

STUDY OF ROLE OF *AbreAtr₁* GENE IN PROTECTIVE MECHANISM AND PATHOGENICITY OF *Alternaria brassicae*, THE CAUSAL AGENT OF LEAF SPOT IN CANOLA, USING REAL TIME PCR*

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Abstract

Alternaria brassicae is one of the most important seed borne pathogens and causal agents of canola leaf spot. In pathogenicity process of *A. brassicae*, *AbreAtr₁* genes correspond to pathogenicity cluster NRPS-ABC transporter and have been introduced as an encoding of pathogenicity factor. The presence of these proteins in organisms prevents the accumulation of toxic compounds caused by them, and protects them against toxins, thus enhancing the growth of pathogen. The role of this gene in growth of *A. brassicae* and amount of pathogenicity of this fungus has not yet been considered. In order to study this role, the amount of *AbreAtr₁* transcription in six isolates of *A. brassicae* was compared by Real time PCR method. The daily growth rate in each isolate in PDA medium and the amount of pathogenicity was also studied. There was a significant difference among isolates at 1% level in the growth rate and amount of pathogenicity. Also, there was a significant difference among isolates in the amount of *AbreAtr₁* transcription. The role of *AbreAtr₁* in protective mechanism of *A. brassicae* against the produced phytotoxins, the correlation between the growth rate, amount of pathogenicity and transcription pattern of *AbreAtr₁* were determined in each isolate. There was a positive relation between growth rate, amount of pathogenicity and transcription pattern of *AbreAtr₁* at 1% level. It is thought that growth and pathogenicity of *A. brassicae* is affected by the ABC transporter encoded by the *AbreAtr₁*. Over-expression of *AbreAtr₁* can lead to reduce toxic effect of secondary metabolites in this pathogen, thus giving more pathogenicity in the isolate.

Keywords: *Alternaria brassicae*, *AbreAtr₁*, ABC transporter, Pathogenicity, Hyphal growth, Canola.

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References

- AGARWAL, A., GARG, G. K., DEVI, S., MISHRA, D. P. and SINGH, U. S. 1997. Ultrastructural changes in *Brassicae* leaves caused by *Alternaria brassicae* and destruxin B. **J. Plant Biochem. Biotechnol.** 6: 25-28.
- AMIRI, B., IBRAHIM, L. and BUTT, T. M. 1999. Antifeedant properties of Destruxin and their potential use with the entomogenous fungus *Metarhizium anisopliae* for improved control of crucifer pest. **Biocontrol Sci. Technol.** 9: 487-498.
- AYER, W. A. and PENA-RODIGUEZ, M. 1987. Metabolites produced by *Alternaria brassicae*, the black spot pathogen of Canola. Part1, The phytotoxic components. **J. Nat. Prod.** 50: 400-407.
- DE WAARD, M. A. Significance of ABC transporters in fungicide sensitivity and resistance. **Pestic. Sci.** 51: 271-275.
- HIGGINS, C. F. 2001. ABC transporters: physiology, structure and mechanism an overview. **Res. Microbiol.** 152: 205-210.
- GENTHNER, F. J., CHANCY, C. A., COUCH, J. A., FOSS, S. S., MIDDAUGH, D. P., GEORGE, S. E., WARREN, M. A. and BANTLE, A. 1998. Toxicity and Pathogenicity Testing of the Insect Pest Control Fungus *Metarhizium anisopliae*. **Arch. Environ. Contam. Toxicol.** 35: 317-324.
- GIULIETTI, A., OVERBERGH, L., VALCKX, D., DECALLONNE, B., BOUILLON, R. and MATHIEU, C. 2001. An overview of real time quantitative PCR: application to quantify cytokinin gene expression. **Methods** 25: 386-401.
- GUILLEMETTE, T. and SIMONEAU, P. 2004. Analysis of nonribosomal peptide synthetase gene from *Alternaria brassicae* and flanking genomic sequences. **Curr. Genet.** 45: 214-224.
- KUCHAREK, T. 2000. *Alternaria* diseases of Crucifers. **Plant Pathol.** 48: 749-755.
- MUKADAN, D. S. and DESHMUKH, K. B. 1977. Effect of different substrates and age of the culture on spore germination in *Alternaria brassicae* and *A. brassicicola*. **Indian Phytopathol.** 30: 374.
- NOURANI, S. L., MINASSIAN, V. and SAFAIE, N. 2008. Identification, pathogenicity and distribution of *Alternaria* spp of canola in Iran. **Iranian J. Plant Path.** 44: 33-36 (In Farsi with English summary).
- PARADA, R. Y., OKA, K., YAMAGISHI, D., KODAMAB, M. and OTANI, H. 2007. Destruxin B produced by *Alternaria brassicae* does not induce accessibility of host plants to fungal invasion. **Physiol. Mol. Plant Pathol.** 71: 48-54.
- PEDRAS, M. S. C., ZAHARIA, L. I. and WARD, D. E. 2002. The destruxins: synthesis biosynthesis, biotransformation, and biological activity. **Phytochemistry** 59: 579-596.
- PEDRAS, M. S. C., ZAHARAI, I. L., GAI, Y., ZHOU, Y. and WARD, D. E. 2001. In planta sequential hydroxylation and glycosylation of a fungal phytotoxin: Avoiding cell death and overcoming the fungal invader. **PNAS.** 98: 747-752.
- READER, U. A and P. BROADA. 1985. Rapid preparation and DNA from filamentous fungi. **Lett. Appl. Microbiol.** 1: 17-20
- STERGIOPOULOS, L., ZWIERS, L. H. and DE WAARD, M. A. 2002. Secretion of natural and synthetic toxic compounds from filamentous fungi by membrane transporters of the ATP-binding cassette and major facilitator superfamily Ioannis. **Eur. J. Plant Pathol.** 108: 719-734.
- THEODOULOU, F. 2002. P6-ABC Transporters in plants and fungi. **Comp. Biochem. Phys. A.** 132: 143-147.