

Monitoring of the SDHI Mutations of *Alternaria solani* in Serbia

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INTRODUCTION

- In recent years, the increasing awareness of the fungicide resistance united many scientists and countries to different strategies to obtain necessary data network required for establishing a programme to effectively monitor fungicide resistance development for economically important pathogens
- Alternaria solani*, causal agent of early blight of potato, has been described as foliar disease which can cause significant yield losses if left uncontrolled (Figure 1)
- Resistance of *A. solani* on fungicides from the SDHI group was detected in 2009 in United States only four years after registration
- In 2014 reduced sensitivity were detected in Europe: Netherlands and Belgium
- In 2015, in Netherlands, Belgium, Germany and Denmark
- In 2016, low frequency of resistant isolates was detected in Poland, Denmark, Italy, Romania and United Kingdom while moderate frequency was detected in Belgium, Germany, Netherlands and Sweden
- In 2016, in Serbia, potato covered around 40.000 ha with, average production of early potato 30-35 t/ha and 25 t/ha for late commercial potato. Potato growing regions are mostly in central and south of Serbia (Leskovac and Čacak)

The objectives of this research were to detect and characterize fungicide sensitivity in populations of *A. solani* in Serbia to the commonly used SDHIs boscalid.

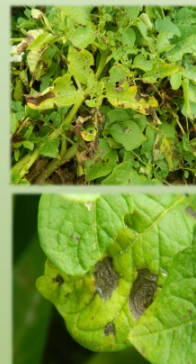


Figure 1. Potato plants showing the symptoms of early blight

MATERIALS AND METHODS

Isolation

- Infected leaf samples showing the typical symptoms were examined under the stereomikroskop. *Alternaria* cultures were identified on the basis of morphological and cultural characteristics and pathogenicity. Isolates were obtained by single spore method of isolation and transferred to V8 medium for 7 days at 23 °C with a 12 h photoperiod

In vitro assay

- Fungicide sensitivity was evaluated as the concentration of technical grade boscalid (98%) at which germination was inhibited by 50% (EC₅₀) compared with growth of nonamended media. Isolates were classified into categories based on sensitivity (Avenot et al., 2008; Gudmestad et al., 2013)
- All statistic calculations were conducted in program IBM SPSS STATISTICS v20

DNA extraction and PCR Method

- Approximately 20 mg of dry weight mycelium were collected and used for DNA extraction according to the manufacturer's instructions of DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA)
- All isolates were used for molecular detection of SDHI resistant isolates. For monitoring the mutations in the SDHI complex which lead to resistance, previously designed primers and multiplex PCR was conducted (Malik et al. 2013)

RESULTS

- In 2016, 91 monospore isolates at 7 locations of commercial potato crops were examined:
- 26,4 % of isolates did not carry any known mutations in AaSdHb, AaSdHc or AaSdHd
- 3,3 % of isolates carried amino acid substitution in AaSdHd
- 63,74 % of isolates carried amino acid substitution in AaSdHc
- 11 % of isolates carried amino acid substitution in AaSdHb

- Based on *in vitro* assay and molecular diagnosis three different phenotypes could be posted (Table 1).

Table 1. EC₅₀ values of *in vitro* boscalid assay and associated phenotypes of *A. solani* isolates

Class	Phenotype	Mean EC ₅₀ values (µg/ml)
1	Sensitive	0.2
2	Low level of resistance	8.5
3	Moderate level resistance	17.7

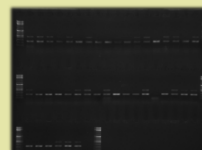


Figure 2. Polymerase chain reaction (PCR) of *A. solani* isolates with primers pairs specific for screening isolates with exchanges in AaSdHb (amplification band of 235 bp, or none), AaSdHc (amplification band of 457 + 235 bp) and AaSdHd (amplification band of 235 + 72 bp)

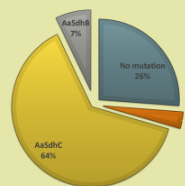


Figure 3. Percentages of *A. solani* isolates carrying the mutations in the AaSdHb, AaSdHc and AaSdHd gene

CONCLUSION

As we know, before 2016, monitoring of *A. solani* isolates have not been done in Serbia. The research provided new information about the occurrence of SDHI mutations responsible for sensitivity shift of *A. solani* isolates from potato plants in several locations in Serbia. These findings will certainly be valuable for informing and supporting preventive and curative treatments on the emergence of resistance in Serbia.

References:

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- Malik, I., Arabatz, S., Pasche, J. S., Bolton, M. D., Patel, J. S., and Gudmestad, N. C. 2014. Molecular characterization and detection of mutations associated with resistance to succinate dehydrogenase inhibiting fungicides in *Alternaria solani*. *Phytopathology*, 104, 40-49.