STUDIES OF MOLECULAR MARKER IN SELECTED TRICHODERMA SPECIES AND THEIR INTERACTION WITH SCLEROTIUM ROLFSII

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ABSTRACT

Experiment was conducted in three parts. (1) *In vitro* antagonistic effect of various species of *Trichoderma* as bio-agents against disease causing *S. rolfsii*. (2) Biochemical characterization of various species of *Trichoderma* and disease causing pathogen *S. rolfsii*. (3) Molecular characterization of various species of *Trichoderma* and disease causing pathogen *S. rolfsii*

Among the 6 *Trichoderma* species, the maximum percent growth inhibition (61%) at 5 DAI and (63.4%) at 10 DAI of fungal pathogen were observed in *T. viride* (NBAII Tv 23) on PDA media followed by *T. harzianum* (NBAII Th1) (55%) at 5 DAI and (56.6%) at 10 DAI. This per cent inhibition was positively correlated with specific activities of lytic enzymes: chitinase, \( \beta-1,3 \) glucanase and protease, and negatively correlated with cellulase and poly galacturonase (PG). *T. viride* was found suitable strain to be used in biological control of plant pathogen *S. rolfsii*.

Mycoparasitism involves morphological changes, such as coiling and formation of appressorium- like structures, which serve to penetrate the host and contain high concentrations of osmotic solutes such as glycerol. This process occurred at different intensities depending on the *Trichoderma* species. Microscopic study showed that *T. viride* was capable of overgrowing and degrading *S. rolfsii* mycelia, coiling around the hyphae with apressoria and hook-like structures. Maximum growth inhibition of pathogen occurred in interacting with *T. viride* (NBAII Tv 23) at 5 DAI, *T. viride* completely destroyed and sporulated the host. *T. viride* (NBAII Tv 23) showed very effectively coiling under a LEICA PHASE microscope followed by
Trichoderma species like T. harzianum (NBAII Th1). These result revealed that the coiling of T. viride on pathogen S. rolfsii start the parasitism earlier. This result revealed that in vitro antagonism of T. viride inhibited the pathogen more effectively.

The RAPD profiles of genomic DNA of Trichoderma species and S. rolfsii demonstrated presence of high level of polymorphism between them. The calculated PIC values for RAPD markers ranged from 0 to 0.911 and dendrogram constructed on the basis of Jaccard's similarity coefficient which illustrated two distinct clusters of 6 species of Trichoderma and S. rolfsii pathogen, and shared 27% similarity. However, the in vitro highest growth (S. rolfsii) inhibitory Trichoderma species - T. viride and T. harzianum were in same group and shared 43.5% similarity.

Among 21 RAPD primers only two primers were selected for designing the SCAR primers. OPC-05 gave a unique fragment of size 900 bp in T. viride species and no amplification in other five species. OPA-16 gave a polymorphic fragments of size 220 bp in T. harzianum. The unique fragment of each species was not present in other species and directly sent as purified PCR product for sequencing.

The designed putatively species-specific SCAR primer pairs for two species of Trichoderma (T. harzianum and T. viride) were used to amplify the genomic DNA of other 5 species. A single and sharp 900 bp band corresponding to the original RAPD fragment was obtained in T. viride with VIR-900 SCAR primer and no amplification was observed in the other six species.

Similarly, single and bright 220 bp band corresponding to the original RAPD fragment was obtained in Trichoderma harzianum with HAR-220 SCAR primer and amplification was not observed in the other six species.

In this study, efforts were made to develop SCAR markers for species identification of Trichoderma. The SCAR marker VIR-900 and HAR-220 are unique to T. viride and T. harzianum can be used for identification of T. viride and T. harzianum from unknown culture collection.