

Original Research Article

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Genetic Variability in the Isolates of *Rhizoctonia solani* Kühn, the Causal Agent of Sheath Blight of Rice by Using RAPD Markers

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ABSTRACT

Keywords

Genetic variability, *Rhizoctonia solani* isolates, RAPD markers, Sheath blight, Rice.

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Thirty two *Rhizoctonia solani* isolates were studied for molecular variability by RAPD technique at the Institute of Agri-biotechnology, University of Agricultural Sciences, Dharwad, India during 2007. The results indicated that, there were differences in RAPD profiles of 32 isolates of *R. solani* with two random primers OPB-15 and OPD-13. The primer OPB-15 and OPD-13 produced 248 PCR products of molecular weight ranging from 300 bp to 600 bp in different isolates with diverse amplicon. To test the resolving ability of these primers, cumulative RAPD profiles generated by the random primers were analyzed by UPGMA. All the isolates shared minimum of 60 per cent and above similarity among themselves. The cumulative analysis of 32 isolates showed four groups at 75 per cent genetic similarity level.

Introduction

Sheath blight of rice caused by *Rhizoctonia solani* Kühn, once a minor disease, has become one of the major diseases inflicting heavy yield losses in most of the Asian countries. Rice sheath blight was first reported from Japan by Miyake in 1910, subsequently it was reported throughout the temperate and tropical rice growing areas. The occurrence of the disease in India was first reported by Paracer and Chahal (1963) from Gurdaspur in Punjab. Now its occurrence is observed in almost all rice growing states of India with an estimated loss of grain yield to the extent of 5.20 to 50 per

cent (Das and Mishra, 1990). Among *R. solani* isolates there exists high variations with respect to cultural, morphological and pathogenic characters. The pathogen produces two types of mycelium, straight and branched, and lobate, of which only the latter type is infectious.

The lesion is covered by lobate mycelium while the straight type may extend beyond without causing infection (Ou *et al.*, 1973). While such variations are to be explored further by studying at pathogenic and molecular level to give logical conclusion.

Hence, there is a need to take up variability studies employing robust sampling and molecular assay methods.

Materials and Methods

Thirty two isolates of *R. solani* were collected from different rice growing areas of India during *kharif* seasons in 2006 and 2007. During the roving survey carried out in *kharif* 2006 and 2007, a large number of sheath blight infected rice samples were collected from different places *viz.*, Tungabhadra Project (TBP) area of Raichur (Sindhnanur, Manvi, Raichur), Bellary (Sirguppa) and Koppal (Gangavati) districts in north eastern Karnataka and Mandya, Mysore, Shimoga in southern Karnataka.

These typical sheath infected samples were subjected to standard tissue isolation technique under aseptic condition using laminar flow to obtain *Rhizoctonia solani* culture. The infected tissue of the sheath were cut into small bits of 1-2 mm size and surface sterilized in 1:1000 mercuric chloride (HgCl₂) solution for one minute and washed repeatedly twice in sterile distilled water to remove the traces of mercuric chloride before transferring them to sterile potato dextrose agar (PDA) slants under aseptic conditions. The slants were incubated at temperature of 27 ±1 °C and observed for fungal growth. The culture, thus obtained was purified by hyphal tip isolation method. In addition isolates of *R. solani* were also obtained from Coimbatore (Tamil Nadu), Hissar (Haryana), Kapurthala and Amritsar (Punjab) and Hyderabad (Andhra Pradesh). The sources and identity of thirty two isolates *R. solani* obtained from different region are presented in table 1.

Hyphal tip isolation

This method was followed for maintaining pure culture, since the fungus is known to be highly heterozygous. Hyphal tip isolation was

done on two per cent water agar plates. Dilute mycelial suspensions (10⁻⁶) were prepared in sterile distilled water. One ml of such suspension was spread uniformly on water agar plates and observed for mycelia under microscope isolated mycelial bit was transferred on PDA plates, under aseptic conditions. The PDA plates were incubated at a temperature of 27±1 °C and observed for fungal growth. No sectoring was observed in any of the plates and all of them were found identical in their growth and colony characters on their respective plate. Hence, it was taken as pure pathogenic culture and was maintained for further studies.

