RESEARCHES REGARDING THE ATTACK OF *SCLEROTINIA SCLEROTIORUM* ON COTYLEDONS OF SOME *BRASSICA NAPUS* L. CULTIVARS

CERCETĂRI PRIVIND ATACUL AGENTULUI PATOGEN *SCLEROTINIA SCLEROTIORUM* ASUPRA COTILEDOANELOR LA CÂTEVA CULTIVARE DE RAPIȚĂ (*BRASSICA NAPUS* L.)

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Abstract. *Sclerotinia sclerotiorum is a major pathogen for the rapessed crop (Brassica napus L.) and for that reason, worldwide, there is a high interest to identify Brassica genotypes with resistance to the pathogen (Sarahan et al., 2008). Field testing to identify resistance in the rapeseed germplasm can be difficult and expensive regarding the necessary time and costs.We aimed to examine the reaction of 40 rapeseed cultivars to the attack of Sclerotinia sclerotiorum, on cotyledons, in controlled enviromental conditions. For this, we have artificially incoluated the cotyledons, with a solution of macerated mycelium (10^4 mycelial fragments / ml), in liquid PDB media (Garg et al., 2008), from an isolate of the pathogen. The rapeseed cultivars responded with a distinct hypersensitive reaction. The results were statistically interpreted. The cotyledon assay proved to be a rapid and useful method to identify the reaction of the Brassica napus cultivars to the attack of Sclerotinia sclerotiorum.*
Key words: *Sclerotinia sclerotiorum,* resistence, artificial infection, cotyledons

Rezumat. *Sclerotinia sclerotiorum este un agent patogen important pentru cultura de rapiță (Brassica napus L.) şi de aceea, la nivel mondial, se doreşte identificarea unor cultivare rezistente la boala produsă de acesta (Sarahan et al., 2008). Testarea rezistenței cultivarelor de rapiță în condiții de câmp se poate dovedi dificilă şi costisitoare din punct de vedere al timpului necesar şi al cheltuielilor. S-a examinat comportarea a 40 de cultivare de rapiță la atacul de Sclerotinia sclerotiorum, pe cotiledoane, în condiții de laborator. Astfel, s-a realizat infecția artificială utilizându-se soluție de miceliu macerat (10^4 fragmente de miceliu / ml), în mediu lichid PDB (Garg et al., 2008), de la un izolat al agentului patogen. Cultivarele luate în studiu au prezentat sensibilitate diferită la atac. Rezultatele obținute au fost interpretate statistic. Metoda de infecție artificială pe cotiledoane s-a dovedit a fi o modalitate rapidă şi utilă de identificare a reacției cultivarelor de B. napus la atacul de Sclerotinia sclerotiorum.*
Cuvinte cheie: *Sclerotinia sclerotiorum,* rezistență, infecție artificială, cotiledoane

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INTRODUCTION

Stem rot, caused by the fungus *Sclerotinia sclerotiorum*, is one of the most important diseases of rapeseed, and can lead to significant losses of yield worldwide (Zhao et al., 2004). The pathogen can attack more than 400 plant species from 75 different families (Bolland et al. 1994). The methods used for controlling this disease include chemical control, cultural control and varietal resistance (Bardin et al., 2001). Selection of the host resistance is the only economic and sustainable means of controlling the disease (Zhao et al., 2004).

Field evaluation for selection of resistant cultivars provides variable results, because the response of the plants is dependent on environmental conditions (Abawi et al., 1979). The results are variable also because under field conditions, the *Brassica* genotypes may differ in their maturity and architecture. In contrast, screening for resistance in controlled environmental conditions (laboratory, green house) is more likely to be solely to physiological resistance, because the disease escape mechanisms are very little involved.

Various controlled environment screening methods have been used to evaluate resistance in *Brassica napus*: petiole inoculation (Zhao et al., 2004, Bradley et al., 2006), detached leaf inoculation (Bradley et al., 2006), oxalic acid assay (Bradley et al., 2006), but they don't positively correlate with the field results.

Some researchers used cotyledon tests in genotypes of alfalfa (Pratt et al., 1998) and soybean (Hartman et al., 2000; Kim et al., 2000, Kull et al., 2003) and the results were correlated to field tests. Due to its economic importance, it's important to develop a reliable screening technique for *Brassica napus*, which can rapidly predict the reaction of the genotypes against *Sclerotinia sclerotiorum*.

MATERIAL AND METHOD

Test conditions. The Brassica napus genotypes used for screening were grown in 38 x 24 x 5 trays, each having 40 cells and containing a compost mixture. Three seeds of each genotype were sown in each cell and thinned to a single seedling per cell after emergence.

A complete randomised block design was used with three replications and two plants per genotype per replication. All experiments were conducted in the growth room, under controlled environmental conditions (18 $\pm 1^{o}$ C during the day and 14 $\pm 1^{o}$ C during the night, with a light intensity of 150 $\mu E/m^2.s$ (Garg et al., 2008).

