Quantitative resistance associated with a very efficient major gene in a single *Brassica napus* genotype increases the potential durability of the resistance to *Leptosphaeria maculans*

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Abstract

The durability of resistance is an important question in plant disease resistance breeding. Resistance to *Leptosphaeria maculans* conditioned by *Rlm6* (MX), which was introgressed from *Brassica juncea* to a susceptible *B. napus* genotype is very high but is not durable. In order to determine the effect of the polygenic resistance of oilseed rape on the potential durability of this major gene, 2 oilseed rape genotypes (Eurol and Darmor) with differences in quantitative resistance at the adult stage were assessed in pairs of near-isogenic lines differing for *Rlm6*. The lines into which the major gene was introgressed (EurolMX and DarmorMX) were highly resistant when evaluated using the standard cotyledon stage test and cotyledon or leaf infections were rarely observed in the field. Cotyledons and leaves of Darmor (polygenic resistance only) displayed high levels of infection (very susceptible) but this genotype displayed partial resistance at the adult plant stage. One field experiment was conducted for 4 years following a protocol developed to increase the selection pressure of *L. maculans* populations on the resistance of *B. napus* genotypes. Each year, 4 separate fields were established in which fungal populations were selected recurrently on residue of 1 of the 4 *B. napus* genotypes (Eurol, EurolMX, Darmor, DarmorMX), which was used as primary inoculum in the next year. MX resistance broke down in the fourth year of the study in the Eurol genetic background when the primary inoculum was recurrently selected on EurolMX. The resistance of DarmorMX, which carries both types of resistance, remained effective in the fourth year of the experiment when the primary inoculum was recurrently selected on the residue of DarmorMX. These results suggest a slower adaptation of the virulent isolates on this line and therefore an effect of the polygenic quantitative resistance on the durability of the resistance.

Key words: Oilseed rape, Blackleg, major gene, fungus adaptation, near isogenic lines.

Introduction

Leptosphaeria maculans is the fungus responsible for Blackleg of oilseed rape, a very damaging disease worldwide (Fitt *et al*., 2006). Many breeding programs aim to improve the level of the cultivar resistance using various resistance sources (Delourme *et al.,* 2006). Two kinds of resistance are described in *Brassica napus.* The first type is major gene which results in hypersensitive reaction expressed from cotyledon stage onwards. This major gene resistance is under gene for gene control and hence is specific to some isolates carrying the corresponding avirulence gene. This resistance can be very high if most of isolates in the fungal populations carry the corresponding avirulence gene. Nevertheless, it also exerts a high selection pressure on the fungal populations and relatively rapidly (3-4 years after the release of the resistant variety) the fungal populations adapt until effectiveness of the new resistance is lost (Rouxel *et al,* 2003; Sprague *et al.,* 2006). The second type of resistance is described as polygenic quantitative resistance, which is effective mainly at adult stage by reducing the size of stem base necrosis; it is considered non specific and hence more durable than major gene resistance. This kind of resistance is strongly affected by environmental conditions and often the resistance is less effective when the conditions are highly conducive to the disease. Among the several major genes available in *B. napus* and other *Brassica* species, a very efficient resistant line (MX) was selected by UMR APBV (INRA, Le Rheu) through interspecific crosses between oilseed rape and brown mustard (Chèvre *et al.,* 1997). The resistance factor segregates as a single major gene designated *Rlm6*. The resistance is efficient under controlled conditions at the cotyledon stage and under field conditions. Field experiments that evaluated its potential durability have demonstrated that this resistance has a short potential durability when it was introgressed into a very susceptible oilseed rape background. Virulent isolates were selected recurrently (Somda *et al*., 1999) and increased within *L. maculans* populations until they overcome the resistance within 3 cropping seasons (Brun *et al*., 2000). It is often recommended to introduce major gene(s) into cultivars with quantitative resistance. Hence, if the major gene resistance is overcome the yield losses of the crop are limited. The question addressed here is whether the quantitative resistance of the line in which the major gene is introgressed can also increase the durability of that gene.

