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ORIGINAL PAPER

The plant genetic background affects the efficiency of the pepper major nematode resistance genes *Me1* and *Me3*

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Abstract
Key message **The plant genetic background influences the efficiency of major resistance genes to root-knot nematodes in pepper and has to be considered in breeding strategies.**
Abstract Root-knot nematodes (RKNs), *Meloidogyne* spp., are extremely polyphagous plant parasites worldwide. Since the use of most chemical nematicides is being prohibited, genetic resistance is an efficient alternative way to protect crops against these pests. However, nematode populations proved able to breakdown plant resistance, and genetic resources in terms of resistance genes (R-genes) are limited. Sustainable management of these valuable resources is thus a key point of R-gene durability. In pepper, *Me1* and *Me3* are two dominant major R-genes, currently used in breeding programs to control *M. arenaria*, *M. incognita* and *M. javanica*, the three main RKN species. These two genes differ in the hypersensitive response induced by nematode infection. In this study, they were introgressed in either a susceptible or a partially resistant genetic background, in either homozygous or heterozygous allelic status. Challenging these genotypes with an avirulent *M. incognita* isolate demonstrated that (1) the efficiency of the R-genes in reducing the reproductive potential of RKNs is strongly affected by the plant genetic background, (2) the allelic status of the R-genes has no effect on nematode reproduction. These results highlight the primary importance of the choice of both the R-gene and the genetic background into which it is introgressed during the selection of new elite cultivars by plant breeders.

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Introduction

Root-knot nematodes (RKNs), *Meloidogyne* spp., are considered as one of the most damaging pathogen in the world (Trudgill and Blok 2001). A reliable way of controlling these polyphagous endoparasitic worms is the use of chemical nematicides. However, the use of such compounds was drastically restricted in the past years because of environmental and public health issues. Now, one of the best alternatives to cope with nematode infestations relies on the deployment of resistance genes (R-genes), which represent an efficient, environmentally safe and economically sustainable method of control (Djian-Caporalino et al. 2009). As a consequence, many breeding programs are being developed in order to introgress the desired R-genes into elite cultivars and/or rootstocks. However, not only the R-gene itself may completely account for the observed

resistance phenotype. Indeed, effects linked to the plant genetic background have been recognized to modify levels of nematode resistance in several crops (Jacquet et al. 2005; López-Pérez et al. 2006; Wang et al. 2008). In addition, in several pathosystems, including plant–nematode interactions, a dosage effect of the R-gene alleles on the pathogen multiplication was shown (Collmer et al. 2000; Jacquet et al. 2005; Chintamanani et al. 2008).

Since resistance sources against RKNs are limited, management of the available R-genes is of crucial importance. As RKNs exhibit noteworthy capacities of adaptation to their environment, including R-genes, the emergence and spread of virulent nematode populations constitute a severe threat to R-gene durability (Castagnone-Sereno 2002, 2006; McDonald and Linde 2002). An R-gene is considered as durable if a cultivar carrying it is widely grown for a long period in an environment favorable to the pathogen spread without developing the disease (Johnson 1984). According to this definition, the durability of R-genes against nematodes is considered relatively high. For instance, it took more than 20 years for RKNs to overcome the tomato R-gene *Mi-1*, the only one to be used in all the modern fresh market and processing resistant tomato cultivars (Williamson 1998), and this gene still remains effective in most agronomic conditions. The potato gene *H1*, a R-gene active against the cyst nematode *Globodera rostochiensis*, has been used for more than 30 years in the UK without being overcome (Fuller et al. 2008). The resistant Prunus rootstock Nemaguard was grown during 50 years before its breakdown by *Meloidogyne* spp. (Williamson and Roberts 2009).

