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Sequence-Related Amplified Polymorphism-PCR Analysis for Genetic Diversity in *Rhizoctonia solani* Populations Infecting Pulse Crops in Different Agro-Ecological Regions of India

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ABSTRACT
Rhizoctonia solani is a destructive fungal pathogen infecting wide range of crop plants including pulses causing wet root rot or web blight disease. The present study was aimed to determine the genetic diversity of *R. solani* populations using Sequence-Related Amplified Polymorphism (SRAP) markers. The SRAP markers were used for genetic diversity analysis of 89 isolates of *R. solani* belonging to 7 Anastomosis Groups (AGs) isolated from different pulse crops representing 21 states from 16 agro-ecological regions of India. Out of 30 SRAP primer combinations evaluated, 16 combinations provided amplification with 100% polymorphism and the primer combinations Me1/Em1and Me1/Em4 provided the highest number of bands (14). The isolates of *R. solani* showed high level of genetic variability and grouped into 7 major clusters at 35% genetic similarity by using unweighted pair group method with an arithmetic average analysis. Bootstrap analysis grouped the isolates into five major clusters at 28% genetic similarity and about 95% isolates shared common sub-grouping patterns in both the analysis. The majority of the isolates representing various AGs were grouped together into different sub-clusters. The molecular clusters did not correspond to agro-ecological regions and crops of the origin of the isolates because of the diversity in the hosts and adopt ability of the pathogen under different environmental conditions prevalent in various parts of the country. First time an attempt was made in the present study to determine the genetic variability of the *R. solani* populations isolated from different pulse crops representing various AGs using SRAP markers.

Key words: SRAP, diversity, *Rhizoctonia solani*, pulse crops, polymorphism

INTRODUCTION

Pulses are main source of protein to vegetarian population of India and also consider a source of income to the farmers due to high market price. It is second important constituent of Indian diet after cereals and can be cultivated in all types of soil under different climatic conditions. Supplementation with cereals, pulses provide perfect mixture of diet with vegetarian protein of high biological values. Pulses are also an excellent feed and fodder for livestock. They have several unique abilities as biological nitrogen fixation, carbon sequestration, low water requirement, soil melioration and capacity to withstand under harsh climate. Therefore, they are being grown in marginal lands. India is the largest producer of pulses in the world, with 24% share in the global production. The important pulse crops are chickpea, pigeon-pea, mungbean, urdbean, lentil and fieldpea. The major pulse-producing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, Karnataka and Andhra Pradesh, which together account for about 80% of the total production. Out of the production constraints responsible for yield gaps in the pulse crops, biotic stresses are considered to be the most important

worldwide. *Rhizoctonia solani* Kühn [teleomorph-*Thanatephorus cucumeris* (Fr.) Donk] is a destructive soil-borne plant pathogen (Dubey *et al*., 2014) infecting a wide range of agricultural and horticultural crops, including legumes, worldwide causing several diseases (Ogoshi,1987; Dubey and Dwivedi, 2000). The pathogen causes considerable yield loss in mungbean, urdbean and other pulse crops in India (Dubey, 2003; Dubey *et al*., 2014).

Presently, the information is available for genome sequences of fungi which made it possible to develop new marker systems. An interesting modified marker technology termed as Sequence-Related Amplified Polymorphism (SRAP) (Li and Quiros, 2001) was similar to RAPD, but it was a preferential random amplification of coding regions in genome are being applied extensively in genetic linkage map construction (Li and Quiros, 2001), genetic diversity analysis (Ferriol *et al*., 2003; Lin *et al*., 2004) and comparative genetics (Li *et al*., 2003) of different species. Furthermore, in the genetic diversity analysis, the information derived from SRAP markers was more concordant to the morphological variability and to the evolutionary history of the morphotypes than that of other molecular markers (Ferriol *et al*., 2003).

