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RESPONSE OF SOYBEAN GENOTYPES TO INFECTION WITH *Phomopsis longicolla* Hobbs

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SUMMARY

Constant increase of acreage under production of soybean raises the issue of disease control (*Glycine max* (L.) Merrill). There are diseases, which almost regularly appear in our agroecological conditions, such as downy mildew (*Peronospora manshurica* (Naum.) Syd. ex Gaum.), and diseases caused by fungus from *Diaporthe/Phomopsis* Complex.

The aim of this investigation was to check the reaction of five domestic soybean genotypes (OS-8, OS-49, OS-101, OS-109 and OS-139) to infection with fungus *Phomopsis longicolla* Hobbs, which causes soybean seed decay. Experiments were carried out through artificial seed infection in wet chamber and in pots with sterile soil. Tested genotypes, maturity group 0-I, are created at the Agricultural Institute of Osijek within the program of soybean breeding. Fungus was isolated from soybean seeds that exhibited symptoms of *Phomopsis longicolla* Hobbs. Soybean seeds were separated from samples that were collected on 10 locations under large-scale production during a period of three years (2000-2002). Evaluation of pathogenicity was done by counting germinated and non-germinated (rotten and healthy) seeds and by determining the germ length and germ necrosis of tested genotypes.

According to results obtained during a wet chamber trial, numbers of germinated and non-germinated seeds among tested genotypes were significantly different (P=0.05). Relation between artificially infected seeds and control in trial showed significant differences for each genotype (P=0.05). All genotypes in control had satisfactory germination and there were no significant differences in number of rotten seeds, germinated seeds and non-germinated seeds. The OS-49 and OS-109 genotypes were proved to be more resistant to infection with *P. longicolla*.

Results from pot trial showed that number of germinated seeds of artificially infected genotypes was significantly different (P=0.05) during whole trial, while all genotypes in control germinated satisfactory, showing no significant differences among themselves. Taking into consideration these results, it can be concluded that the OS-8 genotype is more susceptible to infection with *P. longicolla*. Both trials of artificial infection were successfully completed, resulting in conclusion that there are differences in resistance to infection with this fungus among tested soybean genotypes.

Keywords: soybean (*Glycine max* (L.) Merrill), resistance/tolerance, genotypes, seed, *Phomopsis longicolla* Hobbs, artificial infection

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the oldest and most important cultivated crops in the world. According to FAO data, current soybean growing area is 83,6 million ha with average grain yield of 2.3 t/ha (FAO STAT, 2004). Although soybean is known in our country since 18th century, its intensive production (on around 20 000 ha) started in the 1980-ies (Vratarić and Sudarić, 2000). In 2003, the Republic of Croatia had 48 000 ha of soybean with average grain yield of 2.7 t/ha.

Constant increase of acreage under production of soybean, and intensified soybean production raise the issue of disease control (Aćimović, 1988, Vratarić, 1988, Yorinori, 1994, Sinclair and Hartman, 1999, Vratarić and Sudarić, 2000, Duvnjak, 2001). Around 80% of all soybean diseases are caused by fungi, and the rest of about 20% is caused by bacteria and viruses (Sinclair and Backman, 1989). In soybean large-scale production areas worldwide, occurrence of diseases is a very serious problem, as many of those diseases cause great losses. While occurrence of some diseases is geographically bordered, others can spread in all soybean growing areas. However, damages they cause vary depending on climatic conditions in certain years (Athow, 1987). Generally speaking, some diseases occur regularly each year during vegetation. Some pathogens are very destructive, as they cause serious damages in one year, while they do not occur next year. This fact points out the difference in the economic aspect of diseases. Eventual losses depend on the following factors: cultivar resistance, pathogen, stage of plant development in the moment of infection, intensity of attack, number of attacked (infected) plants, agroecological conditions and management of soybean growing area.

