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Identification of physiological races of *Colletotrichum lindemuthianum* occuring in Slovenia

Identifikacija fizioloških ras glive *Colletotrichum lindemuthianum* v Sloveniji

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Abstract. Bean anthracnose, the most important fungal disease of common bean in Slovenia, is caused by a mitosporic fungus *Colletotrichum lindemuthianum* (Sacc. et Magnus) Briosi et Cav. The most appropriate measure to control the disease is growing of resistant cultivars. Breeding for resistance is limited by the existence of several physiological races of the pathogen and continuous development and introduction of new ones. It is necessary to constantly monitor the occurrence and dynamic of physiological races existing in certain area. With this aim, 47 isolates were obtained from several bean accessions in different bean growing areas in Slovenia. The fungus was isolated from infected pods and leaves. A set of 12 internationally accepted differential bean cultivars was inoculated with spore suspension obtained from single spore isolates. Plant reaction was screened following 1 – 10 severity scale. Four physiological races of the pathogen were identified. Using the binary nomenclature system they were assigned to races 23, 55, 103 and 131. The most frequent and widespread were races 55 and 23, named also lambda and delta. The research will continue by screening the autochthonous bean germplasm for susceptibility towards the existing races.

Key words: bean anthracnose, *Colletotrichum lindemuthianum*, common bean, physiological races

Izvleček. Fižolov ožig, ki je pri nas najpomembnejša glivična bolezen fižola, povzroča gliva *Colletotrichum lindemuthianum* (Sacc. et Magnus) Briosi et Cav. Bolezen preprečujemo predvsem z gojenjem odpornih sort fižola. Žlahtnjenje na odpornost proti tej bolezni je težavno zaradi velikega števila fizioloških ras ter nenehnega razvoja in introdukcije novih ras. Zato moramo stalno spremljati pojav in dinamiko fizioloških ras na določenem območju. S tem namenom smo zbrali 47 primerkov glive *C. lindemuthianum* z različnih območij pridelovanja fižola v Sloveniji. Glivo smo izolirali iz okuženih fižolovih strokov in listov. S pridobljenimi izolati smo okužili 12 diferencialnih sort fižola, ki so mednarodno priznane za identifikacijo fizioloških ras te glive. Testne rastline fižola smo okužili s suspenzijo trosov, pridobljeno iz enotrosnih izolatov. Jakost okužbe smo ocenili po desetstopenjski skali. Odkrili smo štiri fiziološke rase glive in jih na podlagi binarnega nomenklaturega sistema identificirali kot rase št. 23, 55, 103 in 131. Najbolj pogosti in razširjeni sta rasi št. 55 in 23, imenovani tudi lambda in delta. V nadaljnjih raziskavah bomo proučili odpornost domačih akcesij fižola na okužbo s slovenskimi rasami glive *C. lindemuthianum*.

Ključne besede: *Colletotrichum lindemuthianum*, fiziološke rase, fižol, fižolov ožig

Introduction

Bean anthracnose is one of the most destructive diseases in bean growing areas world-wide as well as in Slovenia. It is caused by a mitosporic fungus *C. lindemuthianum* (Sacc. et Magnus) Briosi et Cav. The anamorphic state *Glomerella cingulata* (Stonem.) Spauld et v. Schrenk. f. sp. *phaseoli* can be produced in culture in certain pairings of conidial isolates, but has not yet been found in field conditions (BRYSON & al. 1992). *C. lindemuthianum* has a world wide distribution. It has been recorded on various legumes belonging to the genera *Phaseolus, Vicia, Vigna, Glycine, Pisum* and many others (LENNÉ 1992). Among them, *P. vulgaris* is the most important host, followed by *P. acutifolius var. latifolius* and *Vigna unguiculata.* Other legumes are only slightly susceptible (TU 1992).

C. lindemuthianum shows an extensive intraspecific variability and exists in the form of several physiological races. Pathogenic variability among isolates of *C. lindemuthianum* was first observed by BARRUS (1911). He distinguished two races of the fungus (alpha and beta) by testing their pathogenicity towards 139 bean cultivars. This was the first description of physiological races in a plant pathogenic fungus. Later on extensive variation in the fungus has been found and several new races were described in the local populations of *C. lindemuthianum* throughout the world. Race identification was traditionally based on the nomenclature system using letters of the Greek alphabet. Many other nomenclature systems using local codes were also used, thus rendering the possibility of comparing the research results very difficult. Altogether 13 races, named by letters of Greek alphabet, were described till 1994 and many more were classified using local codes (MELOTTO & al. 2000).

