Zaštita bilja
vol. 59 (1-4), No 263-266, 59-67, 2008, Beograd

UDK 632.35:635.655
Naučni rad

RACE IDENTIFICATION OF *PSEUDOMONAS SYRINGAE* PV. *GLYCINEA* ON COMMERCIAL SOYBEAN VARIETIES IN SERBIA

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Bacterial blight is important and frequent disease of soybean in Vojvodina province. Isolation of the pathogen was carried out from infected soybean leaves of several cultivar grown in Vojvodina province using beef extract and the medium enriched with 5% sucrose.

Only the representative isolates were chosen for further investigation. Pathogenicity of the obtained isolates including referent strain (National Collection of Plant Pathogenic Bacteria, United Kingdom-NCPPB 3318) was proved by inoculating soybean plants in cotyledon stage (Balkan variety), by spraying with bacterial suspension (conc.10^8CFU/ml). Pathogenicity of studied isolates was also confirmed by infiltration on tobacco leaves.

Cultural and biochemical-physiological characteristics were tested.

Differential soybean cultivars: Acme, Chippewa, Flambeau, Harosoy, Lindarin, Merit and Norchief were used to determine physiological race of the bacterium. Young plants were inoculated in two ways: by leaves rubbing with sterile cotton swab dipped into the bacterial suspension, and by spraying leaves under the pressure. Based on the results obtained it was shown that strains which cause bacterial blight of soybeans in Vojvodina, belong to *Pseudomonas syringae* pv. *glycinea* race 4.

Key words: Soyabean, *Pseudomonas syringae* pv. *glicinae*, reses, identification

INTRODUCTION

Bacterial blight caused by *Pseudomonas syringae* pv. *glycinea* is the most common disease of soybean. The pathogen has a worldwide distribution and occurs wherever soyabeans are grown. Disease development is favoured by cool and wet weather. Infected seed and plant debris are the main source of inoculum. Pathogen populations on seedlings, originating from infected seeds, are probably formed by injury cotyledons with abrasive soil particles. These populations become a significant inoculum source for leaves that later develop (Daft & Leben, 1972).

During the years with rainy spring the bacterial blight appears with high intensity (Balaž *et al*. 1995), and cause great damage especially in early maturing cultivars (Vidić & Balaž, 1997).

Seven physiological races of *P. s*. pv. *glycinea* have previosly been distinguished by inoculation of leaves of seven differential cultivars of soybean (Cross et al. 1966). So, there is a need for further characterization of strains of *P. s*. pv. *glycinea* in agroecological conditions of Vojvodina province.

MATERIAL AND METHODS

Isolation procedure

Leaves showing characteristic bacterial blight symptoms were collected from 23 soybean fields located in the nine major soybean-producing localities in Vojvodina region. Leaves were placed in paper bags and transported to the laboratory.

Isolations of the pathogen was caried out from diseased soybean leaves, using standard procedure of smearing macerated tissues across the nutritive surface (Shaad, 1980; Lelliot & Stead, 1987; Arsenijević, 1997). Diseased samples were collected from several localities and different soybean varieties in Vojvodina. Morphological, biochemical and physiological characteristic of investigated isolates was done using convencional methods given by Lelliot *et al*. (1966).

PATHOGENICITY TEST

Hypersensitive reaction (HR) on tobacco leaves

Pathogenicity of obtained isolates were tested on tobacco plants (Nicotiana tabacum L.). Susspension (10^6 CFU/ml) was injected into the intact tobacco leaf with a hypodermic needle (Klement et al., 1990). The referent strain (National

Collection of Plant Pathogenic Bacteria, United Kingdom-NCPPB 3318) was also included.

Pathogenicity on soybean

Soybean seeds (Balkan variety) were sown in plastic boxes with wet and sterile sand covered with plastic lids. After five days, soybean seedlings (cotyledon stage) was used for pathogenicity test. Pathogenicity was done on fully expanded soybean cotyledons by dipping and spraying with suspension of the investigated isolates.

Wounded cotyledons were inoculated by spraying aqueous bacterial suspension (10^8 CFU/ml). The plants were incubated for 48h in the moisture chamber at 25°C, than transferred to the laboratory conditions and observed daily during 15 days.