The *R. solani* isolates were studied for molecular variability by RAPD technique at the Institute of Agri-biotechnology, University of Agricultural Sciences, Dharwad, India. Total genomic DNA from the fungal isolates was extracted by Hexa decyl trimethyl Ammonium Bromide (CTAB) method as follows.

Total genomic DNA extraction

Genomic DNA was extracted by using the CTAB (Hexa decyl trimethyl ammonium bromide, Sigma Chemical Co., St. Louis, USA) method of Lee and Taylor (1990). Then assessment of DNA quality and quantity was done.

Primers and PCR amplification

Thirty Operon oligonucleotide random primers (Operon Technologies, USA) were screened for amplification of genomic DNA of *R. solani*. Among these only two primers OPB-15 and OPD-13 were selected based on their repeatability and used for amplification.

The PCR amplifications were performed by using thermal cycler (Biometra) programmed for initial DNA denaturation at 94 °C for 5 min followed by 45 cycles at 94 °C for 1 min,

45 °C for 1 min 72 °C for 2 min and a final cycle was performed at 72 °C for 7 min. All the amplified DNA products were resolved by electrophoresis on agarose gel (1.5%) in TAE (1 X) buffer, stained with ethidium bromide and photographs were taken by using gel documentation system.

Data analysis

DNA amplicon resulting from PCR were scored for the presence as '1' or absence as '0' at 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 unweighted pair-group method with arithmetic average (UGMA) algorithm in NTSYS-pc (Rohlf, 1993).

Results and Discussion

Out of initial 30 random decamer primers amplified for nucleotide sequence, only two random primers were selected based on their repeatability of polymorphism in all 32 isolates of *R. solani*. Number of countable amplicon produced by these random primers are presented in table 2 and Plate 1.

The differences in RAPD profiles of 32 isolates of *R. solani* with two random primers OPB-15 and OPD-13 are given in table 2. The primer OPB-15 and OPD-13 produced 248 PCR products of molecular weight ranging from 300 bp to 600 bp in different isolates with diverse amplicon.

To test the resolving ability of these primers, cumulative RAPD profiles generated by the random primers were analyzed by UPGMA. All the isolates shared minimum of 60 per cent and above similarity among themselves.

The cumulative analysis of 32 isolates showed four groups at 75 per cent genetic similarity level.

As per dendrogram cluster analysis (Figure 1), all the 32 isolates were grouped into two major clusters such as C1 and C2, wherein cluster C1 consists of 15 isolates and cluster C2 consists of 17 isolates (Table 3). The main cluster C1 has been further grouped into two sub-clusters C1-1 and C1-2. Cluster C1-1 consists of seven isolates namely RS-1, RS-5, RS-9, RS-15, RS-24, RS-16, RS-22 and the sub-cluster C1-2 consists of 8 isolates namely RS-25, RS-21, RS-31, RS-30, RS-14, RS-29, RS-20 and RS-13. The main cluster C2 consists of 17 isolates. The sub-cluster under main cluster C2-1 consists of 10 isolates namely RS-2, RS-3, RS-4, RS-32, RS-18, RS-17, RS-28, RS-23, RS-11 and RS-10, whereas the sub-cluster C2-2 consists of seven isolates namely RS-6, RS-26, RS-7, RS-8, RS-27, RS-19 and RS-12.

Genetic variability in *R. solani* isolates from various places have been studied earlier using RAPD markers. Prasad *et al.*, (2004) reported that *Fusarium oxysporum* isolates (including various identified formae speciales) are grouped along with other formae speciales of *F. oxysporum* in the study. Assessment of molecular variability based on micro-satellite markers using universal rice primers indicated clearly that *F. oxysporum* is heterogeneous, highly diverse and most of the formae speciales have evolutionary biology linked to *F. oxysporum*. In most instances, different species of *Fusarium viz.*, *F. graminearum*, *F. pallidoroseum* and *F. chlamydosporum* were grouped separately showing low genetic similarity to *F. oxysporum*. DNA banding pattern was very specific to species and formae speciales of *F. oxysporum* and other *Fusarium* species obtained in the study were highly useful for the differentiation of *Fusarium* isolates belonging to different species.

