PARTIAL RESULTS REGADING THE EVALUATION OF THE RESISTANCE OF SOME OILSEED RAPE CULTIVARS AT *VERTICILLIUM LONGISPORUM* PATHOGEN

REZULTATE PARTIALE PRIVIND EVALUAREA REZISTENTEI UNOR CULTIVARE DE RAPITA (*BRASSICA NAPUS*) LA AGENTUL PATOGEN *VERTICILLIUM LONGISPORUM*

ARSENE Mădălina-Cristina*[1]*, LIPSA F., LEONTE C., LĂZĂRESCU E.
e-mail: burlacucmadalina@yahoo.com

Abstract. *Oilseed rape (Brassica napus L) is a relative young species species which appeared a few hundred years ago through a spontaneous interspecific hybridization between cabbage (Brassica oleracea L.) and turnip rape (Brassica rapa L.) (Snowdon, 2007). Among the main diseases that can affect the oilseed rape culture we can find also verticillium wilt caused by the pathogen Verticillium longisporum. The mycelium is colonizing the vascular system of the plant and cause his obstruction, so due to the water stress the plant can die. The aim of this study was to evaluate the resistance of 39 oilseed rape cultivars at V. longisporum through artificial infection in laboratory. After the artificial infection, we identified 7 oilseed rape cultivar with resistance at V. longisporum.*
Key words:*Verticillium longisporum, resistance, rapeseed*

Rezumat. *Rapița (Brassica napus L.) este o specie relativ nouă care a apărut în urmă cu câteva sute de ani în urma unei hibridări interspecifice spontane între varză (Brassica oleracea L.) şi nap (Brassica rapa L.) (Snowdon, 2007). Una dintre bolile ce se află în ascensiune în cultura de rapiță este şi veştejirea plantelor produsă de agentul patogen Verticillium longisporum. Agentul patogen invadează sistemul vascular al plantei, obstrucționând fluxul sevei şi în consecință induce o stare de stres afectând productivitatea şi chiar viabiliatatea plantei. Scopul acestui studiu a fost evaluarea rezistentei a 39 cultivare de rapita la agentul patogen Verticillium longisporum prin infectia artificiala in laborator. In urma infectiei artificiale s-au identificat 7 de cultivare rezistente la atacul patogenului.*
Cuvinte cheie:*Verticillium longisporum, rezistență, rapiță*

INTRODUCTION

Verticillium wilt produced by the pathogen *Verticillium longisporum* is considered to be nowadays one of the main diseases of oilseed rape, besides blackleg and stem canker caused by *Phoma lingam* and stem rot caused by *Sclerotinia sclerotiorum* (Enyck, 2007).

After the germination of microsclerotia from the soil, which is induced by root exudates (Mol et al., 1995), the fungus enter in the main root of the plant. From here, the fungus spreads systemically in the vascular system by means of

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

mycelium and conidia released into the transpiration stream (Zhou et al., 2006). When senescence of the plant begins, the fungus leaves its vascular environment and produces ample masses of microsclerotia in the dying plant tissue.

The control of the disease is difficult to make because the microsclerotia can survive in the soil for several years (Schnathorst, 1981; Heale & Karapapa, 1999). Due to the fact that until now there are no available chemicals to prevent this disease, resistant cultivars are required. But, until now, breeding for resistance for both winter and spring type oilseed rape has been severely hampered by the absence of sufficient resistance in commercially available breeding material, however, recently, some promising genotypes of cabbage (*B. oleracea*) and turnip rape *(B. rapa*) with enhanced resistance were identified (Happstadius et al., 2003; Dixelius et al., 2005).

The aim of this study was to test some winter oilseed rape cultivars to the infection with the pathogen *Verticillium longisporum* in order identify some new sources of resistance at *Brassica napus*.

MATERIAL AND METHOD

The resistance tests were performed with *V. longisporum* isolates VL 43 which was provided by the division of Plant Pathology and Plant Protection, Gottingen, Germany. The isolate was chose on the basis were chosen on the basis of results of preliminary virulence tests of several *Verticillium* strains from *B. napus* described by Zeise & von Tiedemann (2002a). Long-term storage of fungi was performed as conidial suspensions in a concentration of 1-3 x 10^6 conidia mL^{-1} in Czapek-Dox medium supplemented with 25% glycerol. The inoculums for the infection were produced by 500 µL of spore stock solutions to 250 mL potato dextrose broth. The cultures were subsequently incubated for 7 days at 23°C on a rotary shaker (100 RPM). After the incubation period, the resulting suspension was filtered through sterile gauze and using a haemocytometer was diluted to 1 x 10^6 spores mL^{-1}.