There are three defense ways against possible plant attackers: resistance, tolerance and avoidance of infection (Parlevliet, 1981). Although tolerance is not the usual expression in breeding, many scientists agree that tolerant

cultivars have significantly lower damages in production in comparison to intolerant cultivars, even when both of them have the same level of infection. They also agree that resistance and tolerance are alternatives in crop protection against extensive yield losses (F e h r, 1987, V r a t a r i ć *et al.*, 1991, B o r o j e v i ć, 1992, N i k s *et al.*, 1993). Previous investigations showed significant variability of soybean genotypes in reaction to pathogens from *Diaporthe/Phomopsis* complex (D i n g h r a *et al.*, 1979, B e r g e r and H i n s o n, 1984). Considering the fact that many of these investigations were conducted on fields, scientists think of them as unreliable, because of the problems caused in recognition of genetic and agroecological factors (A n d e r s o n and B u z z e l l, 1984, K u e n e m a n, 1985).

There are diseases, that almost regularly appear in our agroecological conditions, such as downy mildew (*Peronospora manshurica* (Naum.) Syd. ex Gaum.), and diseases caused by fungus from *Diaporthe/Phomopsis* Complex, in particular seed decay, pod and stem blight and stem canker (C v j e t k o v i ć, 1977, D i m i t r i j e v i ć and J u r k o v i ć, 1982, C v j e t k o v i ć *et al.*, 1983, H e n n e b e r g *et al.*, 1983, J u r k o v i ć and V r a t a r i ć, 1986, J u r k o v i ć *et al.*, 1988, V r a t a r i ć *et al.*, 1988, V r a t a r i ć and S u d a r i ć, 2000, D u v n j a k *et al.*, 2003). This complex is represented by the following fungi: *Phomopsis longicolla* Hobbs – causative agent of soybean seed decay, *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. Var. *caulivora* Athow & Caldwell, the cause of stem canker, *D. phaseolorum* var. *meridionalis* Fernandez, which causes «southern» stem canker and *D. phaseolorum* var. *sojae* (Lehman) Wehmayer, causative agent of pod and stem blight. These pathogens cause numerous symptoms on leaves, stems, pods and grains. The fungi are present in large soybean growing areas all around the world, causing significant yield losses and yield quality decrease. *Phomopsis longicolla* Hobbs, (teleomorf unknown) is differed from other varieties in the complex by its cultural characteristics, morphology and place of infection.

One of the objectives of the breeding program of the Agricultural Institute of Osijek is the creation of cultivars with high quality grain yield. Special attention is thus given to achievement of satisfactory tolerance/resistance to major fungal diseases, which appear in our climatic conditions.

The aim of this investigation was to check the reaction of several soybean genotypes to infection with *Phomopsis longicolla* Hobbs, which is one of the most dangerous soybean pathogen in the world.

MATERIAL AND METHODS

This investigation focuses on five soybean genotypes (OS-8, OS-49, OS-101, OS-109 and OS-139), maturity group 0-I, that were created within the breeding program of the Agricultural Institute of Osijek. Isolation of pathogen

and artificial inoculation were done in the Laboratory for Phytopathology at the Faculty of Agriculture in Osijek.

Fungus was isolated from soybean seed with symptoms of *Phomopsis longicolla* Hobbs, which were separated from samples collected during a period of three years (2000-2002) on the following locations: Nova Gradiška, Magadenovac, Valpovo, Širine, Đakovo, Bilje, Klisa, Bobota, Ovčara, Novi Berak, Tovarnik, Kneževo, Gorjani, Kutjevo and Valpovo. Method described by H o b b s et al., (1985) was applied in investigation of morphological characteristics of pathogen. The PI 027 isolate was used for artificial seed inoculation, because it showed the highest level of pathogenicity in our previous investigations (D u v n j a k, 2004).

