Five years of field experiments on the potential durability of the resistance of highly resistant *Brassica napus* lines to *Leptosphaeria maculans*

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ABSTRACT

A great concern in plant disease resistance breeding schemes is the question of the durability of the resistances selected. It was established that the resistance to *Leptosphaeria maculans* due to *Rlm6* (named MX) introgressed from *Brassica juncea* into a very susceptible *Brassica napus* line possesses a weak potential durability. In order to determine the effect of the genetic background of oilseed rape on the potential durability of the resistance to *L. maculans* due to this major gene, four lines differing for their own levels of resistance at the adult stage were assessed in pairs of near-isogenic lines (NILs) differing for the *Rlm6* gene. The resistance of the lines (named MX) into which the resistance was introgressed was highly efficient at cotyledon stage under controlled conditions and at cotyledon/adult plant stages under field conditions in standard trials. Multi-year field experiments were performed for 5 years according to a protocol adapted to increase the selective pressure of the new resistance on fungus populations. Separate field trials were established in which the populations were selected recurrently on residue of one MX genetic background used as primary inoculum in the next year. The MX resistance broke down in the fourth year of the experiment for two genetic backgrounds. The resistance of one MX line that carries in addition another major gene *Rlm1* was always efficient in the fifth year of the experiment. The MX lines exhibited different behaviours when they faced the virulent populations according to the genetic background on which the virulent fungus populations were selected. These results may suggest a differential adaptation of the virulent isolates on the genetic background and an effect of the pyramiding of major genes.

Key words: Oilseed rape, Blackleg, potential durability, fungus adaptation, plant genetic background.

INTRODUCTION

Leptosphaeria maculans is the fungus responsible for Blackleg of oilseed rape, a very damaging disease worldwide (Gabrielson, 1983). Many breeding programs aim to improve the level of the cultivar resistance using different resistance sources (Rimmer & van den Berg, 1992). A very efficient resistance named MX was selected by UMR APBV (INRA Le Rheu) through interspecific crosses between oilseed rape and brown mustard (Chèvre *et al.,* 1997). The resistance is efficient under controlled conditions at cotyledon stage and under field conditions. The resistance segregates as a single major gene named *Rlm6*. A field experiment performed to evaluate its potential durability demonstrated that this resistance had a short potential durability when it was introgressed into a very susceptible oilseed rape background. Virulent isolates were selected (Somda *et al*., 1999) and they increased within populations to break down the resistance within 3 cropping seasons (Brun *et al*., 2000). The question addressed is to know if the genetic background in which the *Rlm6* gene is introgressed can modify its potential durability.

MATERIALS AND METHODS

Four different couples of near isogenic lines (NIL's) differing for their level of general resistance and for the presence of the *Rlm6* gene (named MX lines) were used : Eurol-EurolMX, Falcon-FalconMX, Samouraï-SamouraïMX, Maxol-MaxolMX. Maxol carries in addition the *Rlm1* gene efficient against French fungus populations where *AvrLm1* isolates were prevalent between 1990 and 1998. Cultivars 'Darmor' with high level of polygenic resistance (Pilet *et al*., 1998), 'Samouraï" with intermediate susceptibility and 'Shogun' with high susceptibility were used as control cultivars.

Two field experiments were established (i) in Centre of France at St Florent, in a traditional oilseed rape cropping region and (ii) in Brittany at Le Rheu. Only one field trial was performed per site the first year of the experiment. It was inoculated with local susceptible oilseed rape stubble. For the following years, 3 series of trials, separated from each other by at least 1 km, were inoculated with stem base residue of EurolMX, FalconMX and MaxolMX collected from the corresponding previous experiment trial, respectively. The experiments were performed according to Brun *et al*., 2000 protocol for inoculating and scoring of the disease.

RESULTS

At the two locations, the resistance of all the MX lines remained efficient for both leaf lesions (data not shown) and crown canker and significantly different from the corresponding NIL without *Rlm6*, until the third year (Table 1 and 2). At the 2 sites, the resistance of some MX lines (EurolMX, FalconMX) broke down in the fourth year. The resistance of MaxolMX was overwhelmed at St Florent when the fungus populations were selected recurrently on FalconMX and MaxolMX plant residue but remained effective when the fungus populations were selected on EurolMX at St Florent and on MaxolMX at Le Rheu. SamouraïMX was highly resistant even in the fourth and the fifth years against virulent populations selected on EurolMX and FalconMX. 'Darmor' remained more resistant than 'Shogun' in most of the trials.

Table 1. Assessment over years of crown canker due to *Leptosphaeria maculans* on MX and recurrent oilseed rape lines, evaluated in three separate field trials inoculated at the autumn with stem canker residue of one of the three MX lines, respectively. Rennes (Ille & Vilaine)