During the experiments plants were watered regularly and wetted with hand sprayer. Check plants were sprayed with distilled water.

Physiological race determination

Reactions of differential soybean cultivars (Acme, Chippewa, Flambeau, Harosoy, Lindarin, Merit and Norchief) were used for race determination of P. s. pv. glycinea according to Cross et al. (1966). The strains were applied onto leaves (in stage of 1/3 -1/2 of fully developed leaf area) of differential cultivars on two ways: a) by rubbing leaves with a sterile cotton swab soaked in an aqueous suspension (Alvarez et al. 1995); b) by spraying leaves with chosen strains under the pressure (Cross et al., 1966; Balaž et al., 1990; Prom & Venette, 1997).

Beggining 3 days after inoculation, plants were examined daily for five days and rated by the system of Cross et al. (1966), in wich leaves with water soaked lesions were considered susceptible and leaves with no hydrosis were designated resistant. Seven separate pathogenic races were distiguished by the reactions of the seven differential varieties (tab. 1).

RESULTS

Isolation of the bacterium

Three days after isolation the colonies on nutrient agar were circular, smooth, shiny and white. Ten isolates were chosen for further investigations (tab. 2). On the basis of this investigation using LOPAT test, it was concluded that all isolates belonged to a Ia fluorescent group.

Table 1 – Reaction of seven pathogenic races of P. s. pv. glycinea on differential soybean varieties (Cross et al. 1966)

Tabela 1 – Reakcija sedam patogenih rasa bakterije P. s. pv. glycinea na diferencijalnim sortama soje (Cross et al. 1966)

Race-Rasa No	Differential varietes – Ddiferencijalne sorte						
	Acme	Chippewa	Flambeau	Harosoy	Lindarin	Merit	Norchief
1	S	R	S	R	R	R	R
2	S	R	S	S	S	I	I
3	S	R	R	S	S	I	I
4	S	I	S	S	S	S	S
5	R	R	R	S	R	S	R
6	R	R	S	R	R	R	S
7	S	R	R	S	R	R	R

R-resistant (otporno), I-intermediate (intermedijarno), S-susceptible (osetljivo)

Table 2 – Bacterial strains obtained from soybean plants
Tabela 2 – Izolati bakterija sa različitih sorti soje

Isolate code Šifra izolata	Variety Sorta	Locality Lokalitet
B2/4	Balkan	Srbobran
B8/2	Balkan	Laćarak
B11/1	Balkan	Čalma
B13/2	Balkan	Begeč
S5/3	Sava	Tovariševo
R9/1	Ravnica	Njegoševo
R9/3	Ravnica	Bačka Topola
R10/6	Rita	Bačka Topola
P15/2	Proteinka	Bijeljina
R12/2	Rita	Subotica

There were no morphological, biochemical and physiological differences between the characters of investigated isolates with original description of Ia pseudomonas fluorescence group given by Lelliot et al. (1966) i Fahy & Hayward (1983).

Pathogenicity tests

Hypersensitive reaction (HR) on tobacco plants

Pathogenicity test on tobacco leaves showed that all tested isolates injected into tobacco leaves caused hipersensitive reaction after 24 h, as the consequence of incompatible between pathogen-host relationship. The rapid death of plant cells leading to necrosis of inoculated tissue with no appearance of usual water-soaked spots.

Pathogenicity on soybean plants

Pathogenicity test of investigated strains showed that all isolates caused greasy and individual spots in all applied method. The first symptoms appeared, three days after inoculation. Investigated strains show different virulence. Later tissue reaction was characteristic for R9/3 isolate inoculated (5 days after inoculation) pointing out to its lower pathogenicity. No differences were detected between isolates B2/5 and R12/2 in their pathogenicity.

Identification of physiological races of the pathogen

The investigated strains caused susceptible reactions in all inoculated soybean differential varietes. Appearance of characteristic water-soaked spots with yellow halo, was observed on plants inoculated by rubbing leaves, 3 days after inoculation (fig. 1).