Materials &Methods

Two pairs of near isogenic lines (NIL's) differing for their level of quantitative resistance and for the presence of the *Rlm6* gene were used: Eurol-EurolMX and Darmor-DarmorMX. Darmor carries a high level of quantitative polygenic resistance (Pilet *et al*., 1998). Samouraï and Shogun, both highly susceptible, were used as control cultivars.

Field experiments were established in Brittany near Le Rheu (Fig. 1). Only one field trial was performed the first year. It was inoculated with local susceptible oilseed rape stubble (2 residues per square meter). For the following years, 4 series of trials (PHO1, PHO2, PHO3, PHO4) each including all the lines assessed, were separated from each other by at least 1 km. They were contaminated with the same number of stem base residue (2 per square meter) of Eurol (PHO1), EurolMX (PHO2), Darmor (PHO3) and DarmorMX (PHO4) collected randomly from the corresponding line grown in the previous trial in each series. The field experiments were performed according to the protocol of Brun *et al*. (2000) for inoculating and scoring of the disease (leaf lesions and crown canker).

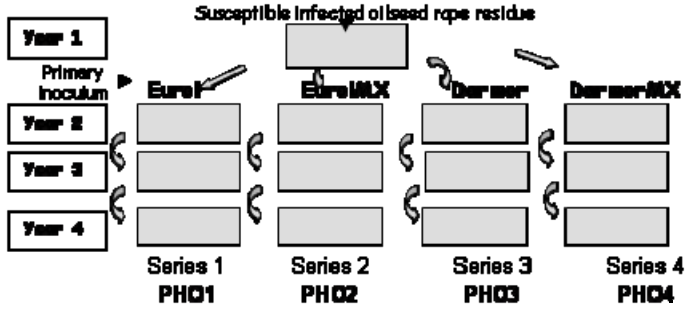

Figure 1. Scheme of the field experiments with the 4 multi-year series: PHO1, PHO2, PHO3, PHO4 recurrently contaminated with residues of Eurol, EurolMX, Darmor, DarmorMX, respectively.

Single ascospore isolates were collected from pseudothecia present on basal part of the residues used as primary inoculum in each of the 4 series of field experiments [i.e. from Eurol (PHO1), EurolMX (PHO2), Darmor (PHO3), DarmorMX (PHO4)]. It was very difficult to find residues carrying pseudothecia on EurolMX and on DarmorMX in 2003 (only 14 and 13 isolates from 14 and 13 plant base residues with pseudothecia, respectively) and for DarmorMX for the following years (9 and 7 isolates collected in autumn 2004 and 2005, respectively). The isolates were analyzed for their virulence pattern in controlled conditions on a differential host set at cotyledon stage to identified 7 avirulence genes (*Avr).*

Results

Avirulence patterns

No isolates carrying *Avr2*, *Avr3* and *Avr9* genes were detected in *L. maculans* populations assessed from the experiments regardless of the line or the year from which the isolates were collected.

In autumn 2002, of the 50 isolates assessed 83.7% carried *Avr6* and *Avr5,* 30.6 isolates carried *Avr1*and 18.4% carried *Avr4* (Fig. 2).

Some variation in frequency of the *Avr* genes was observed among years when the fungal populations were selected from Eurol (PHO1) and Darmor (PHO3). When the residues of EurolMX and DarmorMX were used as primary inoculum (PHO2 and PHO4), the isolates carrying *Avr6* disappeared as a consequence of the use of *Rlm6,* and at the same time isolates with *Avr1* increased. Over the 3 years it was difficult to find pseudothecia of *L. maculans* on DarmorMX; and most of the pseudothecia found on this line belonged to *L. biglobosa.*

Assessment of disease under field conditions

Leaf lesions where scored each year in autumn (data not shown).

