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Plant genetic background increasing the efficiency and durability of major resistance genes to root knot nematodes can be resolved into a few resistance QTLs

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Abstract

With the banning of most chemical nematicides, the control of root knot nematodes (RKNs) in vegetable crops is mainly based on the deployment of single-major resistance genes (*R*-genes). However, these genes are rare and their efficiency is threatened by RKNs capacities of adaptation. In pepper, several dominant *R*-genes are efficient against RKNs, but their efficiency and durability were shown to be increased in partially resistant genetic background. A QTL analysis was performed in such a genetic background, using a $F_{2:3}$ population from the cross between Yolo Wonder, a partially resistant to resistant accession depending on RKN species, and Doux Long des Landes, a susceptible one. The genetic linkage map was constructed from 130 F_2 individuals and the 130 F_3 families were tested for resistance to the three main RKNs species, *M. incognita*, *M. arenaria* and *M. javanica*. Four new major QTLs were mapped into two clusters. The cluster on chromosome P1 includes three tightly linked QTLs with specific effects against each RKN species. The fourth QTL, specific of resistance to *M. javanica*, mapped on the pepper chromosome P9, which is known to carry multiple NBS-LRR repeats with major resistance genes to nematodes and other pathogens. The newly discovered cluster on chromosome P1, displays a broad spectrum of action with major additive effects on resistance. Therefore, it provides innovative potential for breeding new cultivars or rootstocks combining quantitative resistance and major resistance genes and increasing the efficiency as well as the durability of RKNs genetic control.

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

1. Introduction

Root-knot nematodes (RKNs), *Meloidogyne* spp., are major plant pathogens worldwide. They are extremely polyphagous endoparasites able to infest more than 5,500 plant species, among which many field and greenhouse crops *[1]*. Since the use of most chemical nematicides is being prohibited, due to environmental and public health issues, one alternative way to protect crops against these pests is based on the use of resistant cultivars. This method is efficient to control RKNs populations, economically sustainable, health safe and environmentally friendly.

Major resistance genes (*R*-genes) are extensively used in breeding RKN resistant cultivars and/or rootstocks. However, their efficiency is threatened by the capacities of adaptation of RKNs. Indeed, *R*-genes apply a selective pressure on nematode populations and cause a risk of emergence of virulent nematode populations *[2]*, which constitutes a severe limitation to their use.

A previous study showed that a partially resistant genetic background increases R-gene efficiency compared to a susceptible one *[3]*. The major role of the plant genetic background in preventing R-gene from overcoming was reported in other pathosystems *[4, 5, 6]*. In pepper/virus parthosystem, the reason of R-gene increased durability was shown to result from quantitative trait loci (QTLs) which slow down the selection of *R*-gene virulent variants and decrease the pathogen population (e.g., *[7]*). To date, no QTLs were found against RKN in pepper. In that respect, a QTL analysis was performed, as we strongly supposed that the protective effect of the plant genetic background on R-genes agaisnt RKNs is provided by such quantitative resistance factors as well.

2. Material and methods

In this study, a classical QTL analysis was conducted to determine the genetic factors, within the plant genetic background, that may explain the discrepancies in resistance level from a pepper genotype to another. A population of 130 $F_{2:3}$ families, derived from a cross between the resistant to partially resistant (Yolo Wonder) and the highly susceptible (Doux Long des Landes) pepper inbred lines (figure 1), was tested for quantitative resistance to the three main RKNs species (*M. incognita, M. arenaria and M. javanica*).

Genotyping data were collected on the F_2 population from 326 markers, among wich sequence Charaterized Amplified Region (SCAR), Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP). A genetic linkage map was constructed with a LOD score threshold of 3.0 and a maximum recombination fraction of 0.3

Assessement of resistance was performed on the F3 progenies for each RKN species according to a common experimental procedure previously described *[3]*. To estimate the phenotypic value of each F2, the number of egg masses (EMs) was counted for sixteen F3 plants derived from each F2.

QTL detection was performed using Composite Intervalle Mapping. (CIM). A permutation test with 1,000 replicates allowed to empirically determine the genome-wide LOD threshold 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.

Figure 1

The susceptible and resistant pepper cultivars Doux Long des Landes (A) and Yolo Wonder (C) and their respective root systems (B, D). Arrows indicate egg masses.

3. Results

The genetic linkage map constructed with 326 markers provided a new saturated pepper map, among wich one SCAR, 13 SSR and 312 SNP. It comprised 12 linkage groups (LGs), which were assigned to the 12 pepper chromosomes, with an overall length of 1436 cM (table 1).

