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Research Article

## STUDIES ON CHARACTERIZATION OF MOLECULAR VARIABILITY USING RAPD MARKERS IN *RHIZOCTONIA SOLANI* ISOLATED FROM DIFFERENT GEOGRAPHICAL REGIONS OF SOUTH INDIA

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### Abstract

Molecular techniques have become reliable and are highly suitable tools for identifying pathogen species and for genetic variation. The molecular marker is a useful tool for assessing genetic variations. RAPD (Random Amplified Polymorphic DNA) markers have been used to characterize the numerous filamentous fungi collected from different fields of experimental mycology. *Rhizoctonia solani* is a plant pathogenic fungus which cause sheath blight in rice. Present work focused on polymorphic identification and characterization of *Rhizoctonia solani* isolate. Twenty eight samples were collected from different locations of South India and Punjab. *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) were isolated and used polymorphic examination by molecular markers. Molecular analysis was done with OPC-5, OPC-2, OPA-8 and OPA-1 primers and the variability of isolated fungus DNA, allowed the visualization of 265 polymorphic bands with molecular weight ranging from 0.5kb to 20kb significant differences in RAPD profiles of 28 isolates of *R. Solani* were found with two primers OPC-5 and OPC-2. To analyze the resolving ability of these primers, cumulative RAPD profiles generated by the primers were analyzed by UPGMA. The dendrogram constructed using 265 polymorphic bands obtained from 28 isolates with 5 primers was divided into 7 clusters. Based on these results it was concluded that there was a molecular variability among the isolates of *R. solani* was depicted.

**Keywords:** *Rhizoctonia solani*; Rice; Sheath blight; Molecular variability; RAPD

### Introduction

Rice (*Oryza sativa*) is one of the most important crops that provide food for more than half of the world population. It is no longer a luxury food but has become the cereal that constitutes a major source of calories for the urban and the rural (Sasaki and Burr, 2000). India is one of the rice cultivating country and occupies about 23.3% of gross cropped area. *Rhizoctonia solani* Kuhn (teleomorph – *Thanatephorus cucumeris* (Frank) Donk), a ubiquitous pathogen, inflames rice sheath blight, is a fungal disease in the world (Lee and Rush, 1983; Forutan and Rahimian, 1991; Suparyono *et al.*, 2003). Still Indian rice cultivars striving to upbringing sheath blight resistant breeds. However lack of adequate information on the genetic variability of the fungal population occurring, resistant donors not up to the mark and search through the appropriate markers to develop the suitable strategies to distinguish the genetic polymorphism (Neeraja *et al.*, 2002).

Diverse Rice sheath blight isolates has been studied by morphological characterization and pathogenicity testing and also by various molecular techniques reliable and are highly suitable tools for identifying pathogen species and analyzing the genetic variations of microorganisms

(Sundravadana *et al.*, 2011). RAPD (Random Amplified Polymorphic DNA) markers have been successfully used to determine the numerous filamentous fungi species collected from different fields of experimental mycology (Pollastro *et al.*, 2000). RAPD markers are simple, inexpensive and even not required prior information of genetic sequence (Williams *et al.*, 1990) used for estimating genetic polymorphism. It has been used to analyze the rice genotypes by several groups for (i) estimation of the genetic diversity and relatedness among different accessions (Davierwala *et al.*, 2000; Raghunathachari *et al.*, 2000; Porreca *et al.*, 2001; Neeraja *et al.*, 2002; Saker *et al.*, 2005); (ii) detection of duplicates (Virk *et al.*, 1995; Verma *et al.*, 1999) (iii) identification and clarification of various cultivars (Ko *et al.*, 1994; Mackill, 1995; Choudhury *et al.*, 2001) and (iv) determination of the genetic relationship between several species (Bautista *et al.*, 2001; Ren *et al.*, 2003). Understanding the genetic structure of pathogenic fungi is critical for developing appropriate strategies for disease management, however little is known about the genetic structure of *Rhizoctonia* sps. in natural population (Vilgalys and Cubeta, 1994). Thus the present study was undertaken to assess the molecular variability using RAPD

markers to distinguish the isolates collected from four rice growing states of India.

## Materials and Methods

### Fungal Isolates

Twenty eight isolates of *R. solani* were collected from four rice growing states of India. (Mostly South India). The isolates were further purified by hyphal tip culture on acidified water agar (AWA) and were transferred to Potato Dextrose Agar (PDA) slants and maintained at 28°C. These isolates were assigned numbers RS-1 to RS-28.