Two methods of artificial inoculation described by V i d i ć *et al.* (1995) were used to test the differences in resistance among investigated soybean genotypes. The first method was seed inoculation in wet chamber. After two weeks growing on PDA, contents of Petri dishes were mixed with 100 ml of sterile water. Seeds were sterilized in the 1% natrium-hypoclorit solution for three minutes and then twice rinsed in double distilled water. After that, 100 seeds of each genotype were soaked in conidia suspension of fungi strains and left for 24 hours. The trial was set in four replications (four paper beds with 25 seeds each). In control, seeds were soaked for 24 hours in sterile water. Afterwards, the inoculated seeds were transferred to wet paper bed and left in a wet chamber on 25°C temperature, having a 12-hour-long light and dark alternation regime.

The second type of inoculation was the seed inoculation in plastic pots. Soybean seeds (40 of each genotype) were soaked for five minutes in conidia suspension of fungi strains. The inoculated seeds were sown in plastic pots that contained sterilized chernozem soil. Ten seeds were sown per pot. The control variant consisted of seeds that were previously soaked in sterilized water. Trial was carried out in four replications (4 pots per variant). Pots were left on a laboratory bench; soil temperature varied from 18 to 25°C. After 10 days, the number of emerged plants was registered. Wilting of seedlings was observed every 2 days during the following 10 days. The isolation was carried out on the infected seedlings in order to determine the causative agent of wilting.

Statistical data processing was carried out by Statistical Analysis System Version 8.2 (SAS Institute), with analysis of variance (ANOVA) and LSD test (Least Significant Difference test).

RESULTS AND DISCUSSION

Differences were established in response to artificial infection with PI 027 isolate of *Phomopsis longicolla* Hobbs in both of laboratory trials. The trial of infection in wet chamber exhibited germination, which was less intense for all tested genotypes (Table 1).

Table 1. Soybean genotype response to P. longicolla Hobbs infection in wet chamber

Tablica 1. Reakcija genotipova soje na zarazu sa P. longicolla Hobbs u vlažnoj komori

Genotype Genotip	Rotten seed Trulo sjeme		Germinated seed Klijavo sjeme		Germ length (cm) Dužina klice (cm)		Germ necrosis (cm) Nekroza klice (cm)		Non-germinated seed** Neklijavo sjeme**	
OS-8 t*	13	AB	73	D	1.04	C	0.84	BC	27	B
OS-49 t	3	CD	85	C	1.67	C	1.44	A	15	C
OS-101 t	14	A	73	D	1.39	C	1.34	A	27	B
OS-109 t	0	D	88	BC	1.19	C	1.05	B	12	C
OS-139 t	8	BC	56	E	0.81	C	0.79	BCD	44	A
Treatment average Prosjek tretmana	7.60	a	75.0	b	1.22	b	1.09	a	25.0	a
OS-8 c*	0	D	97	A	9.66	A	0.72	CD	3	D
OS-49 c	0	D	99	A	9.44	A	0.53	DE	1	D
OS-101 c	0	D	98	A	7.89	B	0.28	EF	2	D
OS-109 c	0	D	97	A	10.44	A	0.08	F	2	D
OS-139 c	0	D	97	A	8.18	B	0.12	F	3	D
Control average Prosjek kontrole	0.00	b	97.6	a	9.12	a	0.34	a	2.2	b
Average - Prosjek	3.80		86.0		5.17		0.72		13.9	
LSD $_{genotype}$ 0.05	5.11		8.78		1.18		0.29		8.74	
LSD $_{genotype}$ 0.01	6.73		11.54		1.55		0.39		11.49	
LSD $_{treatment}$ 0.05	2.29		3.93		0.53		0.13		3.91	
LSD $_{treatment}$ 0.01	3.01		5.16		0.69		0.17		5.14	

* t – treatment, c - control

* rotten + healthy non-germinated seed

** numbers in columns followed by the same letter do not differ significantly according to LSD test (P=0.05)

Evaluation of pathogenicety was performed by counting the number of germinated and non-germinated (rotten + healthy) seeds. The strongest negative impact of pathogen was noticed on seed germinability of the OS-139 genotype, which had 56 germinated and 44 non-germinated seeds. Number of germinated and non-germinated seeds for this genotype was significantly

different (P=0.05) than all other data in trial. Considering the length of germ, obtained results did not show significant differences, while the highest necrosis level was measures in the OS-49 and OS 101 genotypes (1.44 and 1.34 cm), which was significantly different than in other genotypes. When considering the number of rotten and non-germinated seeds, as well as the number of germinated seeds, the lowest impact of pathogen was recorded in the OS-49 and OS-109 genotypes. According to those results, that genotypes are considered to be more resistant to infection with *P. longicolla*.