In the 2nd and 3rd years the disease pressure was lower on the susceptible line Eurol than in year 1 and 4 (Fig. 3). EurolMX and DarmorMX remained highly resistant (DI<1.0) over years when the recurrent residues used as primary inoculum were from Eurol (PHO1) and Darmor (PHO3). Darmor displayed a high level of resistance to crown canker in both the trials, whereas it was very susceptible to leaf lesions (data not shown).

The use of *Rlm6* lines as primary inoculum in 2003 caused a dramatic decrease in disease level in trial PHO2 (DI=1.1 for Eurol) and in PHO4 (DI=2.4 for Eurol) compared to PHO1 and PHO3 (DI=3.6 for Eurol). When EurolMX residues were used recurrently, the disease increased and no statistical difference between Eurol and EurolMX was observed in the following years. On the contrary, when DarmorMX residues were used the disease level remained very low until the end of the 4th year.

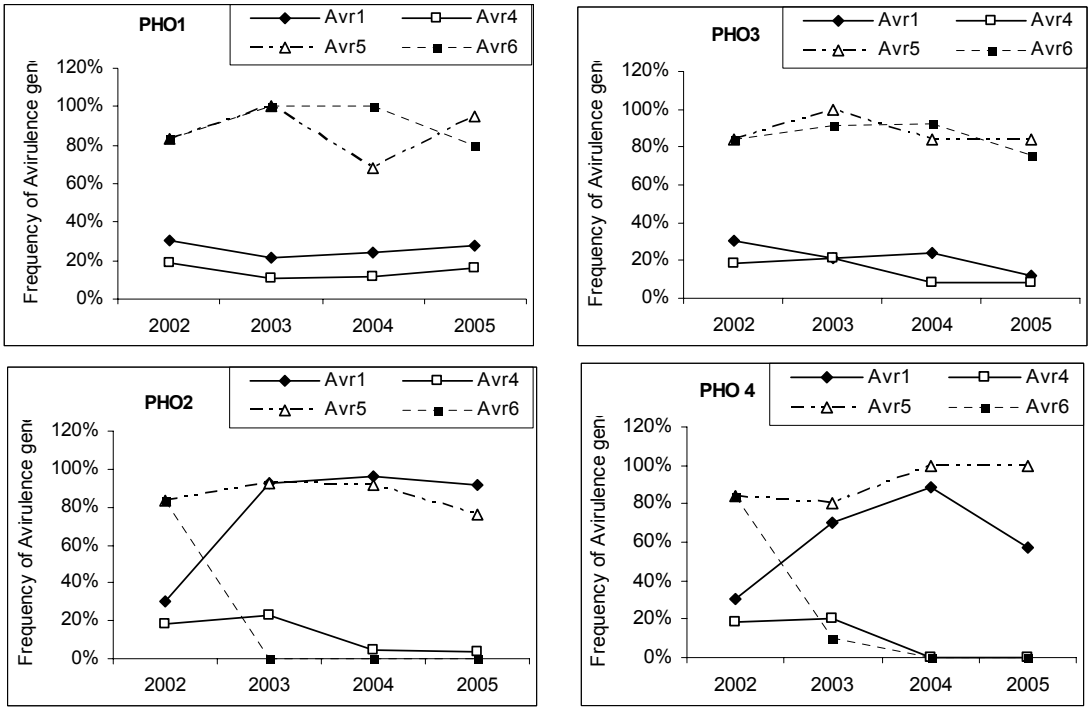

Figure 2. Change in frequency over years of 4 avirulence genes within single ascospore isolates collected every year from the lines used as primary inoculum: residue of Eurol (PHO1), residue of EurolMX (PHO2), residue of Darmor (PHO3), residue of DarmorMX (PHO4).