Bean anthracnose affects all the above ground parts of beans causing dark brown depressed lesions. Affected leaves and stems bear brown lesions that can rapidly spread and girdle the plant. Symptoms are the most conspicuous on pods: rusty brown spots occur first, then sunken dark brown, eye-shaped lesions with brown to reddish edges develop. Black specks (acervuli) occur on the lesions and pink spore masses ooze from them in humid conditions. The disease is transmitted and spread mostly by infected seed. Seed treatment and production of clean seed, crop rotation and sanitation can significantly reduce the extent of the disease. Nevertheless, outbreaks of anthracnose still occur, especially in areas with an extensive use of susceptible local bean accessions. The intensity of the disease depends on weather conditions and can reach the extent of an epidemic in the years with high relative humidity, frequent precipitation and moderate temperature.

The most appropriate measure to control the disease is growing of resistant cultivars. Breeding for resistance against *C. lindemuthianum* has been practised since the beginning of the last century, when disease resistance was demonstrated in certain bean cultivars (BARRUS 1911, 1918). Eight independent dominant resistance genes (*Co*-1 to *Co*-8) were described in common bean (ALZATE-MARIN & al. 1997, YOUNG & al. 1998, GEFFROY & al. 2000). The use of specific resistance genes has not always provided a durable resistance due to the continuous development of new physiological races which are capable of overcoming the resistant germplasm. When selecting for resistance in a particular region, the breeder should carefully choose a gene pair that would confer resistance to all races known in that region (KELLY & MIKLAS 1999).

The aim of our study was to investigate the pathogenic variability of the pathogen and identify the physiological races existing in the bean growing areas in Slovenia.

Materials and methods

Fungal isolates

47 samples were collected from several bean accessions in different bean growing areas in Slovenia. The fungus was isolated from infected pods and leaves on potato dextrose agar (PDA). Sporulation was induced by growing isolates on sterilised bean pods. Single spore isolates were obtained by

spreading spore suspension on PDA plates and isolating individual germinating spores. To prevent a degeneration of single spore isolates during storage, spore suspension of single spore isolates was fixed on sterile filter papers, desiccated on sterilised silica-gel and stored at - 20 °C (FERREIRA & FUEYO 2001).

Differential bean cultivars

Physiological races of the fungus were identified by screening the disease severity (resistant or susceptible reaction) on a selected group of bean varieties - differentials. The reaction of differential cultivars strongly depends on infection techniques, experimental conditions, on host genotype and pathogenicity of the fungal isolate. Different bean cultivars, mostly local ones, were used as differentials and different reaction schemes were applied for assessing the disease severity. Several attempts have been made to select an international set of differential cultivars and to uniform the classification of races. Differential cultivars should be genetically uniform with clear resistant or susceptible reaction to all isolates of the fungus (CHARRIER & BANNEROT 1970, DRIJFHOUT & DAVIS 1989). In 1991, a set of 12 differential bean cultivars was internationally accepted and a binary nomenclature system for identification of *C. lindemuthianum* races was proposed (PASTOR - CORRALES 1991). Using this system, each race is identified by a number, obtained by summing the binary value of all differential cultivars showing a susceptible reaction. Among differential cultivars, four represent the Andean germ plasm (Perry Marrow, Michigan Dark Red Kidney, Kaboon and Widusa) and the others - the Middle American germ plasm (Michelite, Cornell 49242, Mexico 222, PI 207262, To, Tu, Ab136 and G2333).

The seeds of differential bean cultivars used in our study were obtained from CIAT, Columbia. The seeds were surface sterilised with 1 % sodium hypochlorite, left to germinate on moist filter paper and planted in sterilised sand (four seeds per pot). They were kept in greenhouse for two weeks until the development of primary leaves.

Infection trials

A single spore colony of each isolate was grown on PDA for 14 days prior to inoculation. Spores were then scrapped from the plates and used for the preparation of spore suspension. Spore concentration was adjusted to 10^6 - 10^7 per ml prior to inoculation of test plants. Plants with fully expanded primary leaves were infected. Both sides of leaves and stems were inoculated by brushing following the method of TU & AYLESWORTH (1980). Four plants of each cultivar were infected with each isolate and the whole experiment was repeated two to three times. Altogether 1690 inoculations were made. The plants were kept in a growth chamber at the temperature 20 °C and 14 h of light. During the first two days of incubation they were covered with transparent plastic bags to maintain a 100 % relative humidity. The disease expression was scored 8 days after the inoculation. The plant reaction was evaluated qualitatively (resistant or not) and quantitatively using ten- point severity scale (0 - 9). Plants showing no infection or small necrotic lesions originating from hypersensitive reaction, were assigned resistant (score 0 –3), the rest were considered susceptible (score 4 - 9).

