Plant Genetic Background Increasing the Efficiency and Durability of Major Resistance Genes to Root-knot Nematodes Can Be Resolved into a Few Resistance QTLs

Arnaud Barbary, Caroline Djian-Caporalino, Nathalie Marteu, Ariane Fazari, Bernard Caromel, Philippe Castagnone-Sereno* and Alain Palloix

1 INRA, University of Nice Sophia Antipolis, CNRS, UMR 1355-7254, Institut Sophia Agrobiotech, Sophia Antipolis, France, 2 INRA, UR1052, Génétique et Amélioration des Fruits et Légumes, Montfavet, France

With the banning of most chemical nematicides, the control of root-knot nematodes (RKNs) in vegetable crops is now based essentially on the deployment of single, major resistance genes (R-genes). However, these genes are rare and their efficacy is threatened by the capacity of RKNs to adapt. In pepper, several dominant R-genes are effective against RKNs, and their efficacy and durability have been shown to be greater in a partially resistant genetic background. However, the genetic determinants of this partial resistance were unknown. Here, a quantitative trait loci (QTL) analysis was performed on the F2 population from the cross between Yolo Wonder, an accession considered partially resistant or resistant, depending on the RKN species, and Doux Long des Landes, a susceptible cultivar. A genetic linkage map was constructed from 130 F2 individuals, and the 130 F3 families were tested for resistance to the three main RKN species, Meloidogyne incognita, M. arenaria, and M. javanica. For the first time in the pepper-RKN pathosystem, four major QTLs were identified and mapped to two clusters. The cluster on chromosome P1 includes three tightly linked QTLs with specific effects against individual RKN species. The fourth QTL, providing specific resistance to M. javanica, mapped to pepper chromosome P9, which is known to carry multiple NBS–LRR repeats, together with major R-genes for resistance to nematodes and other pathogens. The newly discovered cluster on chromosome P1 has a broad spectrum of action with major additive effects on resistance. These data highlight the role of host QTLs involved in plant-RKN interactions and provide innovative potential for the breeding of new pepper cultivars or rootstocks combining quantitative resistance and major R-genes, to increase both the efficacy and durability of RKN control by resistance genes.

Keywords: Capsicum annuum, Meloidogyne spp., quantitative resistance, major resistance, resistance durability
INTRODUCTION

Root-knot nematodes (RKNs), *Meloidogyne* spp., are major plant pathogens worldwide. These extremely polyphagous endoparasites can infest more than 5,500 plant species, including many field and greenhouse crops (Goodey et al., 1965). Since the banning of most chemical nematicides, due to environmental and public health issues, resistant cultivars have become an increasing important weapon against these pests. This method efficiently controls RKN populations, and is economically sustainable, innocuous to health and environment-friendly. RKN resistance is generally mediated by single, major resistance genes (*R*-genes), which can easily be introgressed into cultivars through back-crossing and phenotypic or marker-assisted selection (MAS). For this reason, major *R*-genes are widely used in the breeding of RKN-resistant cultivars and/or rootstocks. However, their efficacy is threatened by the capacity of RKNs to adapt. Indeed, *R*-genes apply a selective pressure on nematode populations, increasing the risk of virulent nematode populations emerging (Castagnone-Sereno, 2006), and this greatly limits their use. Several management strategies have been developed, to prevent the breakdown of resistance by pathogens. Most of these approaches are based on spatiotemporal management of the deployment of *R*-genes: (i) alternation of different *R*-genes in the crop rotation, (ii) use of mixtures of cultivars with different *R*-genes, or (iii) pyramiding, the introduction of several *R*-genes into the same cultivar (Kiyosawa, 1982; Mundt, 2002; Pink, 2002). The use of such strategies requires several *R*-genes to be available, with no emergence of cross-virulent pathogens. Recent experimental studies have shown that the pyramiding of *R*-genes is the best method for promoting effective, durable RKN resistance in pepper (Djian-Caporalino et al., 2014).

Several recent studies, on very different pathosystems, have shown that the genetic background of the plant greatly influences *R*-gene efficiency, potentially slowing the adaptation of pathogen populations to *R*-gene-carrying cultivars (Palloix et al., 2009; Brun et al., 2010; Fournet et al., 2013; Barbary et al., 2014). In some pathosystems, this greater durability has been shown to result from quantitative trait loci (QTLs), which slow the selection of variants virulent against the *R*-gene and decrease the size of the pathogen population (e.g., Quenouille et al., 2014). Plant genetic background is rarely considered in breeding programs for RKN resistance, despite its contribution to *R*-gene efficiency and durability. Indeed, breeding for resistance with QTLs is more complex and costly than the use of *R*-genes, or (iii) pyramiding, the introduction of several *R*-genes to the same cultivar (Kiyosawa, 1982; Mundt, 2002; Pink, 2002). The use of such strategies requires several *R*-genes to be available, with no emergence of cross-virulent pathogens. Recent experimental studies have shown that the pyramiding of *R*-genes is the best method for promoting effective, durable RKN resistance in pepper (Djian-Caporalino et al., 2014).