Relation between infected seeds and control in trial showed significant differences for each genotype (P=0.05). All genotypes in control had satisfactory germination and there were no significant differences in number of rotten, germinated and non-germinated seeds. However, all tested genotypes exhibited significant differences in germ length and germ necrosis. Compared to other genotypes, the OS-101 and OS-109 genotypes had significantly shorter germ, while the differences in relation to germ necrosis were more complex. Significant differences were not established among data for non-germinated seeds in control.

In this trial, there were no resistant genotypes artificially infected with isolate PI 027 of *P. longicolla*, although there were significant differences in the level of susceptibility. Similar results were obtained by V i d i ć *et al.* (1999). Our results should be examined on field, paying special attention to the impact of climatic conditions on pathogen, as well as on genotype. It is also necessary to investigate different maturity groups of soybean genotypes. According to some authors (W i l c o x *et al.*, 1985), genotypes of late maturity group do not possess genetic resistance, but in case of natural infection they escape the pathogen attack because of incompatibility with of susceptible development stages with optimal conditions for infection. Several authors (M i r a n d a *et al.*, 1980, H i l l *et al.*, 1984, Y a k l i c h *et al.*, 1987) state that seed coat structure could be the reason for higher seed susceptibility to infection with *P. longicolla*. When compared to infected genotypes in control (Table 2), significant differences (P=0.05) were observed in trials with artificially infected soybean seeds in pots.

Differences among tested genotypes were established during the first measurement (10 days after inoculation) and they were retained with small variability during the whole trial. There were also significant differences (P=0.05) observed between each artificially infected genotype and the same one in control, which confirmed the success of infection. When observing inoculated genotypes only, the OS-8 genotype (2.75 plants per repetition during the whole trial) was the most susceptible in comparison with other tested genotypes, while the OS-139 genotype had significantly higher number of survived plants on the 20th day after sowing (7.50 plants per repetition). In control, all genotypes had satisfactory germination without significant differences among them.

Table 2. Soybean genotype response to P. longicolla Hobbs infection in pots

Tablica 2. Reakcija genotipova soje na zarazu s P. longicolla Hobbs u posudama

Genotype Genotip	Number of emerged plants - Broj izniklih biljaka											
	10th day 10. dan		12th day 12. dan		14th day 14. dan		16th day 16. dan		18th day 18. dan		20th day 20. dan	
OS-8 t*	2.75	D	2.75	C	3.00	C	3.00	D	2.75	D	2.75	D
OS-49 t	4.50	C	5.25	B	5.50	B	5.50	C	4.50	C	4.75	C
OS-101 t	4.50	C	5.25	B	5.50	B	5.50	C	4.50	C	4.75	C
OS-109 t	6.00	BC	6.00	B	6.00	B	6.00	BC	5.75	C	5.75	C
OS-139 t	6.75	B	6.75	B	7.25	B	7.50	B	7.50	B	7.50	B
Treatment average Prosjek tretmana	4.90	b	5.20	b	5.45	b	5.50	b	5.00	b	5.10	b
OS-8 c*	9.00	A	9.50	A	9.50	A	9.50	A	9.50	A	9.50	A
OS-49 c	9.00	A	9.00	A	9.25	A	9.50	A	9.25	A	9.50	A
OS-101 c	9.50	A	9.75	A	9.75	A	10.00	A	10.00	A	10.00	A
OS-109 c	9.25	A	9.00	A	9.50	A	9.50	A	9.50	A	9.50	A
OS-139 c	9.00	A	9.00	A	9.75	A	9.75	A	9.75	A	9.25	A
Control average Prosjek kontrole	9.15	a	9.25	a	9.55	a	9.70	a	9.60	a	9.55	a
Average - Prosjek	7.03		7.23		7.50		7.60		7.30		7.33	
LSD $_{genotype}$ 0.05	1.73		1.76		1.85		1.95		1.67		1.66	
LSD $_{genotype}$ 0.01	2.33		2.37		2.49		2.62		2.25		2.24	
LSD $_{treatment}$ 0.05	0.78		0.79		0.83		0.87		0.75		0.74	
LSD $_{treatment}$ 0.01	1.04		1.06		1.11		1.17		1.00		1.00	

