

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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Molecular Markers in Mycology Bhim Pratap Singh, Vijai Kumar Gupta, 2017-01-21 The Kingdom fungi encompass a massive diversity of taxa with wide ranging ecologies life cycles and morphologies ranging from unicellular aquatic chytrids to large mushrooms Before molecular methods came in existence taxonomists considered this Kingdom to be a member of the plant kingdom due to certain life styles like immobility and growth habitats Molecular markers also known as DNA markers facilitated a better alternative method over traditional morphological methods employed for the identification characterization and to understand the evolution of fungi The morphological methods used for identification are mainly dependent on spore color or microscopic features whereas molecular markers are based on DNA polymorphism in the genomic organization Phylogenetic studies reported in last decade based on molecular markers have reshaped the classification system of Kingdom fungi which divided into one subkingdom seven phyla and ten subphyla Recent advances in molecular mycology have opened the way for researchers to identify and characterize novel fungal species from unique environments Mycology is concerned with the systematic study of fungi including their genetic and biochemical properties their use to humans as a source of medicine and food as well as their dangers such as poisoning and infections In the 21st century with the development of DNA sequencing technologies and phylogenetic analysis based on molecular markers new insights into fungal taxonomy were provided This book contains a thorough discussion of molecular characterization and detection of different groups of fungi by using PCR based markers and provides a comprehensive view of the applications and uses of different molecular markers in molecular mycology It also addresses the recent molecular markers employed to solve the problems of identification and discusses current approaches used in molecular characterization and detection of fungi

Plant Biotechnology: Recent Advancements and Developments Suresh Kumar Gahlawat, Raj Kumar Salar, Priyanka Siwach, Joginder Singh Duhan, Suresh Kumar, Pawan Kaur, 2017-05-31 This book presents an overview of the latest advances and developments in plant biotechnology The respective chapters explore emerging areas of plant biotechnology such as RNAi technology fermentation technology genetic engineering nanoparticles and their applications climate resilient crops bio films bio plastic bio remediation flavonoids antioxidants etc All chapters were written by respected experts and address the latest developments in plant biotechnology that are of industrial importance especially with regard to crop yields and post harvest strategies As such the book offers a valuable guide for students educators and researchers in all disciplines of the life sciences agricultural sciences medicine and biotechnology at universities research institutions and biotechnology companies

Diseases of Commercial Crops and Their Integrated Management Amar Bahadur, Pranab Dutta, 2023-12-01 This volume compiles information on different aspects of diseases of commercial crops and their management It contains 17 chapters based on different crops contributed by various authors The book will be helpful for the students pursuing their degree in Agricultural Sciences growers teachers extension personnel and fellow researchers in their respective fields DARE/ICAR

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Sharma,Chakravarthula Manoharachary,Vijai Kumar Gupta,2020-06-18 Fungi are eukaryotic microorganisms that include
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and bioengineered fungi This book provides a valuable resource on the challenges and potential of fungal biotechnology and
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Cocoa Productivity, Quality, Profitability, Human Health and the Environment , *Molecular Characterization of*
Trichoderma Isolates Using SRAPS Rohini Bansode,Sumangala Bhat,2014-07-18 Fungal plant diseases are a major concern in
agricultural food production worldwide Soil borne pathogenic fungi such as Pythium Fusarium Rhizoctonia and Phytophthora
attack most of the economically important crop plants resulting in heavy losses So there is a pressing need to control fungal
diseases to ensure a steady and constant food supply to ever increasing world population In recent years biological control of
plant pathogens has received increasing attention as a promising supplement to chemical control Trichoderma a genus under
Deuteromycotina has gained immense importance as a biological control agent However the identification of isolates to
species level is difficult due to the complexity and closely related characters of the species Therefore developing method to
precisely identify species of Trichoderma and analysis of their molecular diversity among different isolates are important This
book give valuable information on biological control of plant diseases and molecular characterization of species identification
and thus helps in evolving best performing isolates *Ainsworth & Bisby's Dictionary of the Fungi* Geoffrey Clough
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references are given Diagnoses of families and details of orders and higher categories are included for all groups of fungi In
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