In pepper (*Capsicum annuum* L.), several dominant R-genes have been identified and well characterized for their spectrum of resistance against RKNs, i.e., the *Me* genes and the *N* gene (Hare 1956; Hendy et al. 1985a, b; Djian-Caporalino et al. 1999; Thies and Fery 2000). Some of them have been mapped and co-localized in a cluster on the pepper P9 chromosome (Djian-Caporalino et al. 2001, 2007; Fazari et al. 2012). Two of these dominant R-genes, *Me3* and *Me1*, display a broad spectrum of resistance to the three main RKN species, i.e., *M. incognita*, *M. arenaria* and *M. javanica*. Currently, they are being actively exploited in breeding programs. Whereas efficiency of several R-genes against RKNs is temperature-dependent, as for the tomato *Mi-1* gene or the pepper *N* gene (Ammati et al. 1986; Thies and Fery 1998), *Me3* and *Me1* are stable at high temperature (Djian-Caporalino et al. 1999). However, these two genes differ in their mode of action, particularly in the spatio-temporal localisation of the hypersensitive reaction (HR) triggered by RKN penetration into the roots. Indeed, experimental studies have shown that HR occurred early in the epidermis or lately in the cortex, when controlled by *Me3* or *Me1*, respectively (Bleve-Zacheo et al. 1998; Pegard et al. 2005). Interestingly, virulent populations were obtained for *Me3*, both in natural (i.e., in the field) and artificial (i.e., in the laboratory) conditions, whereas, to date, no evidence showed the emergence of *Me1*-virulent populations (Castagnone-Sereno et al. 1996; Djian-Caporalino et al. 2011), which suggests a possible relationship between the mode of action of these R-genes and their durability.

The present work aims at evaluating the influence of the genetic background of pepper genotypes on the expression of the resistance to RKNs conferred by the *Me3* and *Me1* R-genes, using either a susceptible or a partially resistant genetic background. Allele dosage effect of both genes was also tested to evaluate the relevance of hybrid varieties *versus* inbreed lines on R-genes efficiency.

Materials and methods

Plant material

Pepper (*Capsicum annuum* L.) genotypes used in this work were Yolo Wonder (YW), Doux Long des Landes (DLL), DH149 and DH330. These inbred lines were selected for their differential resistance to *Meloidogyne incognita*. DLL is a highly susceptible pepper cultivar; YW is a partially resistant (i.e., shows reduced symptoms) cultivar. DH149 and DH330 are two resistant doubled haploid (DH) lines produced through in vitro androgenesis (Dumas de Vaulx et al. 1981) from the intraspecific F1 hybrids (PM687 × YW) and (PM217 × YW), respectively. DH149 and DH330 carry the single dominant resistance alleles *Me3* and *Me1*, respectively (Hendy 1984). These two genes were chosen in the present study because they differ in the phenotypic expression of the HR induced by nematode infection (Bleve-Zacheo et al. 1998; Pegard et al. 2005).

Each susceptible genotype was crossed with each resistant one. In order to introgress separately the *Me3* or *Me1* alleles into the DLL (very susceptible) or YW (partially resistant) genetic background, the resistant parental lines DH149 and DH330 were crossed with the susceptible parental lines DLL and YW (recurrent parents) and the four F1 hybrids were backcrossed (BC) with their respective recurrent parental lines. Considering *Me3*, a BC1 resistant plant was self-pollinated to obtain BC1-S1 plants which segregated for *Me3*. Considering *Me1*, two backcrosses were performed (BC2) before selfing and generating the BC2-S1 segregating progeny. BC2-S1 [((DH330 × DLL) × DLL) × DLL] was produced to favor the chance of selecting virulent isolates against *Me1* as, up to now, no *Me1*-virulent nematode population has been obtained (Castagnone-Sereno et al. 1996; Djian-Caporalino et al. 2011). We generated BC2-S1 [((DH330 × YW) × YW) × YW] as well in order to compare genotypes which differed

only for their genetic background and to eliminate potential effects due to variable proportion of genetic background surrounding the *Me1* gene.

Plant allelic status determination

Total genomic DNA was isolated from 100 mg of fresh leaf material as described by Fulton et al. (1995). After RNAse treatment, DNA concentration and purity were measured with a NanoDrop 2000 spectrophotometer (Thermoscientific) and adjusted to a final concentration of 20 ng/μL for PCR.

BC1-S1 plants carrying *Me3* were genotyped with SCAR_N, a codominant marker linked to this gene, in order to discriminate *Me3* homozygous susceptible (*Me3*$^+$/*Me3*$^+$), homozygous resistant (*Me3*/*Me3*) and heterozygous (*Me3*/*Me3*$^+$) plants in both DLL and YW genetic backgrounds, according to a standard procedure (Fazari et al. 2012). Similarly, SSCP_PM5, a codominant marker linked to *Me1* was used to genotype the corresponding BC2-S1 plants (Fazari et al. 2012).

