

Molecular characterization of Trichoderma isolates by ISSR Marker

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Abstract: Biocontrol agent *Trichoderma* has attained importance for substitute of chemical pesticides and hence an attempt was intended to corroborate the positive relatedness of molecular and morphological characters with antagonistic ability. Twelve isolates belonging to *Trichoderma harzianum* and *Trichoderma viride* were assessed for their antagonistic effect on *Fusarium oxysporum* F.sp. *lycopersicis* and *Nanthomonas campestris*, *Py.vesicatoria*. *Trichoderma harzianum* isolates were more aggressive than *T. viride* isolates. The dataset generated through morphological characters and ISSR markers showed a comparable output grouping the isolates of *T. viride* in one cluster and all *T. harzianum* isolates in another cluster. It is obvious from the present study that genetic diversity analysis had a positive correlation with the antagonistic ability of *Trichoderma* isolates. Thus an integrated approach of morphological and molecular markers can be employed to identify a superior strain of *Trichoderma* for its commercial exploitation.

Key Words: *Trichoderma*, antagonism, morphological and molecular characters, ISSR.

I. Introduction

Plant disease epidemics have created an ecologically unbalanced system in modern agriculture. Deterrence of such epidemics for the most part achieved through use of chemical fungicides which have greater repercussion on environment and human health. Also progressive con-frontation in a midst of pathogen resistance to accessible chemical plant protectants has engrossed the need of alternative methods of disease control. Fungi of the genus *Trichoderma* are important biocontrol agents of several soil borne phytopathogens (Benítez et al., 2004).

The *Trichoderma* species serves as a potential alter-native to chemical control measure and growing pathogen resistance crop cultivars. *Trichoderma* is easily identified in culture media, which produces large number of characteristics small, green or white conidia, from phialides present on the profusely or meagerly branched conidiophores. However, the identification of isolates to species level is difficult and confusing due to the complexity and closely related characters of the species. Molecular analysis of several strains revealed that classification based on morphological data has been, erroneous to great extent resulting in re-classification of several isolates and species (Samuels, 1996). *Trichoderma* use different mechanism for control of phytopathogens which includes mycoparasitism, out compete pathogenic fungi for nutrients, secretion for antibiotics and fungal cell wall degrading enzymes (Harman et al., 2004; Renio et al., 2008). Furthermore it is difficult to predict the degree of synergism and the behaviour of a biocontrol agent in a natural pathosystem. Thus the present study was to characterize the cryptic species of *Trichoderma* associated with biological control and to establish the relation between bioefficacy and morphological and molecular characters existed if any.

Currently molecular techniques like DNA sequencing (Apple and Gordon, 1996), Random Amplification of Polymorphic DNA (RAPD) analysis (Woo et al., 1996), Restriction Fragment Length Polymorphism (RFLP) analysis (Meyer et al. 1992), internal transcribed sequences (ITS) of the ribosomal DNA (rDNA) analysis (rDNA-ITS1) and universally primed polymerase chain reaction (UP-PCR) have been used to characterize isolates of *Trichoderma* (Cumagun et al. 1996). Inter simple sequence repeats (ISSR) have been used as another effective method to characterize genetic variability. Since the evolutionary rate within ISSR is considerably higher than other types of DNA, the likelihood of finding poly-morphism is greater compared to RAPD (Charlesworth et al., 1994).

II. Material And Methods

Isolation and identification of *Trichoderma*

Soil samples were collected from the different tomato fields in 10 districts of western Maharashtra. A total number of twelve isolates of *Trichoderma* species was isolated and identified on potato dextrose agar (PDA). The identification of *Trichoderma* isolates were confirmed both by colony characters and microscopic characters as given by Rifai (1969).

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