Mapping specific resistance genes to *Leptosphaeria maculans* In rapeseed (*Brassica napus* L.)

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ABSTRACT

Two types of genetic resistance to *Leptosphaeria maculans* are usually distinguished in *Brassica napus*: qualitative total resistance efficient from seedling stage and quantitative partial resistance efficient at adult plant stage. The latter is under the control of many genetic factors that have been mapped through Quantitative Trait Loci (QTL) studies using 'Darmor' resistance (Pilet et al, 1998; 2001). The former is usually ascribed to race-specific resistance controlled by single *Rlm* (Resistance to *L. maculans*) genes. Following genetic characterization of the major resistance genes occurring in the *B. napus* differential set including cultivars 'Glacier', 'Quinta' and 'Jet Neuf', the aim of our study was to map the *Rlm* genes in order to compare their localization to QTL for quantitative adult resistance. Two rapeseed genomic regions were identified as carrying specific resistance genes, different resistance genes being localized in one of them. These two regions do not correspond to regions carrying major QTL for 'Darmor' quantitative resistance.

Key words: *Brassica napus*, *Leptosphaeria maculans*, Specific resistance gene, Mapping

INTRODUCTION

Blackleg caused by *Leptosphaeria maculans* (Desm.) Ces. & de Not. (anamorph *Phoma lingam*) is one of the most economically important disease of oilseed Brassicas worldwide and especially, rapeseed (*Brassica napus* L.). Different sources of resistance to the pathogen have been identified. They are usually classified in two types of resistance: a qualitative, seedling resistance, which is generally ascribed to single-gene race-specific resistance and a quantitative adult-stage resistance, which is controlled by many genetic factors (Pilet et al, 1998; 2001). The former type was first studied using differential sets which discriminate three or six pathogenicity groups (PG) (Mengistu et al, 1991; Kuswinanti et al, 1995). Genetic approaches towards the identification of both avirulence (*Avr*) genes in the pathogen and resistance (*Rlm*) genes in the plant have been developed only recently in *B. napus*. *AvrLm1* and *AvrLm2* correspond to *Rlm1* and *Rlm2* resistance genes present in Quinta and Glacier, respectively (Ansan-Melayah et al, 1998). *AvrLm4* corresponds to *Rlm4* gene present in Jet Neuf (Balesdent et al, 2001). More recently, novel differential interactions were identified between *L. maculans* and *B. napus* cvs. and the new *Avr* genes were termed *AvrLm3* and *AvrLm7*; the *AvrLm5-6* and *AvrLm8* genes corresponding to *L. maculans* / *B. juncea* or *B. rapa* specific interactions, respectively (Balesdent et al, 2002).

In order to get a better knowledge on the genomic organization of blackleg specific resistance genes, we mapped the genes corresponding to the four *Avr* genes (*AvrLm1* to *AvrLm4*) present in the cvs of the differential sets (Quinta, Glacier and Jet Neuf). We studied also a new *L. maculans*/*B. napus* interaction which was identified in (Balesdent et al, 2002) as corresponding to the putative gene pairs *AvrLm7*/*Rlm7*. The relation between all these specific resistance genes and their potential role in adult-plant field resistance are discussed.

MATERIALS AND METHODS

Fungal and plant materials: Fungal isolates and rapeseed parental lines used in this study are listed in Table 1. The genotype of isolates were previously determined (Ansan-Melayah et al,

1998; Balesdent et al, 2001; 2002; Brun, pers. comm.).
Resistance tests at the seedling stage: Inoculations were performed as described in (Williams and Delwiche, 1979). Symptoms were scored 14 to 21 days after inoculation using a 1-9 scale according to lesion size, the occurrence of necrosis or chlorosis and the presence of pycnidia.
Field disease trials: The field disease trials and scoring of blackleg severity were conducted as previously described in (Pilet et al, 1998). The 141 DH lines of the Maxol x S006 population were evaluated at Le Rheu in 1999 in a randomized incomplete block design with two replicates and four blocks per replicate and adjusted mean disease index (DI) were estimated.

Table1: Fungal isolates and *B. napus* segregating populations used.

Cross	type	# plants	isolate name	genotype
Quinta x Score	F2	110	11.26.11	*AvrLm1 avrLm2 avrLm3 avrLm4 AvrLm7*
Quinta x Score	BC	169	v23.2.1	*avrLm1 avrLm2 avrLm3 AvrLm4 AvrLm7*
Glacier x Score	F2	110	14.3.01	*AvrLm1 AvrLm2 avrLm3 avrLm4 AvrLm7*
Glacier x Yudal	F2	189	PHW1245	*AvrLm1 AvrLm2 avrLm3 AvrLm4 AvrLm7*
Maxol x S006	HD	140	v11.1.2	*AvrLm1 avrLm2 avrLm3 avrLm4 AvrLm7*
Maxol x S006	HD	140	19.2.01	*avrLm1 avrLm2 AvrLm3 avrLlm4 avrLm7*
Darmor x Samourai	HD	134	PHW1245	*AvrLm1 AvrLm2 avrLm3 AvrLm4 AvrLm7*
			14.1.01	*AvrLm1 AvrLm2 avrLm3 AvrLm4 AvrLm7*
23-1-1 x Darmor	F2	221	290	*avrLm1 avrLm2 avrLm3 avrLm4 AvrLm7*