However, up to now, the SRAP molecular marker had not been extensively used in the diversity analysis of phytopathogenic fungi. So, in this work, SRAP marker was used to study the genetic variability present in *R. solani* populations representing different Anastomosis Groups (AGs) and isolated from various pulse crops cultivated in different agro-ecological regions of India. Thus, the study showed the results of genetic diversity analysis of 89 *R. solani* isolates using the SRAP markers for the first time which will be of great importance to the genetics study of imperfect and basidiomycetes fungi as well as other plant pathogenic fungi at the molecular level.

MATERIALS AND METHODS

***Rhizoctonia solani* isolates and cultures conditions:** Eighty nine isolates of *Rhizoctonia solani* (including 7 AG testers) isolated from diseased specimens of major pulse crops and weeds present in pulse field covering 16 agro-ecological regions from 21 states of India were used for the genetic diversity analysis. The cultures are being maintained by the authors (Table 1). Stock cultures of the different isolates were prepared on Potato Dextrose Agar (PDA) slants

Table 1: Isolates of *Rhizoctonia solani* used in the present study

States	Districts	Isolate number	Accession number	Anastomosis groups
Andhra Pradesh	Kurnool	RAPG 12	ITCC7543	AG5
	Nizamabad	RAPG 14	ITCC7544	AG2-3
	Warrangal	RAPS 3	ITCC7545	AG1
Assam	Jorhat	RASC 5	ITCC7546	AG2-2
	Nalbari	RASC 8	ITCC7547	AG2-2
	Nalbari	RASC 26	ITCC7548	AG4
	Nalbari	RASC 27	ITCC7636	AG2-3
Bihar	Nalanda	RBRC 1	ITCC7549	AG3
Chhattisgarh	Raipur	RCGM 1	ITCC7550	AG3
Delhi	South West	RDLM 1	ITCC7551	AG3
	South West	RDLM 6	ITCC7552	AG3
	South West	RDLG 3	ITCC7553	AG3
Gujarat	Kheda	RGJC 18	ITCC7590	AG2-2
	Panchmahal	RGJM 24	ITCC7554	AG2-3
	Panchmahal	RGJU 11	ITCC7555	AG3
	Panchmahal	RGJG 2	ITCC7556	AG5
	Panchmahal	RGJG 4	ITCC7557	Undetermined
	Panchmahal	RGJW 15	ITCC7558	AG5
Himachal Pradesh	Solan	RHPF 2	ITCC7559	Undetermined
Haryana	Hisar	RHRC 20	ITCC7560	AG2-3
	Hisar	RHRC 21	ITCC7561	AG4
	Hisar	RHRC 22	ITCC7562	AG2-3
	Hisar	RHRC 28	ITCC7655	AG2-3
	Bhiwani	RHRM 3	ITCC7563	Undetermined
	Karnal	RHRM 4	ITCC7564	AG2-2 LP
	Mahendragarh	RHRG 5	ITCC7565	AG2-3
	Hisar	RHRW 16	ITCC7566	AG1
	Hisar	RHRW 27	ITCC7591	Undetermined
	Hisar	RHRW 32	ITCC7592	AG2-3
Jammu and Kashmir	Samba	RJKM 2	ITCC7593	AG2-3
	Samba	RJKM 8	ITCC7567	AG1
	Kathua	RJKM 15	ITCC7568	AG2-2 LP
	Kathua	RJKU 13	ITCC7569	AG5
Jharkhand	East Singh Bhum	RJHM 1	ITCC7570	AG5
	East Singh Bhum	RJHU 1	ITCC7571	AG1
	East Singh Bhum	RJHC 1	ITCC7594	AG1

