Sensitivity of Winter Rapeseed (*Brassica napus* L.) to Isolates of the Fungus *Leptosphaeria maculans* (Desm.) Ces. et De Not.

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Abstract

The pure cultures of the fungus *Leptosphaeria maculans* were isolated from the rapeseed leaves collected in Moravia. Two isolates were collected on the basis of their ability to produce yellow colour. The isolate producing yellow colour was classified as aggressive, while the isolate without the ability to produce yellow colour was not aggressive. The sensitivity to these isolates was verified in 11 rapeseed genotypes. This test was based on the method from the document Crucifer Genetics Cooperative (information document from the Department of Plant Pathology, University of Wisconsin). This method is based on the inoculation of cruciferous leaflets with suspension from the isolate of the pathogen observed. The scale from 0 to 9 was used for evaluation (0 = no darkening around the wound, 9 = accompanied by profuse sporulation in large, more than 5 mm, lesions with diffuse margins). The infection degree of the aggressive isolate ranged in different genotypes from 1.45 to 4.5, the results of the non-aggressive isolate ranged from 1.0 to 1.5. Based on these results the aggressive isolate was used for further tests of the selected breeding lines. The tests involved selected breeding materials, registered varieties, control varieties with different sensitivity to the isolate of *Leptosphaeria maculans* and control varieties included in state trials. The maximal difference in the sensitivity exhibited by individual genotypes was five degrees.

Keywords: *Leptosphaeria maculans*; *Brassica napus;* resistance

INTRODUCTION

Leptosphaeria maculans (Desm.) Ces. et De Not.1863 belongs to the order *Pleosporales*. *Leptosphaeria maculans* causes phoma stem canker, which can be economically very damaging in many areas of the world, particularly in Europe, Australia and Canada (WEST *et al*. 2001). Yield losses attributable to stem canker are usually less than 10%, but losses of 30 and 50% have also been reported (WEST *et al*. 2001). The colonisation of winter oilseed rape plants and epidemiology of phoma stem canker differed between A/Tox$^+$ and B/Tox^0 *Leptosphaeria maculans* (WEST e*t al*. 2002). High concentrations of rapeseed plants and the incorporation of host plants in a crop rotation shorter than 4 years contribute to the increasing importance of the pathogen in the Czech Republic. The objective of the study is to select plant materials resistant and susceptible to the pathogen *L. maculans*. The selected materials will be used for production of dihaploid lines, which will be a test population for the derivation of molecular markers of resistance to the disease.

MATERIAL AND METHODS

The studies were conducted simultaneously at two institutes – OSEVA PRO Ltd., Research Institute of Oilseed Crops at Opava and AGRITEC, Research, Breeding and Services, Ltd., Šumperk.

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Obtaining an isolate of the fungus *L. maculans* – the isolates were obtained from the collection of leaves of oilseed rape infected by *L. maculans*. The collection was the outcome of the collecting activity in autumn 2001 in a locality of Moravia. From the isolates obtained, the isolate exhibiting a higher degree of infection and higher variability of infection among the particular varieties under study was selected. This isolate was classified as aggressive – Tox^+. The classification of isolates was based on their ability to produce yellow colour and higher ability of pycnidium production. The isolate was denoted 01/2000/LM for study purposes. The isolate was maintained and propagated on culture medium PDA (Potato-dextrose agar).

The selection of the plant material was based on field tests with subsequent testing in the laboratory – tests.

The testing of resistance was performed under laboratory conditions in two stages:

Stage 1: The tests involved 11 genotypes (breeding materials) and 13 registered varieties of oilseed rape. The selection of registered varieties of oilseed rape included also control varieties of state trials of yielding performance of oilseed rape varieties (Orkan, Rasmus, Navajo). As controls of resistance the varieties Glacier, Quinta a Westar were used. In these varieties a different level of sensitivity to *L. maculans* is declared (NEWMAN, BAILEY 1987). On the basis of the test results the most resistant and the most susceptible varieties were obtained. The set also contained newly registered varieties, which were denoted as resistant to *L. maculans*. The control varieties Glacier, Quinta and Westar were also included.

Stage 2: In this stage tests of 10 selected varieties of oilseed rape were performed. Based on the results, from the varieties showing resistance to *L. maculans* the plants with the lowest infection were selected. From the varieties showing susceptibility to *L. maculans* the plants with the highest level of infection were chosen for the preparation of materials for molecular marker studies. The statistical method of evaluation: Multiple comparisons – Method: 95% Tukey-HSD interval (program UNISTAT).