RESULTS

Rlm1 gene present in Quinta and Maxol is localized on LG10: Quinta x Score F2 population was previously used to assess the monogenic inheritance of the resistance in Quinta/*AvrLm1* interaction, determined by *Rlm1* (Ansan-Melayah et al, 1998). Two markers (C02.1375 and O15.1360) were found to be linked to *Rlm1*, which were previously mapped on LG10 of the *B. napus* Darmor-*bzh* x Yudal genetic map (Lombard and Delourme, 2001). The presence of *Rlm1* in Maxol was confirmed using the DH progeny Maxol x S006. *Rlm1* was mapped on the linkage group LG10 at a position corresponding to the one obtained on Quinta x Score cross.
Rlm2 gene present in Glacier and Samourai is localized on LG16: Glacier x Score F2 population was previously used to assess the monogenic inheritance of the resistance in Glacier/*AvrLm2* interaction, determined by *Rlm2* (Ansan-Melayah et al, 1998). Three markers were found to be linked to *Rlm2* but they were not polymorphic on the *B. napus* genetic maps. The presence of *Rlm2* in Samourai was assessed using the DH population derived from the cross Darmor x Samourai. *Rlm2* was mapped on LG16 of the genetic map established on Darmor x Samourai cross (Pilet et al, 2001, Lombard and Delourme, 2001). *Rlm2* was then mapped in a Glacier x Yudal F2 population. Its position corresponds to the one obtained on Darmor x Samourai cross.
Rlm3 gene present in Maxol is mapped on the same group as *Rlm1:* The DH progeny derived from Maxol x S006 was used to study Maxol/19.2.1 interaction. Sixty-one lines were scored as resistant and fifty-six lines as susceptible, which corresponds to a 1:1 segregation ratio (Chi-2= 0.21; P= 0.647). This confirms the hypothesis of a single gene, *Rlm3*, present in Maxol and interacting with *AvrLm3*. *Rlm3* was mapped on the linkage group LG10 previously established for the localization of *Rlm1*, at 30 cM from *Rlm1*.
Rlm4 gene present in Quinta is linked to *Rlm1:* The genetic linkage between *Rlm1* and *Rlm4* suggested in (Balesdent et al, 2001) was confirmed the Quinta x Score testcross population. Their distance was estimated to 3.5 cM.
Rlm7 gene present in 23-1-1 is localized on LG10*:* In the 23-1-1 x Darmor F2 population, 163 plants were scored as resistant and 58 as susceptible, which corresponds to a 3:1 segregation ratio (Chi-2= 0.18; P= 0.67). This confirms the hypothesis of a single gene, *Rlm7*, present in 23-1-1 and interacting with *AvrLm7*. Using markers of the *B. napus* map (Lombard and Delourme, 2001), *Rlm7* was mapped on the linkage group LG10 at a position corresponding nearly to the

one of *Rlm3* and *Rlm4*.

Rlm1 explained a great part of adult plant resistance in our field conditions:

A field experiment was performed at Le Rheu in 1999 on the Maxol x S006 DH population in order to evaluate the effect of *Rlm1* gene on field resistance at adult plant stage. Genotypes were continuously distributed for resistance index but the DH with *Rlm1* were on average more resistant (DI = 2.41) than the ones without *Rlm1* (DI = 4.22). QTL analysis on MT10 linkage group showed that *Rlm1* explained 70 % of the total phenotypic variation.

DISCUSSION

In this paper, we report on the mapping in *B. napus* of several specific resistance genes to *L. maculans*. A more precise genetic characterization of *B. napus* / *L. maculans* interactions was possible through the recent availability of *L. maculans* isolates genetically bred to carry as few *Avr* genes as possible (Balesdent et al, 2001; 2002). Two genomic regions in *B. napus* were identified as carrying specific resistance genes through the study of six *B. napus* / *L. maculans* interactions.

One of these regions on LG10 was identified in four interactions and possibly carries four specific resistance genes: *Rlm1*, *Rlm3*, *Rlm4* and *Rlm7.* Of these, *Rlm1* is clearly distinct from *Rlm3* and *Rlm4*. For the other three genes, our mapping study is not precise enough to know if they correspond to a cluster of tightly linked genes or to a unique gene with different alleles. Interestingly, on the fungus side, an identical situation may exist for the *AvrLm3, AvrLm4* and *AvrLm7* genes (Balesdent et al, 2002). The second genomic region on LG16 carries *Rlm2* present in Glacier and in many other *B. napus* cultivars such as Samourai, Eurol or Bristol.

The efficiency of some of the *L. maculans* specific resistance genes was assessed in the field. The adult resistance in Maxol was mainly explained by the presence of *Rlm1*, which is efficient when isolates harbouring *AvrLm1* are prevalent. Rapeseed cultivars carrying *Rlm1* gene were extensively used at a commercial scale in France but after a rather short period (4 years) their resistance was no more efficient. This could be related to the shift of the *L. maculans* populations with an increase of isolates without *AvrLm1* (Rouxel et al, submitted).

The position of *Rlm2* on LG16 corresponds to a QTL identified for adult plant resistance in Darmor x Samourai DH population (Pilet et al, 2001). Samourai carries the resistance allele at this QTL as well as *Rlm2* gene. Since no French isolate carry *AvrLm2* gene, two hypotheses can be proposed: either *Rlm2* gene has a residual effect at adult plant stage or a gene linked to *Rlm2* is responsible for the part of the variation for resistance at this QTL.

These two regions on LG10 and LG16 identified as carrying specific resistance genes to *L. maculans* do not correspond to the genomic regions carrying the most consistent QTL involved in the quantitative adult plant resistance present in Darmor. They do not correspond either to the regions where interspecific resistance genes were introgressed from *B. juncea* (Chèvre et al, 1997) or from *B. nigra* (Chèvre, unpubl. data). Then, breeding schemes aiming at cumulating intraspecific and/or interspecific major resistance genes and/or QTL within a genotype or in varietal associations can be proposed. Extensive studies on the durability of various resistance genetic factors combinations, on the fitness of some *Avr* genes, on the effect of the genetic background and cultural techniques have to be performed in order to propose an efficient management of all these resistance factors.

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