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Zornitsa B. Stoyanova[1], *Rossitza M. Rodeva*[1*], *Ilija Karov*[2], *Biljana Kovacevik*[2], *Vasilissa I. Manova*[1], *Ralitsa G. Georgieva*[1]

[1] Institute of Plant Physiology and Genetics, 1113 Sofia, Bulgaria
[2] Faculty of Agriculture, University "Goce Delčev", Krste Misirkov bb, 2000 Štip, Macedonia Delcev University – Stip, Macedonia

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *COLLETOTRICHUM COCCODES* ISOLATED FROM PEPPER CULTIVATED IN BULGARIA AND MACEDONIA

ABSTRACT: *Colletotrichum coccodes* has been recognized as one of the causal agents of pepper fruit anthracnose in Bulgaria. Recently, this species has been recorded in pepper fruits in Macedonia. In Bulgaria, the fungus has also been isolated from roots of premature senescent pepper plants but in Macedonia it has not been isolated yet. The purpose of the investigation was to make comparative morphological, cultural and molecular characterization of *C. coccodes* isolates obtained from pepper fruits and roots in Bulgaria and Macedonia. Additionally, a technique was applied to differentiate among the *C. coccodes* isolates obtained from roots and other microsclerotia-producing fungi. On the host tissue, *C. coccodes* developed acervular conidiomata with cup-shaped fruiting bodies accompanied with dark-pigmented, unbranched, thick-walled sterile hyphae called setae. A slimy mass of hyaline, straight, unicellular, fusiform conidia appeared on nutrient media. In a short time, numerous small dark globose setose microsclerotia emerged in the colony starting from its centre and distributing proportionally throughout agar plates. Two PCR primer sets were used to sequences of the ribosomal internal transcribed spacer (ITS1 and ITS2) regions. Single products of ~450 bp and ~350 bp were amplified by the genus-specific (Cc1F1/Cc2R1) and the species-specific primers (Cc1NF1/Cc2NR1), respectively. Morphological, cultural and molecular characterization of the isolates from roots and fruits showed that root rot and fruit anthracnose of pepper were caused by one and the same causal agent determined as *C. coccodes*. The isolates from roots showed rapid mycelial growth, gave rise to numerous minute microsclerotia and produced conidia only on SSPA. SSPA supported significantly more mycelium growth and sporulation than all other media tested and could be recommended for production of large quantity of conidia. No pattern of genetic variation associated with the organ or geographic origin of the isolates was determined.

KEY WORDS: acervuli, fungal isolates, ITS, microsclerotia, pepper, plant pathogens

INTRODUCTION

Colletotrichum coccodes (Wallr.) S.J. Hughes has been recognized as one of the causal agents of pepper fruit anthracnose in Bulgaria (R o d e v a

* Corresponding author: r.rodeva@abv.bg

et al., 2009a; 2009b). Recently, this species has been recorded in pepper fruits in Macedonia (R o d e v a et al., 2012). In Bulgaria, the fungus has also been isolated from roots of premature senescent pepper plants but in Macedonia it has not been isolated yet. The purpose of the work was to make comparative morphological, cultural and molecular characterization of *C. coccodes* isolates obtained from pepper fruits and roots in Bulgaria and Macedonia. The isolates from roots were of great interest for this investigation because, in Bulgaria, the causal agent was and still is recognized as *C. atramentarium* (Berk. & Broome) Taubenh. (B o b e v, 2000; 2009). The pathogen was often detected together with other root-infecting fungal species such as *Macrophomina phaseolina* (Tassi) Goidanich. For that reason, a technique was additionally applied to differentiate among the *C. coccodes* isolates obtained from roots and other microsclerotia-producing fungi.

MATERIAL AND METHODS

Pathogen isolation

Fruits with anthracnose symptoms as well as roots of premature dying plants were sampled from the experimental field, which is in the property of the Institute of Plant Physiology and Genetics (IPPG), Sofia and from commercial fields in the main pepper growing regions in Bulgaria. Infected fruits were also collected from farm fields and plastic greenhouses in pepper growing districts of East Macedonia, including Strumica, Kochani, Kavadarzi and Sveti Nikole. Isolations were made from diseased pepper fruits with typical anthracnose symptoms and from the roots of premature senescent pepper plants bearing microsclerotia. All experimental work was carried out at IPPG.

Morphological and cultural characterization

Potato dextrose agar (PDA) was used for the initial isolations and storage of *C. coccodes*. Twelve isolates were included in the experiment and they were divided into three groups. Group A included isolates obtained from pepper roots in Bulgaria (B8-2, B12-9, B12-13 and K18-1, the latter one from early root infection); Group B included isolates from fruits in Bulgaria (B12-33, B12-45, B12-46 and B12-47) and Group C from diseased fruits taken from Macedonia (MK7-1, MK7-2, MK26-1 and MK26-2). Three nutrient media: PDA, sucrose soy protein agar (SSPA) (Y u et al., 1997) and water agar (WA) were selected in order for the study on morphological and cultural characteristics of the fungal isolates to be carried out. To determine colony growth and morphology, 4 mm plugs were taken from the periphery of actively growing colonies and transferred to the plate centre. Inoculated plates were incubated at 24 ºC. The diameter of each colony was measured 7 and 14 days after inoculation. There were five replicate plates. Isolates were morphologically charac-

terized on the basis of colony growth, color, mycelium density and the characteristics of microsclerotia, acervuli and conidia. Images were acquired using an Olympus BX41 Microscope. Cell^F for Imaging Life Science Microscopy was the acquisition software. At least 30 microsclerotia and 100 conidia of each isolate were measured for cultures grown on the three nutrient media.