Several recent studies, on very different pathosystems, have shown that the genetic background of the plant greatly influences *R*-gene efficiency, potentially slowing the adaptation of pathogen populations to *R*-gene-carrying cultivars (Palloix et al., 2009; Brun et al., 2010; Fournet et al., 2013; Barbary et al., 2014). In some pathosystems, this greater durability has been shown to result from quantitative trait loci (QTLs), which slow the selection of variants virulent against the *R*-gene and decrease the size of the pathogen population (e.g., Quenouille et al., 2014). Plant genetic background is rarely considered in breeding programs for RKN resistance, despite its contribution to *R*-gene efficiency and durability. Indeed, breeding for resistance with QTLs is more complex and costly than the use of *R*-genes. In particular, the introgression of QTLs into elite cultivars must not impair other agronomically important crop traits, such as yield, quality criteria and adaptation, or other physiological characteristics.

In pepper (*Capsicum annuum* L.), several dominant *R*-genes, the *Me* genes and the *N* gene, have been characterized in detail (Hare, 1956; Hendy et al., 1985; Djian-Caporalino et al., 1999; Thies and Fery, 2000). These genes map to a genetic cluster on pepper chromosome P9 (Djian-Caporalino et al., 2007; Fazari et al., 2012). Three of these genes, *Me3*, *Me1*, and *N*, are routinely used in breeding programs. These genes are effective against a wide range of RKN species, including *M. arenaria*, *M. incognita*, and *M. javanica*, the most common species in temperate and tropical areas. They differ in their mode of action: *Me3* and *Me1* are stable at high temperature (Djian-Caporalino et al., 1999), whereas the efficacy of *N* is temperature-dependent (Thies and Fery, 1998). In addition, *Me3* and *Me1* differ in the spatiotemporal location of the resistance response triggered by a nematode attack. *Me3* triggers an early hypersensitive response in the root epidermis at the nematode penetration site, whereas *Me1* triggers a later response in the root vascular cylinder (Bleve-Zacheo et al., 1998; Pegard et al., 2005). It was long thought that there was a relationship between the mode of action of these *R*-genes and their durability, as the emergence of virulent populations has been reported only for *N* and *Me3* (Castagnone-Sereno et al., 1996; Thies, 2011). However, the risk of *Me1*-virulent RKN populations emerging and of the development of multi-virulent populations might increase with the extensive deployment of these resistance genes in agriculture. The efficacy of these genes has been shown to be higher when they are introgressed into a partially resistant background and lower if they are introgressed into a highly susceptible background (Barbary et al., 2014). These same genetic backgrounds were also previously shown to affect the durability of field resistance (Djian-Caporalino et al., 2014). However, no quantitative resistance loci that could be combined with major genes to increase the efficacy and durability of genetic RKN control have yet been identified in the pepper germplasm.

We report here a QTL analysis dissecting the genetic backgrounds previously shown to modulate the efficacy and durability of resistance. An F$_{2:3}$ progeny derived from a cross between a partially resistant (Yolo Wonder, YW) and a highly susceptible (Doux Long des Landes, DLL) pepper inbred line was tested for quantitative resistance to the three main RKN species (*M. incognita*, *M. arenaria*, and *M. javanica*). Four new major QTLs were mapped to two separate clusters. The first, containing one QTL, colocalized with the cluster of *Me* genes on pepper chromosome P9. The second included three QTLs against the three *Meloidogyne* species located on pepper chromosome P1. This new cluster could potentially be used for innovative breeding strategies to increase *R*-gene efficacy and durability for the control of RKNs.

MATERIALS AND METHODS

Plant Material

A population of 130 F$_{2:3}$ families derived from a cross between YW and DLL was used. These pepper cultivars were selected from the INRA pepper germplasm collection at Avignon, France (CRB-Leg, INRA-GAFL), on the basis of their different levels of resistance to nematode species. DLL is highly susceptible to the three main RKN species: *M. arenaria*, *M. javanica*, and *M. incognita*. YW is partially resistant (i.e., low-level symptoms) to *M. incognita* (*Figure 1*), totally resistant to *M. javanica* and has variable levels of resistance to *M. arenaria* (i.e., totally or partially resistant), depending on the RKN isolate considered (Djian-Caporalino et al., 1999). A single F$_1$ hybrid plant was self-pollinated...
The first, *M. incognita* (Morelos isolate), causes disease on DLL, which is susceptible, whereas YW is highly resistant to these two species (Djian-Caporalino et al., 1999). These nematodes were obtained from the INRA Meloidogyne collection maintained at Institut Sophia Agrobiotech in Sophia Antipolis, France. These three *Meloidogyne* species have a mitotic parthenogenetic mode of reproduction. We therefore considered all the second-stage juveniles (J2s) hatching from a single egg mass to constitute a clonal line. Before multiplication, these isolates were specifically identified on the basis of isooesterase electrophoresis (Dalmasso and Berge, 1978) or sequence characterized amplified region (SCAR) PCR (Zijlstra et al., 2000).

