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Morphological and molecular variability studies of *Rhizoctonia solani* isolates of Manipur (India) inciting sheath blight in rice (*Oryza sativa.* L)

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Abstract
Sheath blight, caused by *Rhizoctonia solani* Kuhn, is one of the most devastating diseases incurring huge losses in the production of rice crop. It is important to investigate both morphological and molecular comparative studies among the isolates of *R.solani* The collection of *R.solani* isolates from symptomatic rice plants was done and analyzed for morphological and molecular characterization from root to the tip to have a clear idea on the causal agent and the disease. Morphological studies on growth on *R.solani viz.*, radial growth, mycelial characters, sclerotial characters, growth of fungus at different pH levels and molecular studies using RAPD and ISSR markers were analyzed. Morphological features which includes radial growth (18.95-45mm), sclerotial characters (brown to dark brown), fresh and dry weights (5.08-6.78g and 0.25-0.52 respectively), growth at different pH levels (0-4.30g), growth in different culture media (26.50-44.50mm) had a varied significance. Mycelial compatibility studies of all possible 45 combinations revealed 15 compatible and 30 incompatible isolates by having certain degree of aversion. At deeper levels molecular variability studies were done using molecular markers 10 RAPD and 10 ISSR primers which revealed that isolates were clustered to two clusters at 49% degree of similarity, Similarly, ISSR analysis revealed that isolates were clustered to two clusters at 39% degree of similarity. Our results suggest that, the presence of different races of *R. solani* within the same local geographic regions makes the disease vulnerable and lay huge losses in production.

Keywords: Molecular variability, morphological features, *Rhizoctonia solani*, rice, sheath blight

Introduction
Rice (*Oryza sativa* L.) the world's most widely cultivated and consumed cereal crop, especially to the populace of South Asian countries (Pareja *et al.*, 2011) [14]. The world's populace is expected to reach over 9 billion duly by 2050, on this context the predictions are assumed that total food production will be sufficient only for 60% of the population (FAO 2018) [6]. Around 40,000 different varieties of rice (*Oryza sativa* L.) exist in the world (http://www.riceassociation.org.uk/content/1/18/ types -of-rice.html). China produces largest amount of rice (142.3 million tonnes) followed by India (110.4 million tonnes) (FAO: Rice Market Monitor 2018) [6].

The major constraints involved in the decline of rice production are Diseases, Insects and other abiotic causes. Different pathogens cause different kinds of diseases and imparts decline in the production. The major diseases intervened with rice are Blast, Sheath blight, Brown spot, False smut etc., sheath blight, a biotic stress holds a major hand in decline in the production. The pathogen associated in inciting the disease is *Rhizoctonia solani. Rhizoctonia solani* may inflate the yield loss upto 45% (Margani and Widadi, 2018) [12]. The date back records suggest that the disease was first dates in Japan by Miyake in 1910. Further the spread of the disease was reported throughout temperate and tropical rice growing areas. The incidence of the malady in India was first according to Paracer and Chahal (1963) in Gurdaspur of Punjab region. In India the annual loss incurred by ShB are estimated over 10% (Boukaew and Prasertsan, 2014). The devastating nature of the pathogen induces an average yield loss ranging between 10–30% globally (Xie *et al.*, 2008). The pathogen is responsible for yield loss up to 45% (Margani and Widadi, 2018) [12].

Morphologic and pathogenic variations are familiar in many fungal pathogens. Diversity within rice sheath blight isolates has been studied by morphological characterization and also by using various molecular techniques.

Perhaps, evaluation of the genetic diversity in pathogen isolates has been an initial step towards understanding the population structure. Fungal plant pathogens, in general that do not undergo regular sexual reproduction, in this context mycelial compatibility groups provide an in direct measure of genetic variability among isolates resulting in unrelated gene pools that may display variation because of lack of genetic exchange between MCGs. Anastomosis is important taxonomic tool and isolates belonging to the same anastomosis group may be similar with different or specific species, geographic region or level of pathogenicity (Sneh *et al.*, 1991) [23]. The species is subdivided into anastomosis groups (AG) based on their compatibility for hyphal fusion with known tester isolates. Anastomosis between genetically similar isolates that are compatible, form a fused hyphal network involving fusion of cell wall, cytoplasm and nuclei, whereas genetically distant isolates may form anastomosis but show no changes in the hyphal organization (Kuninaga *et al.*, 2002) [11].