A technique for differentiation of some sclerotial fungi was additionally applied to *C. coccodes* isolates from roots (O s t a z e s k i, 1964). The experimental plot consisted of a Petri dish with WA growth medium onto which four 12-mm-discs of alfalfa leaves were placed. A 6-mm-plug of PDA growth medium colonized with fungal mycelium was inverted on the edge of each leaf disc so that half of the agar block could rest on leaf tissue and half on agar. *M. phaseolina* isolates were involved in the experiment in order to make comparison (R o d e v a et al., 2010). Four replicate plates of each isolate were incubated at 24 ºC.

Molecular characterization

For molecular characterization, the *C. coccodes* isolates were grown in potato dextrose broth and gyrated at 125 rpm for 7-10 days at 24 ± 2 ºC. The mycelia were harvested by filtration, washed in sterile water and lightly squeezed in filter paper. Partially dried mycelium was stored at -80 ºC. Those samples were later used for DNA extraction. DNA was isolated from fungal mycelium by DNeasy Plant mini kit (Qiagen) according to the manufacturer's instructions. Controls included no DNA negative control (mQ water), but they included DNA from one *C. gloeosporioides* and two *C. acutatum* isolates. Briefly, fungal tissue was ground to fine powder in liquid nitrogen and then subjected to lysis and RNase treatment at 60° C for 15 min. Salt-precipitated proteins and polysaccharides were first pelleted by centrifugation for 5 minutes, the resulting supernatant was centrifuged through QIAshredder spin column and the cleared lysate was applied to DNeasy Mini spin column membrane. After 2-step washing, the total genomic DNA was eluted in AE buffer (10mM Tris-Cl, 0.5 mM EDTA, pH 9.0).

C. coccodes isolates were characterized by PCR amplification with both genus and species-specific primers, based on the ITS (internal-transcribed spacer) of ribosomal genes, as described by C u l l e n et al. (2002), with some modifications. PCR amplification was performed as follows: initial denaturation step at 95°C (3 min), 20 cycles at 95°C (45 s), annealing and elongation steps performed at 72°C for 2 min and 15 s, final elongation at 72°C for 5 min. The reactions were performed in 20 µl volume, containing 25 ng total genomic DNA, 1x reaction buffer A (Eurx), 200 µM dNTPs, 0.4 µM primers, 2.5mM $MgCl_2$ and 1.25U Color Taq (Eurx). A total of 2.5µl of each PCR reaction were loaded on 1.6% agarose gels (300ng/ml EtBr) and subjected to electrophoresis in 1xTAE buffer at 60V, for at least 2.5 h. The gels were visualized by UV transillumination, and their electronic images were captured by ImageQuant150 imager (GE Healthcare) and densitometrically analyzed with ImageQuantTL7

software (GE Healthcare) to determine the approximate length of the resulting PCR products.

Statistical analysis

All experimental results were given as mean (M) and standard error of the mean (SEM). The data for colony growth were statistically processed by analysis of variance and calculation of the least significant difference (LSD).

RESULTS

The diseased fruits of pepper showed typical anthracnose symptoms including soft, sunken, round or slightly elongated lesions bearing at first fungal fruiting bodies (acervuli), which extruded gelatinous conidial mass. The size of conidia on natural substrate was (14.9) 18.4±0.2 (22.4) x (3.2) 4.4±0.1 (5.2) µm. In a short time, small dark globose setose structures called microsclerotia emerged on and inside of the diseased pepper fruits (Fig. 1a). The below-ground symptoms caused by *C. coccodes* root attack appeared as large brown to grey lesions on roots covered with microsclerotia and sloughing of the root cortex (Fig. 1b). The above-ground symptoms of affected plants were expressed as chlorotic foliage, wilting and premature plant death. Under the climatic conditions in Bulgaria, the first isolations of *C. coccodes* from roots and fruits were made at the beginning of August. *C. coccodes* was detected on pepper roots either solely or as a disease complex with other root-infecting fungi such as *Verticillium dahliae* Kleb., *Fusarium oxysporum* Schlechtend.: Fr., *F. solani* (Mart.) Sacc., *Rhizoctonia solani* J.G. Kühn and *Macrophomina phaseolina*.

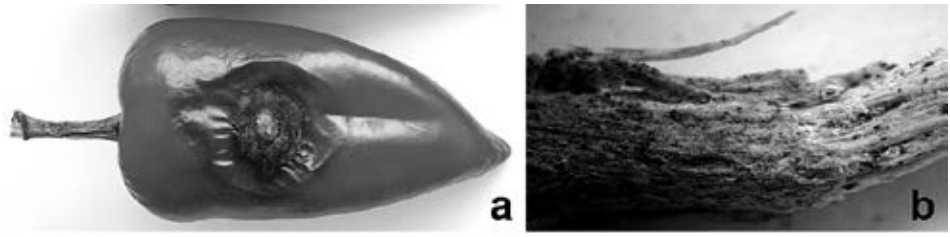

Fig. 1. – Symptoms caused by *C. coccodes*: a. on pepper fruit; b. on pepper root

Overlaying of alfalfa leaves on WA stimulated the development of abundant quantities of *C. coccodes* acervuli, which produced slimy mass of hyaline, straight, unicellular, fusiform conidia together with microsclerotia (Fig. 2a-h). The same technique applied to *M. phaseolina* isolates resulted in producing microsclerotia and great number of pycnidia extruding conidia (Fig. 3a-d).