In order to identify some new sources of resistance, a number of 39 winter oilseed rape cultivars originating from Centre for Genetic Resources Netherlands – CGN were tested by artificial infection with the pathogen *Verticillium longisporum*. Details about the studied material are presented in table1.

As reference control in our experiments, we used Express (less susceptible) and Falcon (highly susceptible). The seeds were two times surface sterilized by immersion in 70% ethanol for 2 minutes. After the sterilization, the seeds were washed with tap water and then sowed in silica sand. After 10 days, the roots of the plans were carefully washed from the sand. Inoculations were performed by cutting 2 cm from the roots and hold them for 30 min in the spore suspension. Plants from the controls were also cut and hold 30 min in tap water. For each cultivar we used 10 plants inoculated and 10 controls.

Table1

Detalils about the studied oilseed cultivars

Nr. Crt.	*Name of accession*	*Country of origin*
1.	Libritta	Germany
2.	Skriverskii	Lithuania
3.	B. napus	Ukraine
	group 1	
4.	Kievskii 216	Ukraine
5.	Kievskii 18	Ukraine
6.	Kombi	Ukraine

7.	SKR. II Kormovoi	Lithuania
8.	Uspekh	Ukraine
9.	Blagodatnyi	Ukraine
10.	Fedorovskii	Ukraine
11.	Snityskii	Ukraine
12.	Diana	Germany
13.	Ksaverovskii	Ukraine
14.	Kodakskii	Ukraine
15.	Lictor	Germany
16.	Liglandor	Germany
17.	Ligora	Germany
18.	Lindora	Germany
19.	Lingot	France
20.	Link	-
21.	Liquanta	Germany
22.	Lirabon	Germany
23.	Lirajet	Germany

24.	Lirakotta	Germany
25.	Lirama	Germany
26.	Lirastern	Germany
27.	Lirektor	Germany
28.	Liropa	Germany
29.	Madora	Germany
30.	Maras	Poland
31.	Marens	France
32.	Marex	Germany
33.	Matador	Sweden
34.	Mirander	Germany
35.	Niederarnbach er	Germany
36.	Norli	Germany
37.	Octavia	-
38.	Olimpiade	Italy
39.	Olymp	Germany

Plantlets were transferred after inoculation in pots into a mixture of sand, peat and compost (1: 1:2) and grown in a climatic chamber at 23^0C with a light/dark cycle of 1/10. Every week we take the disease scores using an assessment key with nine classes as described by Eynck et al 2007 (table 2).

Table.2

Assessment key for scoring disease severity

Score	Symptom development
1	No symptoms
2	Slight symptoms on the oldest leaves (yellowing, bleck veins)
3	Slight symptoms on the next younger leaves
4	About 50% of the leaves show symptoms
5	More than 50% of the leaves show symptoms
6	Up to 50%of the leaves are dead
7	More than 50% of the leaves are dead
8	Only apical meristem is still alive
9	The plant is dead

Because the disease produced by *Verticillium longisporum* reduces the plant growth, the plant height was measured at 28 days after inoculation.

For each accession, area under disease progress curve (AUDPC) was calculated from the disease severity values.

RESULTS AND DISCUSSIONS

After the artificial infection with *Verticillium longisporum* pathogen, the observed symptoms were typical asymmetric yellowing of leaves and the occurrence of black veins (measured as AUDPC value) as well as stunted growth.

The plants from the control variant were also scored in order to take into account the unspecific symptoms occurring during the natural ageing process which varied between accessions.

During the experiment it was observed a large variation of resistance to *V. longisporum* among the tested cultivars. Resistance responses of the tested cultivars along with the oilseed rape controls "Express" and "Falcon" measured by AUDPC values are shown in figure1.