Seedlings were grown until cotyledons were fully expanded (growth stage 1.00, on the scale given by Sylvester-Bradley and Makepeace, 1984).

Genotypes tested. The 40 genotypes tested were provided by the Centre of Genetic Resources of Netherlands.

Inoculum production. A single sclerotium of *Sclerotinia sclerotioru* 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).

S.sclerotiorum was subcultured and maintained in an incubator at 20^{o} Con PDA. Seven agar plug discs (each 5 mm in diameter) were cut from the actively growing margin of a 3-day-old colony and transferred to a 25m ml flask containing 75 ml of a sterilised liquid medium of PDB (potato dextrose broth 24 g, peptone 10 g, H_2O 1 l).

Flasks were rotated on a platform shaker, at 120 rpm / min. After 3 days, colonies were harvested and washed twice with sterilised water (Fig. 1). The fungal mats obtained were transferred to 125 ml of the same liquid medium and the mycelia macerated in a food grinder for 3 min.

The mycelial suspension was then filtered through four layers of cheese cloth and the concentration was adjusted to 10^4 fragments /ml using a haemocytoemeter with the same liquid medium (Garg et al, 2008).

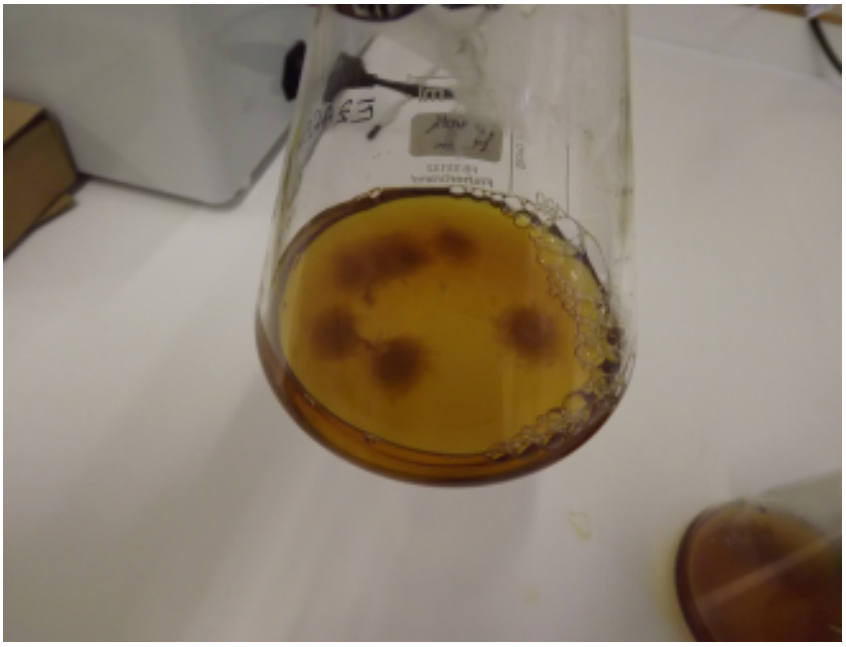

Fig. 1 - Flask containing *Sclerotinia sclerotiorum* colonies, in liquid PDB medium (original)

Inoculations. The inoculation was made when cotyledons were 10 days old. A total of four droplets of mycelial suspension of 10 µl were deposited on every seedling using a micropipette, with a single drop on each cotyledon lobe (Fig. 2). While inoculating, the mycelial suspension must be shaken regularly to maintain the homogeneity of the mixture. A very fine mist of water was sprayed both over cotyledons and on the inside of the lids, with the purpose of maintaining a relative humidity level of 100 %. After the inoculations, the trays covered by lids were placed for 2 days at a low light intensity of 13 µE / m^2 and then returned to the original light intensity (Garg et al, 2008).

Fig. 2 - Rapeseed seedling, artificially inoculated with *Sclerotinia sclerotiorum* (original)

Disease assessment. Typical hypersensitive and or / necrotic lesions were apparent by 1-2 days post-inoculation. At 4 days post-inoculation, the lids were removed and the diameter of the lesions (mm) was measured with a linear ruler (fig. 3).

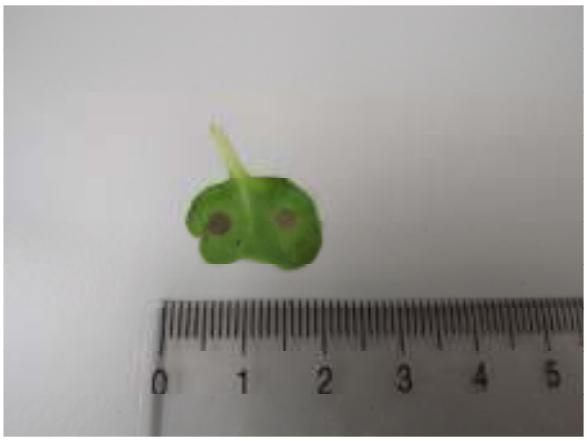

Fig. 3 - Lesions measured on the infected cotyledons (original)

RESULTS AND DISCUSSIONS

Typical necrotic and / or water – soaked lessions appeared on cotyledons of susceptible genotypes infected. The size of the lessions varied between the tested genotypes, from 1,2 for the *Brassica napus* group WOSR cultivar to 4,7 mm for the Lirama cultivar (Fig. 4). From all the tested genotypes, 2 of them (Brassica napus group WOSR and Kodakskii) showed distinct significant differences, and 3 of them (Kievskii 216, Diana and Olymp) presented significant differences compared to the control (the average of all the values for the isolate). For those genotypes, the diameter of the lessions was ≤3.06 mm. Four genotypes (Ligora, Lirama, Madora, Matador)

presented hypersensitive reaction to the isolate used for infection. The other cultivars presented statistical uninsured differences.