The method of infection:

This method is based on inoculation of cruciferous leaflets with suspension from the isolate of the pathogen observed. A scale from 0 to 9 was used for evaluation (DELWICHE 1980). Observe interaction phenotypes on cotyledons 10–12 days after inoculation. Scale:

0 – No darkening around wound like in controls.

1 – Limited blackening around wound, lesion diameter: 0.5–1.5 mm, faint chlorotic halo may be present, sporulation absent.

3 – Dark necrotic lesions, 1.5–3.0 mm, chlorotic halo may be present, sporulation absent.

5 – Non sporulating 3–6 mm lesions, sharply delimited by dark necrotic margin, may show grey-green tissue collapse as in IP 7 a 9 or dark necrosis throughout.

7 – Grey-green tissue collapse 3–5 mm diameter, sharply delimited, non darkened margin.

9 – Rapid tissue collapse at about 10 days, accompanied by profuse sporulation in large, more than 5 mm, lesions with diffuse margins.

RESULTS AND DISCUSSION

The response to artificial inoculation by two isolates of *L. maculans* was studied. In the isolate classified as Tox^+ the difference between the lowest and the highest degree of infection was 3.05. In the isolate classified as Tox^0 the difference between the lowest and the highest degree of infection was 0.5 degrees. Both isolates differed (1) in the ability to produce yellow colour and pycnidia and (2) in the aggressiveness of infection of selected genotypes. On the basis of the results the aggressive isolate Tox^+ 01/2000/LM was used for further studies. During the tests at Stage 1 the co-ordination of methodologies was achieved at both institutes. This concerned the co-ordination of

Table 1. Results of the testing of response of oilseed rape genotypes to artificial inoculation with different isolates of the fungus *Leptosphaeria maculans*

Genotype	Isolate Tox^0		Isolate Tox^+	
	Degree of infection	Denotation of significantly different pairs*	Degree of infection	Denotation of significantly different pairs*
1	1.00	A	1.45	A
2	1.00	A	1.70	A
3	1.00	A	1.75	A
4	1.00	A	1.75	A
5	1.00	A	2.20	AB
6	1.05	A	3.25	BC
7	1.20	A	3.45	CD
8	1.00	A	3.70	CD
9	1.30	AB	4.20	CD
10	1.50	AB	4.20	CD
11	1.05	B	4.50	D

Table 2. Results of the final tests of resistance to *Leptosphaeria maculans* in selected oilseed rape varieties

Opava			Šumperk			Selected genotypes/ S – susceptible R – resistant
Genotype	Degree of infection Tox^+	Denotation of significantly different pairs*	Genotype	Degree of infection Tox^+	Denotation of significantly different pairs*	
Capitol	2.50	A	Capitol	2.50	A	Capitol/R
Glacier	2.55	A	Jesper	2.55	AB	
Westar	2.60	A	Rasmus	2.60	AB	
Cando	2.65	A	Navajo	2.65	AB	
Jesper	2.65	A	Westar	2.65	AB	Jesper/R
Rasmus	3.10	AB	Cando	3.10	ABC	
Orkan	3.15	AB	Quinta	3.15	BCD	Orkan/S
Quinta	3.45	B	Mohican	3.45	CD	
Mohican	3.70	B	Glacier	3.70	D	Mohican/S

*Statistical method of evaluation: 95% Tukey-HSD interval

heat and light regimes. In identical genotypes exhibiting a different level of sensitivity to infection with *L. maculans* in different tests, different levels of infection were reported. The level of infection depended on the day of observation elapsed from the day of artificial inoculation. In studies of resistance to phoma stem canker there were differences observed between field resistance and laboratory resistance in the same genotype (variety Capitol). The selection of genotypes on the basis of natural infection under field conditions was based on the natural infection of the root collar and the stem of the rape plants without any further specification of the pathogen. The selection of genotypes using tests under laboratory conditions was based on the assessment of infection of cotyledons after artificial inoculation with a specific pathogen. Under laboratory conditions two varieties were selected: (1) the variety Capitol, which showed the highest resistance to infection by *L. maculans* and (2) variety Mohican, which showed the lowest resistance to infection by *L. maculans*. In the selected varieties the difference in infection was confirmed as statistically significant at the 95% level of significance at both institutes. The results are given in Tables 1 and 2. Plant breeding materials and field tests were not evaluated in this study.

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