Table 1

Caracteritics of the map from the cross between YW x DLL

Chromosome	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	total
Number of markers	35	22	35	16	21	19	32	22	33	25	38	28	326
Lenght (cM)	205.5	94.9	154.3	93.5	125.3	106.6	130.4	70.3	108.6	106.5	108.4	132.1	1436.4
Mean distance between two disjointed markers (cM)	6.4	5.0	5.1	6.2	6.3	5.6	4.5	3.9	6.0	4.4	3.7	5.3	5.2
Maximum distance between two consecutive markers	25.9	15.7	17.6	14.9	18.6	19.6	14.1	12.3	23.6	14.8	15.6	21.0	

One major resistance QTL, named *Minc-P1*, was detected on pepper chromosome P1 for *M. incognita*. On this chromosome, in the vicinity of *Minc-P1* was detected a QTL for resistance to *M. arenaria* named *Mare-P1*. Regarding *M. javanica*, two QTL were detected. The first one, named *Mjav-P1*, was located on chromosome P1 as well, close to *Minc-P1* and *Mare-P1*. The confidence interval of these three QTLs overlapped. The second QTL, named *Mjav-P9*, was detected at the distal part of the chromosome P9. For all four QTLs, the resistance allele originated from YW. Their positions and effects are resumed in figure 2 and table 2.

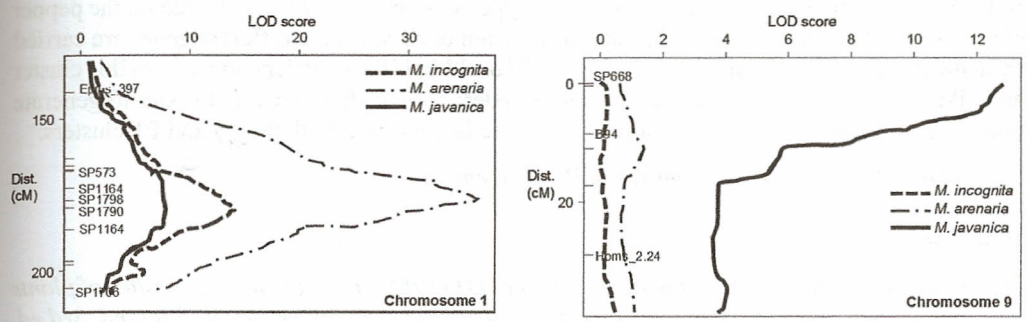

Figure 2

Quantitative trait loci (QTL) for M. incognita, M. arenaria and M. javanica resistance, on pepper chromosome 1 (left, box 1) and chromosome 9 (right, box 2). The log of the likelihood ratio (LOD) score is shown on the x axis. Scaled distances, flanking markers and markers in the confidence interval of the QTLs are given on the y axis.

Table 2
QTL for resistance to the different RKN species in the pepper F2:3 progeny
CI : Confidence interval, defined as a LOD-2 drop-off around the maximum LOD score

RKN species	QTL	Chromosome	Closest marker	Location (cM)	CI	LOD score	R^2
M. incognita	*Minc-P1*	1	SP1790	179.2	173.9 – 188.0	14.1	40.9
M.arenaria	*Mare-P1*	1	SP1798	177.0	168.2 – 182.0	36.5	73.9
M. javanica	*Mjav-P1*	1	SP1790	179.2	164.0 – 192.5	7.7	48.0
	Mjav-P9	9	SP668	1.0	0.0 – 9.8	12.9	52.2

4. Discussion

Albeit broad spectrum *R*-genes are often preferentially used in breeding programs, it was shown that using *R*-genes in an inappropriate pepper genetic background may reduce their efficiency, which may further affect their durability. Indeed, YW proved to be a better genetic background than DLL to reinforce *R-genes* efficiency *[3]* and also to reduce the frequency of *Me3* resistance breakdown compared to DLL *[9]*. The strategy which consists in combining an *R*-gene with a partially resistant genetic background (i.e., carrying QTLs) in order to increase its durability was validated in other pathosystems *[4, 5, 6]*. Quenouille *et al. [8]* suggested that this effect is mainly due to the additional resistance because of QTLs from the genetic background, which decrease the pathogen population and thus the risk of emergence as well as further selection of virulent variants. From that point of view, the new QTLs identified in this study are good candidates for pyramiding with *R-genes* against RKNs. Moreover, it was demonstrated that, under suitable agronomic practice conditions, *R*-gene pyramiding was the best alternative to increase *R-genes* against RKNs durability *[9]*. From our results, *Minc-P1*, *Mare-P1, Mjav-P1* and *Mjav-P9*, thus offer new opportunities for combining major and partial resistance factors together.

From a breeding point of view, the localization of new resistance factors on the pepper chromosome P1 will facilitate their introgression by MAS, in addition to current *R*-genes. Indeed, all the efficient genes against RKNs, mapped until now, are closely linked on the pepper chromosome P9 *[10]*. However, they are in repulsion phases, i.e. the different genes are carried by distinct pepper accessions. *Minc-P1*, *Mare-P1 and Mjav-P1* are independent from this cluster and all carried by the same accession (Yolo Wonder), which should make it easier to generate homozygous plant genotypes harbouring resistance factors from both the P9 and P1 clusters.

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