The initial step to understand population structure is evaluation of morphological and genetic diversity among pathogen isolates. Modern molecular techniques have become more reliable, highly suitable tools for identifying pathogen species and for assessing variation at genetic level within collections and populations. The feasibility to understand genetic diversity of plant pathogens is due to advent of various molecular marker technologies. Keeping these steps to understand the population diversity the objectives of the study are framed to investigate morphological characterization and molecular characterization of *R.solani* isolates collected from different geographical locations of the state Manipur.

Materials and Methods

Sample Collection

An intensive survey was held in *kharif* (September and October, 2017) across rice growing regions of valley districts (Bishnupur, Imphal East, Imphal West and Thoubal) of Manipur. The rice plants showing typical symptoms of sheath blight probably caused by *Rhizoctonia solani* were collected during filed survey and were brought to the laboratory of Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal for further studies. The surveyed locations of valley districts of Manipur are as follows Utlou, Bishnupur Khablock Lamkhai of Bishnupur district, Maklang, Sairemkhun, Kadangband, Heibongpokpi of Imphal West, Tangjeng, Wangjing Mayai Leikai of Thoubal and Tangkham of Imphal East.

Isolation of *Rhizoctonia solani*

The tainted sheaths showing typical symptoms of sheath blight were collected and surface sterilized in 0.5% NaOCl for 1 min., rinsed 3 times in sterile distilled water, dried on filter paper, and plated on to 1.2% water agar. The plates were incubated at 25 ± 2 °C in BOD incubator for 24–48 h. Single hyphal tips growing from plated tissue were transferred to fresh Potato Dextrose Agar (PDA) plates using a sterile dissecting needle after examining the mother culture under stereomicroscope for diagnostic features of *Rhizoctonia* spp. (González *et al.*, 2006) [7]. The isolates were stored at 4°C for future use. The isolates were designated as RS1, RS2, RS3, RS4, RS5, RS6, RS7, RS8, and RS9 respectively. Morphological identification of the isolates was based on cultural characteristics of the *R.solani* and morphology of the mycelial mat and sclerotia formation traits.

Morphological Characters of *R. solani* isolates

The cultures of freshly grown 3–4 days old were used for determining the morphological variability among the isolates. The morphological features like radial growth, mycelial characters, fresh and dry weights of the mycelial and sclerotial characters were analyzed. Radial growth of the virtuous isolate was analyzed on 10 different culture media like Czapek's Dox Agar, Elliots Agar, Modified Potato Dextrose Agar, Oat Meal Agar, Potato Dextrose Agar, Richards Agar, Rose Bengal Agar, Sabouraud's Agar, Water Agar and Malt extract Agar. Whereas, the number of sclerotia produced from each inoculum per Petri plates (90mm), length and diameter of the sclerotia (mm) and weight of sclerotia (mg) was measured after 10–15 days inoculation.

Mycelial Compatibility studies of *R. solani*

Mycelial compatibility was deliberated by incubating paired plates of pathogens, were examined macroscopically after 5 and 10 for the presence of antagonism section at the point of mycelial contact (Punja and Grogan, 1983) [16] and the presence of transparent line in the reaction sector between the colonies. Jaccard's similarity coefficient matrix was developed by the data obtained by assuming compatible isolates as 0 and incompatible as 1 and a dendrogram was constructed based on the Jaccard's similarity coefficient matrix.

Molecular Variability

DNA Extraction

R.solani isolates were grown on PDB medium at 27 ± 1°C in BOD incubator for 7 days. Mycelial mat was harvested. The harvested mycelial mat was cleaned for traces of any media and was subjected for DNA extraction in liquid nitrogen. A standard protocol was assured for DNA extraction mentioned in Hi Media fungal genome extraction kit.