Table.1 Source and identity of *R. solani* isolates from different rice growing regions of India

Sl. No.	State	District	Locality	Isolates
1.	Punjab	Amritsar	Amritsar	RS-1
2.	Punjab	Kapurthala	Kapurthala	RS-2
3.	Haryana	Hissar	Hissar	RS-3
4.	Tamil Nadu	Coimbatore	Coimbatore	RS-4
5.	Andhra Pradesh	Hyderabad	DRR, Hyderabad	RS-5
6.	Karnataka	Mandya	VC Farm	RS-6
7.			Kirangur	RS-7
8.			B.R.Koppalu	RS-8
9.			Thubinakere	RS-9
10.			Shivalli	RS-10
11.			Kalenahalli	RS-11
12.			M.N.Katte	RS-12
13.			B.Hosalli	RS-13
14.		Raichur	Manvi	RS-14
15.			Neermanvi	RS-15
16.			Kalmala	RS-16
17.			Rampura	RS-17
18.		Koppal	Gangavathi	RS-18
19.			Vaddaratti	RS-19
20.		Bellary	ARS, Siruguppa	RS-20
21.			Desnur	RS-21
22.			Ibrahimpur	RS-22
23.			Dadesugur	RS-23
24.			Thekkalakote	RS-24
25.			Halekote	RS-25
26.			Raravi	RS-26
27.			Araliganur	RS-27
28.			K.Sugur	RS-28
29.		Mysore	T.N.Pura	RS-29
30.			Gargeswari	RS-30
31.		Shimoga	Abbalagere	RS-31
32.			Kommanahal	RS-32