Table 1: Continue

States	Districts	Isolate number	Accession number	Anastomosis groups
Kerala	Palakkad	RKLC 1	ITCC7572	AG2-3
	Palakkad	RKLC 4	ITCC7573	Undetermined
Karnataka	Raichur	RKNM 8	ITCC7574	AG3
	Dharwad	RKNG 9	ITCC7575	AG1
Madhya Pradesh	Satna	RMPM 9	ITCC7576	AG3
	Satna	RMPM 10	ITCC7577	AG3
	Satna	RMPM 13	ITCC7578	AG2-3
	Tikamgarh	RMPM 23	ITCC7579	AG4
	Damoh	RMPG 28	ITCC7580	AG2-3
	Damoh	RMPG 29	ITCC7581	AG1
	Damoh	RMPP 30	ITCC7582	AG4
Maharashtra	Nagpur	RMHM 3	ITCC7583	AG2-3
	Akola	RMHM 6	ITCC7584	AG5
	Pune	RMHG 24	ITCC7595	AG1
	Pune	RMHP 21	ITCC7596	AG2-2
Orissa	Mayurbhanj	RORC 9	ITCC7631	AG3
Punjab	Ludhiana	RPBC 1	ITCC7632	AG3
	Firozpur	RPBM 17	ITCC7637	AG4
	Ludhiana	RPBU 5	ITCC7633	AG4
	Ludhiana	RPBU 7	ITCC7634	AG3
	Firozpur	RPBR 18	ITCC7635	AG5
Rajasthan	Jaipur	RRJM 7	ITCC7646	AG3
	Sriganganagar	RRJG 1	ITCC7782	AG5
	Sriganganagar	RRJG 3	ITCC7647	Undetermined
Tamil Nadu	Erode	RTNU 1	ITCC7648	AG1
	Coimbatore	RTNG 5	ITCC7649	Undetermined
Uttarakhand	U.S. Nagar	RUKM 10	ITCC7650	AG2-2
	U.S. Nagar	RUKM 8	ITCC7651	Undetermined
	U.S. Nagar	RUKU 4	ITCC7652	AG3
Uttar Pradesh	Mirzapur	RUPC 95	ITCC7767	Undetermined
	Kanpur	RUPM 42	ITCC7768	AG2-3
	Varanasi	RUPM 83	ITCC7769	AG1
	Mirzapur	RUPU 58	ITCC7770	AG2-2
	Lalitpur	RUPU 82	ITCC7771	AG1
	Kushinagar	RUPU 23	ITCC7772	AG3
	Varanasi	RUPU 84	ITCC7773	AG2-2
	Kushinagar	RUPU 20	ITCC7774	AG2-3
	Kushinagar	RUPU 18	ITCC7775	AG2-3
	Kushinagar	RUPU 50	ITCC7776	AG2-3
	Kushinagar	RUPK 8	ITCC7777	Undetermined
	Jhansi	RUPG 103	ITCC7778	AG5
	Jhansi	RUPG 106	ITCC7653	AG3
	Mirzapur	RUPP 93	ITCC7779	AG5
	Jhansi	RUPL 104	ITCC7654	Undetermined
West Bengal	24 paragans	RWBC 1	ITCC7780	AG3
	Midnapur	RWBC 4	ITCC7781	AG5
International testers		RS 6	BBA62990	AG1
		RS 7	BBA62999	AG5
		RS 8	BBA63002	AG4
		RS 9	BBA63008	AG3
		RS 11	BBA69570	AG2-2
		RS 14	BBA71917	AG2-2 LP
		RS 15	BBA71921	AG2-3

ITCC: Indian type culture collection, IARI, New Delhi, BBA: Federal Biological Research Center for Agriculture and Forestry, Germany

(potato 200, glucose 20 and agar 20 g L^{-1}, pH 7.0). After being incubated at 25°C for 5 days, these strains were stored at 4°C for future use. The cultures were grown in liquid Potato Dextrose Broth (PDB) medium (20 g L^{-1}; Hi-media) at 120 rpm in an orbital shaker at 25°C for 7 days. The mycelium was harvested by filtration and washed for three times with sterilized double-distilled water.

DNA extraction: Total DNA was extracted from modified CTAB method (Murray and Thompson, 1980; Dubey and Singh, 2008). The DNA pellets were re-suspended in 200 μL TE buffer (10 mmol L^{-1} tris hydrochloric acid and 1 mmol L^{-1} sodium EDTA, pH 8) and analyzed by electrophoresis at 5 V cm^{-1}, on a 1.0% agarose gel in 0.5×TBE buffer (45 mM Tris-borate and 1 mM EDTA at pH 8.0) stained with

0.5 μg mL^{-1} ethidium bromide. Quality and quantity of DNA were estimated by spectrophotometer and stored at -20°C in small aliquots.