RAPD and ISSR Marker Analysis

The variability tests of 9 isolates of *R.solani* were subjected to RAPD and ISSR fingerprinting. A total of 10 RAPD and 10 ISSR primers were used to determine the variability among the isolates. PCR reactions were performed using a 2Xthermalcycler (Thermo Electron Corporation, USA). The PCR reaction was set up in a 25µl reaction volume with 5.0µl of 5x Taq buffer (with 4.0 µl of $MgCl_2$), 0.5µl dNTPs (10mM dNTPs mix), 0.6µl of primers, 0.2µl of 3U of Taq polymerase and 200ng template DNA. The reproducibility of the amplification was confirmed by repeated PCR with a similar set of ingredients and condition. For each experiment, negative controls were taken with sterile water in place of the template. The thermal cycler program consisted of an initial denaturation for 4min at 94 °C, followed by 45 (RAPD) and 35 (ISSR) cycles of denaturation at 94 °C for 1min, optimized annealing temperature for 1min, and extension at 72 °C for 2min with a final extension of 72°C for 10min. The PCR amplicons were resolved on 2.5% Agarose gel in 1x TAE buffer by electrophoresis at $90Vcm^{-1}$. DNA ladder of 100bp was used as a standard for estimation of molecular weight of each band. Coefficient matrix was generated by using Jaccard's Coefficient values for each primer comparing among the samples (Jaccard 1908). Two dendrogram based

on RAPD and ISSR primers were generated from the matrix (NTSYS PC, Version 2.2).

Results

Morphological Identification

All the *R. solani* isolates collected from rice growing regions of valley districts of Manipur exhibited morphological characteristics specific to Rhizoctonia solani. The hypal branching of the isolates had a branching at right angles and a septum was present in the branch of hype near the point of origin with a single constriction at the branching. The identified isolates were designated as RS1, RS2, RS3, RS4, RS5, RS6, RS7, RS8 and RS9 respectively.

Morphological Variability

Morphological characters of *R. solani* isolates were studied on PDA media. Morphological characters like radial growth, color of the colony, texture, growth pattern of mycelia were studied. Potato Dextrose Broth was used to record fresh and dry weights of mycelia respectively. All the readings are represented in respective tables.

Variation in colony radius of *R. solani* isolates on PDA showed after 48h of incubation. Highest radius (45.00mm) of the colony was revealed by the isolate RS1 covering the entire plate. The second highest (40.45mm) colony radius was noticed in RS7 and minimum by RS3 (38.50mm). Variability on radial growth was observed among the isolates is shown in Table 1.

The morphological characters like color, appearance and margins of the colony were rendered in Table 1. Three distinct colors were noticed among the isolates of *R.solani.* isolates *viz.*, Creamy White (RS1, RS4, RS6 and RS9), Brown color (RS3 and RS5) and Dark Brown (RS2, RS7 and RS8). The character, mycelial appearance among nine isolates was varied, six of them i.e., RS1, RS3, RS4, RS5, RS6 and RS7 showed fluffy appearance. The other isolates RS2, RS8 and RS9 showed flat appearance. The colony margins of the isolates RS1, RS2, RS3, RS5, RS6 and RS7 showed regular pattern of mycelial margins whereas, RS8 and RS9 showed irregular pattern respectively. Significant variation on mycelial fresh weights and dry weights among the nine isolates of *R.solani* was observed and are rendered in Table 1. The fresh weights ranged from 5.27- 6.78g, the dry weights ranged from 0.25 to 0.54g.