Nematode material

The *M. incognita* Morelos isolate, from the collection maintained at INRA research centre in Sophia Antipolis, was used in this study. It is avirulent towards both *Me3* and *Me1* genes. Because of the mitotic parthenogenetic mode of reproduction of *M. incognita* (Triantaphyllou 1985), all the second-stage juveniles (J2s) that hatched from a single egg mass were considered as a clonal line. Prior to multiplication, this isolate was specifically identified according to its isoesterase electrophoretic pattern (Dalmasso and Bergé 1978) and/or by SCAR PCR (Zijlstra et al. 2000).

Experimental procedures and evaluation

Two inoculation experiments were conducted independently. In the first one, the *M. incognita* avirulent isolate Morelos was inoculated to the BC1-S1 plants carrying *Me3* in either DLL or YW genetic background. In the second one, the same isolate was inoculated to BC2-S1 plants carrying *Me1* in either DLL or YW genetic background. Both experiments were conducted to determine the influence of the genetic background and the allele dosage effect on R-gene efficiency and their possibility to be overcome.

For each experiment, pepper seedlings were sown individually in 9 cm plastic pots containing steam-sterilized sandy soil covered by a 1 cm layer of loam. At least 20 replicates (individual plants) were performed for each control genotype (i.e., DLL, YW, DH149, DH330 and each F1) and 120 BC1-S1 and BC2-S1 plants were grown for experiments with *Me3* and *Me1*, respectively. This was done to ensure to obtain at least twenty replicates of each genotype (homozygous susceptible, homozygous resistant and heterozygous). The whole experiments were conducted in a climatic chamber maintained at 24 °C (±2 °C) with a 12 h light cycle and a relative humidity of 60–70 %. Six- to seven-week-old plants (4–6 true leaves) were inoculated with a water suspension of 5,000 hatched second-stage juveniles (J2s) obtained in a mist chamber, from previously inoculated susceptible tomato roots (cultivar Saint Pierre). Such a high-inoculation pressure (5,000 J2s) was chosen in order to increase the probability of R-gene breakdown as a lower pressure proved to be inefficient (Castagnone-Sereno et al. 1996).

Six to seven weeks after inoculation (i.e., a duration that allowed completion of the nematode life cycle), plants were harvested, carefully washed individually with tap water, and stained for 10 min in a cold aqueous solution of eosin yellow (0.1 g/l water), to specifically stain egg masses (EMs) (Roberts et al. 1990). The roots were rinsed and examined under a magnifying glass. The number of EMs was counted for each plant and the average number of EMs was calculated for the different genotypes. In addition, for each genotype, the frequency of plants exhibiting more than five EMs in relation to the number of inoculated plants was computed.

Statistical analysis

All the statistical analyses were performed using the free software R (http://www.r-project.org/). First, to check the good fit of the expected segregation of the BC-S1 populations, a χ^2 test was performed. In order to investigate a possible effect of the genetic background and/or a dosage allele effect, non-parametric tests were further applied to compare the number of EMs of the different genotypes. When the Kruskal–Wallis test was significant, Wilcoxon–Mann–Whitney bilateral tests with a significance level at $\alpha = 0.05$ were carried out using Bonferroni correction.

Results

Experiment involving the *Me3* R-gene

Homozygous susceptible (*Me3*$^+$/*Me3*$^+$), homozygous resistant (*Me3*/*Me3*) and heterozygous (*Me3*/*Me3*$^+$) BC1-S1 plants were sorted using SCAR_N, a codominant marker linked to *Me3* (Fig. 1). With both recurrent parents DLL and YW, the observed segregation of *Me3* fitted the expected segregating ratio as revealed by a χ^2 test at $\alpha = 0.05$ (Table 1). All the genotypes were infested with a high-pressure inoculum (5,000 J2s) of *M. incognita* Morelos avirulent isolate. The Kruskal–Wallis test revealed that

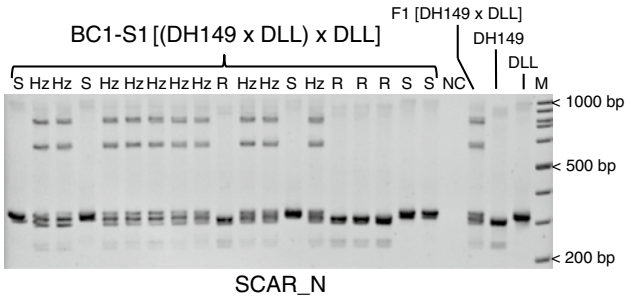

Fig. 1 Example of screening of BC1-S1 pepper progenies in DLL genetic background and controls with the SCAR_N codominant marker, linked to the *Me3* major resistance gene. *BC1-S1* backcross 1 self-pollinated, *F1* hybrid F1, *Hz* heterozygous resistant (*Me3*/*Me3*$^+$) plant, *S* susceptible homozygous (*Me3*$^+$/*Me3*$^+$) plant, *R* resistant homozygous (*Me3*/*Me3*) plant, *NC* negative control (H_2O), *DLL* Doux Long des Landes (susceptible genotype), *YW* Yolo Wonder (partially resistant genotype), *DH149* Double Haploid 149 line (resistant genotype), *M* size marker

