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CHARACTERIZATION OF *DIAPORTHE/PHOMOPSIS* SPP. FROM PLUM TREES BY SDS-PAGE

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INTRODUCTION

The ascomycete genus *Diaporthe* Nitschke, and their anamorphs *Phomopsis* (Sacc.) Bubák contains a large number of cosmopolitan plant pathogens, many of which incite blights, cankers, die-backs, rots, spots, and wilts on a wide range of host including economically important crops (Uecker, 1988). During last ten years the die-back of young plum trees (*Prunus domestica* L.) was noticed in many localities in Western Serbia. *Phomopsis* isolates recovered from necrotic cambium tissues were examined morphologically, by pathogenicity tests and DNA sequencing, but could not be identified at the species level (except of *Diaporthae eres*, isolate SL-U-4). Distinct differences in colony appearance, and morphology of conidiomata, conidiogenous cells and conidia were observed among all *Diaporthe/Phomopsis* spp. (Živković, 2008).

Electrophoretic analysis of total cell proteins by one-dimensional protein patterns provides a rough measure of the number and physicochemical properties of gene products. A proteomic approach could be a solution for better understanding the variation in virulence in a fungal pathogen population (Huang and Mahoney, 1999).

Since polypeptide pattern diversity of *Diaporthe/Phomopsis* populations has not been investigated so far, a study with this aim was conducted on 12 isolates collected from Western Serbia. Protein gel electrophoresis may provide important information about genetic variation of *Diaporthe/Phomopsis* spp. originating from plum trees.

Figure 1a,b: The branches and trunk of plum trees with canker and die-back symptoms.

Table 1. Isolates of *Diaporthe/Phomopsis* spp. from plum trees used for SDS-PAGE analysis.

Isolate code	Species	Cultivar	Location	Year of isolation
Sl-1*	Phomopsis sp.	stenlej	Valjevo	2004
Sl-M	Phomopsis sp.	čačanska rodna	Osečina	2004
Sl-T-1	Phomopsis sp.	čačanska rodna	Lipolist	2005
Sl-B-2	Phomopsis sp.	stenlej	Belanovica	2004
Sl-Br-2	Phomopsis sp.	čačanska rodna	Belanovica	2004
Sl-K-1	Phomopsis sp.	čačanska lepotica	Koceljeva	2006
Sl-K-3	Phomopsis sp.	čačanska lepotica	Koceljeva	2006
Sl-K-4	Phomopsis sp.	čačanska lepotica	Koceljeva	2006
Sl-K-5	Phomopsis sp.	čačanska lepotica	Koceljeva	2006
Sl-K-7	Phomopsis sp.	čačanska lepotica	Koceljeva	2006
Sl-K-8*	Phomopsis sp.	čačanska lepotica	Koceljeva	2006
Sl-U-4*	Diaporthe eres	čačanska rodna	Ub	2006

* The isolates were identified by CBS Fungal Biodiversity Centre

MATERIAL AND METHODS

Fungal cultures

Diaporthe/Phomopsis spp. were isolated from branches and trunk of plum trees with canker and die-back symptoms (Figure 1a,b). Twelve representative monoconidial isolates were selected for further studies (Table 1.). The strain JP-3, (*Phomopsis perniciosa*) from apple fruit was used as a control.

Preparation of protein extracts

Mycelium production of *Diaporthe/Phomopsis* spp. was carried out by culturing the fungi in 100 ml of potato dextrose broth (PDB) and incubating them at 25°C for 7 days. Mycelial mats were then filtered under vacuum on a Büchner funnel, rinsed with distilled water and blotted dry.

SDS-PAGE analysis

SDS-PAGE was performed by the method described by Laemmli (1970), and modified according to Sambrook et al. (1989). Electrophoresis was carried out at constant current of 0.12 kV through the stacking gel, and 0.15 kV through the separation gel at room temperature. After electrophoresis the gel was stained in 0.1% (w/v) Coomassie Brilliant Blue R250, and then distained in a distaining solution (25% methanol, 7% acetic acid, and 68% H_2O).

Data analysis

The similarity and relationship between the protein traces of isolates were expressed in a dendrogram derived by means of the simple matching coefficient, and unweighted pair group method with arithmetic averages algorithm using STATISTICA 5 software.

RESULTS

Since the difficulties of distinguishing *Phomopsis* species are well known, due to the wide host range of some species and their morphological plasticity, isolates from plum trees were characterized by total cell protein profiles using SDS-PAGE. There were considerable differences in protein profiles of tested isolates at 170 - 17 kDa region (Figure 2). A dendrogram of the total cell protein profiles of *Diaporthe/Phomopsis* spp. is shown in Figure 3. Numerical analysis revealed clearly three distinct clusters at a difference level of 29%. Isolate Sl-U-4 (*D. eres*), formed the first separate cluster with the largest percentage difference (33.5%). The level difference of members of the cluster 2 and 3 changed between 4.8% and 24%. *D. eres* showed a genetic distance ranging from 0.334 to 0.524. This fungus exhibited a significant difference in protein expression.

CONCLUSION

The electrophoretic analysis of total cell proteins showed that each isolate had characteristically distinctive protein band patterns. The similarities and distinctions indicated that population of different *Diaporthe/Phomopsis* spp. from plum trees were characterized by expressive genetic variability.

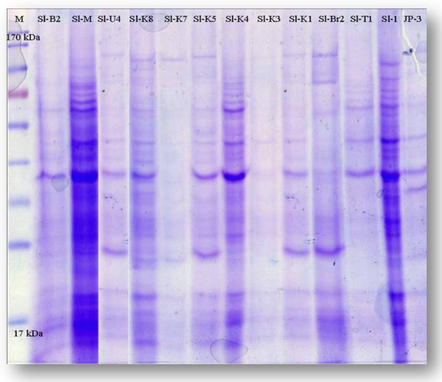

Figure 2. SDS-PAGE of total cell proteins of *Diaporthe/Phomopsis* spp. (Line M: Marker, Fermentas; Page Ruler Prestained Protein Ladder SM 0697).

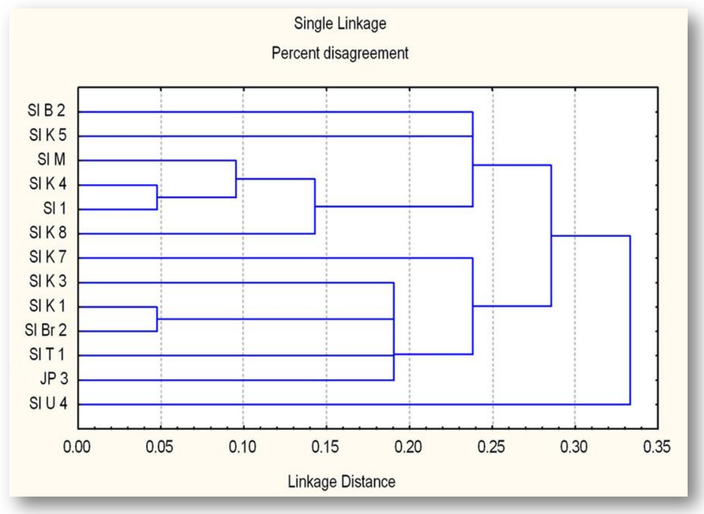

Figure 3. Dendrogram based on unweighted pair group method with arithmetic averages algorithm of the protein patterns of *Diaporthe/Phomopsis* spp. from plum trees.

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