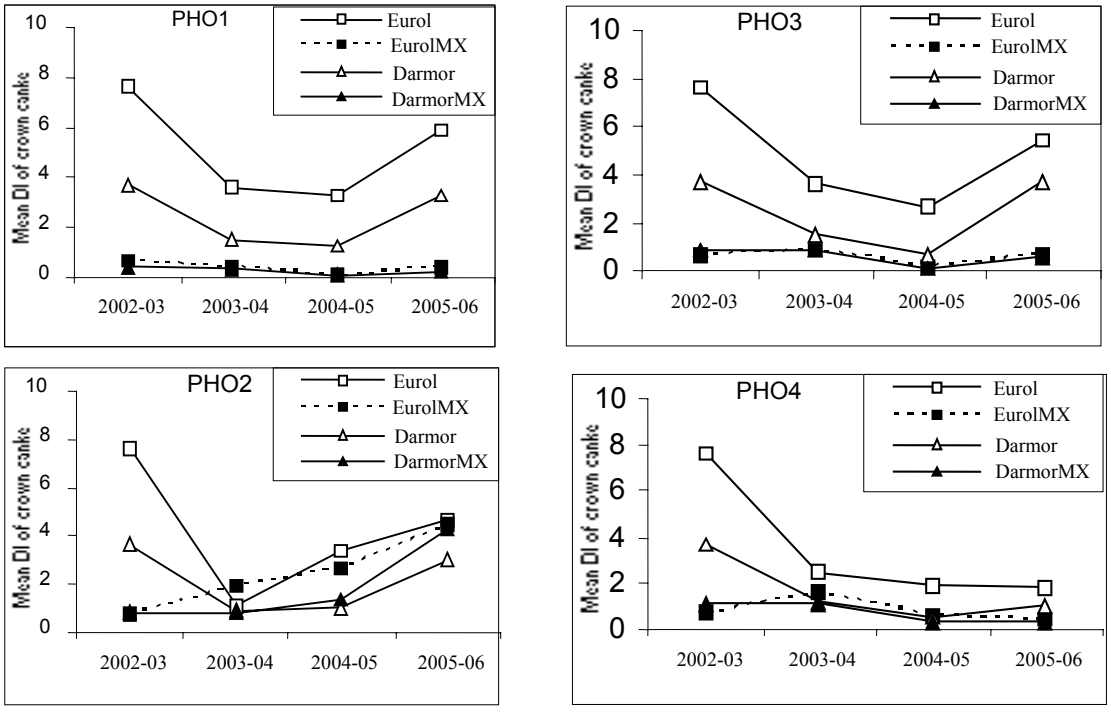

Figure 3. Evolution over years of the mean disease indices of crown canker (0=healthy plants, 9=all plants broken) for 2 pairs of near isogenic lines differing for the *Rlm6* (MX) major gene introduced into lines with (Darmor), or without (Eurol) quantitative polygenic résistance. *Leptosphaeria maculans* populations were selected recurrently on the residue of each of the 4 lines (PHO1=residue of Eurol, PhO2=residue of EurolMX, PHO3=residue of Darmor, PHO4=residue of DarmorMX).

Discussion

This study demonstrated that the introduction of the major resistance gene *Rlm6* into a line possessing a high level of polygenic quantitative resistance (Darmor) increased the durability of the resistance compared to its introduction in a susceptible background (Eurol). Therefore, among the different hypotheses given by Brun *et al*., (2000) to explain the superior durability of *B. nigra* resistance compared to *B. juncea* resistance, both introduced into *B. napus* lines with or without

quantitative resistance, the quantitative resistance may play an important role.

The frequency of avirulence genes was stable, except in 2004 for *Avr5*, over years for *L. maculans* populations selected on cultivars (Eurol and Darmor) without selective pressure on the isolates. On the contrary, for the genotypes possessing *Rlm6,* the *Avr6* isolates were counter-selected. Surprisingly, the frequency of *Avr1* isolates increased at the same time. Perhaps this increase was due either to chance and/or to better fitness of *Avr1avr6* isolates than *avr1avr6* isolates.

