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ABSTRACT

For diversification of specific resistance genes to *Leptosphaeria maculans*, *Brassica rapa* (AA, 2n=20) populations were screened. One resistant plant was selected within a forage population. A BC_1 progeny was produced after two crosses of this plant to a susceptible *B. rapa* doubled haploid line and screened with six isolates. Resistance to different isolates proves to be under mono or oligogenic control. Resistance genes are organized in a cluster. The same *B. rapa* plant was crossed twice to 'Darmor' oilseed rape variety, susceptible at the cotyledon stage but partially resistant at the adult stage. The BC_1 oilseed rape progeny was analyzed with two isolates and showed the same results as in *B. rapa* BC_1 progeny. From 2n=38 resistant BC_1 plants, two additional backcrosses were performed in which the different resistant genes were separated through recombination. The results will be analyzed according to breeding for a durable resistance.

Key words: *Brassica napus, Brassica rapa*, blackled resistance, specific resistance genes

INTRODUCTION

Blackleg caused by *Leptosphaeria maculans* is one of the most damaging diseases of oilseed rape (*Brassica napus* L., AACC, 2n=38). Several sources of resistance have already been described: they are either total and specific, efficient from the cotyledon stage and under mono or oligogenic control (Chèvre *et al.* 1996, 1997; Balesdent *et al.* 2002) or partial, efficient at the adult stage, and under polygenic control (Pilet *et al.* 1998). Combination of different resistances is likely to be the only issue to produce material durably resistant. Nevertheless, a diversification of resistance genes is needed since various single ascospore isolates were found to be virulent at the cotyledon stage on almost all major resistance genes even those introduced from related species, *Rlm10* from *B. nigra* (Brun *et al.* 2001) or *Rlm6* from *B. juncea* (Somda *et al.* 1999). We found different specific resistance genes into a *B. rapa* variety. The genetic analysis within *B.rapa* and after introduction into an oilseed rape variety is presented.

MATERIALS AND METHODS

Plant materials: One resistant plant, C1.3 was selected within an old French forage *B.rapa* variety. It was crossed twice to a susceptible doubled haploid line, Z1 (kindly provided by AAFC, Canada) to produce F1 and from one F1 plant the BC1 progeny. The same C1.3 plant was crossed twice to a winter oilseed rape variety, 'Darmor'. From the five BC1 resistant plants selected, crosses were performed with two oilseed rape variety either 'Darmor' or 'Yudal', a Korean variety.

Fungus materials: Six isolates were used. Some of the avirulent genes (AvrLm) they carried, were identified on a differential set with the known resistance genes (table 1).

Resistance tests at the seedling stage. After inoculations performed on cotyledons as described by Williams and Delwiche 1979, symptoms were scored at 14 and 21 days using a 1 (resistant)-11 (susceptible) scale according to lesion size, the occurrence of necrosis or chlorosis and the presence of pycnidia. Each plant was tested with two isolates, one on each cotyledon.

Cytogenetic studies. Flow cytometry and meiotic behavior analyses were performed as described by Chèvre *et al.* (1996) and by Eber *et al*. (1997).

RESULTS

Genetic analyses in the BC1 progeny within *B.rapa*

Screening with S7 and R2 isolates revealed that resistance to S7 isolate is conferred by two dominant independent genes (121R:46S) whereas resistance to R2 isolate is controlled by one dominant gene (88R:72S). The low number of recombinant plants indicated that the resistance genes are linked (table 1). Additionally, at least one S7 resistance gene is closely linked to the R2 resistant gene as no recombinant susceptible to S7 isolate and resistant to R2 isolate was observed.

Resistance to each of the other isolates tested (MX4.3 and 290, S14 and p27d) is controlled either by one gene in distortion or by two partially linked genes. Whatever the test, the low frequency of recombinant plants indicated that resistance genes are linked to one another (table 1).

Table 1: Number of plants in the BC1 progeny from *B. rapa* crosses showing a resistant-resistant (RR), a resistant-susceptible (RS), a susceptible-resistant (SR), or a susceptible-susceptible (SS) phenotype after testing with both isolates, one on each cotyledon

	Phenotypes			
Combination of isolates per plant	RR	RS	SR	SS
S7(*AvrLm1+6+7*) / **R2** (*AvrLm7+10*)	47	9	0	19
MX4.3 (*AvrLm1+7+10*) / **290** (*AvrLm6+7+10*)	102	7	0	52
S14 (*AvrLm* not tested) **/ p27d** (*AvrLm1+6+7+10*)	53	1	0	31