there was a significant effect of the plant genotype on the number of EMs ($\chi^2 = 363.71$, $df = 10$, p value $<10^{-3}$). Consequently, the mean values of the different genotypes were compared with each other to determine which one(s) provided the best efficiency against RKNs (Fig. 2). As expected, DLL was very susceptible and exhibited a high number of EMs (mean of 1579.7 EMs/plant) whereas YW was partially resistant and showed a moderate one (mean of 462.4 EMs/plant). For both genotypes, EMs were detected on the root system of all inoculated plants (100 % of infected plants). DH149 confirmed its resistant status with a mean number of 4.4 EMs/plant and 17 % of plants affected. The plants of the susceptible BC1-S1 (*Me3*$^+$/*Me3*$^+$) genotypes were all infected and they exhibited numerous EMs, but less than their respective susceptible parents DLL and YW. The EM number of susceptible BC1-S1 (*Me3*$^+$/*Me3*$^+$) plants in the DLL genetic background was much higher than in the YW one (490.9 and 116.1 EMs/plant, respectively). There was no significant differences between the average EM number of the two F1 hybrids (DH149 × DLL) and (DH149 × YW) which appeared resistant (0.9 and 0.2 EMs/plant, respectively). Different results were obtained with the BC1-S1 plants heterozygous or homozygous resistant at the *Me3* locus. In the DLL genetic background, the number of EMs was significantly much higher than in the DH149 one (30.8 and 49.1 EMs/plant, respectively) and the rate of infected plants was important (68 and 61 %, respectively). In the YW genetic background, the number of EMs of the BC1-S1 plants, heterozygous or homozygous resistant at the *Me3* locus did not significantly differ from DH149 (0.5 and 1.0 EMs/plant, respectively) and both genotypes had a similar rate of infected plants (2 and 9 %, respectively). Comparing *Me3* heterozygous to homozygous resistant BC1-S1 plants within the same genetic background (DLL or YW) did not reveal significant differences.

Experiment involving the *Me1* R-gene

PM5_SSCP, a codominant marker linked to *Me1*, was used to sort the segregating BC2-S1 progenies in both DLL and YW genetic backgrounds (Fig. 3). In both progenies, the observed segregation ratios at the *Me1* locus fitted the expected ones at $\alpha = 0.05$ (Table 2). A high-inoculation pressure with 5,000 J2s of the avirulent *M. incognita* Morelos isolate was applied to each plant. A significant effect of the plant genotype on the number of EMs was detected by the Kruskal–Wallis test ($\chi^2 = 237.22$, $df = 10$, p value $<10^{-3}$). Thus, the efficiency of the different genotypes against nematodes was evaluated by comparing their mean values (Fig. 4). The parental controls were consistent with the expected results, i.e., a high number of EMs for DLL and a moderate one for YW (513.2 and 218.8 EMs/plant, respectively) and a value of 0.9 EMs/plant for DH330, consistent with a resistant genotype. All DLL and YW plants were infected, conversely to DH330. Whatever their genetic background, homozygous susceptible BC2-S1 (*Me1*$^+$/*Me1*$^+$) genotypes showed a substantial number of EMs (115.2 EMs/plant in DLL genetic background

Table 1 Observed segregation ratio of the *Me3* alleles in pepper progenies with DLL (upper part) or YW (lower part) genetic background from a self-pollinated heterozygous resistant backcross 1 plant

Genetic background	Allelic status at the *Me3* locus	Number of plants	χ^2 (1:2:1)
BC1-S1 [(DH149 × DLL) × DLL]	Homozygous susceptible *Me3*$^+$/*Me3*$^+$	19	$X^2 = 4.1538$
	Homozygous resistant *Me3*/*Me3*	23	$df = 2$
	Heterozygous *Me3*/*Me3*$^+$	62	p value = 0.1253
BC1-S1 [(DH149 × YW) × YW]	Homozygous susceptible *Me3*$^+$/*Me3*$^+$	35	$X^2 = 3.2182$
	Homozygous resistant *Me3*/*Me3*	22	$df = 2$
	Heterozygous *Me3*/*Me3*$^+$	53	p value = 0.2001

