RESEARCHES REGARDING THE ATTACK OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY ON RAPESEED USING THE COTYLEDON ASSAY

CERCETĂRI PRIVIND ATACUL DE *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY LA RAPIȚĂ UTILIZÂND METODA DE INFECȚIE PE COTILEDOANE

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Abstract. *Sclerotinia sclerotiorum (Lib.) de Bary is a major pathogen for the rapeseed crop (Brassica napus L.). The aim of the study was the assessment of 130 rapeseed cultivars regarding the response to the attack of white rot, using a cotyledon assay, in controlled enviromental conditions. In order to evaluate the response of the cultivars, we have artificially inoculated the cotyledons, with a solution of macerated mycelium (10^4 mycelial fragments / ml), in liquid PDB media, following the protocol described by Garg et al.(2008). After three days, the diameter of the lesions was measured. From the 130 cultivars, 39 had smaller lesions than the control. The cotyledon assay proved to be a rapid method to identify the reaction of the rapeseed cultivars to the attack of Sclerotinia sclerotiorum (Lib.) de Bary.*
Key words: *Sclerotinia sclerotiorum, rapeseed, lesions, attack*

Rezumat. *Sclerotinia sclerotiorum (Lib.) de Bary este un patogen important al culturii de rapiță (Brassica napus L.).scopul acestui studiu a fost testarea a 130 de cultivare de rapiță în ceea ce privește atacul de putregai alb, utilizând metoda de infecție artificială pe cotiledoane, în condiții de mediu controlat. Pentru a evalua răspunsul cultivarelor, cotiledoanele au fost inoculate artificial, cu o solutie de miceliu macerat (104 fragmente de miceliu / ml), în mediu lchid PDB, după protocolul descris de Garg și colab. (2008). După trei zile, a fost măsurat diametrul leziunilor apărute. Din cele 130 de cultivare, 39 au înregistrat leziuni mai mici decât cele ale martorului. Metoda de infecție pe cotiledoane s-a dovedit a fi o emtodă rapidă de identificare a reacției cultivarelor de rapiță la atacul de Sclerotinia sclerotiorum (Lib.) de Bary.*
Cuvinte cheie: *Sclerotinia sclerotiorum, rapiță, leziuni, atac*

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, is one of the most important pathogens of rapeseed, and can cause significant losses of yield worldwide (Zhao et al., 2004).

There have been used various controlled environment screening methods, in order to evaluate the resistance of *Brassica napus* to white rot: petiole inoculation (Zhao et al., 2004, Bradley et al., 2006), detached leaf inoculation

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(Bradley et al., 2006), oxalic acid assay (Bradley et al., 2006), but they don't positively correlate with the field results.

Due to its economic importance, it's crucial to find genotypes with improved tolerance to *Sclerotinia sclerotiorum* (Lib.) de Bary,

MATERIAL AND METHOD

Test conditions. The *Brassica* 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 randomized 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 130 genotypes tested were provided by the Centre of Genetic Resources of Netherlands.

Inoculum production. A single sclerotium of *Sclerotinia sclerotiorum* was surface sterilized 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 sterilized 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 sterilized 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).

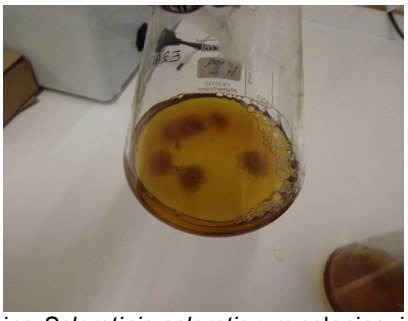

Fig. 1 - Flask containing *Sclerotinia sclerotiorum* colonies, in liquid PDB medium

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

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

RESULTS AND DISCUSSIONS

Typical necrotic lessions appeared on cotyledons of susceptible genotypes infected. The size of the lessions varied between the tested genotypes, from 0,37 mm for the *Libritta* cultivar to 10,04 mm for the *Liberator* cultivar (fig. 4, fig. 5), with an average considered as control of 4,73 mm. From all the tested genotypes, 39 had smaller lessions than the control.

