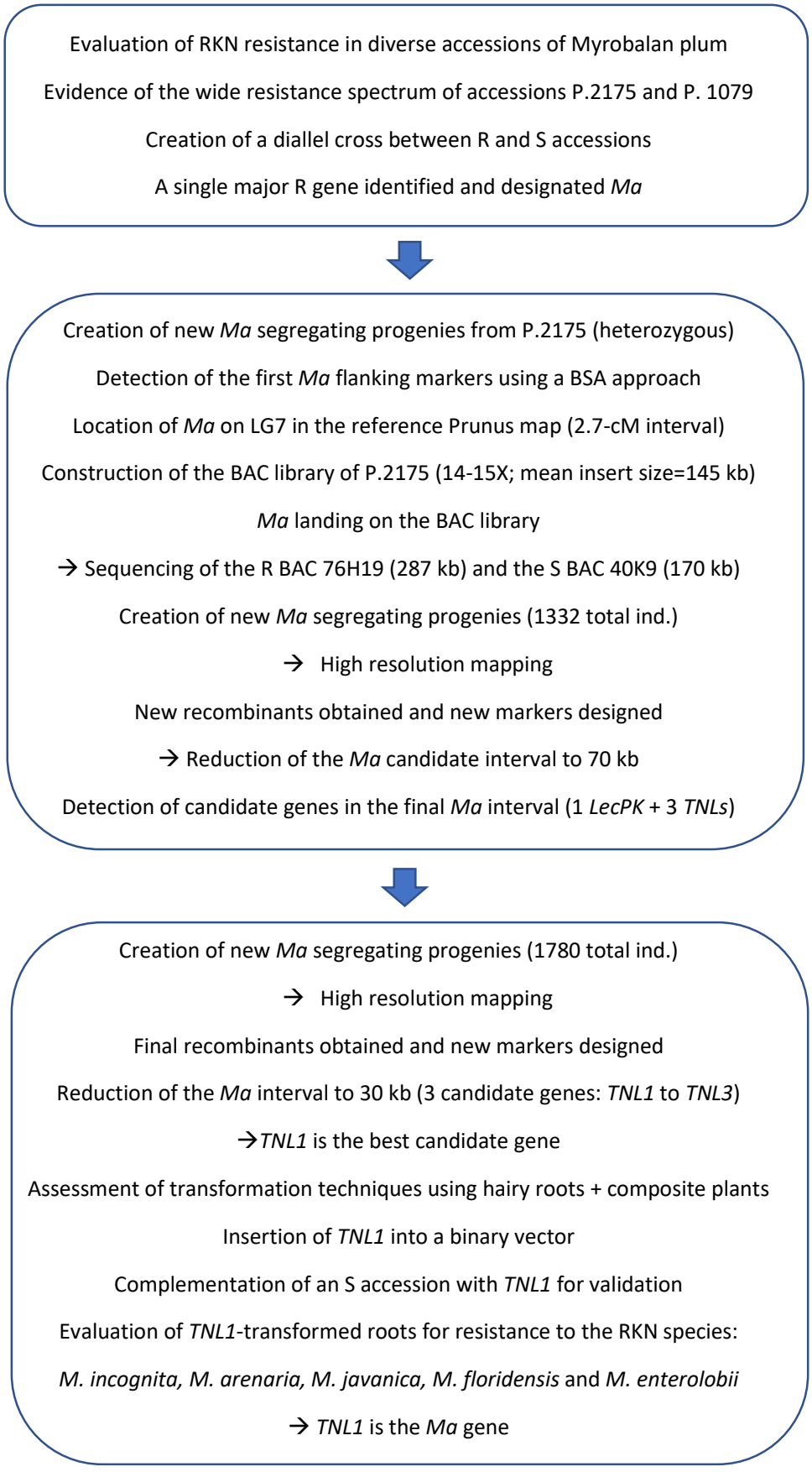

Figure 1. From the plant to the gene: the steps of the RKN resistance study in Myrobalan plum.