SRAP markers and polymerase chain reaction: The SRAP technique is a PCR-based marker system employing a combination of two primers, a forward primer of 17 bases and a reverse primer of 18 bases, which consisted of preferential amplification of Open Reading Frames (ORFs). The primers were composed of a core sequence of 13-14 bases, where the first 10 or 11 bases starting at the 5' end were 'Filler' sequences of no specific constitution followed by the sequence CCGG in the forward primer and AATT in the reverse primer. Three selective bases were added to the 3' ends of the primers. A variation in these three selective nucleotides generated a set of primers sharing the same core sequence. The forward primer preferentially amplified exonic regions. The reverse primer preferentially amplified intronic regions. The observed polymorphism fundamentally originated in the variation of the length of these introns, promoters and spacers, both among individuals and among species (Li and Quiros, 2001). In the present study, 30 different combinations of SRAP primers (Sun *et al*., 2006) were employed using five forward primers and six reverse primers (Table 2).

The polymerase chain reaction mixture (25 μL) consisted of 50 ng template DNA, 1.0 U *Taq* polymerase, 2.0 mM of $MgCl_2$, 0.4 mM of each of the dNTPs and 7.5 pmol of each primer in 1×reaction buffer (Bangalore Genei, India). For amplification, first five cycles were run at 94°C for 1 min, 35°C for 1 min and 72°C for 1 min, for denaturing, annealing and extension, respectively. Then the annealing temperature was raised to 50°C for another 35 cycles. Amplification products were analyzed by electrophoresis in 2% agarose gel in 1×TAE buffer at 110 V for 1 h. Gels were stained with ethidium bromide (1 μg mL^{-1}) and observed under UV light (Bio Rad™ gel doc system). The primers that gave reproducible and scorable amplifications were used for the analysis.

Scoring and data analysis: Relatedness among 89 isolates of *R. solani* was estimated by means of scorable DNA bands amplified from different SRAP markers. Each band was considered as character and was scored as either present (coded as 1) or absent (coded as 0). Bands were reproducible and scored in identical positions for each time. Cluster analysis employing Unweighted Pair Group Method with an Arithmetic Average (UPGMA) algorithm was performed using NTSYS-PC (v. 2.01) to produce the different dendrogram (Rohlf, 1998). Bootstrap analysis was also performed using Winboot software for all the SRAP primers which amplified the *R. solani* isolates.

Table 2: Sequences of the forward and reverse SRAP primer combinations used in this study

Forward primers (5'-3')	Reverse primers (5'-3')
Me1 TGAGTCCAAACCGGATA	Em1 GACTGCGTACGAATTAAT
Me1 TGAGTCCAAACCGGATA	Em1 GACTGCGTACGAATTAAT
Me3 TGAGTCCAAACCGGAAT	Em3 GACTGCGTACGAATTGAC
Me4 TGAGTCCAAACCGGACC	Em4 GACTGCGTACGAATTTGA
Me5 TGAGTCCAAACCGGAAG	Em5 GACTGCGTACGAATTAAC
	Em6 GACTGCGTACGAATTGCA

RESULTS

A total of 30 different combinations of SRAP primers were employed using five forward and 6 reverse primers (Table 2). Among all the combinations only 16 combinations of the primers revealed the polymorphisms between the isolates of *R. solani* and no monomorphic band was observed (Table 3). The primers amplified all the isolates of *R. solani* and the isolates were highly variable in respect of their banding profiles. The number of fragments amplified using each primer combination was ranging from 9-14 with an average of 11 polymorphic bands per combination of primers and band size of 0.1-1 kb. All 172 bands generated with 16 primers combinations were polymorphic showing high range of variability with 100% polymorphism (Table 3). The primer combination Me1/Em1 and Me1/Em4 showed maximum polymorphism and a representative gel picture of primer Me2/Em4 is given (Fig. 1).