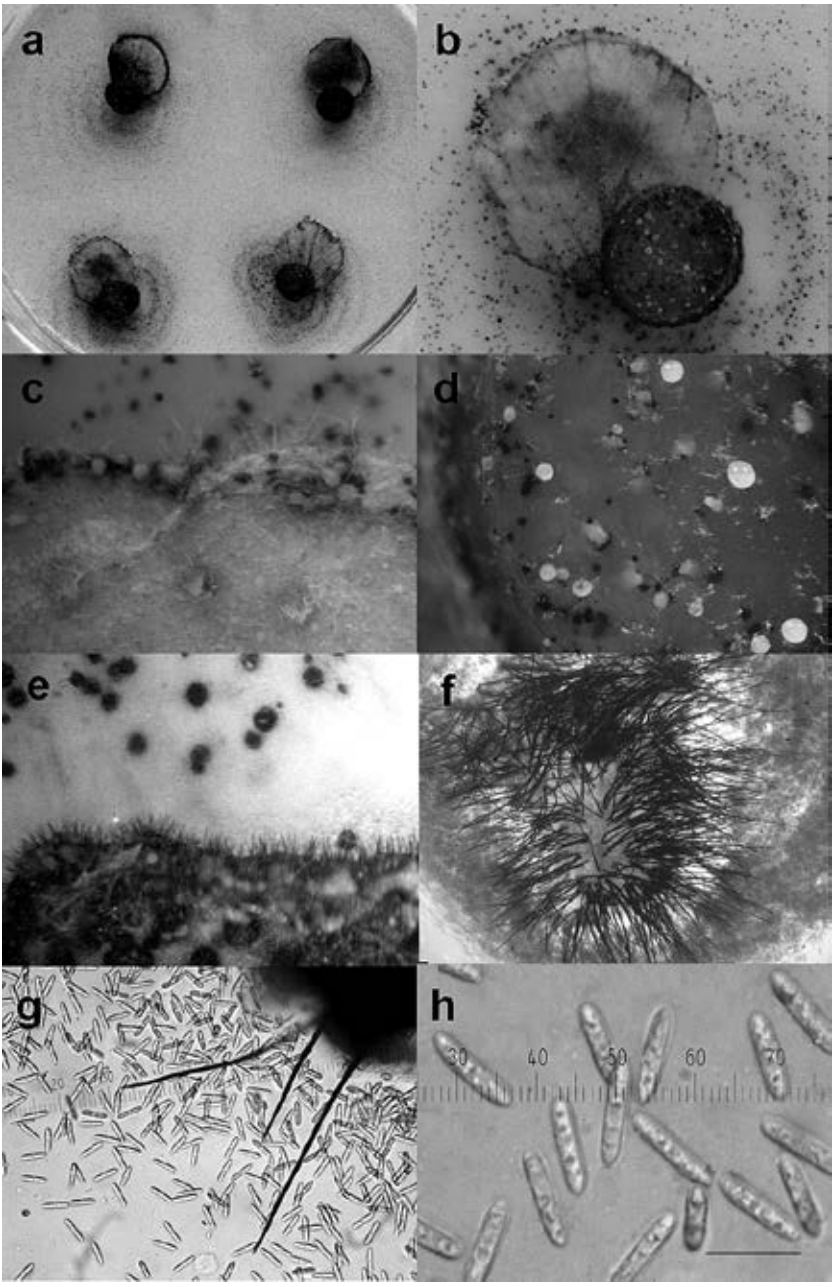

Fig. 2. – Sporulation of *C. coccodes* on alfalfa leaf discs: a. Experimental design on water agar plate; b. Acervuli and microsclerotia on and around the leaf disc and mycelial plug; c. Periphery of leaf disc with acervuli and slimy conidial mass; d. Reverse side of agar plug with droplets of conidial jelly; e. Increased number of acervuli and dark setae on the leaf disc; f. Acevulus accompanied with great number dark-pigmented setae; g. Acervulus, setae and conidia, x 160; h. Conidia, x 400, scale bar = 20 μm

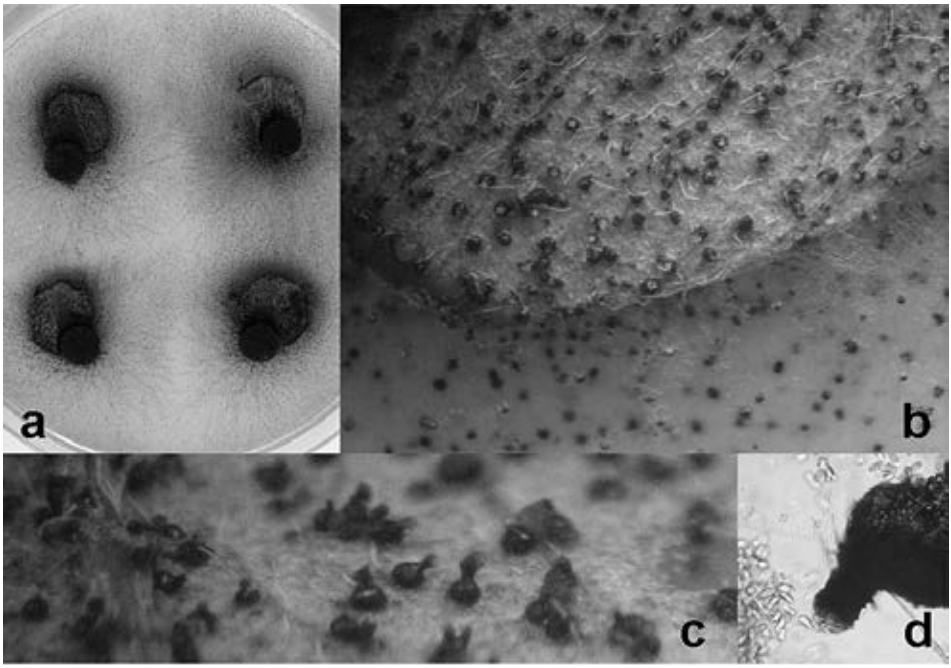

Fig. 3. – Sporulation of *M. phaseolina* on alfalfa leaf discs: a. Experimental design on water agar plate; b. Pycnidia and microsclerotia on and around the leaf disc; c. Pycnidia with short necks and ostioles; d. Pycnidium extruding conidia