### DNA Extraction, Genotyping of Molecular Markers, and Linkage Map

Genomic DNA was isolated from the young leaves of both parents, the F1 and the individuals of the mapping population, as described by Fulton et al. (1995). After RNAse treatment, the concentration and purity of DNA were assessed with a NanoDrop 2000 spectrophotometer (Thermo Scientific), and the final DNA concentration was adjusted to 20 ng/µL for PCR.

The F2 mapping population was genotyped with 58 markers previously used in other populations: one B94 SCAR marker (Djian-Caporalino et al., 2007), 13 simple sequence repeat (SSR) markers previously mapped in pepper (Alimi et al., 2013), 44 single-nucleotide polymorphism (SNP) markers from Nicolai et al. (2012). In addition, 272 new SNP markers were provided by Syngenta Seeds. We used these markers to construct a genetic linkage map with Mapmaker software version 3.0b (Lander et al., 1987), using a LOD score threshold of 3.0 and a maximum recombination fraction of 0.3. Distances between markers were calculated with the Kosambi mapping function. For each linkage group (LG), marker order was checked with the ‘ripple’ command and markers were retained only if the LOD score value was greater than 2.0. The LGs were assigned to pepper chromosomes on the basis of the positions of SSR and SNP markers common to the genetic maps for pepper published by Alimi et al. (2013) and Quenouille et al. (2014).

### Experimental Procedures for Evaluating Nematode Resistance

Resistance was assessed on the F3 progenies. For each RKN isolate, 16 F3 seeds per F2:3 family were sown individually in 9 cm plastic pots containing steam-sterilized sandy soil covered with a 1 cm layer of loam. F3 plants were split evenly (i.e., eight plants per F3) between two growth chambers maintained at 24°C (±2°C) with a 12-h light:12-h dark cycle and a relative humidity of 60–70%. Parental lines, the F1 progeny and two resistant controls (HD149 and HD330) were included in the experimental design. The 130 F2:3 families and controls were randomly arranged within each growth chamber. Six to seven-week-old plants (4–6 true leaves) were each inoculated with 500 freshly hatched J2s suspended in water, for experiments with *M. arenaria* and *M. javanica*, and with 1,000 J2s suspended in water for experiments with *M. incognita*. This difference in inoculum was based on the behavior of YW with respect to the species used (resistant or partially resistant). It has been shown that a higher inoculum density is required to reveal the differences between the partially resistant parent (YW) and the highly susceptible parent (DLL). J2s were obtained in a mist chamber, from previously inoculated susceptible tomato roots (cultivar Saint Pierre). Six to seven weeks after inoculation (i.e., a period long enough for completion of the nematode life cycle), plants were harvested, carefully washed individually with tap water and frozen at −20°C until scoring. Before analysis, the roots were thawed and stained by incubation for 10 min in a cold aqueous solution of eosin (0.1 g/l water), for the specific staining of egg masses (EMs). The roots were rinsed and examined under a magnifying glass. The number of EMs was counted for each plant.
and the median number of EMs per plant for each F₂ family (and for the control genotype) was determined, for estimation of the phenotypic value of each F₂.

**Statistical Analyses**

R software¹ was used for descriptive statistics. Analyses of variance (ANOVA) were carried out for each phenotypic trait (i.e., for resistance to each RKN species), to estimate genotypic/environmental effects. For each phenotypic trait, narrow-sense heritability (h²) was estimated with the formula

\[ h^2 = \frac{s^2_G}{s^2_G + s^2_E + s^2_N/n} \]

where \( s^2_G \) corresponds to the genotypic variance and \( s^2_E \) to the environment variance (including block, interaction, and error effects) and \( n \) is the number of replicates per F₂,₃ family (two growth rooms). Additive and dominance effects were calculated as described by Stuber et al. (1987). The normality of phenotype distributions was assessed with Shapiro–Wilk tests (\( a = 0.05 \)).

Quantitative trait loci analyses were performed with the R/qtl package of R software (Broman et al., 2003). QTLs were detected by regression analysis, SIM, CIM, and non-parametric interval mapping (model = “np” in the R/qtl package) for the non-Gaussian phenotype distributions, although the residues were normal, making it possible to carry out a regression analysis to estimate additive and \( R^2 \) values. All the methods yielded similar results (the same QTLs at the same positions; data not shown), although the QTL peaks were slightly less sharp with the non-parametric procedure. A permutation test was performed with 1,000 replicates to determine the genome-wide LOD threshold empirically at the 5% probability level, for each phenotypic trait individually. The LOD threshold was estimated at 3.6 for the three traits. For each QTL, the confidence interval (CI) was defined as a 2-LOD drop-off around the maximum LOD score. \( R^2 \) coefficients were calculated with the ‘fitqtl’ function of R/qtl.

**RESULTS**

**Linkage Map**

Four of the 330 markers tested on the F₂ mapping population remained unlinked. The genetic linkage map was therefore constructed with 326 markers: 13 SSRs, 312 SNPs, and 1 SCAR. Markers displaying significant deviation from the expected Mendelian ratio of 1:2:1 were retained (indicated by asterisks in Supplementary Data 1). The map comprised 12 LGs, covering an overall length of 1436 cM (Supplementary Data 1). The map comprised 12 LGs, covering an overall length of 1436 cM (Supplementary Data 1). The map comprised 12 LGs, covering an overall length of 1436 cM (Supplementary Data 1). The map comprised 12 LGs, covering an overall length of 1436 cM (Supplementary Data 1).