4.3. Histological Mechanisms of RKN Resistance

Histological resistance mechanisms have been studied in plum and peach. *Ma*-resistant plums have no galls, and no swollen larval nematode stages are observed, regardless of the RKN species considered. In those plums, the early penetration *of M. arenaria* J2s was shown to be similar in resistant and susceptible (S) (=lacking the *Ma* resistance gene) accessions [33]. In both the R and S accessions studied, J2s enter the elongation zone above the root cap, migrate downwards to the apical meristematic region and move then upwards in the direction of the stele [32]. In susceptible tissues, J2s complete their life cycle, whereas in resistant tissues, a hypersensitive-like response (HLR) of the root cells blocks the nematode development in its filiform state, either in the apical meristematic region or in the stele. Severe damage due to J2s in the apical meristem induces the development of new subterminal rootlets [32,33], which is an active reaction to the necroses of terminal root cells.

In peach, studies on the histological mechanisms of resistance in the rootstocks Nemaguard and Nemared have been conducted. In both rootstocks, which are considered resistant to certain *M. javanica* populations despite the formation of small galls, a protective 'walling off' process isolating young giant cells from neighboring cells has been observed. The giant cells then became necrotic and collapsed, thereby preventing the maturation of the females, and this nematode restriction was accompanied by callose deposition within the compact cell layer around the infection site [59–61].

4.4. Other Features of Resistance in Prunus: Durability, Stability at High Temperature and Relationship with Other Pests and Pathogens

An important feature for breeding RKN-resistant *Prunus* cultivars is the level of durability conferred by the R genes. This is a key trait since the perennial nature of trees increases the risk of resistance breakdown. Durable resistance, as defined by Johnson [62], is resistance that remains effective during extensive use in agriculture, over a long period, in an environment conducive to the disease. In interspecific *Prunus* hybrids, the resistance conferred by *Ma* alone, *RMia* alone or by a combination of *Ma* and *RMia*, has not been overcome when challenged, under greenhouse conditions, by continuous *M. incognita* inoculum pressure for up to four years. By contrast the resistance conferred by *Mi-1* in tomato broke down after only one to two years [63]. Additionally, the effect of temperature on resistance expression has also been assessed. The *Ma* heterozygous-resistant accession P. 2175 (*Ma/ma*)and the *Ma* homozygous-resistant accession P. 1079 (*Ma/Ma*) were not affected by a continuous temperature of 32 °C, which showed that *Ma* resistance is stable at high temperatures [64].

The relationship between resistance to RKNs and other pests and pathogens of the *Prunus* spp. has been studied towards the soil bacterium *Agrobacterium. tumefaciens* and towards the root endoparasitic nematode *Pratylenchus vulnus*. *Agrobacterium tumefaciens* is responsible for a very common disease in the roots of stone fruit crops, the crown gall disease. *Meloidogyne* spp., when present, facilitate the expression of crown gall symptoms in RKN-susceptible rootstocks. The *Ma* gene has been shown to have a protective effect against the expression of bacterial symptoms in the presence of RKN infections, as no nematode or bacterial symptoms have been observed in *Ma*-resistant materials [65]. This effect of *Ma* and presumably of other RKN R genes against *Meloidogyne*-transmitted crown gall is an additional argument for gene introgression into *Prunus* rootstocks.

The root-lesion nematode (RLN) *P. vulnus* is an endoparasitic species that differs from *Meloidogyne* spp. with its migratory behaviour at all its developmental stages. A study by Stalin et al. [66] illustrated that the *Ma* gene does not confer resistance to the RLN *P. vulnus*, which could be expected given the completely different mode of parasitism of the two nematode genera.

5. High-Resolution Mapping and Cloning of the *Ma* Locus

The *Ma* gene, the first RKN R gene characterized in a perennial, has remarkable biological properties in comparison with the *Mi1-2* tomato R gene. As reported earlier the resistance that *Ma* confers is high and durable, whatever the temperature, and controls all the RKN species attacking the *Prunus* spp. These remarkable features have justified a positional cloning strategy in order to reveal its structure and function. Within stone fruit crops, it is also the first R gene to be cloned [67,68]. Successive steps to reach this objective are summarized hereafter and in the second part of Figure 1.