All isolates grew quickly on PDA (Tab. 1, Fig. 4). Colonies of group A were dark gray with pale rose periphery; the reverse was dark gray. The mycelium was scarce. Numerous small microsclerotia were ordered in concentric rings darker in the colony centre. The isolate K18-1 showed well pronounced radial stripes. The growth of colonies belonging to group B was slower on PDA. The colonies were dark gray with pinkish to salmon periphery; the reverse was grayish with ochre to salmon periphery with well expressed concentric rings. Gray creeping mycelium that covered the colony surface with irregularly distributed light gray to white floccose patches (isolates B12-33 and B12-47). Microsclerotia showed to have bigger dimensions and were more aggregated. Colonies of group C had similar growth rate as those of group B. The isolates varied in morphology. Isolate MK7-1 gave rise to greenish gray, densely textured colonies with slightly expressed concentric and radial zones and pale rose irregular periphery; the reverse was light brown with dispersed aggregates of microsclerotia. Isolate MK7-2 showed well pronounced concentric rings and radial stripes like the isolate K18-1. A big central part was covered by gray velutina mycelium; the reverse was dark gray. Isolate MK26-1 grew quickly on PDA with well-defined concentric rings of microsclerotia and pale ochre to greenish periphery; the reverse was the same. Isolate MK26-2 was similar to the isolates of group B.

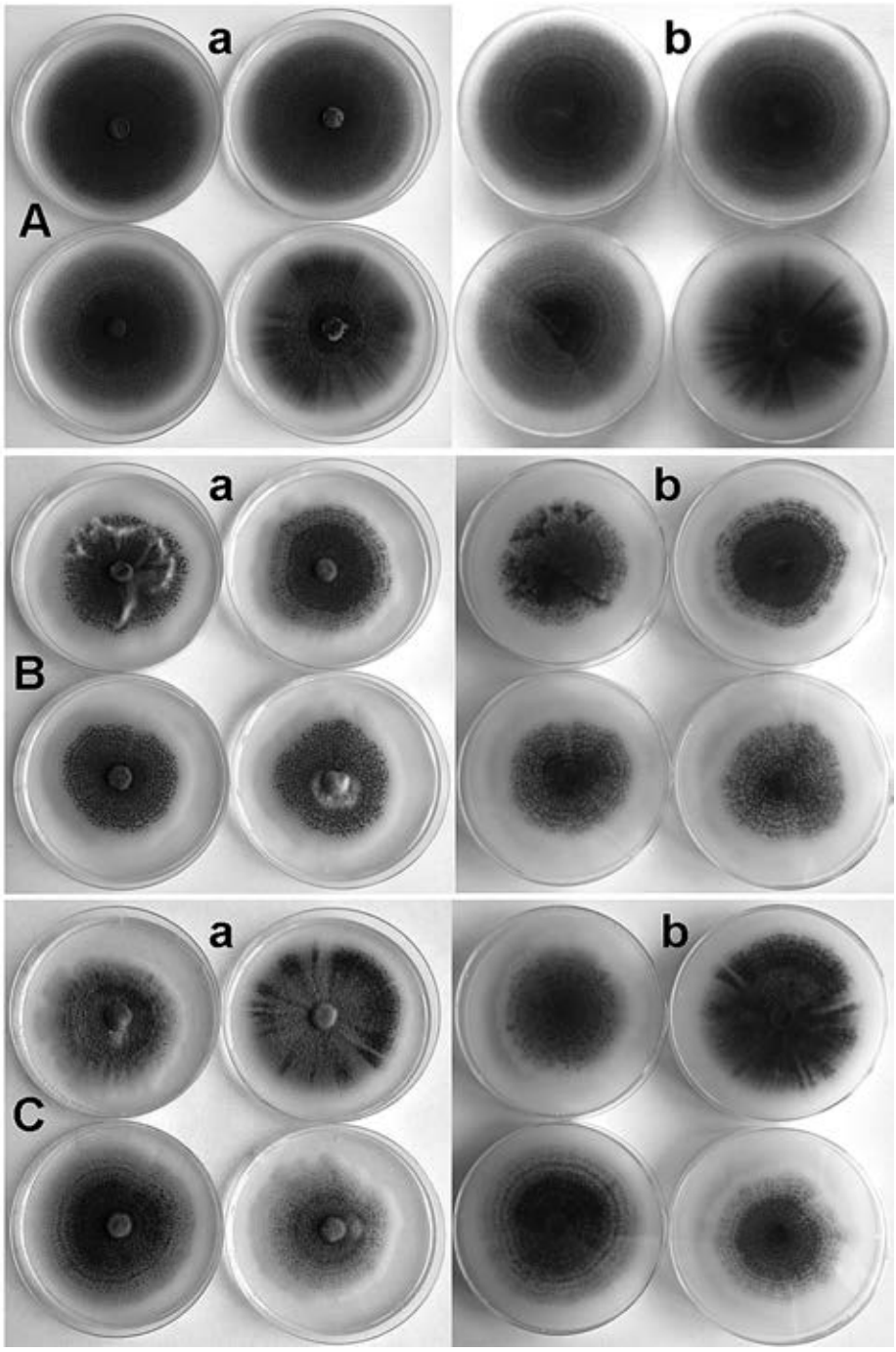

Fig. 4. – Colony morphology of the isolates of group A, B and C on potato-dextrose agar after 14 days of incubation: a. above side; b. reverse side