**Segregation of Resistance to the Three RKN Species**

The frequency distributions of resistance to *M. incognita*, *M. arenaria*, and *M. javanica* in the F₂,₃ families are shown in Figure 2. For *M. incognita*, the effects of both genotype and block were significant (\( p\)-value = 0.000653 and \( p\)-value < 2.00e⁻¹⁶, respectively) as revealed by ANOVA. The regression of F₃ values from block 1 over block 2 was significant (\( R_{Pearson} = 0.32, p = 0.00027 \)) with higher values in block 1. Individual EM data were therefore adjusted by multiplying the data from block 2 by the regression coefficient (value: 1.4). This linear adjustment removed the block effect and the data were pooled for further analyses. The phenotypes of the control pepper lines were consistent with the expected results (Table 1): YW presented a lower infestation rate than DLL (mean of 250 and 330 EMs/plant, respectively). The F₁ progeny was skewed toward DLL (319 EMs/plant), with a \( d/a \) ratio of –0.72, indicating additive to partly dominant inheritance in favor of susceptibility. A Shapiro–Wilk test showed that the values for the F₂,₃ families were normally distributed (\( W = 0.99, p\)-value = 0.2029; Figure 2A). The \( h^2 \) value was 0.48.

For the experiment with *M. arenaria*, ANOVA revealed a significant effect of genotype (\( p\)-value = 3.26e⁻¹⁵), but no significant block effect (\( p\)-value = 0.0805). YW and DLL were resistant and susceptible (mean values of 5 and 69 EMs/plant, respectively), as expected (Table 1). The F₁ phenotype was skewed toward YW (14 EMs/plant), with a \( d/a \) ratio of 0.72, indicating that resistance was additive to partly dominant in favor of resistance. The values for the F₂,₃ families were not normally distributed, as confirmed by a Shapiro–Wilk test (\( W = 0.92, p\)-value = 8.63e⁻⁰⁷; Figure 2B). The distribution was skewed toward resistance. Neither logarithmic nor square-root transformation resulted in normality (data not shown). Resistance to *M. arenaria* was highly heritable, as \( h^2 \) was 0.76.

For the experiment with *M. javanica*, ANOVA showed a significant effect of genotype (\( p\)-value < 2.00e⁻¹⁶) but no significant block effect (\( p > 0.183 \)). YW was highly resistant and DLL was susceptible, as expected (means of 1 and 131 EMs/plant, respectively; Table 1). The F₁ displayed an intermediate phenotype, with skewing toward YW (mean of 17 EMs/plant), with a \( d/a \) ratio equal to 0.75, indicating that resistance was mostly dominant, but slightly additive. The Shapiro–Wilk test indicated that the data for the F₂,₃ families were not normally distributed (\( W = 0.66, p\)-value = 2.80e⁻¹⁵; Figure 2C). Neither logarithmic nor square-root transformation yielded normality. Heritability was high for resistance to *M. javanica* (\( h^2 = 0.87 \)).

**Mapping QTLs for Resistance to the Three Meloidogyne Isolates**

Simple interval mapping (SIM), composite interval mapping (CIM) and non-parametric (“np” or Kruskal–Wallis) analyses were performed to identify QTLs for resistance. However, as CIM and non-parametric analyses did not improve QTL detection, only the results for SIM are shown. Only one QTL for resistance to *M. incognita* was detected on pepper chromosome P₁, with a LODmax at 179.2 cM and an \( R^2 \) value of 40.9, corresponding to 85% of the heritability (\( h^2 = 0.48 \); Figure 3; Table 2). This QTL was named *Minc-P1*. The \( d/a \) ratio of –0.38 at the closest marker (SP1790) indicates a mostly additive effect of this QTL, with a partial dominance effect for susceptibility.

¹http://www.r-project.org/
FIGURE 2 | Frequency distribution of the resistance to different root-knot nematode (RKN) species of the pepper F2,3 families: (A) Meloidogyne incognita; (B) Meloidogyne arenaria; and (C) Meloidogyne javanica. Arrows indicate the position of each parent and the F1 in the phenotypic distribution, with their values indicated in brackets. YW, Yolo Wonder; DLL, Doux Long des Landes; F1, (YW × DLL).

TABLE 1 | Summary of root-knot nematode (RKN) reproduction capacity in the parental lines, F1 and F2,3 progeny (130 F2,3 families) from the pepper cross (YW × DLL), for different RKN species.

| RKN species       | Parents | F1 | F2,3 families |
|-------------------|---------|----|--------------|
|                   | YW      | DLL| Maximum      |
| Meloidogyne incognita | 250     | 330 | 319          |
| Meloidogyne arenaria | 5       | 69  | 14           |
| Meloidogyne javanica | 1       | 131 | 17           |

Reproduction capacities were evaluated as the median number of egg masses (EMs) on 16 plants for each genotype. YW, Yolo Wonder (resistant or partially resistant genotype); DLL, Doux Long des Landes (susceptible genotype); F1, hybrid F1; SD, standard deviation; $h^2$, broad-sense heritability ($h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_E^2 / n)$).

