

IN VITRO ANTIFUNGAL ACTIVITY OF RECOMBINANT CHITINASE PROTEIN AGAINST CORYNESPORA CASSIICOLA INFECTING HEVEA BRASILIENSIS

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Corynespora leaf disease caused by *Corynespora cassiicola* has emerged as a major disease of rubber in South East Asia. During plant-pathogen interactions, various novel proteins called pathogenesis related (PR) proteins which play a major role in plant disease resistance mechanism are induced. The chitinase (PR3) is one of the most widely studied groups of PR proteins in plants. *C. cassiicola* induced chitinase from *Hevea brasiliensis* was characterized in the present study. The cDNA was developed from the RNA of *C. cassiicola* infected leaves of the clone GT 1. A 978 bp chitinase gene was obtained from *H. brasiliensis* and over expressed in the pET 32a⁺ expression system. The *in vitro* studies of purified recombinant *Hevea* chitinase showed antifungal activity against *C. cassiicola*. The chitinase gene expression in *H. brasiliensis* during *C. cassiicola* infection was quantified through qPCR and increased expression of chitinase transcripts was observed. In clone GT1 chitinase gene was induced up to 24th hour after *C. cassiicola* infection and eventually it came down in later hours, where as in clone RRII 105 chitinase level had been lesser in induced plants than control. The polyclonal antibody was raised with the recombinant chitinase and the induced clone GT I showed a prominent band in western blot, while a minor band was observed in RRII 105 in induced condition.

Keywords: *Corynespora cassiicola*, Chitinase, *Hevea brasiliensis*, Recombinant protein

INTRODUCTION

Corynespora leaf disease of rubber (*Hevea brasiliensis*) caused by *Corynespora cassiicola* is now considered to be a serious problem in all rubber growing countries in South East Asia. In India it is a serious threat for rubber plantations in South Karnataka and adjacent places of Kasaragod district of Kerala (Rajalakshmi and Kothandaraman,

1996). Chemical control is being followed to control the disease in the field (Marju *et al.*, 2002). As rubber trees are deciduous in nature, fungicide application has to be repeated every year. Repeated application of fungicide may lead to resistance build up in pathogen in addition to other environmental problems. Protection against disease by inducing the resistance mechanism in

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