Results and discussion

Isolates of *C. lindemuthianum* from *Phaseolus vulgaris* grown in Slovenia were assigned to four physiological races based on their pathogenicity towards 12 differential bean cultivars (Table 1). Most of the isolates were pathogenic to differential cultivars originating from Andean gene pool. Among them, the cultivar Michigan Dark Red Kidney was the most liable to infection and showed a highly susceptible reaction to all isolates. By contrast differential cultivars originating from Middle American gene pool were relatively resistant with the exception of the cultivar Michelite, which showed a moderately susceptible reaction to most of the isolates.

Using the binary nomenclature system the races of *C. lindemuthianum* from Slovenia can be identified as races 23, 55, 103 and 131. The majority of isolates (25) represented race 55, 16 isolates represented race 23, and only 6 isolates represented races 103 and 131.

Table 1: Reaction of differential bean cultivars to races of *C. lindemuthianum* from Slovenia
Tabela 1: Reakcija diferencialnih kultivarjev fižola pri okužbi s slovenskimi rasami glive *C. lindemuthianum*

Race	Differential bean cultivars											
	1	2	4	8	16	32	64	128	256	512	1024	2048
131	S_{5-7}	S_9	R_0	R_0	R_3	R_3	R_2	S_{4-5}	R_3	R_0	R_2	R_0
103	R/S_4	S_9	S_9	R_0	R_3	S_8	R/S_4	R_2	R_3	R_0	R_2	R_0
23	S_7	S_9	S_9	R_0	S_8	R_{2-3}	R_2	R_1	R_3	R_0	R_2	R_0
55	S_7	S_9	S_9	R_0	S_8	S_{5-7}	R_2	R_2	R_2	R_0	R_2	R_0

Legend: Differential bean cultivars: 1- Michelite, 2 - Michigan Dark Red Kidney, 4 - Perry Marrow, 8 - Cornell 49242, 16 - Widusa, 32 - Kaboon, 64 - Mexico 222, 128 - PI 207262, 256 - TO, 512 - TU, 1024 - AB 136, 2048 - G 2333; R - resistant reaction, S - susceptible reaction, numbers indicate severity of symptoms (ten- point severity scale).
Legenda: Diferencialni kultivarji fižola: 1- Michelite, 2 - Michigan Dark Red Kidney, 4 - Perry Marrow, 8 - Cornell 49242, 16 - Widusa, 32 - Kaboon, 64 - Mexico 222, 128 - PI 207262, 256 - TO, 512 - TU, 1024 - AB 136, 2048 - G 2333; R - odporen, S - občutljiv, številke kažejo jakost okužbe po desetstopenjski skali

As has already been mentioned, many different systems of race classification are used in the studies of pathogenic variability in *C. lindemuthianum*. The results are difficult to compare since different bean cultivars are used as differentials. Nevertheless, comparing our results with other studies of *C. lindemuthianum* race composition in Europe in the past decades (CHARRIER & BANNEROT 1970, DRIJFHOUT & DAVIS 1989, GOTH & ZAUMEYER 1965, KRUGER & al. 1977, YERKES & ORTIZ 1956) we can conclude that races 55 and 23 fully correspond with the races lambda and delta. The race lambda has been first reported in Europe in 1974 from the Netherlands and the race delta in 1953 from Germany (HUBBELING 1974, FRANDSEN 1953). Both races are very virulent, showing high level of pathogenicity towards many bean cultivars. The minority of isolates tested in our study belonged to races 103 and 131. They partly correspond with the races gamma and beta. Their reaction to differential cultivars, especially to the cultivar Michelite, slightly differs from the reaction described in other studies; Michelite was moderate to susceptible in our study, while it was resistant in most other studies (ALAM & RUDOLPH 1993, FERNÁNDEZ & AL. 2000, GOTH & ZAUMEYER 1965, YERKES & ORTIZ 1956).

The existence of a large number of *C. lindemuthianum* races and a continuous emergence of the new ones are very important aspects of the control of bean anthracnose. It is necessary to constantly monitor the race composition in a certain area and screen the local bean germplasm for susceptibility towards the existing races. With this aim we intend to infect 27 local bean accessions with isolates representing all the four races of the pathogen occurring in Slovenia. Preliminary results of infection with three isolates of the delta race revealed that only 4 local accessions were resistant to the anthracnose pathogen, the rest were susceptible with a level of infection ranging from 5 to 8.

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