5.1. Positional Cloning and Functional Characterization of the Ma Gene

5.1.1. High-Resolution Mapping, BAC Library Construction and Chromosome Landing

Molecular mapping of the *Ma* locus began with the detection of two SCAR (sequence characterized amplified region) markers, SCAL19 and SCAFLP2, tightly linked to *Ma* [69,70] using a BSA (bulked segregant analysis) approach. *Ma* was then mapped on the chromosome 7 of the reference European *Prunus* map in a 2.7 cM interval between the SSR (simple sequence repeat) pchgms6 and SCAL19 markers [71]. A total of 1332 individuals revealed 31 individuals recombining between SCAL19 and pchgms6 in this interval [71]. The recombinant individuals allowed a finer location of the gene, as *Ma* co-segregated with the SCAFLP2 marker and was separated from SCAFLP3 (proximal) and SCAFLP4 (distal) by a single and 11 recombination events, respectively.

The Myrobalan accession P. 2175, heterozygous for *Ma*, was used for the construction of a DNA BAC library comprising 30,720 clones with a mean insert size of 145 kb and a 14–15x *Prunus* haploid genome coverage. A few BAC clones from the library allowed to land on the *Ma* spanning interval and to construct the resistant and susceptible physical contigs using PCR screening with codominant markers. Additional microsatellite markers were designed from BAC subcloning or BAC end sequencing. Finally, in the resistant contig, a single 287-kb BAC clone (BAC 76H19) was shown to carry the *Ma* gene (=R dominant allele); this BAC clone contained two flanking markers on each side of the gene as well as two cosegregating markers [71]. BAC 40K9 (170 kb) was shown to carry the S recessive allele (*ma*).

5.1.2. Evidence of the *TNL* Cluster Containing *Ma*

Complete sequencing of BAC 76H19 allowed new SCAR and SSR markers to be placed on the sequence and the determination of a physical interval of 174 kb containing *Ma* [72]. The new markers SSR2 and SCAFLP4, manually designed within this interval, reduced the region containing the gene to 70 kb with four candidate genes: one lectin protein kinase (*LecPK*) and three TIR-NB-LRRs (*TNLs*). In order to locate *Ma* more precisely, a final round of mapping using 1780 additional segregating individuals was undertaken. Among these, some carried the *RMia* gene from the Nemared peach and were separated from those carrying *Ma* by resistance assays using *M. floridensis*. A total of 19 new recombinants were then genotyped for nine SCAR or SSR markers designed within the 70-kb candidate interval. The phenotype of five recombinant genotypes located within this interval and evaluated for *M. floridensis* reduced the final candidate region to the 32-kb sequence encompassing a cluster of three *TNL* genes *(TNL1* to *TNL3)*.

The sequence of the susceptible BAC 40K9, 170 kb long, encompassing the S allele of the *Ma* candidate region [71], allowed for the identification of the three *TNLs* (*tnl1* to *tnl3)* orthologous to the resistant haplotype *(TNL1* to *TNL3)*. A prediction of intron/exon structures showed that *TNL2* was a pseudogene (deletion of a single base leading to a frameshift mutation) and that *TNL3* was truncated in its LRR repeat motif and was much shorter. Thus, *TNL1* appeared to be the best candidate gene.

5.1.3. Validation of the Final Candidate *TNL1* and Evidence of the Remarkable Structure of the *Ma* Gene

The complete cDNA sequence of this best candidate revealed a gene size of 6147 bp (without UTRs) and a predicted protein of 2048 aa [72]. A *TNL1* sequence of 15 kb containing the 10 kb genomic sequence and a 5 kb native promotor sequence was cloned. Validation of the *TNL1* candidate gene was then performed using hairy roots (*A. rhizogenes*), and several transformation events of a susceptible clone [73] were resistant to all RKNs tested, which unequivocally proved that *TNL1* was the *Ma* gene. Thus, the *Ma* gene is a *TNL* consisting of nine exons separated by introns of variable sizes. The first four exons code, respectively, for the protein domains TIR, NB, NLL and LRR, the so-called 'NLL' exon (NBS-LRR Linker), encoding a sequence linking the very different structures of NB and LRR domains. The last five exons, designated as post-LRR (PL) domains because of their location in C-terminal position in the gene, encode five modules, separated by introns containing microsatellites [72]. The similarities noted between these PL domains indicate that they are repeated exons of approximately 200–250 amino acids.

The addition of these five PL exons to the gene structure makes *Ma* a *TNL* twice as large as the so-called classical *TNLs*. A further study showed that *TNL* genes with multiple PL domains are rare in peach and the other plants screened. Moreover, the five-PL domain pattern is probably unique to *Ma* and its orthologues within *Prunus* and closely related genera from the Rosaceae and was probably inherited from the common ancestor of these plants in the subfamily Spiraeoideae. To date, *Ma* is the longest *TNL* R gene cloned in plants [74].