Similarly, only one QTL for resistance to M. arenaria was detected and mapped to chromosome P1 (Mare-P1) with a LODmax at 177.0 cM, close to Minc-P1. This QTL, with an $R^2$ value of 73.8 (97% of the $h^2$ value) and a $d/a$ ratio of 0.28, acts as a major additive QTL, with a weak dominance effect in favor of resistance.

Two QTLs for resistance to M. javanica were detected. The first, Mjav-P1, was located on chromosome P1 with a LODmax at 178.0 cM, an $R^2$ of 31.9 and a $d/a$ ratio of 0.29, indicating a mostly additive effect. The second QTL, Mjav-P9, was detected on the distal part of chromosome P9 (closest marker SP381) with an $R^2$ of 52.4 and a $d/a$ of 0.73, indicating a mostly dominant effect in favor of resistance. Together, Mjav-P1 and Mjav-P9 explained 61.2% of the phenotypic variance, corresponding to 71% of the $h^2$ value.

All the resistance-conferring alleles at these four QTLs originated from the partially resistant parent YW.

Looking for Recombinant Individuals Within the P1 QTL Cluster

F2 individuals with recombinant genotypes for the QTL cluster on chromosome P1 were surveyed, focusing on their genotypes for the markers and the phenotypes of their F3 progenies (homogeneous resistant, homogeneous susceptible, or segregating). Four F2 individuals were found to be recombinant for the alleles at the markers within the P1 QTL cluster (recombination between SP1790 and SP1798). Two F2,3 progenies for these individuals clearly corresponded to phenotypic recombinants in terms of their resistance to M. arenaria and M. incognita, as attested by the phenotypes of the F3 progenies (Table 3). Resistance to M. javanica was less informative, probably due to the major effect of the second locus Mjav-P9.

DISCUSSION

The pepper genetic map constructed in this study with 130 F2 plants from the cross between YW and DLL comprised 12 LGs, consistent with the known number of chromosomes in pepper, and it covered a total length of 1436 cM, similar to previous maps for pepper (Lefebvre et al., 2002; Paran et al., 2004; Wu et al., 2009). However, as only a few of the previously used markers proved to be polymorphic between the parental lines YW and DLL, new SNPs had to be developed to complete the map. The sequences supporting the SNPs targeting the QTLs are provided in Supplementary Data 2. This new mapping population was developed because it was shown in previous studies that R-genes are more effective and durable against RKN attacks when introgressed into the YW genetic background than when introgressed into the DLL genetic background (Barbary et al., 2014; Djian-Caporalino et al., 2014). This difference is thought to result from the partial resistance alleles carried by YW, which seem to protect the R-genes. This new map was
constructed to identify these resistance factors and associated molecular markers, which should constitute valuable resources for further MAS. The 130 F$_{2:3}$ [YW × DLL] families were tested against the three main RKN species, *M. incognita*, *M. arenaria*, and *M. javanica*. The QTL analyses identified four new major QTLs affecting reproductive capacity, located in two separate clusters. No minor-effect QTL was detected, and the phenotypic variance explained by the major QTLs for each resistance trait closely fitted the $h^2$ values (71–97%), indicating that almost all the genetic variance was explained by these major QTLs. Three of these QTLs were grouped on pepper chromosome P1, with overlapping CIs, in a 30 cM region. The YW alleles at these QTLs each confer resistance to a single species of RKN: *M. incognita*, *M. arenaria*, or *M. javanica*. These are the first QTLs conferring resistance to RKNs to have been detected in pepper, but QTLs conferring resistance to *Meloidogyne* spp., have already been mapped in soybean (Li et al., 2001), cotton (Shen et al., 2006), and cowpea (Muchero et al., 2009). This is also the first report of nematode resistance factors mapping to a genomic location other than chromosome P9 in pepper (i.e., on chromosome P1). All the RKN R-genes previously identified in pepper mapped to a cluster on P9 (Djian-Caporalino et al., 2007; Fazari et al., 2012). It is unclear whether Minc-P1, Mare-P1, and Mjav-P1 on P1 are all part of a single gene with a broad spectrum of action, or whether they belong to separate genes forming a new cluster, as observed on

**TABLE 2** | Quantitative trait loci (QTLs) for resistance to the different RKN species in the pepper F$_{2:3}$ progeny.

| RKN species | QTL | Chr. | Location (cM) | Closest marker | LOD | CI$^b$ | Additive effect (a) | Dominance effect (d) | d/a ratio | $R^2$ |
|-------------|-----|------|--------------|---------------|-----|-------|-------------------|--------------------|-----------|------|
| *M. incognita* | Minc-P1 | 1 | 179.2 | SP1790 | 14.1 | 173.4–184.0 | –55.7 | 21.1 | –0.38 | 40.9 |
| *M. arenaria* | Mare-P1 | 1 | 177.0 | SP1798 | 36.6 | 173.4–179.0 | –24.5 | –6.8 | 0.28 | 73.8 |
| *M. javanica* | Mjav-P1 | 9 | 1.0 | SP381 | 12.9 | 0.0–6.4 | –19.1 | –13.9 | 0.73 | 52.4 |

$^a$Chromosome. $^b$CI: Confidence interval, defined as a LOD-2 drop-off around the maximum LOD score. Negative values for additive (a) or dominance (d) effects indicate that the allele from the resistant parent decreases the phenotype value.