All colonies on SSPA grew very quickly, occupying the whole surface of Petri dish in 14 days (Tab. 1, Fig. 5). Each group expressed different morphology pattern. Colonies of group A had dark greenish-gray central part with microsclerotia in concentric rings, followed by a large pinkish to salmon actively sporulating zone; the reverse was the same. Colonies of group B had dense texture and were spotted with sectors of light and dark gray mycelium. Big salmon drops of conidia were seen through the mycelium mainly around the colony centre. Colonia of group C developed less microsclerotia and less mycelium in irregular darker sectors. They produced big quantity of conidia, which appeared as large drops or flood of cream-colored jelly.

On WA, all colonies were loosely textured with similar characteristics (Tab.1, Fig. 6). They were transparent and consisted mainly of microsclerotia dispersed deeply in the media. No conidia were found. The isolates belonging to the group B and C had the same morphology (Figures are not shown).

Tab. 1 – The colony growth (mm) of *C. coccodes* isolates on three nutrient media (PDA, SSPA, WA) after 7 and 14 days of incubation

Isolates (Factor A)	*7 days*			*Average per isolate*	*14 days*			*Average per isolates*
	Nutrient media (Factor B)				Nutrient media (Factor B)			
	PDA	SSPA	WA		PDA	SSPA	WA	
B8-2	50.0±0.7	54.0±0.4	31.6±1.9	**45.2**	80.0±0.0	85.0±0.0	80.0±0.0	**81.7**
B12-9	49.6±0.6	54.6±0.8	33.4±1.2	**45.9**	80.0±0.0	85.0±0.0	66.4±2.5	**77.1**
B12-13	48.0±0.6	52.8±0.4	29.2±0.5	**43.3**	80.0±0.0	85.0±0.0	50.0±0.0	**71.7**
K18-1	49.8±0.2	51.0±0.4	31.0±1.1	**43.9**	80.0±0.0	85.0±0.0	73.2±2.0	**79.4**
B12-33	36.2±2.0	58.4±2.8	28.2±1.0	**40.9**	60.8±2.1	85.0±0.0	75.2±1.1	**73.7**
B12-45	38.6±1.4	52.4±0.3	32.4±0.4	**41.1**	59.4±2.3	85.0±0.0	48.4±1.3	**64.3**
B12-46	37.4±1.8	60.8±1.0	28.8±0.5	**42.3**	59.8±1.0	85.0±0.0	55.0±1.1	**66.6**
B12-47	36.4±2.4	53.2±1.2	34.6±0.4	**41.4**	58.8±2.0	85.0±0.0	51.2±1.4	**65.0**
MK7-1	35.0±1.1	48.6±1.0	31.2±1.4	**38.3**	54.2±1.5	85.0±0.0	50.8±0.5	**63.3**
MK7-2	47.4±0.8	54.4±1.2	29.4±0.7	**43.7**	77.2±1.0	85.0±0.0	53.6±1.1	**71.9**
MK26-1	45.6±2.2	54.4±0.7	32.0±1.4	**44.0**	72.0±2.1	85.0±0.0	58.2±0.8	**71.7**
MK26-2	35.2±0.5	50.2±0.7	30.2±0.9	**38.5**	56.2±1.2	85.0±0.0	62.0±2.1	**67.7**
Average per media	42.4	53.7	31.0		68.2	85.0	60.3	
LSD	A	B	A x B		A	B	A x B	
0.05	1.7	0.9	3.0		1.6	0.8	2.8	
0.01	2.3	1.2	3.9		2.1	1.0	3.7	
0.001	2.9	1.5	5.0		2.7	1.3	4.7	

On PDA, all isolates from fruits (group B and C) produced hyaline, straight, unicellular, fusiform conidia with average size of 15.0 x 4.7 µm (Tab. 2). They appeared as a slimy mass mainly in the colony periphery, where the fungus was actively growing. In colonies of root isolates (group A), conidia were not found at the end of experiment. Pale rose periphery, where conidia

