

# VARIATION IN AGGRESSIVENESS OF *PHOMA MACDONALDII* ISOLATES FROM THREE BALKAN COUNTRIES AND UKRAINE

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## ABSTRACT

*Phoma macdonaldii* is a ubiquitous pathogen, affecting sunflower by premature leaf senescence, stem cortical tissue necrosis and basal stem girdling. Sunflower genotypes expressing partial resistance have been reported. Currently, disease has reemerged as a threat to sunflower production in some sunflower cropping areas. To estimate variability in pathogen population, sunflower stems with disease symptoms from Serbia, Turkey, Romania and Ukraine were sampled. Total of 54 isolates was used for inoculation of four inbred lines differing in resistance to phoma black stem. Results from scoring of sunflower inbred lines seven days after inoculation showed significant differences in disease severity measured by percentage of cotyledon petiole necrosis. Significant difference was detected both among isolates and genotypes. Isolates were segregated in five clusters. Five isolates were found to be highly aggressive based on disease severity. The least aggressive were seven isolates, producing mild symptoms on all tested genotypes. Majority of tested isolates lead to complete necrosis of inoculated plant part of the most susceptible genotype and mild symptoms of other three genotypes. Isolate aggressiveness was not correlated with geographic origin. In conclusion, significant variability among pathogen isolates was confirmed with several isolates distinguished as highly aggressive. This research could assist in breeding process for resistance to phoma black stem.

**Keywords:** sunflower, *Phoma macdonaldii*, aggressiveness

## INTRODUCTION

Diseases are a major constraint in sunflower production. Phoma black stem, caused by pathogenic fungus *Phoma macdonaldii*, is widely distributed disease, usually considered to have limited impact on sunflower yield and quality (Gulya et al., 1997). The most distinguishing symptoms of disease appear in form of black lesions on stem, elliptical in shape and commonly 5-10 cm in length (Marić and Schneider, 1979). Symptoms can develop at stem base and in time girdling of stem may result in premature ripening (Donald et al., 1987).

Severity of disease depends on sunflower genotype and up to date no complete resistance to phoma black stem has been found. Sunflower genotypes significantly differ in susceptibility (Roustee et al., 2000a; Bert et al., 2004). Tolerant genotypes were found in cultivated and wild sunflowers (Darvishzadeh et al., 2010; Larfeil et al., 2010). Disease development differs spatially and temporally as a result of influence of environmental factors and cultural practices (Sessau et al., 2010; El Sayed and Marić, 1981). In addition, variability among sunflower genotypes is complemented with differences in isolate aggressiveness

(Roustae et al., 2000b). Most recently, virulence variability of pathogen was reported in Argentina (Lazzaro *et al.*, 2012).

The objective of this study was to determine aggressiveness of *P. macdonaldii* isolates collected in Serbia and compared these results with aggressiveness of isolates from three countries where sunflower is extensively cultivated.

## MATERIAL AND METHOD

*P. macdonaldii* isolates were collected in 2012, across three regions in Serbia, and received from Ukraine, Romania and Turkey. Four sunflower inbred lines (CMS-1-122, ROD-DI-111, VL-A-8, DOP-32-08), differing in resistance to phoma black stem were selected based on previous research (Dedić *et al.*, 2012). Sunflower seed was surface sterilized in 1% solution of NaOCl and sown in plastic containers 9x9x9 cm in size, and filled with peat. Four plants grown in each container served as replication. Experiment was set in three replications. Temperature during experiment was maintained at 22/18 °C during 16/8 h photoperiod. Inoculation of plants, with fully developed first pair of leaves, was done following method described by Roustae *et al.* (2000b). Cotyledon petioles were inoculated with 20 µl of *P. macdonaldii* picnospore suspension, concentration  $10^6$  picnospores/ml. Seven days after inoculation disease severity was assessed using scale 1-9 (Roustae *et al.*, 2000b). For each line and isolate median value was calculated and isolates were clustered using software PAST (Hammer *et al.*, 2001).

## RESULTS AND DISCUSSION

Results after inoculation of four inbred lines shows significant difference in susceptibility to disease with median values ranged from 1 to 9 (Figure 1). Significant variation of disease severity was observed among isolates expressed in large interquartile range particularly for inbred lines ROD-DI-111 and VL-A-8.

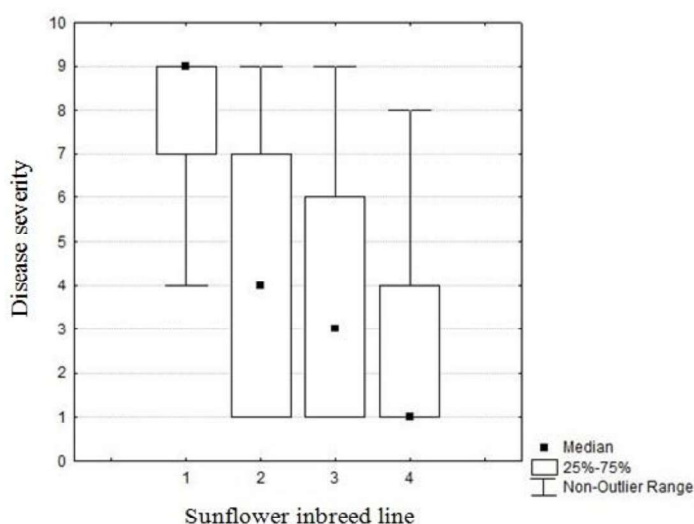


Figure 1. Median values and variation of Phoma black stem severity for all tested isolates on inbred lines CMS-1-122 (1), ROD-DI-111 (2), VL-A-8 (3) and DOP-32-08 (4)