Eighty nine isolates of the pathogen representing 7 Anastomosis Groups (AGs) were grouped into 7 major

Table 3: Sequence related amplified polymorphism primer combinations and their polymorphism obtained in this study

Primer combinations	Total number of bands	Number of polymorphic bands	Polymorphism (%)
Me1/Em1	14	14	100
Me1/Em2	13	13	100
Me1/Em3	9	9	100
Me1/Em4	14	14	100
Me1/Em5	9	9	100
Me2/Em1	12	12	100
Me2/Em2	12	12	100
Me2/Em3	13	13	100
Me2/Em4	10	10	100
Me2/Em5	10	10	100
Me2/Em6	10	10	100
Me3/Em2	10	10	100
Me3/Em3	7	7	100
Me3/Em4	9	9	100
Me3/Em5	9	9	100
Me4/Em1	NA	NA	NA
Me4/Em2	NA	NA	NA
Me4/Em3	11	11	100
Me4/Em4	NA	NA	NA
Me4/Em5	NA	NA	NA
Me5/Em1	NA	NA	NA
Me5/Em2	NA	NA	NA
Me5/Em3	NA	NA	NA
Me5/Em4	NA	NA	NA
Me5/Em5	NA	NA	NA
Me6/Em1	NA	NA	NA
Me6/Em2	NA	NA	NA
Me6/Em3	NA	NA	NA
Me6/Em4	NA	NA	NA
Me6/Em5	NA	NA	NA

NA: Not amplified

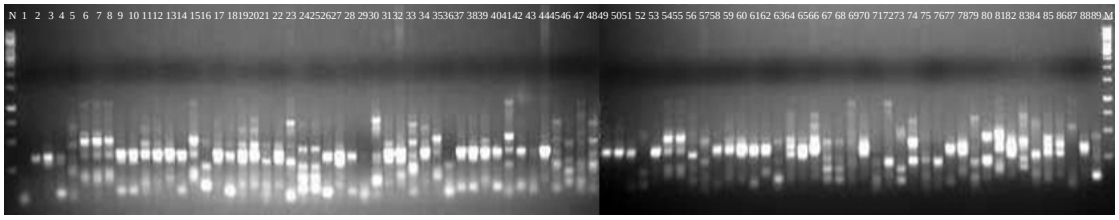

Fig. 1: DNA profile generated by SRAP primer combination of Me2 and Em4. M marker-1 kb. Lanes 1-12: AG 1, 13-20: AG 2-2, 21-23: AG 2-2LP, 24-41: AG 2-3, 42-59: AG 3, 60-66: AG 4, 67-78: AG 5 and 79-89: Unknown AG isolates of *Rhizoctonia solani*

clusters at 35% similarity coefficient using UPGMA analysis (Fig. 2). The first cluster had three isolates of AG 1 each from Andhra Pradesh (RAPS3; AG1), Haryana (RHRW16; AG1) and Jammu and Kashmir (RJKM18; AG1). The second cluster had 28 isolates representing 5 AGs, namely, AG1, AG2-2, AG2-2LP, AG2-3 and AG3 originated from Uttar Pradesh, Haryana, Delhi, Jharkhand, Uttarakhand, Gujarat, Jammu and Kashmir, Assam, Madhya Pradesh, Bihar and Maharashtra. The third clusters had 6 isolates from 3 AGs, namely, AG1, AG2-2 and AG2-3 originated from 4 states, namely Madhya Pradesh, Haryana, Gujarat, Maharashtra and Tamil Nadu. Thirteen isolates originated from Andhra Pradesh, Assam, Maharashtra, Punjab, Haryana, Rajasthan, Uttarakhand, Delhi, Kerala and Gujarat representing 3 AGs, namely, AG2-3, AG3 and AG5 along with 2 isolates of undetermined AG were grouped into the fourth cluster. The fifth cluster had 29 isolates from 3 AGs, namely, AG3, AG4 and AG5 along with 9 isolates of undetermined AG originated from 15 states representing almost all parts of the country. Nine isolates from 6 states belongs to 4 AGs, namely, AG3, AG2-3, AG3 and AG5 were grouped in the sixth cluster. The seventh clusters had only one isolate from Madhya Pradesh (RMPM23; AG4).