### RAPD Analysis of *Rhizoctonia solani* Isolates Collected from Rice Fields

RS<sub>1</sub> to RS<sub>28</sub> isolates of *R. solani* used in this study to distinguish using the PCR protocol described by Williams *et al.*, (1990) with the help of random primers. Molecular variability analyzed by RAPD technique in twenty-eight isolates of *R. solani*. Total genomic DNA from the fungal isolates was extracted by Cetyl Trimethyl Ammonium Bromide (CTAB) method as follows.

### Data Analysis

DNA fingerprints were scored for the presence '1' or absence '0' of bands of various molecular weight sizes in the form of binary matrix. Data were analyzed to obtain Jaccard's coefficients among the isolates by using NTSYS-pc (version 2.0; Exeter Biological Software, Setauket, NY). Jaccard's coefficients were clustered to generate dendrograms by using the SAHN clustering programme,

selecting the UPGMA algorithm in NTSYS-pc (Rohlf, 1993). The similarity values were subjected to Principal Coordinate Analysis.

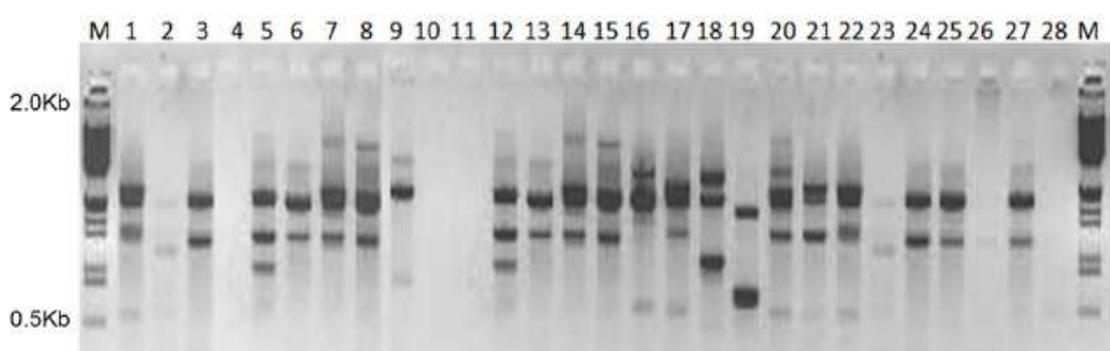
## Results and Discussion

### DNA Profile Analysis

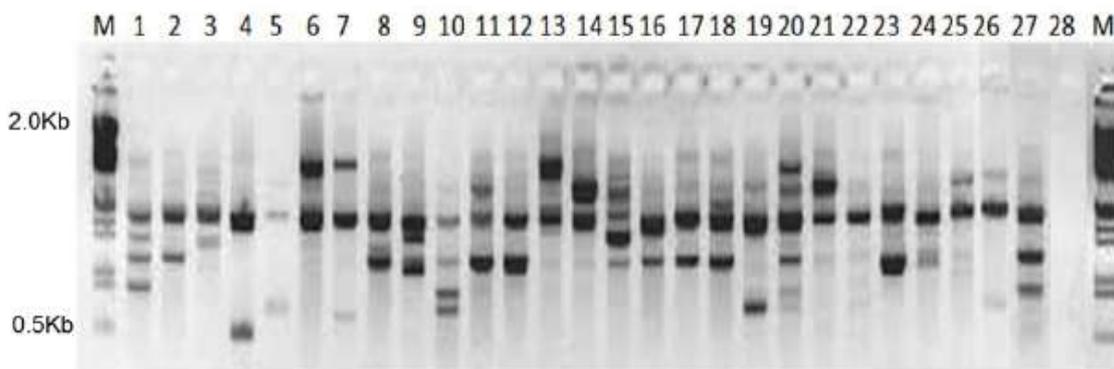
All the twenty-eight isolates of *R. solani* from rice were studied for molecular variability. In the preliminary study, *R. solani* was amplified with 30 primers, out of 30 primers four primers (OPC-5, OPC 2, OPA-8, and OPA-11) were selected based on polymorphic nature of the amplified *R. solani*. Subjected 28 *R. solani* produces polymorphism in the form of bands that reflected by the number of countable bands produced by these primers (Table: 1). Significant differences in RAPD profiles of 28 isolates of *R. solani* were found with two primers OPC 5 and OPC 2. The primers OPC-5, OPC-2, OPA-8, and OPA-11 produced 265 PCR products of molecular weight ranging from 0.5 kb to 2.0 kb in different isolates with diverse finger printing patterns (Fig. 1- 4).