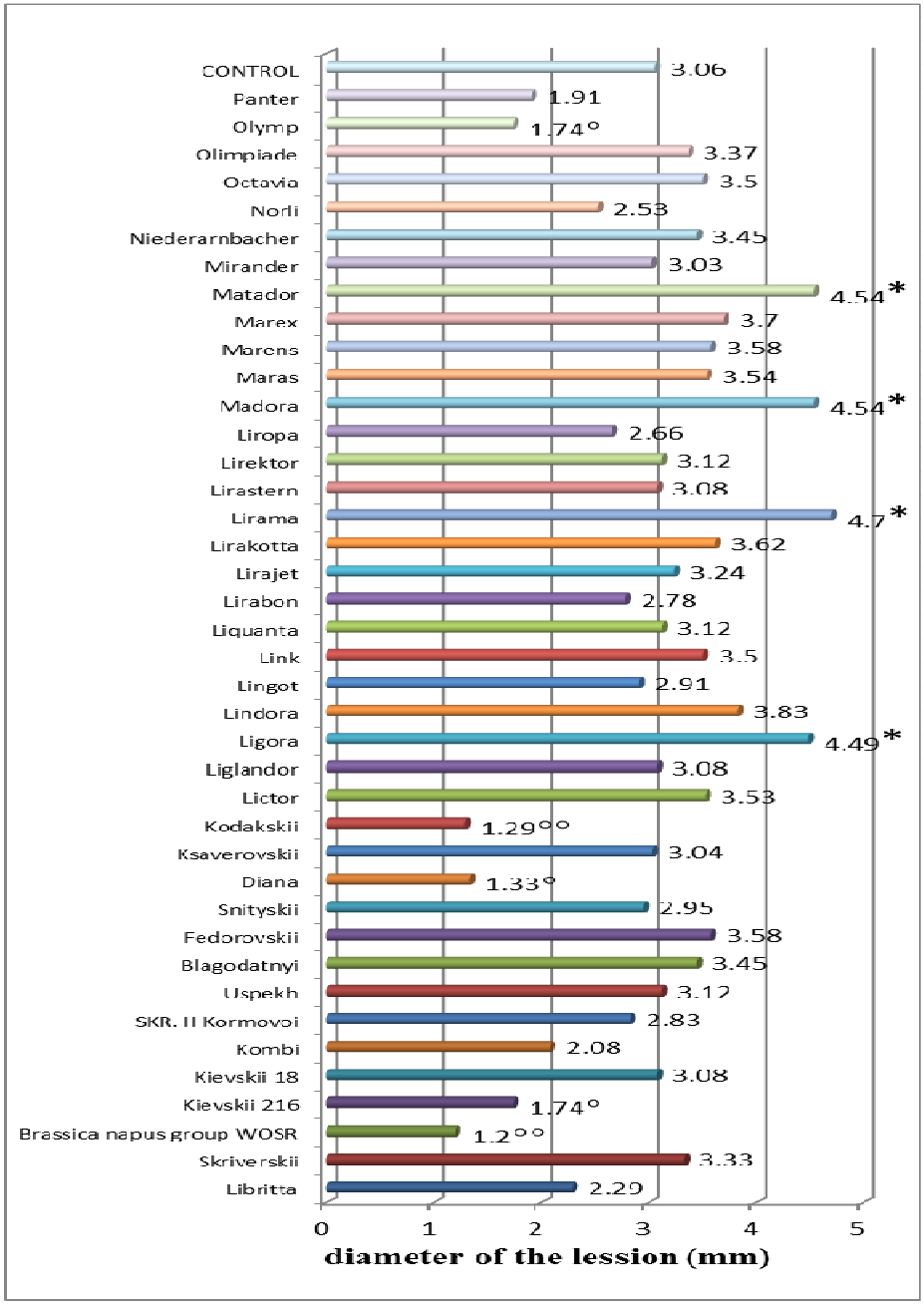

Fig. 4 - Diameter of the lession measured on the cotyledons

CONCLUSIONS

1. The cotyledon assay proved to be a reliable method of screening rapeseed genotypes for the resistance to *Sclerotinia sclerotiorum*.

2. From the 40 genotypes tested, only 2 presented a better resistance to the pathogen, compared to the average.

3. Four cultivars showed hypersensitive reaction to the artificial infection.

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