**TABLE 3** | Recombinant genotypes within the cluster of QTLs on chromosome P1 containing Minc-P1 and Mare-P1.

| F$_{2:3}$ family | Alleles (YW versus DLL) at the markers | Phenotype$^*$ (EMs) |
|-----------------|-----------------------------------|------------------|
| | SP1164 | SP1798 | SP1790 | SP1781 | M. arenaria | M. incognita |
| 17 | YW/YW | YW/YW | YW/DLL | YW/DLL | R (3) | He (310) |
| 22 | YW/DLL | YW/DLL | DLL/DLL | m.d. | He (13) | S (478) |
| 29 | DLL/DLL | DLL/DLL | YW/DLL | m.d. | S (48) | S (398) |
| 66 | m.d. | YW/DLL | DLL/DLL | DLL/DLL | S (37) | He (306) |

Alleles at the marker: YW, Yolo Wonder (resistant allele); DLL, Doux Long des Landes (susceptible allele); m.d., missing data. *Observed segregation in the F$_3$ family: R, all F$_3$ plants resistant, S, all F$_3$ plants susceptible, He, segregation of susceptible, and resistant plants within the F$_3$ family. The value in brackets is the median number of EMs per plant for each F$_{2:3}$ family.
P9 for Me3, Me1, and N, which have different spectra of action against RKN species (Hendy et al., 1985; Thies and Fery, 2000). However, our results provide two lines of evidence in support of these QTLs belonging to different genes within a cluster. Firstly, for the Mare-P1 and Mjav-P1 QTLs, the resistant YW allele displayed partial dominance, whereas partial dominance of the susceptible allele from DLL was observed for Minc-P1, suggesting different modes of action. Secondly, F2 individuals displaying genetic recombination between the markers at the peak values of the Minc-P1 and Mare-P1 loci were detected and the phenotypes of the corresponding F3 families confirmed recombination in the F2 plant, with a homozygous resistant or susceptible genotype at one QTL and a heterozygous genotype at the other QTL (Table 3). Despite their very tight linkage, these QTLs thus probably belong to different genes conferring different specificities against RKN populations. These findings provide further evidence that broad-spectrum quantitative resistance can result from the combination of race-specific resistance factors.

An additional major QTL, Mjav-P9, with a major dominant effect for resistance against M. javanica, was mapped to pepper chromosome P9, close to the B94 marker. The detection of a new resistance factor at this site was not unexpected, because the P9 genomic region also carries the Me and N genes (Fazari et al., 2012), together with the R-gene Bs2, which confers resistance to the bacterial pathogen Xanthomonas campestris pv. Vesicatoria (Mazourek et al., 2009), and QTLs for resistance to potyvirus PVY (Wang et al., 2008) and to the oomycete Phytophthora capsici (Thabuis et al., 2004). The genomic sequence of this P9 chromosomal region also has a high density of NBS-LRR genes from the Bs2 subclass (82 genes), highlighting the “explosive expansion of the pepper genome” relative to those of other Solanaceae species (Kim et al., 2014). This expansion, which probably involved tandem duplications, resulted in diversification and a clustering of the R-genes on chromosome P9, and the Mjav-P9 QTL, which has a mostly dominant effect, probably belongs to this cluster.

Broad-spectrum R-genes are often preferentially used in breeding programs, but previous studies have shown that the use of R-genes in an inappropriate genetic background may decrease their efficacy, in turn affecting their durability. The strategy of combining an R-gene with a partially resistant genetic background (i.e., a background carrying relevant QTLs) to increase its durability has been evaluated and validated in other pathosystems (Palloix et al., 2009; Brun et al., 2010; Fournet et al., 2013). Quenouille et al. (2012) suggested that this effect was due mostly to the additional resistance conferred by QTLs from the genetic background, decreasing the size of the pathogen population and, thus, the risk of emergence and of the further selection of virulent variants. For interactions between pepper and RKNs, YW proved to be a better genetic background than DLL for strengthening the efficacy of Me1 or Me3 (Barbary et al., 2014) and reducing the frequency of Me3 resistance breakdown (Djian-Caporalino et al., 2014). However, the genetic determinants of this partial resistance had never been characterized. The new QTLs identified in this study (i.e., Minc-P1, Mare-P1, Mjav-P1, and Mjav-P9) are, thus, good candidates for pyramiding with Me1, Me3, or N, providing new opportunities for combining major and partial resistance factors together in pepper cultivars.