**Table: 1:** Number of loci amplified by RAPD primers in the isolates of *Rhizoctonia solani*.

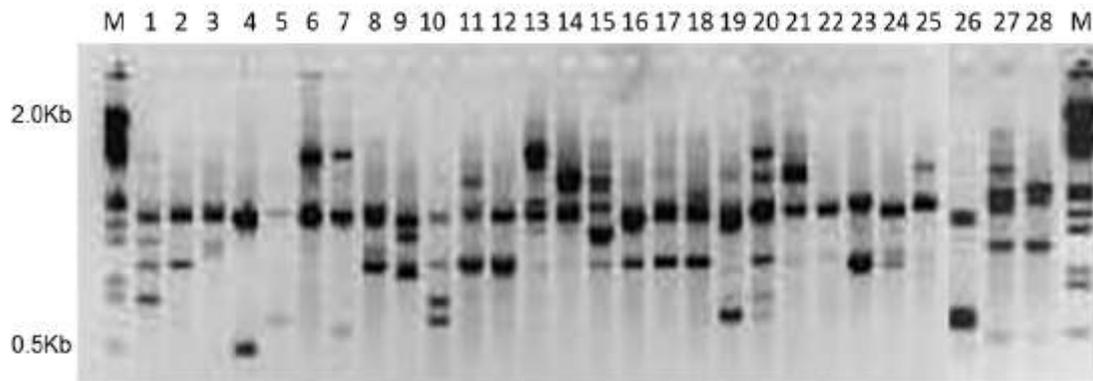
Primers	Number loci amplified
OPC 5	82
OPC2	55
OPA8	71
OPA11	57
Total loci amplified	265



**Fig: 1:** Banding pattern of Random amplified polymorphic DNA (RAPD) obtained from 28 isolates of *R. solani* (Lane 1 to 28) M=100 bp ladder with primer OPC 5.



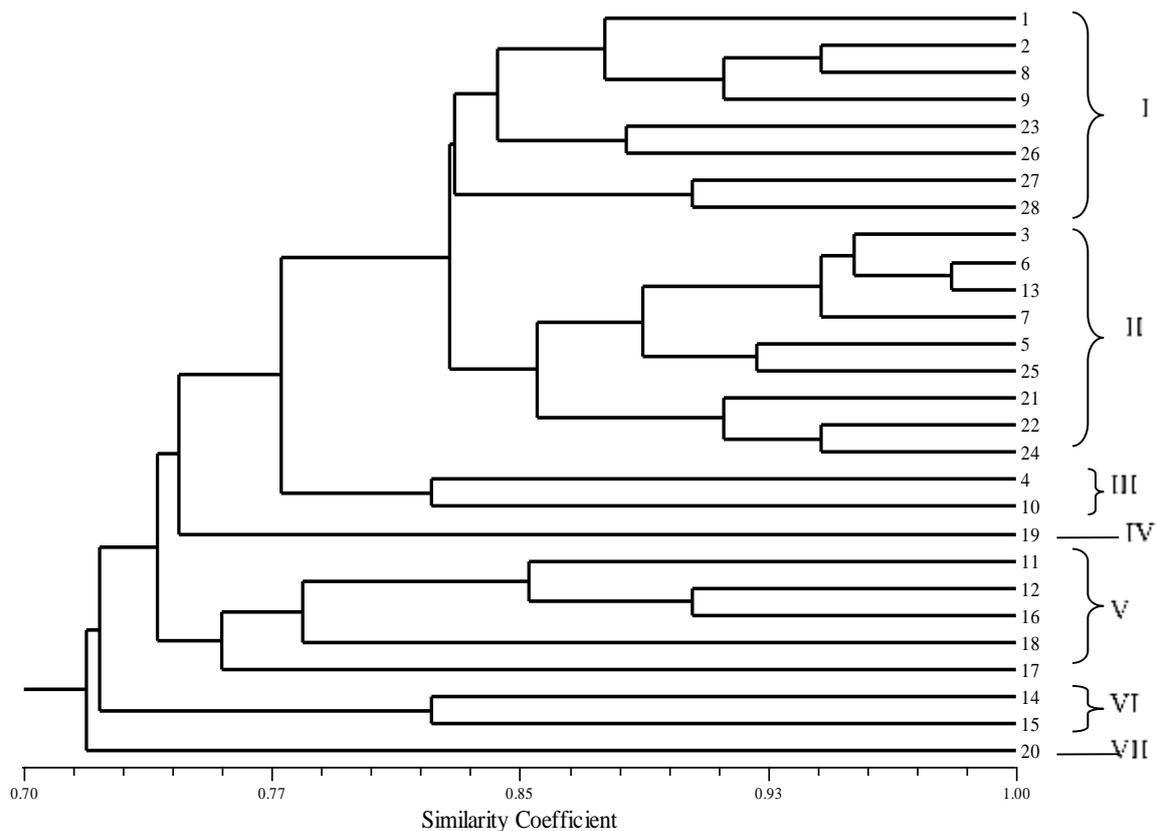
**Fig: 2:** Banding pattern of Random amplified polymorphic DNA (RAPD) obtained from 28 isolates of *R. solani* (Lane 1 to 28) M=100 bp ladder with primer OPC 2.