Isolates were clustered in four distinct groups based on disease severity of inoculated inbred lines (Figure 2). The first group was consisted of five isolates which produced the most severe symptoms and consequently were considered to be highly aggressive. Out of this five isolates origins of two is same region in Serbia (SRB-R1S52, SRBR1S21), another two were sampled in Ukraine and one in Romania. The second group has two isolates which were characterized by high disease severity on inbred lines CMS-1-122 and DOP-32-08 and mild symptoms on other two inbred lines. The third group consisted of five low aggressive isolates. Isolates from the fourth and the largest group expressed high disease severity on genotype CMS-1-122, moderate disease severity on genotypes ROD-DI-111 and VL-A-8, and low disease severity on genotype DOP-32-08.

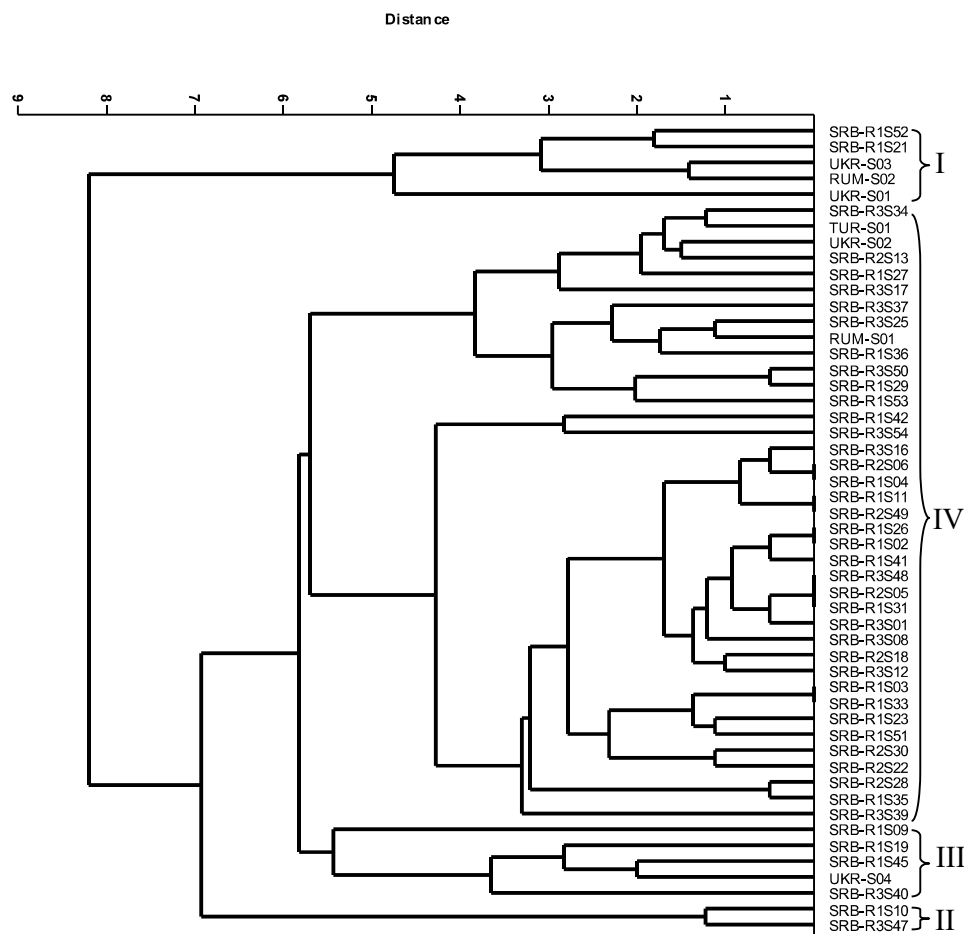


Figure 2. Dendrogram showing similarity and clustering of *P. macdonaldii* isolates based on their aggressiveness on four sunflower inbred lines

Considerable variability in aggressiveness was confirmed as a result of this research. Majority of tested isolates have similar pattern in disease severity on selected genotypes. However, a group of both high and low aggressive isolates was distinguished along with two isolates able of producing severe symptoms on the most resistant genotype. Isolates not originated from Serbia were not clustered based on aggressiveness. Most of these isolates proved to be highly aggressive. However, number of isolates from other countries was small

and this conclusion needs to be verified on larger sample of pathogen population from that areas. Difference in aggressiveness among *P. macdonaldii* proved in this research was also confirmed by other researchers. Most data comes from France where this disease is considered highly damaging (Mirleau-Thebaud *et al.*, 2011). Larfeil *et al.* (2002) determined five pathotypes based on stark differences in disease severity, following inoculation of ten sunflower inbred lines. Rostae *et al.* (2000a) presented considerable variability among isolates in various traits including aggressiveness. Similar differences were found after testing isolates of pathogen in Argentina (Lazzaro *et al.*, 2012). In addition, highly significant genotype-isolate interaction in *P. macdonaldii* – sunflower pathosystem was reported (Darvishzadeh *et al.*, 2007; Maleki and Darvishzadeh, 2014).

In conclusion, patterns of reaction of four inbred lines to disease revealed differences in aggressiveness, with 5 out of 54 tested isolates regarded as highly aggressive. Isolates with similar aggressiveness did not cluster according to geographic origin. Determination of pathogen variability will provide selection of *P. macdonaldii* isolates suitable for breeding programs.

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