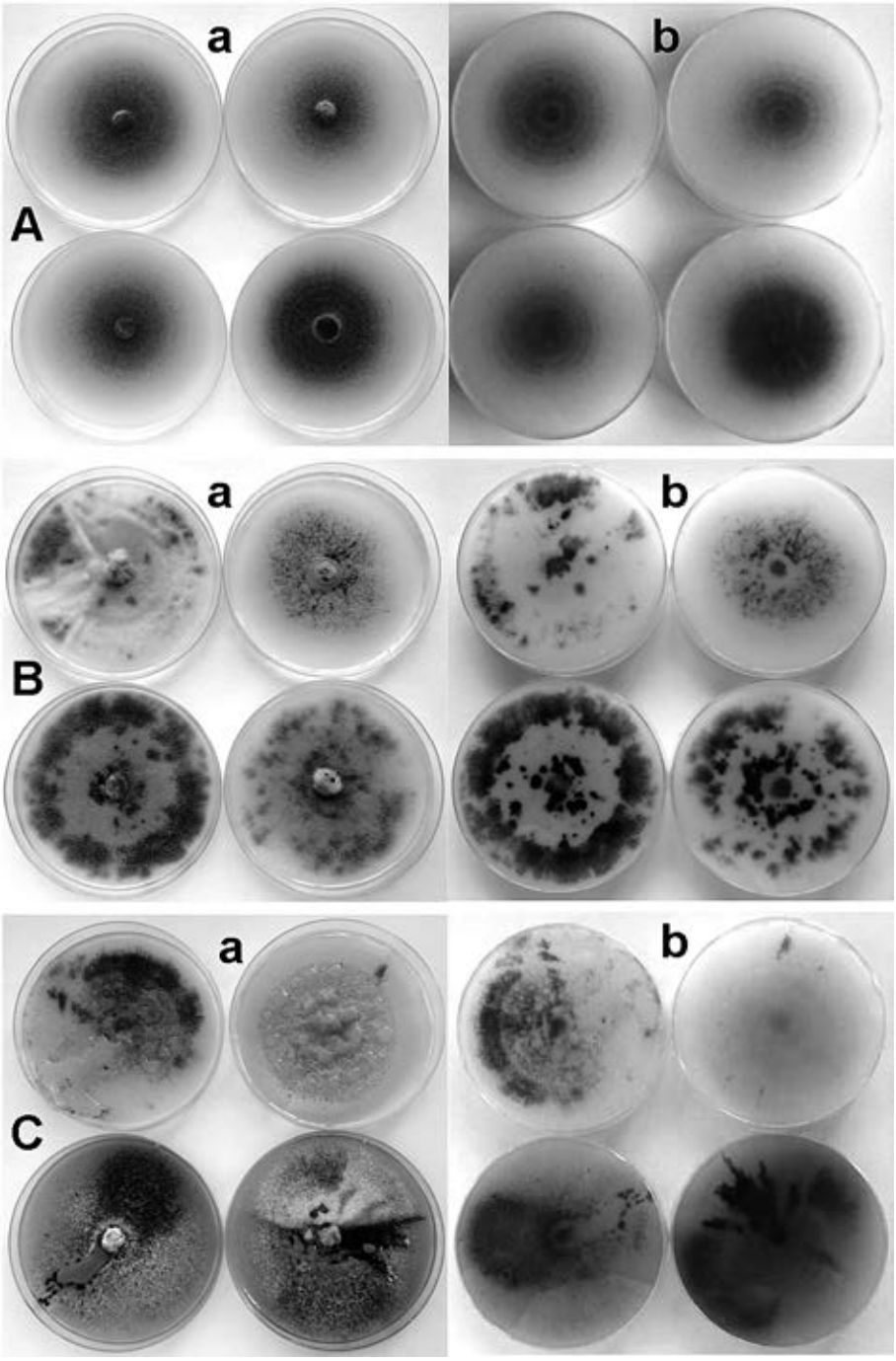

Fig. 5. Colony morphology of the isolates of group A, B and C on sucrose-soy protein agar after 14 days of incubation: a. above side; b. reverse side.

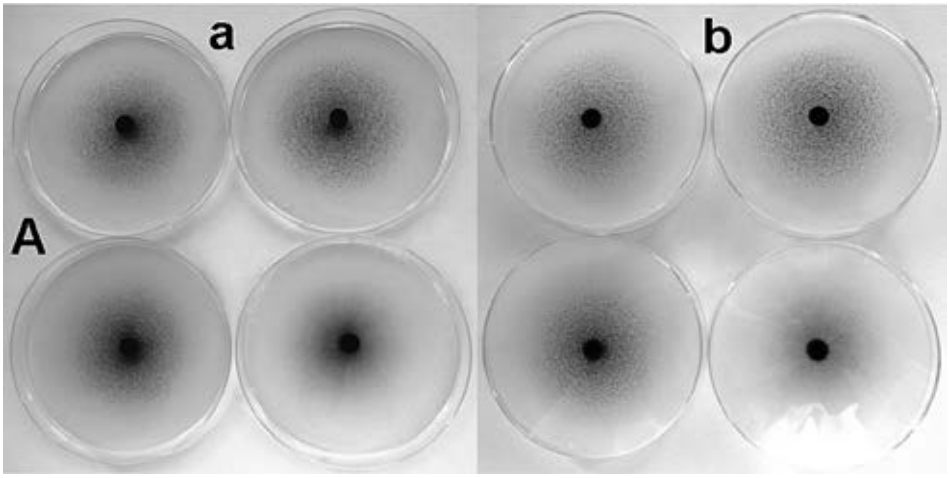

Fig. 6. – Colony morphology of the isolates of group A on water agar after 14 days of incubation: a. above side; b. reverse side.

could be found, was recorded in the first days of cultivation, but these colonies grew quickly occupying almost the whole surface of Petri dish after 15 days of cultivation. All isolates sporulated extremely well on SSPA. The conidial mass was accumulated in large drops or even flood of cream-colored jelly giving a strange yeast-like morphology to the colonies (group B and C). The average size of conidia was 13.3 x 4.7 µm. No conidia were found on WA.

Tab. 2 – The size (µm) of *C. coccodes* conidia on two nutrient media (PDA and SSPA)