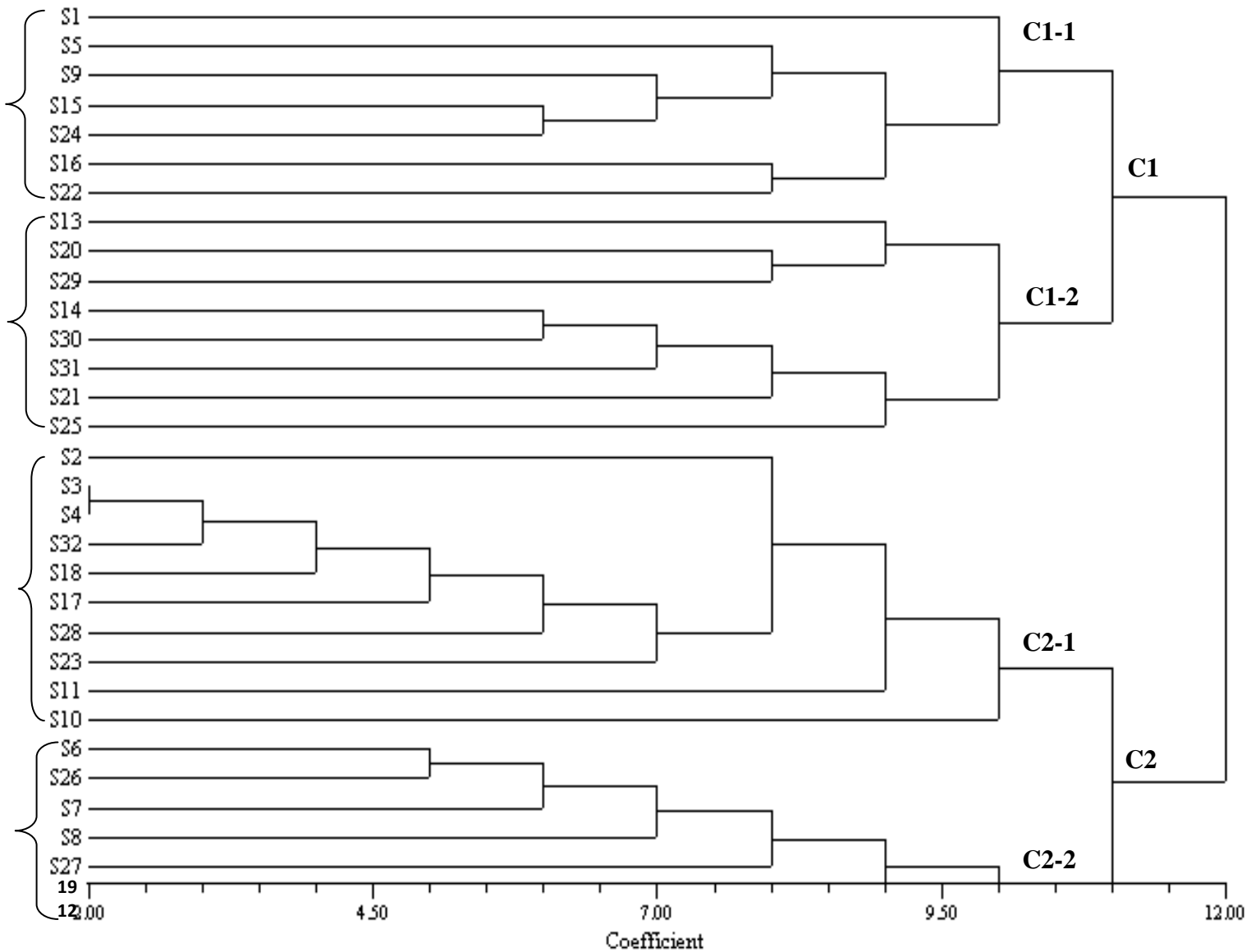
Table.2 Number of loci amplified by two RAPD primers in isolates of *Rhizoctonia solani*

Random primer	Number of loci amplified
OPB-15	126
OPD-13	122
Total loci amplified	248

Table.3 Grouping of *Rhizoctonia solani* isolates of rice using cluster analysis based on RAPD data

Cluster		Isolate number
		Cluster analysis
C1	C1-1	RS-1, RS-5, RS-9, RS-15, RS-24, RS-16, RS-22
	C1-2	RS-25, RS-21, RS-31, RS-30, RS-14, RS-29, RS-20, RS-13
C2	C2-1	RS-2, RS-3, RS-4, RS-32, RS-18, RS-17, RS-28, RS-23, RS-10, RS-11
	C2-2	RS-6, RS-26, RS-7, RS-8, RS-27, RS-19, RS-12