* t – treatment, c - control

** numbers in columns followed by the same letter do not differ significantly according to LSD test (P=0.05)

Both trials showed differences in resistance of tested soybean genotypes to infection with *P. longicolla*. This fungus is the most important soybean seed pathogen in the world. As it is recently discovered in our country, it is necessary to continue investigation and to test newly created soybean lines and cultivars in order to distinguish differences in their resistance to this pathogen. Moreover, the results of laboratory trials described in this paper should be investigated on field and then included in our breeding program as a standard method.

REAKCIJA GENOTIPOVA SOJE NA ZARAZU SA *Phomopsis longicolla* Hobbs

SAŽETAK

Zaštita od bolesti jedan je od osnovnih problema koje donosi povećanje proizvodnih površina pod sojom (*Glycine max* (L.) Merrill). Nekoliko bolesti javlja se gotovo redovito u našim agroekološkim uvjetima: plamenjača (*Peronospora manshurica* (Naum.) Syd. ex Gaum.) i bolesti uzrokovane gljivama iz *Diaporthe/Phomopsis* kompleksa.

Cilj ovoga istraživanja bio je provjeriti reakciju pet domaćih genotipova soje (OS-8, OS-49, OS-101, OS-109 i OS-139) na zarazu sa *Phomopsis longicolla* Hobbs, uzročnikom truleži sjemena soje, kroz pokuse sa umjetnom zarazom sjemena u vlažnoj komori i posudama sa sterilnom zemljom. Testirani genotipovi, grupa zriobe od 0-I, stvoreni su u okviru oplemenjivačkog programa soje na Poljoprivrednom institutu Osijek. Gljiva je izolirana iz sjemena soje sa simptomima koji odgovaraju zarazi sa *Phomopsis longicolla* Hobbs i izdvojenog iz uzoraka prikupljenih tijekom tri godine (2000-2002) sa 10 lokacija iz široke proizvodnje. Procjena patogenosti mjerena je brojem klijavih i neklijavih (trula+neklijava zrna) zrna, dužine klice i nekroze klice testiranih genotipova.

Prema dobivenim rezultatima iz pokusa u vlažnoj komori, broj klijavih i neklijavih zrna testiranih genotipova se značajno razlikovao (P=0.05). Odnos između umjetno zaraženih zrna i kontrole u pokusu pokazuju značajne razlike sa svaki genotip (P=0.05). Svi genotipovi u kontroli imali su zadovoljavajuću klijavost i nisu zabilježene značajne razlike između broja trulih, klijavih i neklijavih zrna. Među testiranim genotipovima, OS-49 i OS-109 mogu se smatrati otpornijim na zarazu sa *P. longicolla*.

Rezultati pokusa u posudama pokazuju da je broj zrna umjetno zaraženih genotipova bio značajno različit (P=0.05) tijekom cijelog pokusa dok su svi genotipovi u kontroli niknuli zadovoljavajuće i među njima nije bilo značajnih razlika. Prema dobivenim rezultatima, genotip OS-8 može se smatrati osjetljivijim na zarazu sa *P. longicolla*.

Oba pokusa pokazuju uspješnost umjetne zaraze, kao i razlike u otpornosti testiranih genotipova soje na zarazu ovom gljivom.

Ključne riječi: soja (*Glycine max* (L.) Merrill), otpornost/tolerantnost, genotipovi, zrno, *Phomopsis longicolla* Hobbs, umjetna zaraza

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