RESEARCHES REGARDING THE ATTACK OF *SCLEROTINIA SCLEROTIORUM* ON THE *BRASSICA NAPUS* LEAVES

CERCETĂRI PRIVIND ATACUL AGENTULUI PATOGEN *SCLEROTINIA SCLEROTIORUM* PE FRUNZELE DE RAPIȚĂ (*BRASSICA NAPUS* L.)

CALISTRU Anca – Elena[1], LEONTE C.[1], LĂZĂRESCU E.[1], LIPŞA F.[1], LUPU Ancuța- Elena[1]
e-mail: ancaelenacalistru@gmail.com

Abstract. *Sclerotinia sclerotiorum is one of the rapeseed pathogens that causes important yield losses (Saharan et al., 2008). Until now, there weren't identified resistant cultivars to this pathogen. There were examined 20 rapeseed cultivars, in controlled enviromental conditions, using the detached leaf assay (Bradley et al., 2006), with mycelium cultivated on PDA, from 2 diferent isolates of the pathogen. The diameter of the leasions was measured, and the results were statistically analysed. The cultivars responded differently, depending on the isolate used.*
Key words: *Sclerotinia sclerotiorum,* artificial infection*, Brassica napus*

Rezumat. *Sclerotinia sclerotiorum se numără printre agenții patogeni ai rapiței care produce pierderi de producție importante (Saharan et al., 2008). Până în prezent, nu au fost identificate cultivare rezistente la boala produsă de acesta. Au fost evaluate 20 de cultivare de rapiță, în condiții de laborator, utilizându-se metoda de infecție artificială pe frunze detașate (Bradley et al., 2006), cu miceliu cultivat pe mediu PDA, de la 2 isolate ale agentului patogen. A fost măsurată dimensiunea leziunilor produse în urma infecției, iar rezultatele au fost prelucrate statistic. Cultivarele s-au comportat diferit, în funcție de izolatul utilizat.*
Cuvinte cheie: *Sclerotinia sclerotiorum,* rezistență*, Brassica napus*

INTRODUCTION

Stem rot of oilseed rape, caused by the fungus *Sclerotinia sclerotiorum* is one of the most important diseases of the crop and leads to high losses of production worldwide. Depending of the environmental conditions, the yield losses can get up to 100% (Sarahan et al., 2008).

No oilseed rape cultivars are marked as having resistance to this disease. According to Garg et al., (2008), strategies for selecting resistant host are considered the most economic and sustainable control means. To develop resistant or tolerant genotypes, oilseed rape breeders have focused on morphological (e.g. stem diameter, Li et al., 2006; or epicuticular wax, Skoropad and Tewari, 1977) and physiological (e.g. phytoalexins, Toal and

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

Jones, 1999, or oxalate oxidase enzyme, Dong et al., 2008) traits of host genotypes to improve resistance to stem rot in oilseed rape (Rahmanpour et al., 2011).

Several methods have been used to identify resistance to *Sclerotinia sclerotiorum* in rapeseed. They include screening against oxalic acid, which is a fairly well-known pathogenicity factor for the pathogen (Cessna et al., 2000), petiole inoculation (Zhao et al., 2004; Bradley et al., 2006), leaf inoculation; stem inoculation (Chaocai, 1995; Li et al., 2006), and more recently, cotyledon assay (Garg et al., 2008). Variability of responses of oilseed rape germplasm to *Sclerotinia* stem rot using different methods and experiments is common (Wegulo et al., 1998).

Field evaluations for resistance to *Sclerotinia sclerotiorum* are important; however, problems can be associated with field evaluations. Disease pressure may not be uniform in a field situation, which may lead to wrong results.

The objective of this study was to characterize the level of resistance to *Sclerotinia sclerotiorum* on 20 cultivars of rapeseed, using a detached leaf assay.

MATERIAL AND METHOD

The tested rapeseed genotypes were provided by the Centre of Genetic Resources of Netherlands.

For the artificial infection, there were used 2 isolates of Sclerotinia sclerotiorum, one collected from Germany (Giessen) and one from the Romania (Ezareni).

For the inoculum production, a single sclerotium of *Sclerotinia sclerotiorum* was surface sterilised in 1% (v/v) sodium hypochlorite and 70 % ethanol for 4 min followed by two washes in sterile distilled water for 1 min (Clarkson et al., 2003).

The sclerotium was cut in half and placed on potato dextrose agar (PDA). The fungus was subcultured and maintained in an incubator at 20^{o} Con PDA. For the inoculation, were used 3 days-old colonies.

Young, fully expanded leaves were detached from plants grown in the controlled environmental room and transferred to the laboratory.

There were used 6 leaves for every cultivar, 5 for infection and 1 for control.