Plant genotypes	First year	Second year			Third year			Fourth year			Fifth year		
	1[1]	1[2]	2[3]	3[4]	1[2]	2[3]	3[4]	1[2]	2[3]	3[4]	1[2]	2[3]	3[4]
Darmor	1.1de	2.3d	2.4bc	2.2d	3.6d	3.6d	3.5c	1.2f	2.5c	2.6c	3.7c	2.6c	2.1cd
Shogun	5.6a	7.2a	6.6a	5.8a	5.9b	5.7c	5.3ab	2.8d	4.5abc	4.4b	6.8a	4.6a	5.1a
Samouraï	2.3c	6.9a	6.4a	4.9b	4.9c	7.2ab	6.0a	2.9d	3.8c	4.3b	6.2ab	4.1ab	3.3bc
SamouraïMX	0.9de	-	-	-	0.7f	0.4e	0.2d	0.6g	0.9d	0.2d	0.7d	1.1d	2.0cd
Vivol	0.6e	-	-	2.8d	-	-	-	-	-	-	-	-	-
Eurol	3.3b	**5.1b**	-	-	**5.8b**	7.0ab	5.3ab	**3.7c**	-	-	**5.5abc**	-	-
EurolMX	0.8de	**4.1c**	-	-	**2.5e**	2.9d	0.3d	**4.3bc**	-	-	**6.8a**	-	-
Falcon	1.7cd	3.8c	**3.5b**	3.9c	5.1bc	**6.0bc**	4.8b	2.0e	**3.7c**	4.4b	-	**3.0bc**	2.1cd
FalconMX	0.6e	-	**1.3cd**	-	2.7e	**2.5d**	0.2d	-	**4.1bc**	-	-	**4.1ab**	-
Maxol	1.0de	-	-	**3.9c**	4.0d	5.1c	**4.8b**	-	-	**6.3a**	-	-	**3.0bc**
MaxolMX	0.6e	-	-	**2.2d**	0.9f	0.4e	**0.3d**	-	-	**0.4d**	-	-	**1.1d**

[1] : trial inoculated with susceptible oilseed rape stubble
[2]1 :trial inoculated with EurolMX stubble from the previous trial used as primary inoculum at the autumn.
[3]2 : :trial inoculated with FalconMX stubble from the previous trial used as primary inoculum at the autumn.
[4]3 : :trial inoculated with Maxol MX stubble from the previous trial used as primary inoculum at the autumn.
[5]0 to 9 : Mean disease index of crown canker, 0 : healthy to 9 : plants totally broken.
[6]4 : figures with the same letter are not significantly different (Newman & Keuls : 0.05%).

Table 2. Assessment over years of crown canker due to *Leptosphaeria maculans* on MX and recurrent oilseed rape lines, evaluated in three separate field trials inoculated at the autumn with stem canker residue of one of the three MX lines, respectively. St Florent (Cher)

Plant genotypes	First year	Second year			Third year			Fourth year		
	1[1]	1[2]	2[3]	3[4]	1[2]	2[3]	3[4]	1[2]	2[3]	3[4]
Darmor	1.5[5]a[6]	2.2c	2.8b	2.7a	3.4bc	2.7b	1.3ab	2.3ab	2.8b	3.8ab
Shogun	6.3c	5.3e	6.3d	6.7c	4.1bc	3.6b	2.1b	3.7c	3.9c	4.4b
Samouraï	4.8b	5.1e	4.4c	5.6bc	**-**	-	-	**-**	-	-
Eurol	5.4bc	**5.1e**	4.1c	5.9bc	**4.7cd**	5.1c	2.4b	**4.3c**	4.5c	4.5b
EurolMX	0.7a	**0.8b**	1.5a	2.8a	**1.7a**	1.1a	1.2ab	**4.1c**	4.2c	3.8ab
Falcon	4.3b	3.5d	**3.3b**	4.6b	4.1bc	**2.9b**	2.0b	3.1bc	**2.8bc**	4.0ab
FalconMX	0.8a	0.3a	**1.7a**	2.1a	2.8ab	**1.5a**	1.5ab	3.1bc	**3.4c**	3.3a
Maxol	1.5a	4.8e	3.4b	**4.6b**	5.6d	5.5c	**4.3c**	3.9c	4.4c	**4.6b**
MaxolMX	0.7a	-	-	**-**	1.5a	1.2a	**0.7a**	1.7a	2.8b	**3.9ab**

[1] : :trial inoculated with susceptible oilseed rape residue at the autumn
[2]1 : :trial inoculated with EurolMX stubble from the previous trial used as primary inoculum at the autumn.
[3]2 : :trial inoculated with FalconMX stubble from the previous trial used as primary inoculum at the autumn.
[4]3 : :trial inoculated with MaxolMX stubble from the previous trial used as primary inoculum at the autumn.
[5]0 to 9 : Mean disease index of crown canker, 0 : healthy to 9 : plants totally broken.
[6]4 : figures with the same letter are not significantly different (Newman & Keuls : 0.05%).

DISCUSSION

Our results obtained under field conditions validated the field experiment protocol to evaluate the potential durability of new major resistance genes before their commercialisation and therefore it could be used to generate data for modelling the adaptation of fungus populations to resistant plant genotypes over years and/or to validate a model of durability. Particularly, rapeseed cultivars carrying one major resistance gene (*Rlm1*) extensively used at a commercial scale in France recently lost their resistance effectiveness after a similar 4 year short crop period as in our field experiments (Rouxel *et al.,* 2003).

The dynamics of the resistance breaking down of some MX lines are similar at the 2 sites, located very far from each other, when there was no efficient major genes in addition to *Rlm6* as EurolMX and FalconMX. Nevertheless, when the line carries another major gene as *Rlm1* in MaxolMX the 2 genes might work as gene pyramiding depending on the initial fungus populations. A surprising result was the behaviour of SamouraïMX which doesn't carry any known major gene efficient against local populations except *Rlm6* and it was nevertheless highly resistant against virulent EurolMX and FalconMX populations. These results suggest a high adaptation ability of the virulent fungus populations to the genetic background on which they were selected.

The comparison of the initial population structure and of virulent populations selected by each of the MX line in the fourth year and in the 2 locations for avirulent/virulent genes might give explanation about the differences existing between these populations and give explanation about the effect of the genetic background.

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