In terms of plant breeding, the location of the newly identified resistance factors on pepper chromosome P1 should facilitate their introgression by MAS, alongside current R-genes. Indeed, all the resistance genes effective against RKNs mapped to date are closely linked on pepper chromosome P9 (Fazari et al., 2012). However, they are in repulsion phases, with the different genes carried by different pepper accessions. Minc-P1, Mare-P1, and Mjav-P1 are independent of this cluster and all are carried by the same accession (YW). This should make it easier to generate homozygous plant genotypes harboring resistance factors from both the P9 and P1 clusters. Breeders could also make use of Minc-P1, Mare-P1, and Mjav-P1, which do not act as fully dominant R-genes and may act through different resistance mechanisms. He et al. (2014) reported two QTLs affecting two different processes in RKN attack on cotton plants: root galling and egg production. These QTLs provided strong resistance to RKNs when combined in the same genotype. We are currently investigating this aspect in our pathosystem, by performing histological time-course studies to explore the spatiotemporal induction of plant responses conferred by these new resistance factors in pepper. In particular, the kinetics of J2 invasion in the roots, and the timing and location of cell necrosis (if any) will be investigated. On the basis of our preliminary observations, we hypothesize that the newly identified QTLs will induce defense reactions different from the classical HR triggered by the major R-genes Me1 and Me3. We therefore anticipate that the successful combination of these qualitative and quantitative resistance factors into elite cultivars will provide new opportunities for enhanced and durable RKN resistance in pepper.

**AUTHOR CONTRIBUTIONS**

AB, PC-S, CD-C, AP, and BC conceived the project, contribute to technical work, to data treatment and article writing, AF and NM organized and performed technical work.

**ACKNOWLEDGMENTS**

We thank Anne-Marie Sage-Palloix and Ghislaine Nemouchi for plant breeding and for providing F3 families seeds. We also acknowledge Delphine Angella, Chami Kim, Cristina Martin Jimenez, and Pierre Bautheac for technical assistance. This work was financially supported by the French Ministry of Higher Education and Research (MESR) and the seed companies Gautier Semences, Rijk Zwaan France, Sakata Vegetables Europe, Syngenta Seeds, Takii France, and Vilmorin & Cie in the frame of a CIFRE contract.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2016.00632
REFERENCES

Alimi, N. A., Bink, M., Dieleman, J. A., Magan, J. J., Wubs, A. M., Palloix, A., et al. (2013). Multi-trait and multi-environment QTL analyses of yield and a set of physiological traits in pepper. Theor. Appl. Genet. 126, 2597–2625. doi: 10.1007/s00122-013-2160-3

Barbary, A., Palloix, A., Fazari, A., Marteu, N., Castagnone-Sereno, P., and Djian-Caporalino, C. (2014). The plant genetic background affects the efficiency of the pepper major nematode resistance genes Me1 and Me3. Theor. Appl. Genet. 127, 499–507. doi: 10.1007/s00122-013-2235-1

Brom, K., Wu, W. H., Sen, S., and Churchill, G. A. (2003). QTL mapping in experimental crosses. Bioinformatics 19, 889–890. doi: 10.1093/bioinformatics/btg112

Brun, H., Chevre, A. M., Fitt, B. D., Powers, S., Besnard, A. L., Ermel, M., et al. (2005). Selection for Meloidogyne incognita virulence against resistance genes from tomato and pepper and specificity of the virulence/resistance determinants. Eur. J. Plant Pathol. 120, 585–590. doi: 10.1007/BF02177026

Dijian-Caporalino, C. (2014). The plant genetic background affects the efficiency of the pepper major nematode resistance genes Me1 and Me3. Theor. Appl. Genet. 127, 499–507. doi: 10.1007/s00122-013-2235-1

Fournet, S., Kerlan, M. C., Renault, L., Dantec, J. P., Rouaux, C., and Montarry, J. (2013). Multi-trait and multi-environment QTL analyses of yield and a set of physiological traits in pepper. Theor. Appl. Genet. 127, 1343–1351. doi: 10.1007/s00122-014-2302-2

Hendy, H., Pochard, E., and Dalmasso, A. (1985). Transmission héréditaire de la résistance aux Meloidogyne portée par deux lignées de Capsicum annuum: études de descendances d’homozygotes issues d’androgénèse. Agronomie 5, 93–100. doi: 10.1051/agro:19850201

Kim, S., Park, M., Yeom, S. I., Kim, Y. M., Lee, J. M., and Lee, H. A. (2014). Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat. Genet. 46, 270–278. doi: 10.1038/ng.2877

Kiyosawa, S. (1982). Genetics and epidemiological modeling of breakdown of plant disease resistance. Annu. Rev. Phytopathol. 20, 93–117. doi: 10.1146/annurev.py.20.090182.000521

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., et al. (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1, 174–181. doi: 10.1086/575800

Lefebvre, V., Piéger, S., Thabuis, A., Caranta, C., Blattes, A., Chauvet, J. C., et al. (2002). Towards the saturation of the pepper linkage map by alignment of three intraspecific maps including known-function genes. Genome 45, 839–854. doi: 10.1139/g01-053

Li, Z., Jakkula, L., Hussey, R. S., Tamulonis, J. P., and Boerma, H. R. (2001). SSR mapping and confirmation of the QTL from the P96354 conditioning soybean resistance to southern root-knot nematode. Theor. Appl. Genet. 103, 1167–1173. doi: 10.1007/s001220100672

Mazourek, M., Cirulli, E. T., Collier, S. M., Landry, L. G., Kang, B. C., Quirin, E. A., et al. (2009). The fractionated orthology of Bt2 and Bt2a supports shared syntenic disease resistance in the Solanaceae. Genetics 182, 1351–1364. doi: 10.1534/genetics.109.101022

Mucedo, L. B., Matthews, W. C., Diop, N. N., Bhat, P. R., Wanamaker, S., Ehlers, J. D., et al. (2009). QTL mapping of root-knot nematode resistance in cowpea (Vigna unguiculata) using EST derived SNP markers. J. Nematol. 41, 361–362.

