272Molecular and phenotypic characterization of near isogenic lines at QTL for quantitative resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus* L.)

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Abstract

The most common and effective way to control phoma stem canker (blackleg) caused by *Leptosphaeria maculans* in oilseed rape (*Brassica napus*) is through the breeding of resistant cultivars. Specific resistance genes have been identified in *B. napus* and related species but in some *B. napus* cultivars, the resistance is polygenic (mediated by Quantitative Trait Loci), postulated to be race non-specific and durable. The genetic basis of quantitative resistance in the French winter oilseed rape 'Darmor', derived from 'Jet Neuf', has been studied in two genetic backgrounds (Pilet et al. 1998, 2001) and QTL stably involved in blackleg resistance across year and genetic backgrounds were identified. Near-isogenic lines (NILs) were produced in the susceptible background 'Yudal' for three of these QTL using marker-assisted selection. New molecular markers were developed through various strategies and mapped in these QTL regions. They were used to characterise the derived NILs for the length and homozygosity of the 'Darmor' introgressed segment. Different representatives of each NIL have been experimented in blackleg disease field trials and assessed for their level of crown canker in comparison to the recurrent line 'Yudal'. This work provides valuable material that can be used to study the mode of action of genetic factors for *L. maculans* quantitative resistance.

Key words: Oilseed rape, *Brassica napus*, stem canker, quantitative resistance, QTL

Introduction

Blackleg (stem canker) of oilseed rape (*Brassica napus* L.), caused by the fungus *Leptosphaeria maculans* (Desm.) Ces. et de Not., is a serious disease in most of rapeseed producing countries (West *et al*., 2001; Fitt *et al*., 2006). In Europe, since the mid 1980's, the levels of stem canker have fluctuated, may be due to inter-year variations in climatic conditions and pathogen populations, resistance level of cultivated varieties. As chemical control is not enough efficient, efforts focus on research programs aiming at a genetic control of the disease.

In oilseed rape, several sources of resistance to blackleg are known (Delourme *et al*., 2006). Many studies on the inheritance of resistance have been done at both seedling and adult plant growth stages. Two types of resistance are usually distinguished. The first type is a qualitative resistance, which is expressed from the seedling to the adult plant stage in cotyledons and leaves and is generally considered as single-gene race specific resistance. The second type is a quantitative adult-plant resistance, which is a partial resistance mediated by many genes. Quantitative resistance is considered to be race non-specific and more durable than qualitative resistance.

The genetic basis of quantitative resistance in the French winter oilseed rape 'Darmor', derived from 'Jet Neuf', has been studied. In the 'Darmor-*bzh*' × 'Yudal' cross, Pilet et al. (1998) identified a total of ten QTL for resistance, of which four were associated with decreased stem canker severity and decreased plant death in both seasons of field experiments. Analysis of progeny derived from a 'Darmor' × 'Samourai' cross, consisting of one DH population and a number of $F_{2:3}$ families, identified six QTL in the DH population and four QTL in the $F_{2:3}$ families (Pilet et al., 2001). Out of a total of sixteen loci detected in the four cultivars, only four QTL were common to the 'Darmor-*bzh*' × 'Yudal' and 'Darmor' × 'Samourai' crosses.

Near isogenic lines (NILs) for three QTLs were initiated in order to validate the effect of individual QTL in the susceptible background 'Yudal'. Two of these QTL were stably detected in the two DH populations (on linkage group 3 and 11) and the other one (on linkage group 5) was specific to 'Darmor-*bzh*' × 'Yudal' cross but was detected across the two-year trials.

Material and Methods

Backcrosses to 'Yudal', the susceptible parent, were initiated from doubled haploid (DH) lines of the segregating population derived from the cross 'Darmor-*bzh*' × 'Yudal'. The DH lines were chosen with the genotyping data to carry the targeted QTL and to be as close as possible to 'Yudal' genetic background. Backcrosses were made until BC2 and BC3 generations and then selfing was performed to get NILs homozygous at the QTL. Screening of the plants was made using markers located in the QTL regions and markers to control the genetic background. For each QTL, dwarf and tall lines were derived. Molecular markers were developed through various strategies and mapped in these QTL regions. They were used to characterise the derived NILs for the length and homozygosity of the 'Darmor' introgressed segment.