Genetic analyses in the BC1 progeny obtained from crosses to *B.napus*

The three F1 resistant plants showed the expected genomic structure (AAC, 2n=29). Their average meiotic behavior (8.83 univalents + 9.99 bivalents + 0.04 quadrivalents) indicated that the two A genomes paired regularly. Flow cytometry analyses showed that the number of chromosomes segregated in the BC1 progeny obtained after backcrossing the F1 AAC plants to *B.napus.* A higher frequency of plants close to 38 chromosomes as oilseed rape was produced when oilseed rape was used as female (Figure 1)

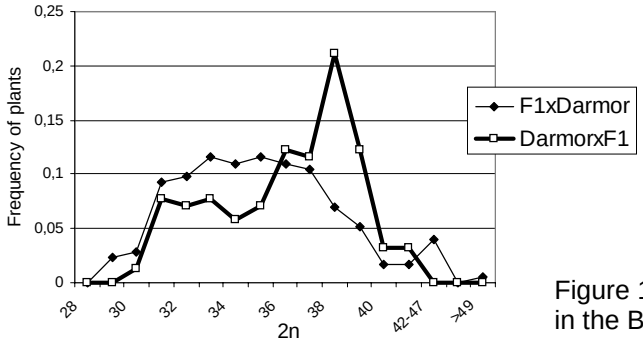

Figure 1: Segregation of the chromosome number in the BC1 progeny from AAC F1 hybrids

Pathological test was performed on the whole BC1 progeny with S7 and R2 isolates, each on one cotyledon. As in the *B.rapa* progeny, resistance to S7 segregated as two dominant independent genes (61R:17S) whereas resistance to the R2 isolate was conferred by one dominant gene (30R:28S). Among the 45 plants phenotyped with S7 and R2 isolates, 11 recombinant plants, resistant to one isolate and susceptible to the other, were observed but only one plant was susceptible to S7 isolate and resistant to R2 isolate. This result indicates at least three genes are involved and two of them linked.

Five resistant BC1 plants showing 37 or 38 chromosomes were selected and backcrossed to oilseed rape. Whatever the mother plant, resistance to the R2 isolate still segregated as one single gene. Only one BC2 progeny gave the same result as in the previous generation i.e. two independent dominant genes for the resistance to S7 isolate. In the other BC2 plants, the resistance to S7 isolate segregated as one dominant gene either closely linked to the resistance to the R2 isolate in three progenies (0 or 1cM) or more distant from R2 gene (23.6cM) in the fourth progeny.

Three 2n=38 BC2 plants resistant to both isolates were selected. In all cases, the resistance to each isolate, S7 and R2, was conferred by one dominant gene. The R2 and S7 resistance

genes linked in the BC2 progeny were totally linked in the BC3 generation whereas a distance of 17cM was found between the two genes in the other progeny.

DISCUSSION

The C1.3 *B.rapa* plant contained different resistance genes. According to the avirulent genes currently identified in the six isolates used, some resistance could correspond to *Rlm1*, *Rlm6 Rlm7* and *Rlm10*. Some of them have only been described in related species i.e. *B.nigra* (*Rlm74,* Chèvre *et al.* 1996) and *B.juncea* (*Rlm6*, Chèvre *et al.* 1997). Molecular mapping (data not shown) indicated that all these genes are linked.

Crossing the same *B.rapa* plant to an oilseed rape variety allowed the production of F1 resistant plants (AAC) with a meiotic behavior close to the expected one, 10 bivalents between the A genomes and 9 univalents from the C genome. The segregation of resistance genes carried by the *B.rapa* A genome can be therefore analyzed in the progeny. The distribution of the chromosome number at the BC1 generation is the same as the one reported by Namai (1987). A higher frequency of plants with 2n=38 was obtained when the female parent was the oilseed rape plant. However, no difference was observed for the segregation of resistance genes which was the same as the one observed in the *B. rapa* progeny. Only the genetic distances between resistance genes and molecular markers were modified in the BC1 hybrids (data not shown). In the following BC generations we showed that it is possible to separate the two genes involved in the resistance to the S7 isolate; one of them is closely linked to the gene conferring resistance to the R2 isolate. From the BC4 resistant plants, doubled haploid line will be produced and tested with different isolates carrying different *AvrLm* genes in order to characterize all the resistance genes. Additionally, the size and location of the cluster as well as the variation in genetic distances among the generations will be defined by molecular mapping.

We showed that it is possible to introduce in oilseed rape resistance genes from one of its progenitor, *B.rapa.* Different varieties will be produced with different specific resistance genes alone or combined in varieties carrying a polygenic partial resistance. The impacts of these different genetic constructions on the efficiency and durability of blackleg resistance will be studied under field conditions.

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