**Fig. 3:** Banding pattern of Random amplified polymorphic DNA (RAPD) obtained from 28 isolates of *R. solani* (Lane 1 to 28) M = 100 bp ladder with primer OPA 8.



**Fig. 4:** Banding pattern of Random amplified polymorphic DNA (RAPD) obtained from 28 isolates of *R. solani* (Lane 1 to 28) M=100 bp ladder with primer OPA 11.



**Fig. 5:** Dendrogram constructed with UPGMA clustering method with 28 isolates of *R. solani* based on polymorphism obtained with RAPD primers. The scale below the dendrogram represents genetic similarity coefficients calculated according to Jaccard (1908). The branches are labeled by isolates number.

### Cluster Analysis

UPGMA, were used to analyze to produce the cumulative RAPD profiles to quantify the resolving ability of these primers. The dendrogram constructed using 265 polymorphic bands obtained from 28 isolates with 4 primers was divided into 7 clusters (Fig: 5). RAPD analysis of *R. solani* isolates revealed that, all the isolates shared 78 percent and above similarity. Cluster I consisted of 8 isolates (RS-1, RS-2, RS-8, RS-9, RS-23, RS- 26, RS-27, RS-28) have similarity values ranged from 78 to 94.4%. All these isolates except RS-1, RS-27 and RS-28 were collected from North coastal zone of Andhra Pradesh. Cluster II is formed from 9 isolates (RS-3, RS-6, RS-13, RS-7, RS-5, RS-25, RS-21, RS-22, RS-24) and they shared highest genetic similarity values ranged from 86-97%. Among 3 isolates (RS-21, RS-22, RS-24) from Tamilnadu and the remaining were collected from coastal Andhra Pradesh. Cluster III consisted of 2 isolates Rs-4 and Rs-10 shared genetic similarity of 80.4 %. Cluster IV only one isolate Rs-19 have genetic similarity of 76.9%. Cluster V is formed from 5 isolates (RS-11, RS-12, RS-16, RS-18, RS-17) of Karnataka state. They shared the genetic similarity between 74.7 and 89.4%. Cluster VI consisted of two Karnataka isolates (Rs-14 and Rs-15) shared genetic similarity of 80.6%. Cluster VII consisted of only one isolate Rs-20 collected from Punjab did not share any of the groups and shared a genetic similarity of 72.8 %.

### Discussion

In the present study, RAPD markers were employed to assess the genetic diversity among 28 isolates of *Rhizoctonia solani* from different rice growing states of India. Although the use of molecular markers to study the genetic diversity and relationships among the *R. solani* isolates has been reported (Duncun *et al.*, 1993; Davierwala *et al.*, 2000; Porreca *et al.*, 2001; Neeraja *et al.*, 2002; Runhua *et al.*, 2002; Saker *et al.*, 2005; Banerjee *et al.*, 2012).

The use of molecular marker to study the genetic diversity relationships among populations from different as well as the same geographical regions (Sundravadana *et al.*, 2011). The results of our present study indicate significant level of variation among the 28 isolates of *R.solani*. The range of variation estimated is from 72.8 to 97% among the 28 isolates. Most of the isolates collected from similar agro ecological location clustered together in the present study. This lends support to the findings of Toda *et al.* (1999); Sharma *et al.* (2005) and Banerjee *et al.* (2012).

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