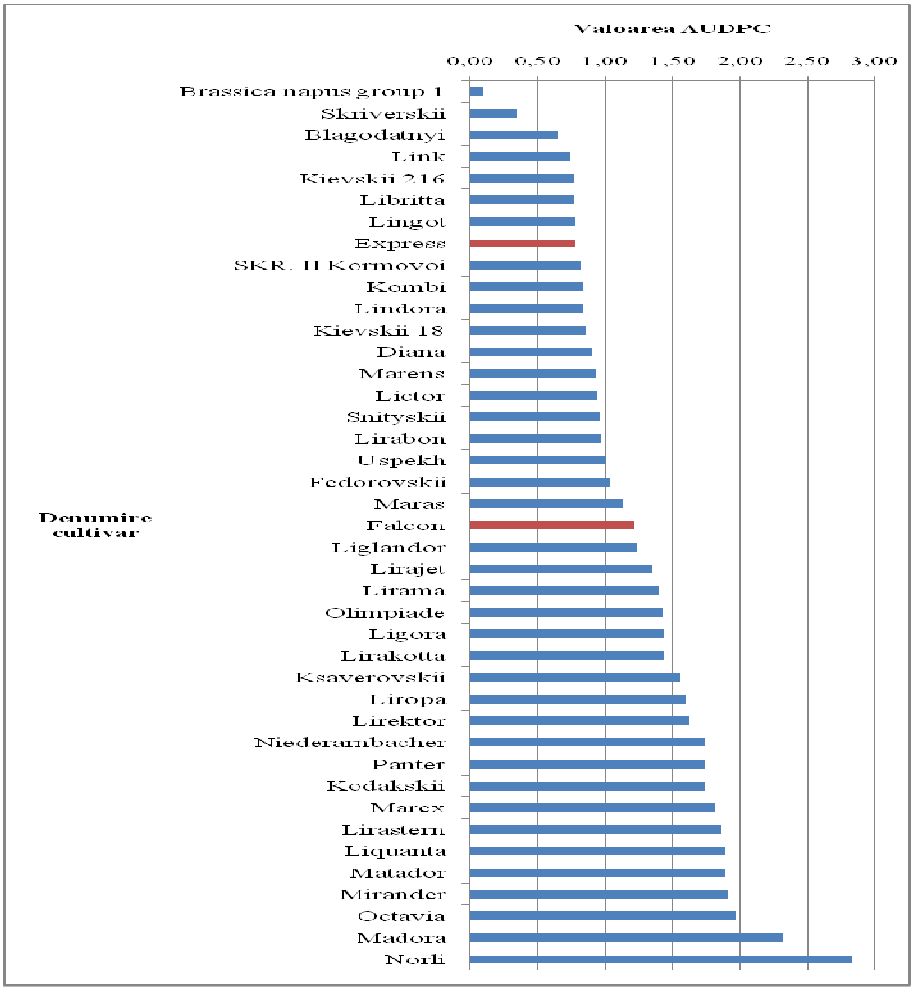

Fig. 1 - The resistance response of the tested oilseed rape cultivars to *Verticillium longisporum*

After the artificial infection of the 39 tested cultivars at *Verticillium longisporum* the AUDPC recorded values between 0,10 („*Brassica napus* Group 1”) and 2,83 („Norli”). The most resistant cultivars proved to be „Brassica napus Group 1”, „Skriveskii”, „Blagodatny”, „Link”,” Kievskii 216”, „Libritta” și „Lingot”, with AUDP values smaller than “Express”cultivar. A medium resistance showed 12 cultivars with AUDPC values situated between „Express” and „Falcon”.