BC1-S1 backcross 1 self-pollinated, *DH149* Double Haploid 149 line (resistant genotype), *DLL* Doux Long des Landes (susceptible genotype), *YW* Yolo Wonder (partially resistant genotype)

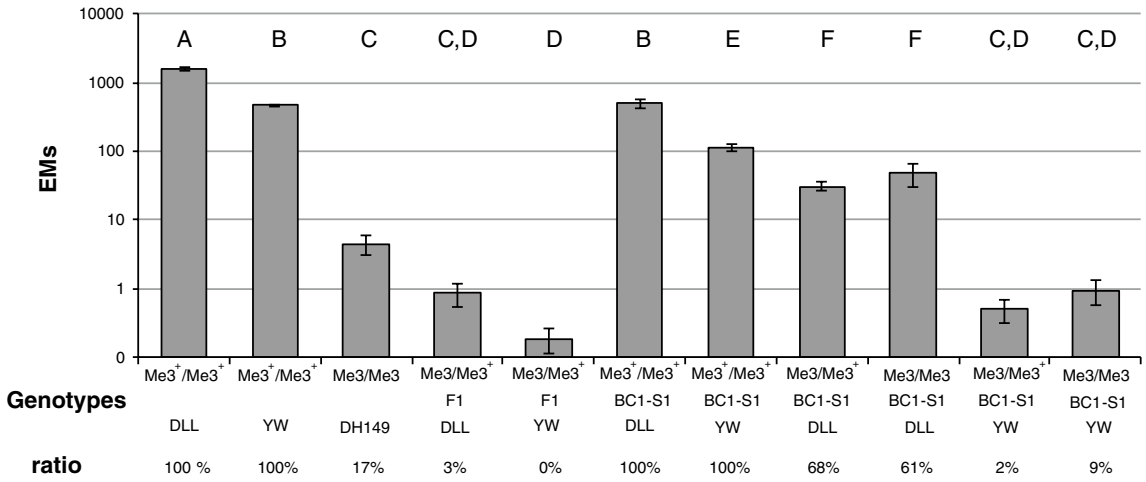

Fig. 2 Average number of egg masses/plant (EMs) on a $\log_{10}$ scale and ratio of plants exhibiting more than five egg masses in relation to the number of inoculated plants (ratio) of different pepper genotypes inoculated with a high-pressure inoculum (5,000 J2s) of an avirulent isolate of *M. incognita*. *Me3*$^+$/*Me3*$^+$ homozygous susceptible at the *Me3* locus, *Me3*/*Me3* homozygous resistant at the *Me3* locus, *Me3*/*Me3*$^+$ heterozygous at the *Me3* locus, *BC1-S1* backcross 1 self-pollinated, *F1* hybrid F1, *DLL* Doux Long des Landes (susceptible genotype or genetic background), *YW* Yolo Wonder (partially resistant genotype or genetic background), *DH149* Double Haploid 149 line (resistant genotype), *Bar* standard error. *Different letters* mean significant differences (Wilcoxon–Mann–Whitney bilateral tests at $\alpha = 0.05$ after Bonferroni correction)

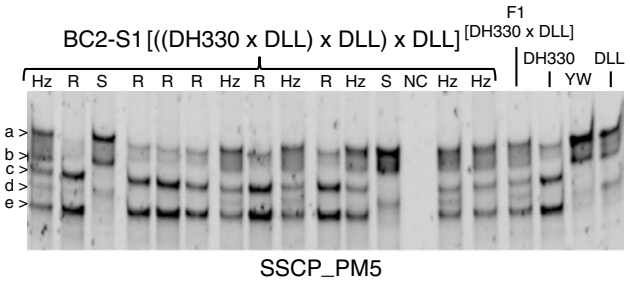

Fig. 3 Example of screening of BC2-S1 pepper progenies in DLL genetic background and controls with the SSCP_PM5 codominant marker, linked to *Me1* major resistant gene. *BC2-S1* backcross 2 self-pollinated, *F1* hybrid F1, *Hz* heterozygous resistant (*Me1*/*Me1*$^+$) plant; *R* resistant homozygous (*Me1*/*Me1*) plant; *S* susceptible homozygous (*Me1*$^+$/*Me1*$^+$) plant; *NC* negative control (H_2O), *DH330* Double Haploid 330 line (resistant genotype), *DLL* Doux Long des Landes (susceptible genotype); *YW* Yolo Wonder (partially resistant genotype); *a, b, d* specific bands to DLL; *c, e* specific bands to DH330