The dendrogram derived from the data on amplified products obtained with 16 primer sets by using bootstrap analysis showed different levels of genetic similarity among the populations of *R. solani*. At 28% genetic similarity, the isolates were grouped into five major clusters. The first major cluster consisting of 81 isolates was further sub-divided into 13 sub-clusters. Majority of the sub-clusters had AG specific isolates originated from different parts of the country along with their respective AG tester isolates. The second and third clusters had only 2 isolates in each representing AG2-3 and AG3, respectively. The fourth cluster had 3 isolates belonging to AG3 and AG5 whereas, the fifth cluster had only one isolate of AG4 (Fig. 3). About 95% isolates showing similar sub-clustering by using both types of analysis.

DISCUSSION

Genetic diversity analysis of *R. solani* populations with Sequence Related Amplified Polymorphism (SRAP) markers using UPGMA and bootstrap indicated that the isolates were highly variable in their genetic makeup. More or less similar clustering patterns were obtained in both the analysis. The AG specific sub-clustering was common in both the analysis with a few exceptions might be due to genetic variation in the isolates of the same AG. The groups generated by UPGMA and bootstrap analyses were not corresponding to the place of origin, agro-ecological region and host of the isolates. Thus, the polymorphism of *R. solani* was very rich that may relate with their diverse growing environments, wide host range and genetic background. In the present study, first time attempt was made to explore the possibility of SRAP markers for diversity analysis of fungal plant pathogens. Earlier to this, the SRAP was used for diversity analysis to resolve the taxonomy of plants and other fungi. Sun *et al*. (2006) successfully demonstrated the utility of SRAP markers for taxonomic analysis of 31 different *Ganoderma* strains. The results revealed the genetic diversity of *Ganoderma* strains and their correlation with geographic environments. It has also been suggested that the SRAP markers could be used in the taxonomic analysis of fungi.

Ma *et al*. (2010) studied the genetic diversity of *Tricholoma matsutake* using SRAP technique. A total of 129 strains from 13 geographical locations in Northeastern China were amplified by using 12 primer pairs. Abundant genetic variation was detected within the individual populations. The analyses showed significant positive correlation between genetic distance and geographical distance and no correlation between genetic distance and altitudinal differences among the populations could be established. In the present study also, high level genetic variability among the populations of *R. solani* was observed. The present study also supported by Chang *et al*. (2012), who observed the genetic variations among and within native *Saccharum spontaneum* populations collected from Sichuan, China. Ren *et al*. (2012) also revealed a large genetic variation in 20 strains of endophytic fungi using SRAP markers. They concluded that the SRAP technology is more efficient than traditional morphological identification. It was found that SRAP markers could more really reflect the genetic diversity of endophytic fungal strains and also could be used as a method for identification of endophytic fungi. Zhang *et al*. (2014)