Fig. 1 – Symptoms on leaves (Acme variety)
Sl. 1 – Simptomi na listovima (sorta Acme)

Symptoms on plants inoculated by spraying appeared after 48 to 72 h (fig. 2). Daily observation of symptoms show tendency of increasing number and size of spots on soybean plants. According to table 1. all investigated strains were compatible with the seven soybean cultivars and were designated race 4.

DISCUSSION

After spray inoculation, all investigated isolates caused typical bacterial blight symptoms (with yellow halo), which confirmed their pathogenicity. Appearance of hyper sensible reaction on tobacco leaves also pointed out to pathogenic characteristics of tested bacterial isolates. Klement (1963) mentioned that pathogenicity of obtained bacterial isolates can be most reliably checked on host plant, as well as on the basis of hypersensitive reaction of tobacco plants.

The susceptible reaction of different soybean cultivar showed that all investigated strains from Vojvodina province were race 4. These results was expected because Balaž et al. (1990) noted the dominance of P. syringae pv. glycinea race 4 in soybeans grown in Vojvodina province.

First data about investigation of physiological races of P. s. pv. glycinea strains, were obtained by Cross et al. (1966). Fett and Sequeira and Basu also reported the predominance of race 4 in Winsconsin, Iowa and eastern Ontario. Races 3 and 5 were found in limited numbers in North Dakota. AboMoch et al. (1995), loc. cit. Balaž & Vidić (2001)., concluded by testing population of bacterium P. s. pv. glycinea in Europe, (isolates collected in France, Germany, Hungary, Poland, Italy, Ukraine and former Yugoslavia) that race 4 was the most dominant. Prom and Venette (1997) testing 164 isolates originating from 170 most cultivated soybean from North Dakota pointed out that race 4 was prevalent (63%) in relation to other (race 6 – 22%; race 2 – 7%; race 3 – 0.3%, and race 5 – 0.1%). The pathogenicity of 199 isolates of P. s. pv. glycinea obtained from five provinces in China was studied on 7 soybean varietes, during 1990-94. Eight separate pathogenic races (1, 2, 4, 5, 9, 10, 11, 12) were distinguished by the reactions of the 7 different soybean varietes. Race 4 was also dominant (56%) in China (Gao Jie, 1998). According to Sinclair (1999), there are four dominant genes controlling resistance to P. s. pv. glycinea (Rpg1, Rpg2, Rpg3 and Rpg4). The resistance of soybean to this pathogen is also affected by a host of other factors within the plants, one being an increased production of ethylene that reduces the virulence of the bacterium.

Based on this findings, it can be concluded that the bacterial blight of soybean must be continuosly monitored and studied in the future and that cultivars with reduced susceptability to P. s. pv. glycinea should be developed in order to improve soybean production in this regard.

LITERATURE

Abo-Moch F., Mavridis A., Rudolph K. (1995): Determination of races of Pseudomonas syringae pv. glycinea occuring in Europe. Journal of Phytopathology, Vol. 143, No. 1. pp. 1-5.

Alvarez E., Braun E. J., McGee D. C. (1995): New Assays for Detection of Pseudomonas syringae pv. glycinea in Soybean Seed. Plant Diseases, vol. 79, No.1.

Arsenijević, M. (1997): Bakterioze biljaka. S print. Novi Sad.

Balaž J., Arsenijević M., Vidić M. (1990): Bakteriološke karakteristike i fiziološke rase Pseudomonas syringae pv. glycinea (Coerper) Young, Dye et Wilkie parazita soje. Zaštita bilja, vol. 41 (4), br. 194: 423-429. Beograd.

Balaž F., Tošić M., Balaž J. (1995): Zaštita biljaka-bolesti ratarskih i povrtarskih biljaka. Novi Sad.

Balaž J., Vidić, M. (2001): Iznalaženje otpornijih genotipova soje prema dominantnim rasama Pseudomonas syringae pv. glycinea, 219-239. In: Primena sistema gen za gen. Ed. Bošković J., Bošković M. Veternik.

Cross J. E., Kennedy B. W., Lambert J. W., Cooper R. L. (1966): Pathogenic races of the bacterial blight pathogen of soybeans, Pseudomonas glycinea. Plant Dis. Rep. 50:557-560.