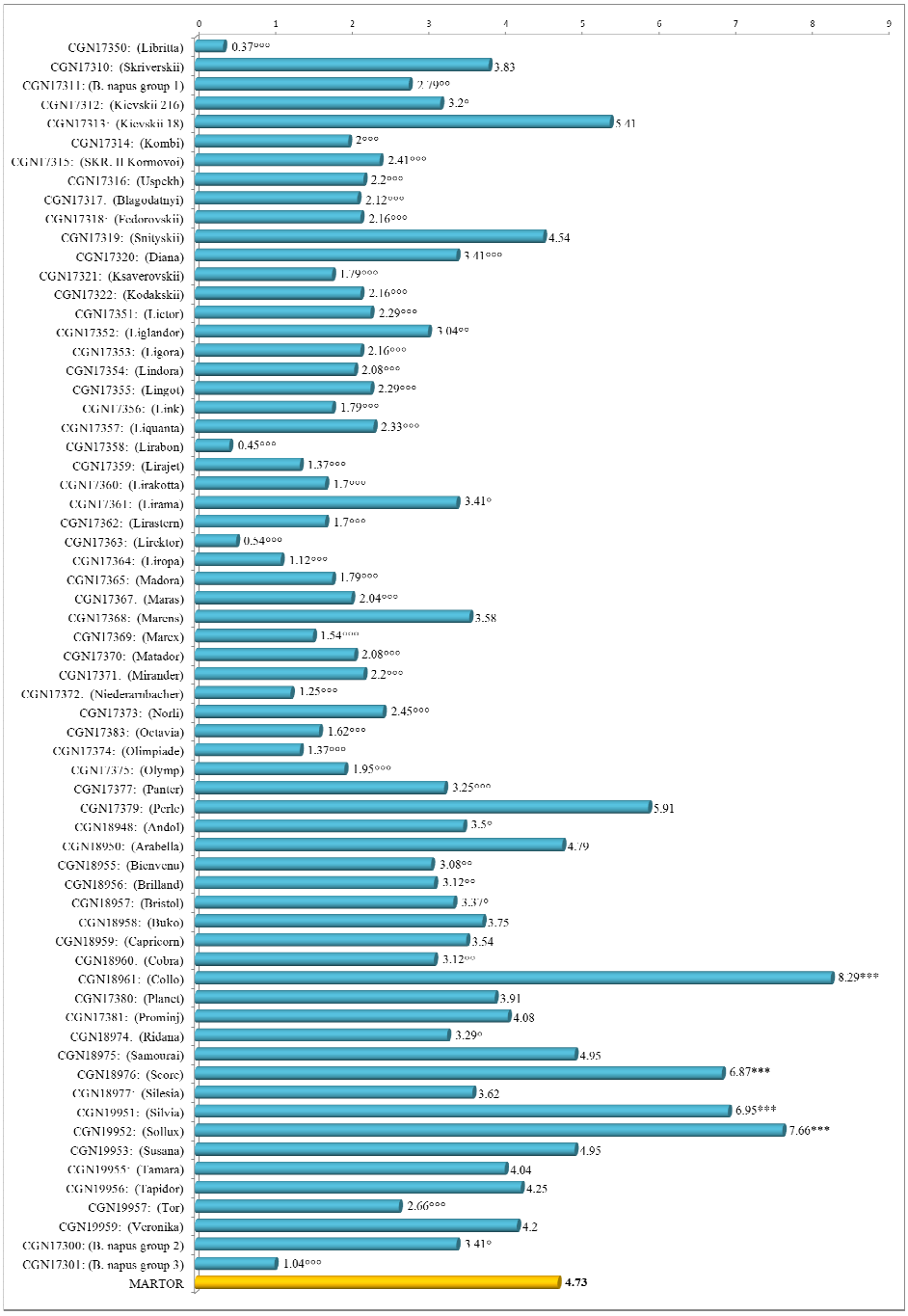

Fig. 4 - Diameter of the lession measured on the cotyledons for the cultivars1-65