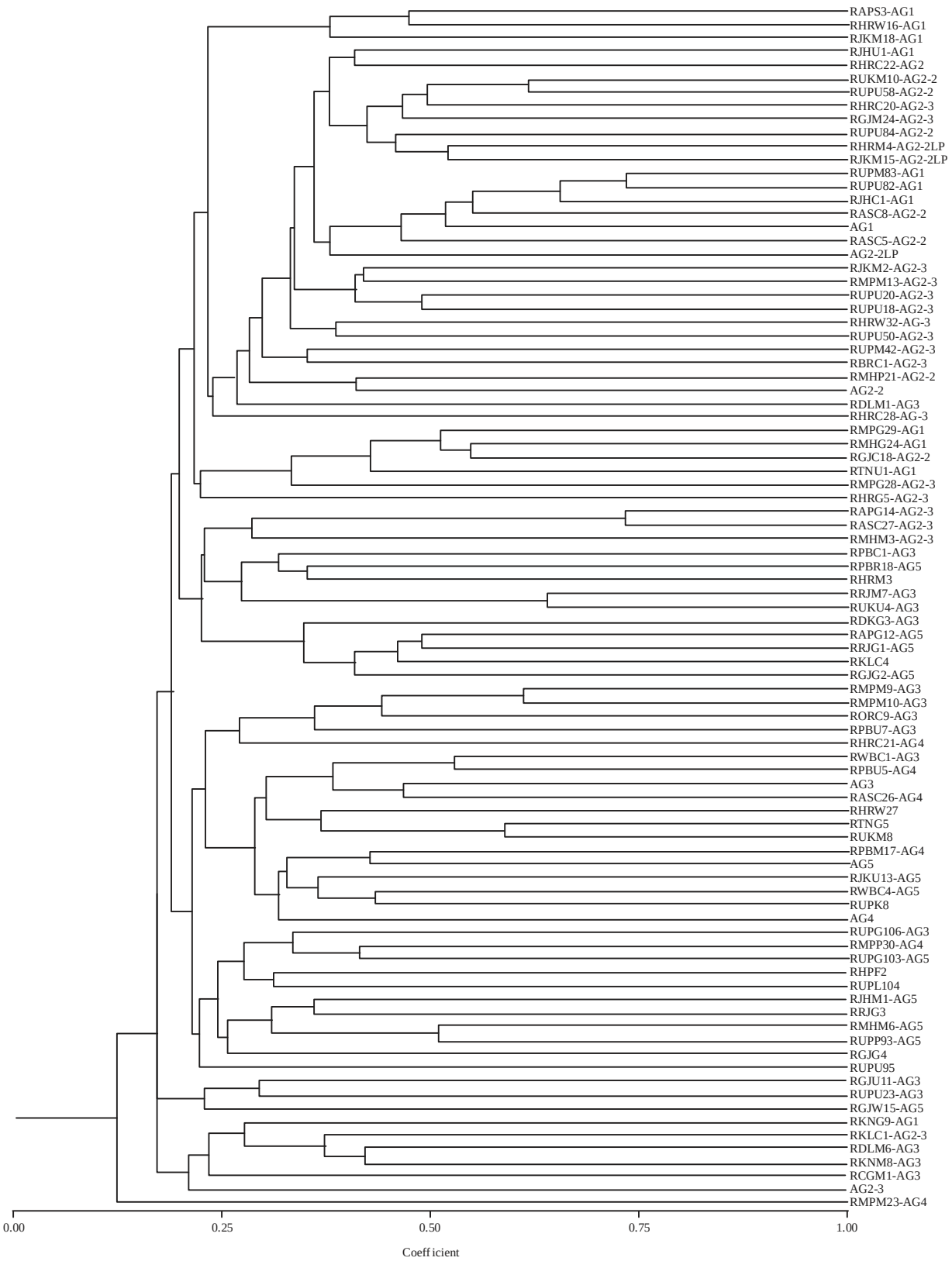

Fig. 2: Dendrogram derived from combined analysis of 16 SRAP primers combinations for 89 *Rhizoctonia solani* isolates using UPGMA. The bottom scale represents the percentage of Jaccard's similarity coefficient