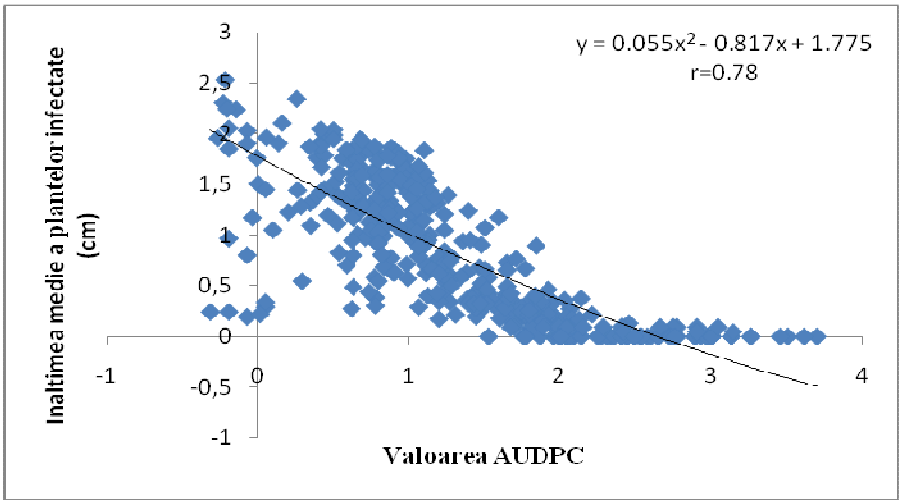

Fig. 2 - Correlation between the AUDPC values and the plant height for the 1-39 rape cultivars

Because one of the symptoms that occur in the case of the infection of with *V. longisporum* is the plant stunting (Enyck, 2007), for each infected plant, at 28 dpi, it was measured the plant height.

In order to show the influence of the attack of the pathogen upon the morphological traits of the plant, it was calculated the correlation between the AUDPC values and plant height (figure 2). The correlation coefficient was positive with a value of 0,78 indicating that the plant height is smaller when the infection is strong.

CONCLUSIONS

The present spread of *V. longisporum* in European oilseed rape production areas with a long history of cultivation of this crop requires great efforts to develop cultivars with total or at least partial resistance to this pathogen (Enyck, 2007).

For this reason a large numbers of plant accessions and breeding progenies need to be screened for resistance. The aim of this study was to test a number of 39 oilseed rape cultivars to the pathogen *Verticillium longisporum* in order to identify some new sources of resistance in *Brassica napus*.

The *Brassica napus* cultivars tested in this study showed a large variation of resistance to the pathogen *V. longisporum*. In this case, we identified seven cultivars with greater resistance to the pathogen than the Express cultivar. These genotypes can be a valuable source of resistance for the future studies for in the breeding process for obtaining some resistant commercial oilseed cultivars.

***Acknowledgments**: The financial support for our work is from the project POSCCE ID714/1268 funded by EU.*

REFERENCES

1. **Cociu V., Oprea Şt., 1989 -** *Metode de cercetare în ameliorarea plantelor pomicole.* Ed. Dacia, Cluj, p. 29, 124-129.
2. **Dixelius, C., Happstadius, I., Berg, G., 2005 -** *Verticillium wilt on Brassica oil crops – a swedish perspective*, Journal of the Swedish Seed Association, nr.115, p. 36-48.
3. **Eynck, C, Koopmann B, Grunewaldt-Stöcker G, Karlovsky P, von Tiedemann A., 2007 -** *Differential interactions of Verticillium longisporum and Verticillium dahliae with Brassica napus detected with molecular and histological techniques,* European Journal of Plant Pathology, nr.118, p. 259-274**.**
4. **Happstadius, I., Ljungberg, A., Kristiansson, B., Dixelius, C., 2003** - *Identification of Brassica oleracea germplasm with improved resistance to Verticillium wilt*. Plant Breeding nr.122, p. 30–34**.**
5. **Heale, J.B., Karapapa, V.K., 1999 -** *The Verticillium threat to Canada`s major oilseed crop:* Canola, Canadian Journal of Plant Pathology, nr.21, pag. 1-7.
6. **Mol, L., Scholte, K., 1995 -** *Formation of microsclerotia of Verticillium dahliae Kleb. On various plant parts of two potato cultivars,* Potato Research, nr.38, p. 143-150**.**
7. **Schnathorst, W.C., 1981 -** *Life cycle and epidemiology of Verticillium*. In: Mace, M.E., Bell, A.A., Beckmann, CH (editor) Fungal Wilt Diseases of Plants, ed. Academic Press, New York, p. 81-111.
8. **Zeise, K., von Tiedemann, A., 2002a -** *Host specialization among vegetative compatibility groups of Verticillium dahliae in relation to Verticillium longisporum*, Journal of Phytopathology, vol.150, p. 112-119.
9. **Zhou, L., Hu, Q., Johansson, A., and Dixelius, C., 2006 -** *Verticillium longisporum and V. dahliae: infection and disease in Brassica napus*, Plant Pathology, nr. 55, p. 137–144.