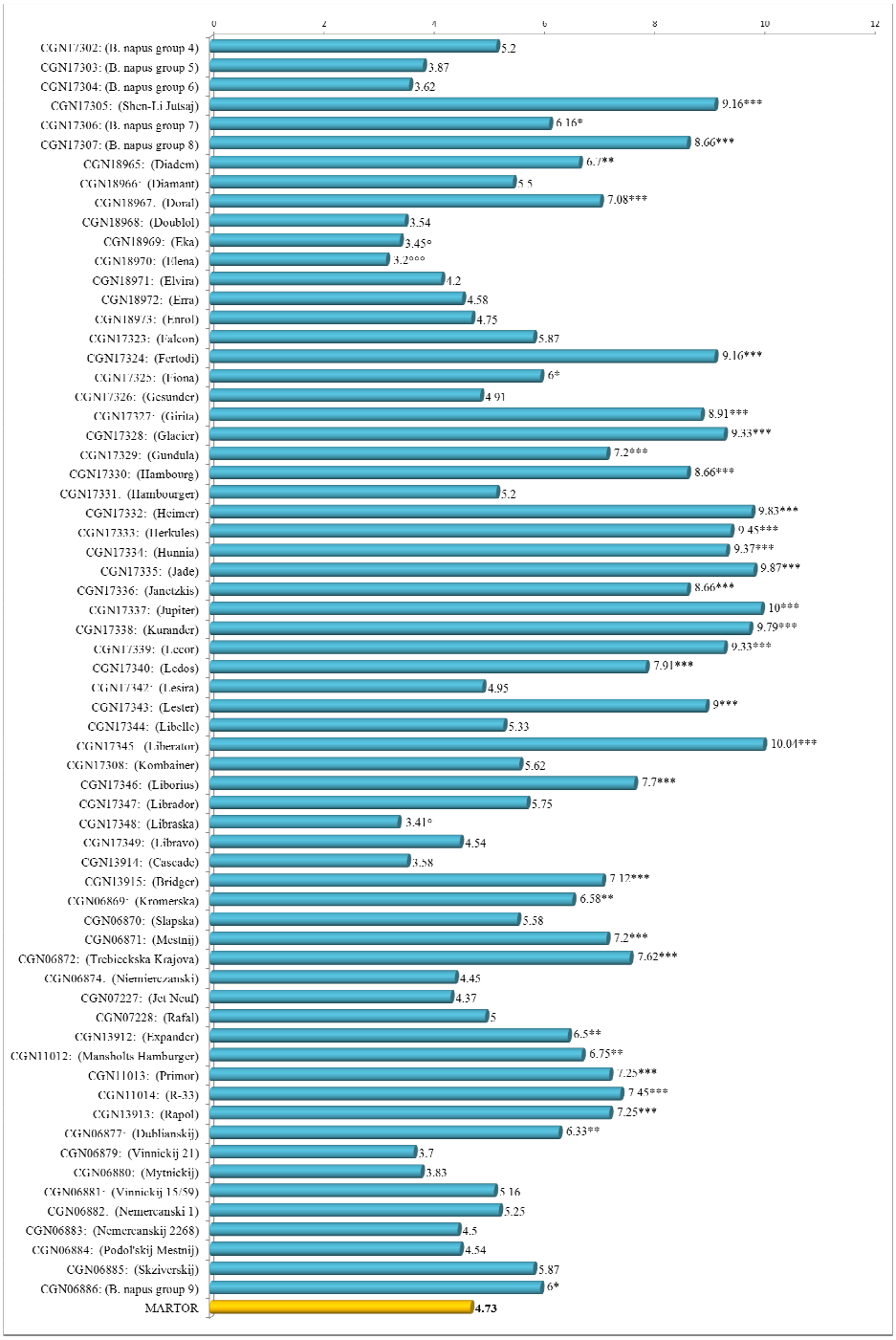

Fig. 5 - Diameter of the lession measured on the cotyledons for the cultivars 65-130

CONCLUSIONS

From the 130 genotypes tested, 39 presented a better resistance to the pathogen, compared to the control. Those cultivars can be used by plant breeders in order to identify resistance genes to the attack of *Sclerotinia sclerotiorum* (Lib.) de Bary.

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