Different representatives of each NIL were experimented in blackleg disease field trials and assessed for their level of stem canker in comparison to the recurrent line ‘Yudal’. The field disease trials and scoring of blackleg severity (G2 disease index) were conducted as previously described in Pilet *et al.*, (1998). NILs for the three QTLs were evaluated at one location with three replications (INRA Le Rheu) in 2004-05. NILs for QTL5 and QTL11 were evaluated at four locations in 2005-06 (INRA Le Rheu, CETIOM Grignon, PIONEER Génétique Blois & EURALIS Semences Blois). Eight replications were performed in each location to increase the precision of the tests. In order to have a good comparison to ‘Yudal’, which is a very susceptible line, the disease assessment was performed between 16th and 30th of May according to the location.

Results and Discussion

A total number of 39 NILs were produced (31 tall lines and 8 dawrf lines). The characterization of these NILs with the molecular markers that were developped in the QTL regions led to an estimation of the length of the ‘Darmor’ introgressed segment for each QTL (QTL3 : 20 – 47 cM; QTL5 : 30 – 52 cM ; QTL11 : 28 – 39 cM).

These 39 NILs were experimented in 2004-05 at one location. The figure 1 shows the disease level assessed on some of these lines. The disease level of the controls is low because of the date of the disease assessment. The comparison of the mean G2 disease index obtained for the different NILs and ‘Yudal’ showed that:

- The *Bzh* dwarf gene has a significant effect on G2 disease index as previously detected in the ‘Darmor-*bzh*’ × ‘Yudal’ DH population (Pilet *et al.*, 1998).
- No significant effect on G2 disease index was detected for QTL3.
- A significant effect was detected for QTL5 for 3 NILs out of 9.
- A significant effect was detected for QTL11 for 9 NILs out of 11.

For QTL3, a new QTL detection performed with an updated genetic map on the ‘Darmor-*bzh*’ × ‘Yudal’ cross showed that there were potentially two QTL located on the linkage group DY3. Only one of these QTL was retained in the produced NILs. The effect of the other QTL which seems to have a bigger effect on G2 disease index could not be assessed.

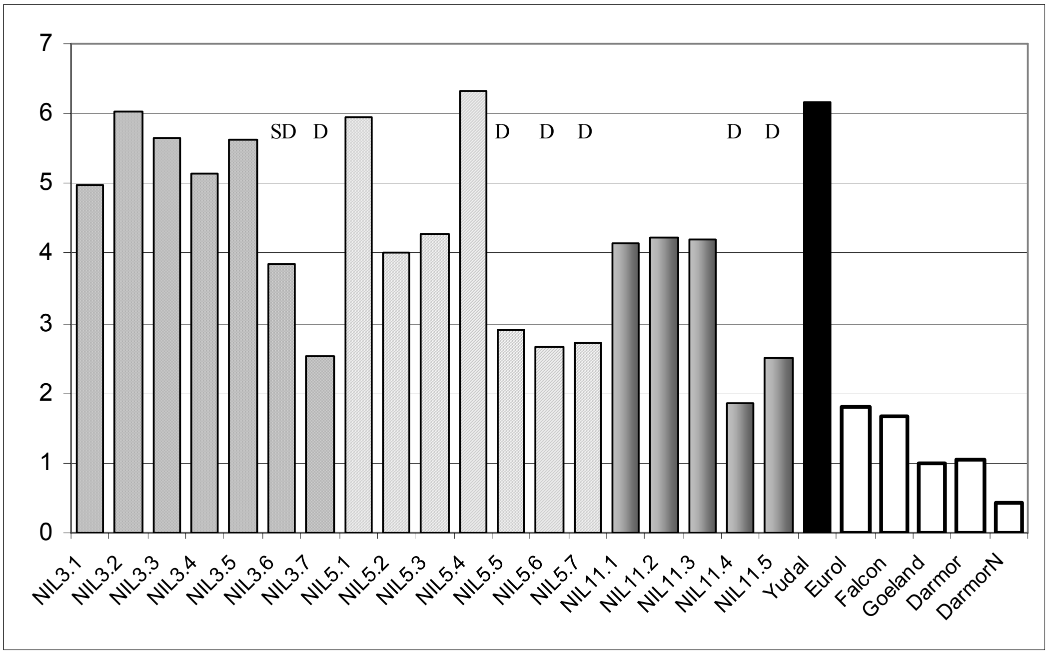

Figure 1: G2 disease index of NILs for QTL3, QTL5 and QTL11 in comparison to ‘Yudal’ and controls (‘Eurol’, ‘Falcon’, ‘Goeland’, Darmor, Darmor-bzh) in one location (INRA Le Rheu).