and 116.2 in YW one), although less than their respective susceptible recurrent parent DLL and YW. As for susceptible genotypes, EMs were present on all the BC2-S1 (*Me1*$^+$/*Me1*$^+$) plants. The F1 (DH330 × DLL) hybrid genotype displayed a very high rate of infected plant (90 % of them) and exhibited a significantly higher number of EMs (18.6 EMs/plant) than its resistant parent DH330. On the contrary, the F1 (DH330 × YW) hybrid genotype exhibited no EMs at all. Among BC2-S1 genotypes heterozygous or homozygous resistant at the *Me1* locus, the rate of infected plants was higher on genotypes with DLL genetic background (54 and 27 %, respectively) than on genotypes with YW genetic background (15 and 7 %, respectively). Concerning the number of EMs, both homozygous resistant (*Me1*/*Me1*) and heterozygous (*Me1*/*Me1*$^+$) BC2-S1 in YW genetic background behaved as the resistant parental line DH330. In the DLL genetic background, the heterozygous BS2-S1 (*Me1*/*Me1*$^+$) showed a significantly higher number of EMs compared to YW genetic background, as observed from the F1 hybrids, but this background effect was not significant in resistant homozygous individuals. In the DLL as well as in the YW genetic backgrounds, BC2-S1 plants carrying one or two resistant alleles at the *Me1* locus were not significantly different from each other for the number of EMs.

Discussion

In order to explore the effect of the plant genetic background on R-gene efficiency, *Me3* and *Me1* were introgressed into a susceptible (i.e., DLL) or a partially resistant (i.e., YW) pepper genetic background. Compared with the donor resistant parental lines, the DLL or YW genetic background surrounding the R-gene was increased of 50 % in F1 hybrids, of 75 % in BC1-S1 plants and of 87.5 % in BC2-S1 plants. The different genotypes were challenged with a high-inoculation pressure of *M. incognita* and their ability to resist to this pathogen was evaluated. The main observation was that plants with *Me3* or *Me1* in a

Table 2 Observed segregation ratio of the *Me1* alleles in pepper progenies with DLL (upper part) or YW (lower part) genetic background from a self-pollinated heterozygous resistant backcross 2 plant

Genetic background	Allelic status at the *Me1* locus	Number of plants	χ^2 (1:2:1)
BC2-S1 [((DH330 × DLL) × DLL) × DLL]	Homozygous susceptible *Me1*$^+$/*Me1*$^+$	30	$\chi^2 = 0.9091$
	Homozygous resistant *Me1*/*Me1*	30	$df = 2$
	Heterozygous *Me1*/*Me1*$^+$	50	p value = 0.6347
BC2-S1 [((DH330 × YW) × YW) × YW]	Homozygous susceptible *Me1*$^+$/*Me1*$^+$	27	$\chi^2 = 0.64$
	Homozygous resistant *Me1*/*Me1*	27	$df = 2$
	Heterozygous *Me1*/*Me1*$^+$	46	p value = 0.7261

BC2-S1 backcross 2 self-pollinated, *DH330* Double Haploid 330 line (resistant genotype), *DLL* Doux Long des Landes (susceptible genotype), *YW* Yolo Wonder (partially resistant genotype)