Daft G.C., Leben C. (1972): Bacterial blight of soybeans: seedling infection during and after emergence. Phytopathology, 62 (10):1167-1170. View Abstract.

Fahy P. C., Hayward A. C. (1983): Media and Methods for Isolation and Diagnostic Test (in: Plant Bacterial Diseases. A Diagnostic Guide. Ed. by Fahy, P. C., Persley, G. J.) Academic Press.

Fett W. F., Sequeira L. (1981): Further characterization of the physiological races of Pseudomonas glycinea. Can. J. Bot. 59:283-287.

Gao Jie (1998): Physiological specialization of the bacterial blight pathogen of soybeans Pseudomonas syringae pv. glycinea. Journal of Jiling Agricultural University 20: 10-12.

Klement, Z. (1963): Rapid detection of the pathogenicity of phytopathogenic Pseudomonads. Nature, 199-300.

Klement Z., Rudolph K., Sands D. C. (1990): Methods in Phytobacteriology. Akademiai Kiado. Budapest.

Lelliott, R. A., Billing, E., Hayword, A. C. (1966): A determinative scheme for the fluorescent plant pathogenic Pseudomonads. Journal of Applied Bacteriology, 29, 470-489.

Lelliott R. A., Stead D. E. (1987): Methods for the Diagnosis of Bacterial Diseases of Plants. British Society for Plant Pathology Blackwell Scientific Publications, Oxford, London. UK.

Prom L. K., Venette J. R. (1997): Races of Pseudomonas syringae pv. glycinea on commercial soybeans in eastern North Dakota. Plant Disease 81: 541-544.

Schaad N. (1980): Laboratory guide for identification of plant pathogenic bacteria. APS Press, St. Paul, Minnesota, USA.

Sinclair, J. B. (1999): Bacterial Diseases, p. 5-10. In: Compedium of soybean diseases. Ed. Hartman, G. L., Sinclair, J. B., Rupe, J. C. APS Press. USA.

Vidić M., Balaž J. (1997): Reakcija genotipova soje prema Pseudomonas syringae pv. glycinea. Zaštita bilja, 220: 119-125, Beograd.

(Primljeno: 03.12.2008.)
(Prihvaćeno: 31.03.2009.)

IDENTIFIKACIJA RASA *PSEUDOMONAS SYRINGAE* PV. *GLYCINEA* NA KOMERCIJALNIM SORTAMA SOJE U SRBIJI

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REZIME

Bakteriozna pegavost soje čiji je prouzrokovač Pseudomonas syringae pv. glycinea (Coerper) Young, u Srbiji se javlja redovno i to često u jakom intenzitetu. Izolacija patogena vršena je sa obolelih delova lista soje različitih sorti. Izolacije su vršene na podlogu od mesnog ekstakta (MPA) i podlogu obogaćenu saharozom (NSA). Patogenost izolata je dokazana pozitivnom hipersenzitivnom reakcijom (HR) na duvanu. Provera patogenosti dobijenih izolata, kao i referentnog izolata ove bakterije (NCPPB 3318), dokazana je i inokulacijom mladih biljaka soje u fazi kotiledona (sorta Balkan), prskanjem i potapanjem u suspenziju bakterija.

Odgajivačke i biohemijsko-fiziološke odlike ispitane su standardnim bakteriološkim metodama.

Pripadnost dobijenih izolata fiziološkim rasama određena je korišćenjem diferencijalnog sortimenta soje: Acme, Chippewa, Flambeau, Harosoy, Lindarin, Merit i Norchief. Mlade biljke su inokulisane na dva načina: prskanjem pod pritiskom i povredom listova sunđerom natopljenim suspenzijom bakterija. Zaključeno je da izolati bakterije P. s. pv. glycinea, sa soje u Vojvodini, pripadaju rasi 4.

Ključne reči: Soja, *Pseudomonas syringae* pv. *glicinae*, rase, identifikacija

(Received: 03.12.2008.)
(Accepted: 31.03.2009.)

Plant Protection, vol. 59 (1-4), No 263-266, 59-67, 2008, Belgrade