D= dwarf lines; SD=semi-dwarf line

In 2005-06, only some tall representative NILs for QTL5 and QTL11 were experimented in four locations (figure 2). As in 2004-05, the disease level of the controls is low because of the date of the disease assessment. Despite different levels of disease, the results of the four trials were very consistant and showed that:

--- No significant effect on G2 disease index was detected for QTL5. The two NILs (NIL5.2 and NIL5.3) which were significantly less susceptible than ‘Yudal’ in 2004-05 at Le Rheu location were still less susceptible than ‘Yudal’ in 2005-06 at this same location but the difference was not significant. These two lines were not less susceptible in the other locations.

--- A significant effect was detected for QTL11 in all locations.

--- The G2 index difference between ‘Yudal’ and NILs with QTL11 was estimated on average at 1.4 (1.1 to 2.3 according to the location).

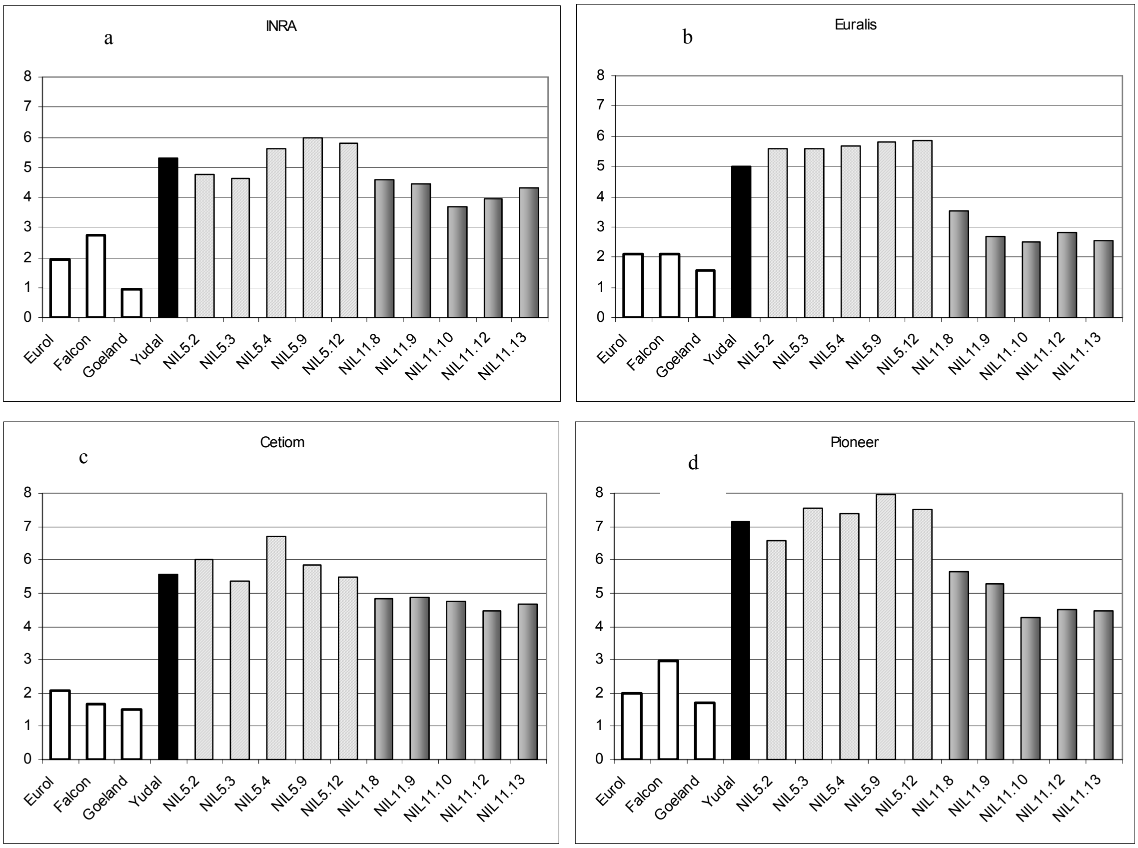

Figure 2: G2 disease index of NILs for QTL5 and QTL11 in comparison to 'Yudal' and controls ('Eurol', 'Falcon', 'Goeland') in four locations (a: INRA Le Rheu; b: Euralis Blois; c: CETIOM Grignon; d: Pioneer Blois)

These results provide valuable material that can be used to study the mode of action of genetic factors for *L. maculans* quantitative resistance, especially the one underlying QTL11. New NILs are being produced for the QTL on linkage group 3 that could not be assessed in this study and pyramiding of some QTls will be carried on in order to test their cumulative effect and to test for potential epistatic effect.

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