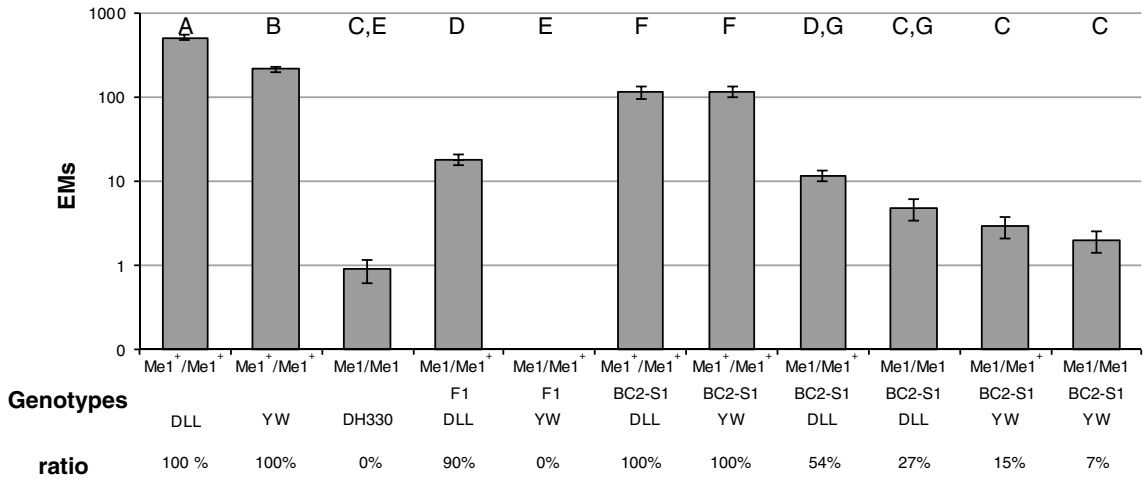

Fig. 4 Average number of egg masses/plant (EMs) on a $\log_{10}$ scale and ratio of plants exhibiting more than five egg masses in relation to the number of inoculated plants (ratio) of different pepper genotypes inoculated with a high-pressure inoculum (5,000 J2s) of an avirulent isolate of *M. incognita*. *Me1*$^+$/*Me1*$^+$ homozygous susceptible at the *Me1* locus, *Me1*/*Me1* homozygous resistant at the *Me1* locus, *Me1*/*Me1*$^+$ heterozygous at the *Me1* locus, *BC2-S1* backcross 2 self-pollinated, *F1* Hybrid F1, *DLL* Doux Long des Landes (susceptible genotype or genetic background), *YW* Yolo Wonder (partially resistant genotype or genetic background), *DH330* Double Haploid 330 line (resistant genotype), *Bar* standard error. *Different letters* mean significant differences (Wilcoxon–Mann–Whithney bilateral tests at $\alpha = 0.05$ after Bonferroni correction)

susceptible genetic background were more easily attacked than in a partially resistant one. This genetic background effect was highly significant for *Me3* in both homozygous resistant and heterozygous BC1-S1 and in heterozygous BC2-S1 (and F1) for *Me1*. The production of EMs was higher when the part of susceptible genetic background surrounding the R-gene was increased. Unexpectedly, we observed variable levels of EM production on the DLL and YW control plants in the two experiments (Figs. 2, 4). After careful analysis of the experimental conditions, we identified variations in the composition of the commercial substrate used in both experiments, in terms of organic matter content, as the probable factor explaining such variations of nematode reproduction. However, as the differences between the susceptible and resistant plants within each experiment were significantly reproducible, and as no direct comparison was performed between the two experiments, DLL and YW indeed constitute valid controls in both experiments. Our results are in agreement with studies on other pathosystems. Influence of the plant genetic background on R-gene efficiency to nematodes was shown in tomato (López-Pérez et al. 2006) and cotton (Wang et al. 2008). It was also demonstrated that the genetic background was able to modulate the expression of an R-gene in rice, conferring more or less resistance efficiency against a bacteria (Zhou et al. 2009). Further research needs to be conducted to determine the genetic factors, within the plant genetic background, that may explain the discrepancies from a pepper genotype to another. It is likely that partial resistance factors (resistance QTLs) in YW genetic background, but absent in DLL, explain the infection rate difference observed between the two lines. These partial

resistance factors may also explain the differences observed between the YW versus DLL resistant backcross lines (BC1-S1 for *Me3* and BC2-S1 for *Me1*). In these backcross lines harboring the YW genetic background, the R-genes were probably unaffected by a high-inoculation pressure because the partial resistance factors added a protective role to the R-genes and strengthened their efficiency. On the opposite, the absence of partial resistance factors surrounding the R-genes in DLL genetic background may have weakened the resistance and favored the development of nematodes. To date, no QTLs were found against RKN in pepper. In that respect, a QTL analysis is currently ongoing on this biological material, as we strongly suppose that the protective effect of the plant genetic background on R-genes is provided by such quantitative resistance factors.