Mundt, C. C. (2002). Use of multiline cultivars and cultivar mixtures for disease management. Annu. Rev. Phytopathol. 40, 381–410. doi: 10.1146/annurev.phyto.40.110101.143723

Nicolai, M., Pisani, C., Bouchet, J. P., Vuylsteke, M., and Palloix, A. (2012). Discovery of a large set of SNP and SSR genetic markers by high-throughput sequencing of pepper (Capsicum annuum). Theor. Mol. Biol. 11, 2295–2300. doi: 10.1242/2012.August.13.3

Palloix, A., Ayme, V., and Moursy, B. (2009). Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. New Phytol. 183, 190–199. doi: 10.1111/j.1469-8137.2009.02827.x

Paran, I., Rouppe Van Der Voort, J., Lefebvre, V., Jahn, M., Landry, L., Van Schriek, M., et al. (2004). An integrated genetic linkage map of pepper (Capsicum spp.). Mol. Breed. 13, 251–261.

Pegard, A., Brizzard, G., Fazari, A., Soucaze, O., Abad, P., and Dijian-Caporalino, C. (2005). Histological characterization of resistance to different root-knot nematode species related to phenolics accumulation in Capsicum annuum. Phytopathology 95, 158–165. doi: 10.1094/PHYTO-95-0158

Pink, D. (2002). Strategies using genes for non-durable disease resistance. Euphytica 124, 227–236. doi: 10.1023/A:1015638718242

Quenouille, J., Montarry, J., Palloix, A., and Moursy, B. (2012). Farther, slower, stronger: how the plant genetic background protects a major resistance gene from breakdown. Mol. Plant Breed. 14, 109–118. doi: 10.1111/j.1364-3703.2012.00834.x

Quenouille, J., Paulhauc, E., Moursy, B., and Palloix, A. (2014). Quantitative trait loci from the host genetic background modulate the durability of a resistance gene: a rational basis for sustainable resistance breeding in plants. Heredity (Edinb.) 112, 579–587. doi: 10.1038/hdy.2013.138

Shen, X. L., Van Beecelaere, G., Kumar, P., Davis, R. F., May, O. L., and Chee, P. (2006). QTL mapping for resistance to root-knot nematodes in the M-120 RNR upland cotton line (Gossypium hirsutum L.) of the Auburn 623 RNR source. Theor. Appl. Genet. 113, 1539–1549. doi: 10.1007/s00122-006-0401-4

Stuber, C. W., Edwards, M. D., and Wendel, J. F. (1987). Molecular marker- facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. Crop Sci. 27, 639–648. doi: 10.2135/cropsci1987.0011183x0027000000006x

Thabuis, A., Lefebvre, V., Bernard, G., Daubé, A. M., Phaly, T., Pochard, E., et al. (2004). Phenotypic and molecular evaluation of a recurrent selection program for a polygenic resistance to Phytophthora capsici in pepper. Theor. Appl. Genet. 109, 342–351. doi: 10.1007/s00122-004-1633-9
Thies, J. A. (2011). Virulence of *Meloidogyne incognita* to expression of N gene in pepper. *J. Nematol.* 43, 90–94.

Thies, J. A., and Ferry, R. L. (1998). Modified expression of the N gene for southern root-knot nematode resistance in pepper at high soil temperatures. *J. Am. Soc. Hortic. Sci.* 123, 1012–1015.

Thies, J. A., and Ferry, R. L. (2000). Characterization of resistance conferred by the N gene to *Meloidogyne arenaria* Races 1 and 2, *M. hapla*, and *M. javanica* in two sets of isogenic lines of *Capsicum annuum* L. *J. Am. Soc. Hortic. Sci.* 125, 71–75.

Wang, L. H., Zhang, B., Caranta, C., Mao, S., and Palloix, A. (2008). Molecular markers assisted selection for three QTLs resistant to PVY in pepper (*Capsicum annuum* L.). *Acta Hortic.* 35, 53–58.

Wu, F., Eannetta, N. T., Xu, Y., Durrett, R., Mazourek, M., Jahn, M. M., et al. (2009). A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus *Capsicum*. *Theor. Appl. Genet.* 118, 1279–1293. doi: 10.1007/s00122-009-0980-y

Zijlstra, C., Donkers-Venne, D., and Fargette, M. (2000). Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2, 847–853. doi: 10.1163/156854100750112798

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Barbary, Djian-Caporalino, Marteu, Fazari, Caromel, Castagnone-Sereno and Palloix. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.