Isolates	Length		*Width*	
	PDA	SSPA	PDA	SSPA
B8-2	–	(9.0)**14.8±0.3**(19.8)	–	(4.0)**5.1±0.1**(6.4)
B12-9	–	(10.7)**15.0±0.7**(16.6)	–	(4.5)**5.4±0.1**(5.9)
B12-13	–	(11.8)**14.7±0.6**(22.2)	–	(4.6)**5.6±0.1**(7.4)
K18-1	–	(9.1)**13.9±0.4**(25.9)	–	(3.6)**4.9±0.1**(6.1)
B12-33	(11.0)**14.9±0.6**(19.2)	(8.5)**11.3±0.4**(20.7)	(4.2)**4.8±0.1**(6.1)	(3.6)**4.5±0.1**(5.4)
B12-45	(11.9)**17.5±0.5**(23.0)	(10.6)**13.7±0.4**(16.3)	(3.2)**4.8±0.1**(6.6)	(3.5)**4.4±0.1**(5.4)
B12-46	(10.3)**12.0±0.3**(14.3)	(8.3)**14.6±0.3**(22.0)	(4.0)**4.6±0.1**(5.3)	(3.7)**4.9±0.1**(6.1)
B12-47	(11.2)**14.0±0.4**(18.6)	(7.6)**11.6±0.2**(18.6)	(3.8)**4.9±0.2**(6.2)	(3.2)**4.4±0.1**(6.3)
MK7-1	(9.4)**15.8±1.0**(25.7)	(7.2)**12.7±0.4**(24.8)	(3.4)**4.2±0.1**(5.0)	(3.3)**4.5±0.1**(6.0)
MK7-2	(8.0)**13.7±0.5**(21.2)	(8.8)**12.9±0.5**(19.2)	(3.4)**4.7±0.1**(5.9)	(2.9)**4.3±0.1**(5.4)
MK26-1	(15.0)**16.8±1.1**(18.8)	(8.4)**12.0±0.3**(16.5)	(4.0)**4.9±0.4**(6.1)	(2.9)**4.2±0.1**(5.5)
MK26-2	(10.8)**15.0±0.6**(19.2)	(9.5)**12.4±0.3**(20.0)	(3.6)**4.7±0.2**(5.8)	(3.2)**4.3±0.1**(6.0)
Average	15.0	13.3	4.7	4.7

Tab. 3 – The size (μm) of *C. coccodes* microsclerotia on three nutrient media (PDA, SSPA, WA)

Isolates	PDA	SSPA	WA	Average
B8-2	(128) **175±4** (237)	(72) **107±2** (139)	(35) **58±2** (85)	113
B12-9	(63) **100±2** (134)	(58) **95±3** (154)	(44) **67±3** (96)	**87**
B12-13	(64) **96±2** (145)	(63) **92±3** (140)	(43) **72±3** (100)	**87**
K18-1	(38) **58±2** (100)	(44) **84±5** (131)	(13) **21±2** (39)	**54**
B12-33	(248) **307±8** (412)	(58) **105±6** (173)	(48) **79±4** (117)	164
B12-45	(134) **223±10** (296)	(113) **148±6** 197	(57) **98±5** (133)	**156**
B12-46	(102) **137±4** (174)	(73) **108±4** (176)	(55) **84±3** (120)	**110**
B12-47	(114) **150±5** (205)	(83) **114±6** (161)	(49) **92±7** (125)	**119**
MK7-1	(153) **215±11** (309)	(46) **66±4** (113)	(45) **73±5** (129)	118
MK7-2	(113) **159±7** (239)	(40) **65±4** (107)	(35) **50±3** (70)	**91**
MK26-1	(218) **283±10** (393)	(67) **102±4** (174)	(38) **74±4** (123)	**153**
MK26-2	(164) **192±8** (272)	(58) **73±1** (110)	(64) **94±4** (138)	**120**
Average	175	97	72	

All isolates produced microsclerotia on all of the nutrient media (Tab. 3). Those were small dark globose setose bodies which emerged in the colony starting from its centre and distributing proportionally throughout agar plates. Culture media influenced the size of microsclerotia. On PDA, microsclerotia were more aggregated and bigger in size. On SSPA, they had intermediate size with the exception of isolate K18-1 and MK-1. The entire colonies on WA consisted of numerous, smallest in size, microsclerotia that immersed in the agar plate. The isolates of group A had the smallest microsclerotia, while the isolates from group B had the largest one.

PCR amplification with genus-specific primers (Cc1F1/Cc2R1) gave one single band of ~450 bp in all isolates analyzed (*C. coccodes, C. acutatum* and *C. gloeosporioides*) confirming that they belong to the genus *Colletotrichum* (Figure is not shown). The nested primer set Cc1NF1/Cc2NR1 amplified one single PCR band of ~350 bp only in the reactions containing DNA from *C. coccodes* isolates as a template and as it was expected from the literature (C u l l e n et al., 2002) (Fig. 7). When DNA isolated from *C. gloeosporioides* and *C. acutatum* was utilized as a template, no amplification band was visible on the gel because of the lack of homology between Cc1NF1 primer and the respective rDNA region in those species.

DISCUSSION

As it is illustrated the present study, *C. coccodes* may, by itself, cause root rot and should be considered as a pathogen causing primary wilt disease. In mixed infections and in different combinations with vascular wilt agents