The second year of the experiment in the trials where *Rlm6* plant residues were used as primary inoculum the disease decreased dramatically. Indeed, only 16.7% of the isolates were able to infect EurolMX and DarmorMX lines in the 1st year. However, this phenomenon was expressed only for a very short time when *Rlm6* was introduced into a susceptible background (Eurol), then the disease increased rapidly. On the contrary, the resistance remained effective when coupled with quantitative resistance of DarmorMX, at least until the 4th year. These results indicate that the polygenic quantitative resistance of Darmor increased the efficiency of both types of resistance.

Jet Neuf was cultivated as the only cultivar for several years (from 1977 to 1983), in France and in Europe without erosion of its resistance. Jet Neuf carries quantitative resistance (Pilet *et al*., 1998) and the high durability of its resistance was generally attributed to its quantitative nature. Jet Neuf also carries the major gene *Rml4*. Few *Avr4* isolates (less than 10% in average) are presently detected within *L. maculans* populations in France and EU countries (Stachowiak *et al*., 2006). Nevertheless, we can assume that before the cultivation of Jet Neuf the *Avr4* isolates could have been more frequent than presently reported. As a result, has the quantitative resistance been protected from erosion by this supposed efficient major gene? Therefore, a crucial question remaining to be addressed is to know whether the *L. maculans* populations are able to adapt to quantitative resistance by increasing their aggressiveness.

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References

Brun, H., Levivier S., Ruer D., Somda I., Chèvre A.M., Renard M. 2000. A field method for evaluating the potential durability of new resistance sources: application to the *Leptosphaeria maculans/Brassica napus* pathosystem. Phytopath. 90: 961-966.

Chèvre A.M., Barret P., Eber F., Dupuy P., Brun H., Tanguy X., Renard M. 1997. Selection of stable *Brassica napus- B. juncea* recombinant lines resistant to Blackleg (*Leptosphaeria maculans*) 1: Identification of molecular markers, chromosomal and genomic origin of the introgression. Theor. Appl. Gen. 95**:** 1104-1111.

Delourme R., Chèvre A.M., Brun H., Rouxel T., Balesdent M.H., Rimmer R., P. Salisbury. 2006. Major genes and polygenic resistance to *L. maculans* in oilseed rape. *European Journal of Plant Pathology* 114: 41–52

Fitt B. D. L., Brun H., Barbetti M. J. and S. R. Rimmer. 2006. World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). *European Journal of Plant Pathology* 114: 3-15.

Pilet, M.L., R. Delourme, N. Foisset and M. Renard, 1998, Identification of QTL involved in field resistance to light leaf spot (*Pyrenopeziza brassicae*) and blackleg(*Leptosphaeria maculans*) in winter rapeseed (*Brassica napus* L.). Theor. Appl. Gen., 97, 398-406.

Rouxel T, A. Penaud , L. Jain , X. Pinochet , H. Brun , L. Gout, R. Delourme, J. Schmit, Balesdent M-H. 2003. A ten-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape. *European Journal of Plant Pathology* 109: 871-881.

Somda I., Delourme R., Renard M., Brun H. 1999. Pathogenicity of *Leptosphaeria maculans* isolates on a *Brassica napus-Brassica juncea* recombinant line. Phytopath. 89: 169-175.

Sprague S.J., Balesdent M., Brun H., Hayden H.L., Marcroft S.J., X. Pinochet, Rouxel T., B. J. Howlett. 2006. Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence of populations of *Leptosphaeria maculans* in France and Australia. *European Journal of Plant Pathology* 114: 33–40.

Stachowiak A., Olechnowicz J., Jedryczka M., Rouxel T., Balesdent M. H., Happstadius I., Gladders P, Akinwunmi Latunde-Dada and N. Evans. 2006. Frequency of avirulence alleles in field populations of *Leptosphaeria maculans* in Europe. *European Journal of Plant Pathology* 114: 67-75.