These leaves were placed in trays with gauze covering the petiole and kept in darkness, at 22 -24^{o} C and humidity of 70 – 80 %.

On each leaf, there were put 2 plug discs of PDA medium with mycelia near the main vein.

On the control leaf there were put 2 discs of PDA medium, without mycelia.

The diameter of the lesions was measured 24 h, 48 h and 72 h after inoculation (fig. 1).

Fig. 1 - Tray with leaves inoculated with PDA discs with mycelia (original)

RESULTS AND DISCUSSIONS

For the artificial infection with the Giessen isolate, typical necrotic lessions appeared on the leaves of susceptible genotypes. The size of the lessions varied between the tested genotypes (fig. 2), from 14.3 mm for the Link cultivar, to 42,56 mm for the Panter cultivar. 7 cultivars (Skriverskii, *Brassica napus* WOSR 1, SKR. II Kormovoi, Link, Liquanta, Silex and *Brassica napus* group WOSR 2) presented very significant differnces comapred to the control of 30.56 mm (the average of the values for the isolate), which means that these cultivars have a better tolerance against the pathogen. 2 cultivars (Lirektor si Capricorn) had distinct significant differences and the Lirakotta cultivar presented significant difference compared to the control. These cultivars presented a lower tolerance against *Sclerotinia sclerotiorum*. The Kodakskii, Matador, Olymp, Panter, Sollux and Veronika cultivars proved to be hypersensitive, having distinct significant values comapred to the control. The other cultivars presented statistical uninsured differences.

For the artificial infection with tht Ezareni isolate, the diameter of the lessions varied from 11.43 mm for the Kodakskii cultivar to 36.56 mm for the SKR. II Kormovoi cultivar (fig. 3). 5 cultivars (Kodakskii, Link, Lirakotta, Matado and Capricorn) had highly significant differences, compared to the control of 26.97 (the average of the values for the isolate), which means that these cultivars had a better tolerance against the pathogen. The Liquanta cultivar had a significant difference compared to the control.