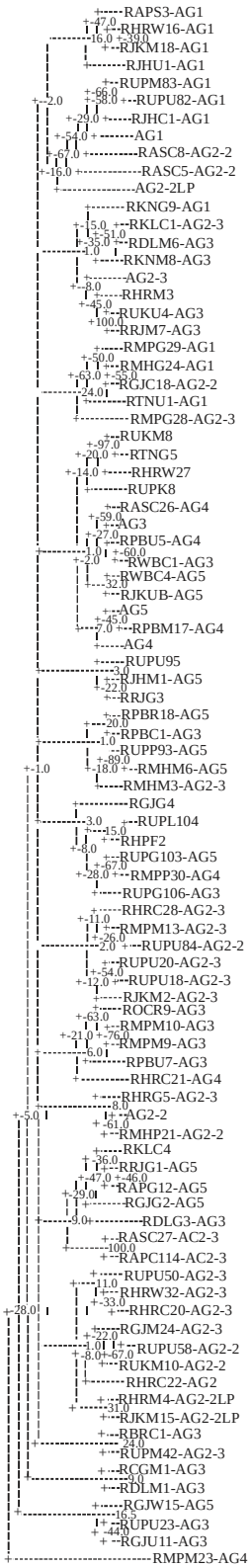

Fig. 3: Dendrogram derived from combined analysis of 16 SRAP primers combinations for 89 *Rhizoctonia solani* isolates using boot step analysis. Numbers at the forks show the percentage of time the group consisting of the isolates which are to the right of that fork occurred

analyzed the genetic diversity of endangered *Polyporus umbellatus* using SRAP and clearly indicated the suitability of markers for diversity analysis. Dubey *et al*. (2012) analyzed the genetic diversity of similar set of *R. solani* isolates with different molecular markers namely, Universal Rice Primers (URPs), Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers. The majority of the isolates representing various AGs were grouped together into different sub-clusters. At genetic level, the isolates did not correspond to agro-ecological regions or states and crops of the origin because of the wide host range and adopt ability of the pathogen under different environmental conditions. The present finding is accordance with these results, indicating the suitability of SRAP for diversity analysis.

SRAP markers were also used to analyzed the genetic diversity of different fungal pathogens (Pasquali *et al*., 2010; Que *et al*., 2012; Xu *et al*., 2014; Dinolfo *et al*., 2015). Pasquali *et al*. (2010) first time used SRAP technique to analyse the molecular variability of fungal pathogen *Puccinia striiformis* f. sp. *tritici* and identified the polymorphisms among the isolates. Que *et al*. (2012) assessed molecular variation among *Sporisorium scitamineum* isolates causing sugarcane smut by SRAP markers. The molecular variation of *S. scitamineum* was associated with geographic origin and there was no evidence of co-evolution between sugarcane and the pathogen. Xu *et al*. (2014) observed considerable genetic variation among the isolates of *S. scitamineum* and the environmental heterogeneity has played an important role for high degree of variation. They reported that the genetic difference in the population of the pathogen depends to a large extent on the heterogeneity of their habitats and long-term adaptations of pathogens to their ecological environments. In the present study also several AG specific isolates from the same place clustered together clearly indicates the variability in the population of *R. solani* due to adoption and variation in the host in particular area. The present results are also in accordance with the observations made by Dinolfo *et al*. (2015) used SRAP markers for *Fusarium poae* genetic variability. The molecular analysis showed high intraspecific variability within *F. poae* isolates and a partial relationship was revealed between variability and the host/geographic origin.

The present study clearly indicated that the SRAP markers are suitable for genetic diversity analysis of *R. solani*. The bootstrap analysis clearly showed that the *R. solani* population in India broadly originated from similar ancestors as out of 89 isolates (including 7 tester isolates), 81 grouped in a single cluster with 28% genetic similarity. Although, AG wise sub-clustering was obtained. The variation in the population might be due to diversification of crops and variation in the environmental conditions in different agro-ecological regions of the country. More or less similar, AG specific sub-grouping pattern was obtained with UPGMA analysis.

CONCLUSION

The populations of *Rhizoctonia solani* belonging to 7 Anastomosis Groups (AGs) isolated from different pulse crops representing 21 states from 16 agro-ecological regions of India showed high level of variability during genetic diversity analysis using SRAP markers. The markers provided 100% polymorphism showed suitability for genetic diversity analysis of phytopathogenic fungi. The genetic groups did not correspond to origin of the crops and agro-ecological regions might be due to wide range of the hosts and adaptability of the pathogen under divers environmental conditions present in various parts of the country but partially corresponding to the Anastomosis Groups (AGs) of the pathogen. The present study deals first attempts on the use of SRAP markers for genetic diversity study of plant pathogenic fungus *R. solani.*

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