5.2. High-Resolution Mapping of the Almond RMja Gene, a Ma Orthologue

An F2 almond progeny of approximately 1500 individuals from a cross between *P. dulcis* cv. Alnem 1, a homozygous carrier of the *RMja* gene (*RMja/RMja*), and *P. dulcis* cv. Lauranne, which is susceptible to *M. javanica* (*rmja/rmja*), was used. Simultaneously, SSR and SNP (single nucleotide polymorphism) markers flanking the *Ma* gene locus were generated from the peach genome and were used to genotype all individuals. Two flanking markers with a single recombinant and thus closely linked to *RMja* were identified. These data show that, in the peach genome [50], the *RMja* gene was located in an interval of less than 100 kb. This window spans a cluster of four *TNLs* in peach that contains the orthologue of the *Ma* gene. In order to obtain a reliable sequence of the region containing *RMja* in almond, the BAC libraries of the Alnem 1 and Lauranne parental accessions were generated. Two BAC clones flanking the gene in Alnem 1 were identified, sequenced and used to generate an assembled sequence of 336 kb containing the *RMja* gene. The location of markers from the peach genome on the Alnem 1 sequence revealed the presence of only three genes in the *RMja* candidate interval, two of which have an altered structure.

A complementary bioinformatic analysis associated with expression data allows for the conclusion that the *RMja* gene is the ortholog of the *Ma* gene in almond. The *RMja* gene has an architecture which is highly similar to *Ma*. Nevertheless, the polymorphism, located mainly in the PLs domains, might explain the difference in the resistance spectrum of the two genes. More generally, the comparison of the *TNL* cluster sequences in almond (from BAC sequences [53]), in peach (from the peach genome [50] and in plum (from the BAC sequences [71,72]) allowed for the proposed hypotheses on the genetic mechanisms that led to the shaping and evolution of the particular structures in this cluster.

6. Conclusions and Perspectives

Research on resistance to root-knot nematodes in stone fruit crops began early in the USA, and data obtained there was completed later by diverse studies conducted in other Mediterranean countries (Israel, France, Spain, etc.). Beyond the three RKN species identified before the 1980s, new species have been described that have a more restricted geographical distribution but can be destructive when present. In each of the *Prunus* species most severely affected by these pests, i.e., the peach, the almond and the plum, at

least one resistance source has been characterized and the cognate major genes identified and mapped. Studies on the key features of the genes have been conducted; in particular, the resistance spectrum of *Ma, RMia* and *RMja* now considers the array of six predominant RKN species attacking stone fruit crops. Such information will facilitate the creation of complete-spectrum resistance in *Prunus* rootstocks. It also opens the way for breeders to pyramid several resistance layers to *M. arenaria* and *M. ethiopica* (*Ma* + *RMia* + *RMja*), *M. javanica* and *M. enterolobii* (*Ma* + *RMja*), and *M. incognita* (*Ma* + *RMia*), while *M. floridensis* is the sole species controlled by *Ma* alone (Table 1).

The high-resolution mapping of the *Ma* locus revealed the unprecedented structure of the gene and of one of its alleles (*RMja*). The fact that *Ma* controls all *Prunus* RKN species might underpin an original function of the gene. This function presumably renders it able to recognize, directly or indirectly, either a wide range of effector molecules differing between each of the diverse RKN species controlled, or a single or a few effector molecules that are common to all of them [13]. Another important point is that three *Ma* allelic (= orthologous) sequences with different resistance spectra are now available. In line with these data, research will have to fully consider comparative genomics within the *Ma* locus to identify the resistance determinants (sequences and/or motifs) and in particular, those involved in the resistance specificity. Methods and model studies published recently open the way in this objective [75–77]. The noteworthy C-terminal part of the gene with a huge post-LRR region (=5 repeated PL domains) makes it the longest R gene ever cloned. A recent work has shown that integrity of the single PL domain often carried by *TNL* genes such as *RPS4* (Arabidopsis) or *N* (tobacco) is required for function [78], even though its role is still ignored. Consequently, deciphering whether and, in the presumed positive case, how this 5-PL region is involved in the remarkable biological properties of *Ma* remains a real challenge for the future.

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