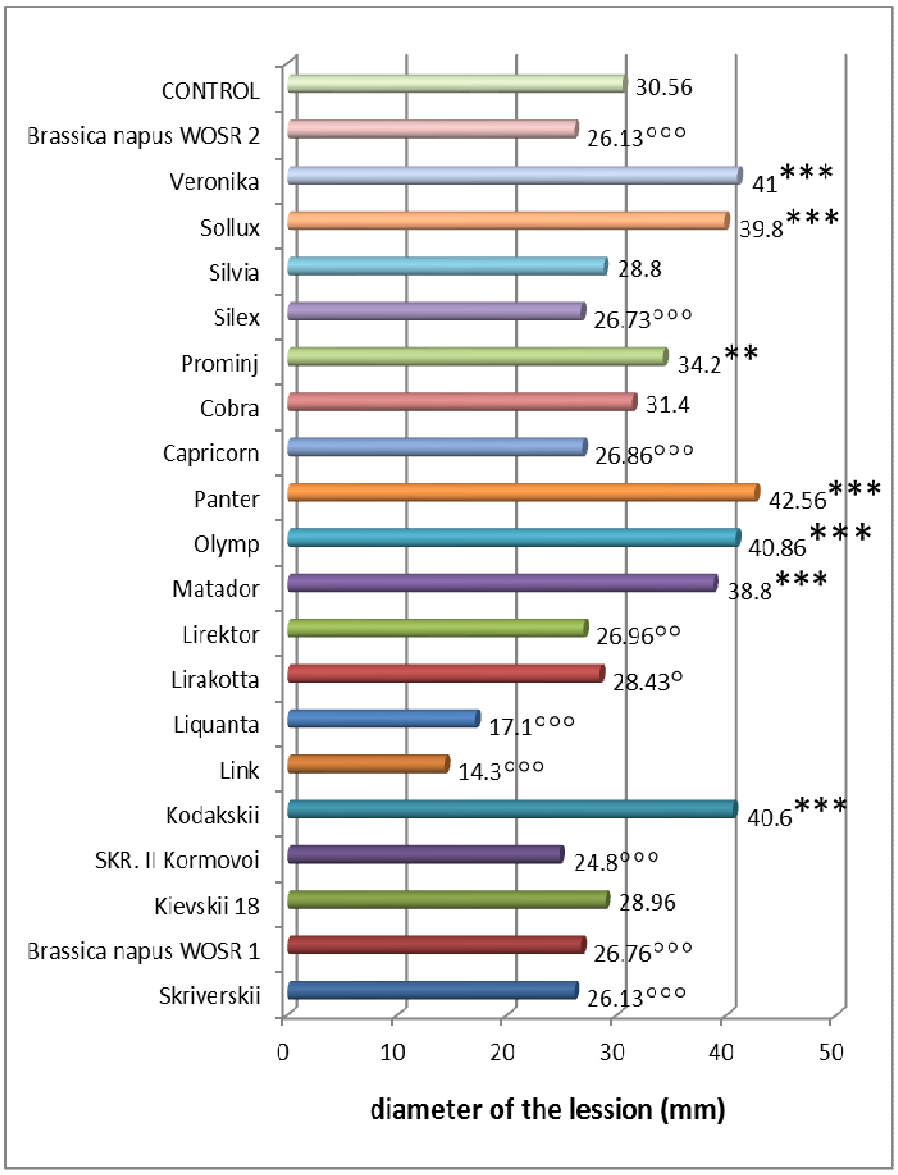

Fig. 2 - Diameter of the lesions for the artificial infection for the Giessen isolate

8 cultivars (Skriverskii, *Brassica napus* WOSR 1, Kievskii 18, SKR. II Kormovoi, Panter, Cobra, Veronika and *Brassica napus* WOSR 2) proved to be very sensitive to the artificial infection with the Ezareni isolate, having highly significant differences compared to the control. The Lirektor cultivar presented sensitive reaction, with a significant difference compared to the control.

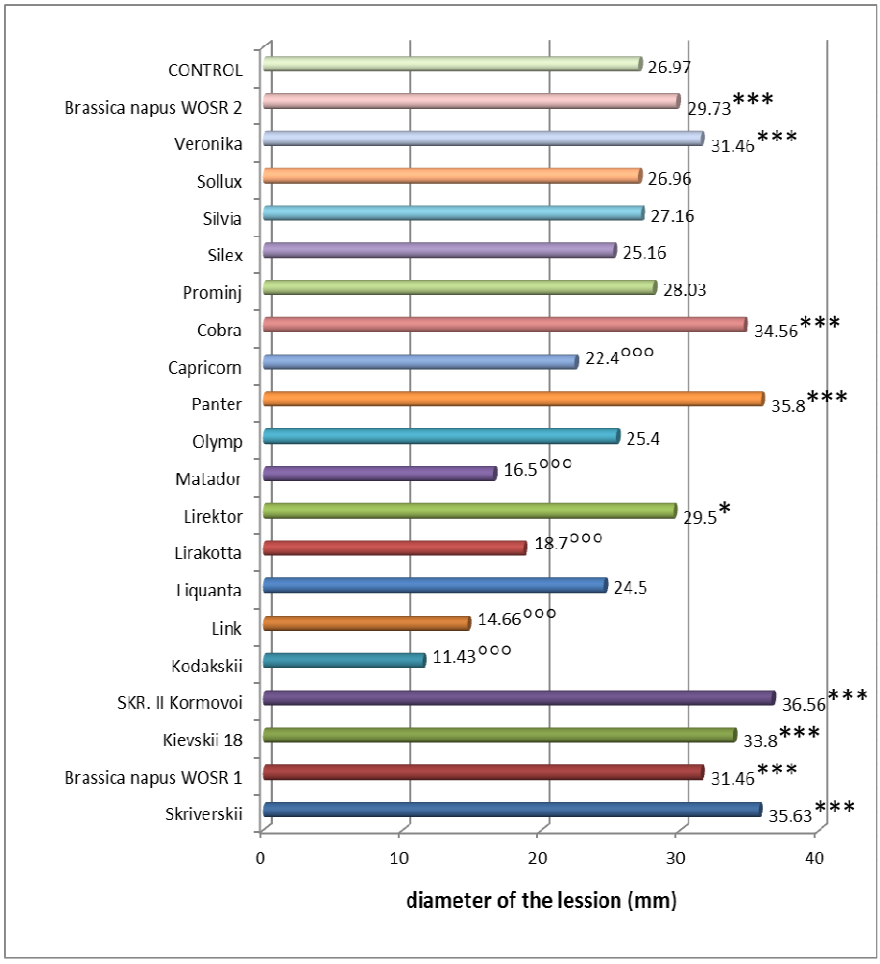

Fig. 3 - Diameter of the lesions for the artificial infection for the Ezareni isolate

CONCLUSIONS

1. For the artificial infection with the Giessen isolate, 7 cultivars (Skriverskii, *Brassica napus* WOSR 1, SKR. II Kormovoi, Link, Liquanta, Silex and *Brassica napus* group WOSR 2) presented better tolerance against *Sclerotinia sclerotiorum*.

2. For the Ezareni isolate, 5 cultivars (Kodakskii, Link, Lirakotta, Matado and Capricorn) presented better tolerance against the pathogen

3. The detached leaf assay proved to be a good screening method for the resistance against *Sclerotinia* stem rot in rapeseed.

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