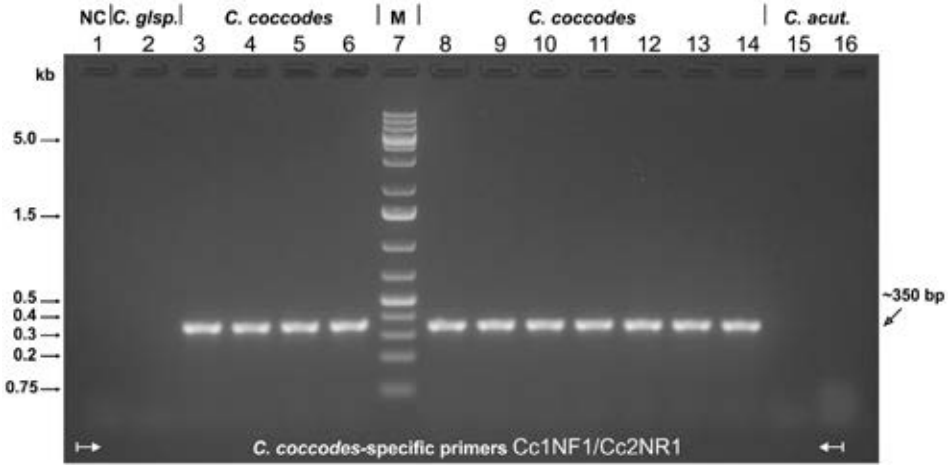

Fig. 7. – Identification of *C. coccodes* in genomic DNAs isolated from different *Colletotrichum* species. PCR amplification with *C. coccodes*-specific primers Cc1NF1/Cc2NR1: Lane 1: NC – Negative control mQ water; Lane 2: *C. gloeosporioides* isolate; Lanes 3-6: *C. coccodes* isolates from pepper roots; Lane 7: DNA marker GeneRuler 1kb+ (Fermentas); Lanes 8-14: *C. coccodes* isolates from pepper fruits; Lanes 15-16: *C. acutatum* isolates.

such as *V. dahliae* and *F. oxysporum* and other root-infecting fungi such as *F. solani*, *R. solani*, *M. phaseolina*, this pathogen could be responsible for the early dying syndrome of pepper. Morphological, cultural and molecular characterization of the isolates from roots and fruits showed that root rot and fruit anthracnose of pepper were caused by one and the same causal agent determined as *C. coccodes*. *C. atramentarium* could be considered only as an obsolete synonym. The isolates originated from roots showed rapid mycelial growth, gave rise to numerous minute microsclerotia and produced conidia only on SSPA.

Molecular identification of *C. coccodes* with species-specific primer was a successful method for the confirmation of species. Some morphological differences were observed between the isolates on PDA and predominantly on SSPA. ITS region was successfully amplified for the isolates of *C. coccodes,* but additional methods of identification were required in order for genetic diversity to be revealed.

SSPA supported mycelium growth and sporulation more significantly than all other media tested. Isolates from group B and especially from group C released extremely large quantity of conidia confirming the results of Y u et al. (1997). WA supported no conidia development and significantly less mycelium than all other media tested, but the isolates, especially those obtained from roots, produced more microsclerotia.

CONCLUSION

Morphological, cultural and molecular characterization of the isolates from roots and fruits showed that root rot and fruit anthracnose of pepper were caused by one and the same causal agent determined as *C. coccodes*. The isolates from roots showed rapid mycelial growth, gave rise to numerous minute microsclerotia and produced conidia only on SSPA. SSPA supported mycelium growth and sporulation more significantly than all other media tested and could be recommended for the production of large quantity of conidia. No pattern of genetic variation associated with the organ or geographic origin of the isolates was determined.

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МОРФОЛОШКА И МОЛЕКУЛАРНА КАРАКТЕРИЗАЦИЈА ИЗОЛАТА *COLLETOTRICHUM COCCODES* ИЗОЛОВАНИХ ИЗ ПАПРИКЕ УЗГАЈАНЕ У БУГАРСКОЈ И МАКЕДОНИЈИ

Зорница Б. Стојанова[1], Росица М. Родева[1],
Илија Каров[2], Биљана Ковачевиќ[2],
Василиса И. Манова[1], Ралица Г. Георгијева[1]

[1] Институт за физиологију и генетику биљака, 1113 Софија, Бугарска
[2] Пољопривредни факултет, Универзитет „Гоце Делчев", Крсте Мисирков бб, 2000 Штип, Македонија

Резиме

Colletotrichum coccodes се јавља као један од узрочника антракнозе у плодовима паприке у Бугарској. Недавно је потврђено њено присуство и на плодовима паприке из Македоније. У Бугарској је ова гљивица изолована из корена прерано сазрелих биљака али у Македонији нису забележена слична запажања. Циљ овог рада је да се изврши компаративна морфолошка, културолошка и молекуларна карактеризација изолата *C. coccodes* добијених из плодова и корена паприке узгајане у Бугарској и Македонији. Даље, ова техника је примењена за утврђивање разлика између изолата *C. coccodes* добијених из корена и осталих гљивица које могу да формирају микросклероције. На ткиву домаћина, *C. coccodes* је развила ацервуларне конидиомате са плодоносним телом у облику шоље са тамно обојеним, неразгранатим, стерилним хифама са дебелим зидом које се зову сете. На хранљивој подлози, развиле су се праве, једноћелијске, вретенасте конидије састављене од слузасте хијалинске масе. За кратко време појавио се велик број ситних, тамних, глобуларних, чекињастих микросклероција почевши од центра колоније и пропорционално се ширећи преко агарне плоче. Два сета PCR прајмера је коришћен за секвенционирање рибозомалних интерних (ITS1 и ITS2) регија. Појединачни одзиви на ~450 bp и ~350 bp су појачани коришћењем род-специфичних (Cc1F1/Cc2R1) и врста-специфичних (Cc1NF1/Cc2NR1) прајмера, респективно. Морфолошка, културолошка и молекуларна карактеризација изолата из корена и плодова паприке је показала да је узрочник трулежа корена и антракнозе плодова један те исти патоген, *C. coccodes*. Изолати из корена су показали способност брзог мицеларног раста и стварања великог броја ситних микросклероција а конидије су производили само на SSPA. На SSPA подлози је добијен значајно већи мицеларни раст и интензитет спорулације у односу на остале испитиване подлоге и може се препоручити за производњу већих количина конидија. Није запажена никаква генетска варијација у изолатима у односу на биљни орган из којег су изоловани или географско порекло биљака.

КЉУЧНЕ РЕЧИ: ацервуле, изолати гљивице, ITS, микросклероција, паприка, биљни патогени

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