The second objective of this study was to evaluate an eventual dosage effect of the *Me3* and *Me1* alleles on the reproductive potential of RKN. Heterozygous and homozygous genotypes at the R-gene locus, in the same genetic background and at the same level of introgression, exhibited the same level of resistance. Thus, the number of alleles of the R-gene did not significantly influence the nematode production of EMs. This result indicates that there is no dosage effect of the *Me3* and *Me1* alleles on nematode reproduction. This finding is in agreement with other studies on the dosage allele effect of several other R-genes against RKN (Bost and Triantaphyllou 1982; Cap et al. 1993; Thies and Fery 2002; Cortada et al. 2009). Seemingly, other studies raised opposite conclusions (Tzortzakakis et al. 1998; Jacquet et al. 2005). However, it is noteworthy that in the studies quoted above, authors tested the homozygous *versus* heterozygous status of the R-gene in non-homogenous genetic backgrounds. Conversely, we took care of this issue in our own study, and results confirmed that it is important to consider these parameters when investigating dosage allele effect of a R-gene. Indeed, in most cases, comparing a F1 genotype (i.e., heterozygous) with the corresponding homozygous BC-S1 genotype would have led to conclude that there was a dosage allele effect, but this assertion is invalidated when comparing homozygous to heterozygous BC-S1 genotypes, which differ only for the allelic status of the R-gene. The difference observed between the F1 and the homozygous BC-S1 genotype was due to the proportion of genetic background surrounding the R-gene, not the number of alleles. The homogeneity of the genetic background is very often disregarded in dosage allele studies, whereas it is of major importance.

In the present study, the crucial role of the plant genetic background in the resistance to RKNs was clearly demonstrated using the *M. incognita*/pepper pathosystem as a model. It impacts the RKN reproduction of avirulent nematode populations. This point can have direct practical implications on breeding strategies. It is of major importance for breeders to take into account the genetic background into which they introgress major R-genes, in order to increase their efficiency and likely improve the lifetime of new elite varieties released on the market. The assumption was made that quantitative differences in level of resistance were due to QTLs. Even if harder to breed, they are considered to be more durable than typical R-gene mediated resistance (Parlevliet, 2002). However, most of the time, QTLs provide partial resistance and only reduce the level of symptoms. In addition, pyramiding QTLs with a major R-gene may result in different issues. It may have a positive effect as providing total and durable resistance (Paillard et al. 2012) or only increasing the level of resistance of the plant (Richardson et al. 2006; Jahier et al. 2009; Tan et al. 2010). On the opposite, it may have no effect (Tan et al. 2009; Riedel et al. 2011). These examples illustrate the complexity of using QTLs as many interactions could occur when exploiting this kind of resistance. Several hypotheses were proposed to explain molecular basis of quantitative disease resistance (Poland et al. 2009; Kou and Wang 2012). Thus, one of the best alternatives to avoid nematode damages without impairing *Me3* and *Me1* efficiency would be to combine them with partial resistance factors. This strategy would take simultaneous advantage of these R-genes, which provide total resistance to the three main RKN species, and of QTLs, which theoretically reduce the level of infestation. Pyramiding a qualitative resistance gene with quantitative resistance alleles resulted in a highest level of resistance to stripe rust in barley (Castro et al. 2003; Rossi et al. 2006). In addition to increase resistance efficiency, we suspect that a partially resistant genetic background may have a protective role on *Me3* and *Me1* and may prevent them from being quickly overcome. One might expect that the reduction of the nematode reproduction due to the partially resistant genetic background surrounding *Me3* or *Me1* may decrease the risk of resistance breakdown by RKNs and may increase the durability of these R-genes. This hypothesis is supported by several studies on different pathosystems which proved that the durability of R-genes was dependent on the plant genetic background into which they were introgressed (Palloix et al. 2009; Brun et al. 2010; Fournet et al. 2012). We succeeded in getting a *Me3*-virulent laboratory-selected line as described by Jarquin-Barberena et al. (1991) and we demonstrated that, once overcome, *Me3* was inefficient (data not shown). The same result was obtained with field *Me3*-virulent populations (Djian-Caporalino et al. 2011). It confirms that management of R-genes against nematodes in a sustainable way is of prime importance.

Another point that can have direct practical implications on breeding strategies relies on the absence of dosage effect of the *Me3* and *Me1* alleles on the reproductive potential

of RKNs. Consequently, since the proportion of hybrids in commercial cultivars has been increasing, taking advantage of the possibility to cumulate R-genes against different pathogens in a heterozygous status, using *Me3* and *Me1* in hybrid varieties is not an issue, as long as they are introgressed into a suitable genetic background.

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Ethical standards The authors declare that the experiments comply with the current laws of the country in which they were performed.

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