Fig.1 Dendrogram of 32 isolates of *Rhizoctonia solani*



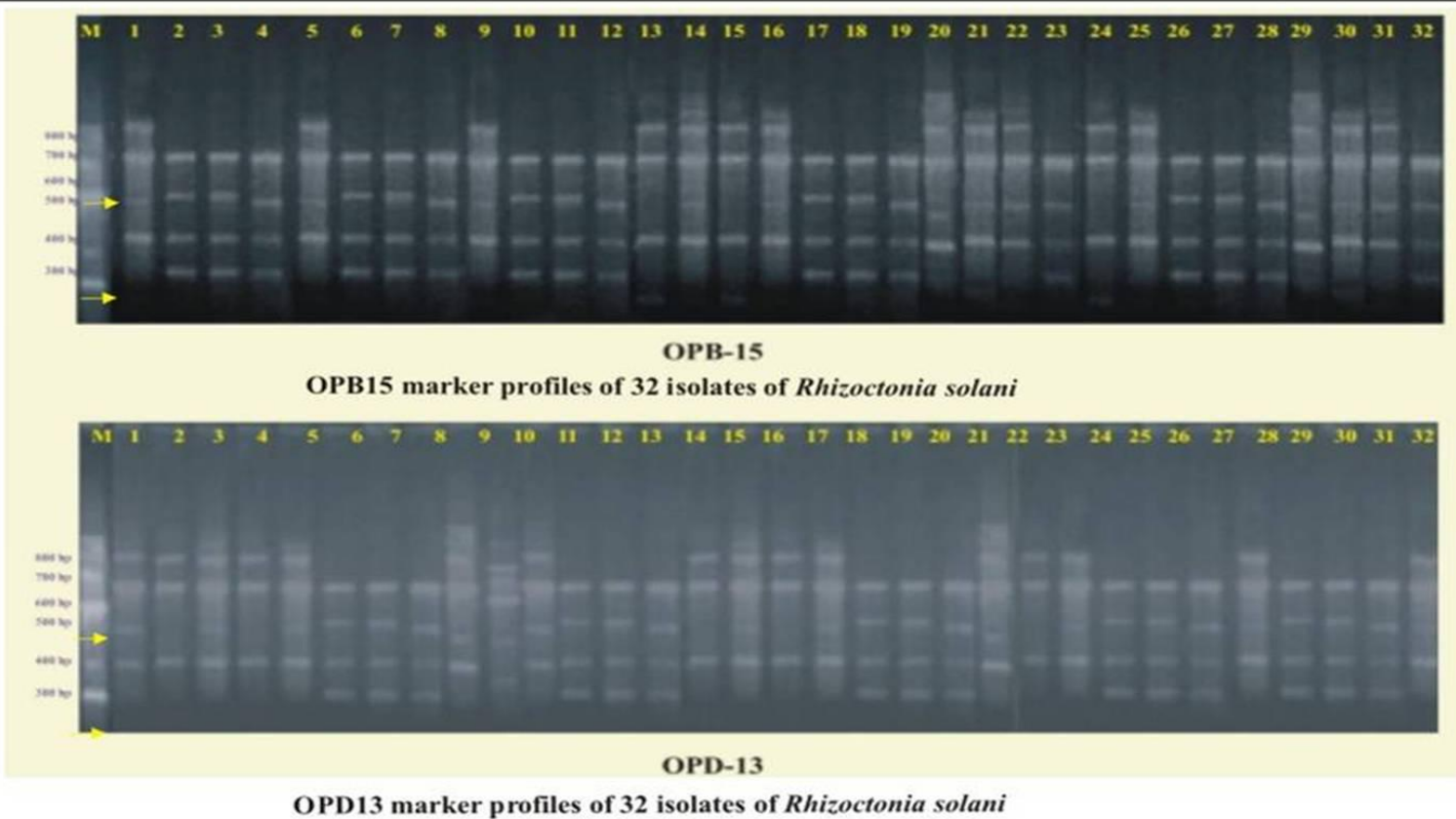


Plate 1 : Molecular variability of 32 isolates of *R. solani* by RAPD analysis

The lack of a perfect state, wide intra-field variability and compatible fusion among morphologically and virulent wise dissimilar isolates indicated heterokaryosis as the major mechanism for creating inter-field variability in *R. solani* (Singh *et al.*, 2003).

The results of our study are similar in lines with those obtained by Kistler (1997) and Gherbawy (1999) who suggested that certain *F. oxysporium* isolates within a formae speciales may be genetically less similar to each other than those isolated as nonpathogenic strains or those isolated as a group in the other formae speciales (Vilgalys and Cubeta, 1994; Banniza *et al.*, 1996; Kistler, 1997; Koenig *et al.*, 1997; O'Donnell *et al.*, 1998; Neeraja *et al.*, 2002 ; Runhua *et al.*, 2002).

From the present investigation, it was concluded that, all the isolates of *R. solani* of rice shared minimum of 60 per cent and above genetic similarity among themselves. The cumulative analysis of 32 isolates showed four groups at 75 per cent genetic similarity level. This shows the genetic variability among the *R. solani* isolates collected from different geographical regions of India.

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