Table 1: Mycelial and Sclerotial characters of *R. solani* on PDA

Isolates	Mean Radial growth (mm)* at different incubation periods.		Mycelial characteristics of *R.solani* isolates on PDA			Mycelial weight (g)*		Sclerotial characters					
	24 hrs	48 hrs	Colony colour	Appearance	Margins	Fresh	Dry	Color	Growth Pattern	Shape	Texture	100 sclerotial Weight (mg)*+	Size in diameter (mm)*,-
RS1	14.14	45	Creamy White	Fluffy	Regular	6.78	0.52	Brown	Scattered	Irregular	Coarse	39.1	2.85
RS2	8.21	37.91	Dark Brown	Flat	Regular	5.24	0.25	Brown	Cluster	Globose	Coarse	24.9	5.35
RS3	9.81	38.5	Brown	Fluffy	Regular	5.54	0.46	Dark Brown	Cluster	Globose	Fine	30.3	3.46
RS4	7.92	34.91	Creamy White	Fluffy	Regular	5.22	0.41	Brown	Cluster	Globose	Coarse	28.5	4.36
RS5	9.95	34.12	Brown	Fluffy	Regular	5.52	0.54	Light Brown	Scattered	Globose	Coarse	39.5	3.83
RS6	5.62	33.83	Creamy White	Fluffy	Regular	5.08	0.4	Dark Brown	Scattered	Irregular	Fine	42.9	2.73
RS7	8.33	40.45	Dark Brown	Fluffy	Regular	6.23	0.34	Dark Brown	Scattered	Globose	Coarse	20.2	3.35
RS8	13	40.37	Dark Brown	Flat	Irregular	5.27	0.28	Dark Brown	Scattered	Globose	Fine	38.4	3.78
RS9	5.54	18.95	Creamy White	Flat	Irregular	6.37	0.47	Brown	Cluster	Irregular	Fine	23.7	3.01
SE(d)±	0.4	1.53	--	--	--	0.07	0.02	--	--	--	--	2.1	0.33
$CD_{(0.05)}$	0.85	3.23	--	--	--	0.15	0.04	--	--	--	--	4.42	0.71

*Mean of three replications, +Mean of 100 sclerotial weights in each replication, -Mean of 50 sclerotial sizes in each replication

Three distinct colors were observed on characters of sclerotia among the isolates (Table 1) Light Brown (RS5), Brown color (RS1, RS2, RS4 and RS9) and Dark brown (RS3, RS6, RS7 and RS8). With respect to sclerotial shape, two types of shapes were observed among *R.solani* isolates *viz.*, Irregular (RS1, RS6 and RS9) and Globose (Regular) (RS2, RS3, RS4, RS5, RS7 and RS8). In context to texture of sclerotia, coarse texture comprising of isolates RS1, RS2, RS4, RS5 and RS7 and fine texture comprising isolates RS3, RS6, RS8 and RS9 was observed. Isolates showed coarse texture, isolates showed fine textured sclerotia. The growth pattern of sclerotia on PDA was observed to be in various positions *viz.*, scattered, centre, in concentric manner, at the walls of petri dishes, and sometimes on the lid of petri dish. Isolates RS1, RS5, RS6, RS7 and RS8 showed scattered pattern of sclerotial formation, isolate RS2 had its sclerotial formation at the centre, isolates RS4 and RS9 showed their sclerotial formation in concentric manner. Isolate RS2 had showed its sclerotial pattern on the walls and lid of the petri dish. Varied significance was observed among the test weights of sclerotial bodies of isolates. Isolate RS6 showed highest sclerotial test weight of 42.90mg. The least sclerotial weight was showed by isolate RS7 with 20.20 mg respectively.

The isolates had a significant difference among size of sclerotial bodies produced (Table 1). Highest size was found in isolate RS2 with 5.35mm and the least sclerotial size of 2.73mm was observed in isolates RS6. Sclerotial weights ranged from 42.9 - 20.2 mg among *R.solani* isolates which had a varied significance.

Growth of *R. solani* was analyzed at different pH levels. Test fungus was grown in PDB. Growth of fungus was analyzed by taking weights of fresh mycelial mat. Significant variation was observed in growth of test fungus at different pH levels. Highest growth was observed at pH6 with 4.30g (2.19)*, followed by pH7&8 with 4.08g (2.14)*. Mycelial growth of 3.79g (2.07)*, 3.67g (2.04)*, 3.40g (1.97)*, 2.58g (1.75)*,

2.46g (1.72)*, 2.43g (1.71)*, 2.30g (1.67)* was shown by pH 5, 9, 4, 11, 10, 3 and 12 respectively. No growth of test fungus was observed in case of pH2.

Ten different media were tested to perceive best culture media for mycelial growth and sclerotial formation of *R.solani*. A significant variation in radial growth was found (Table 2, Plate 1) of the pathogen after 48h of incubation. It revealed that Modified Potato Dextrose Agar, Potato Dextrose Agar and Czapek's Dox agar showed maximal radial growth of 44.5mm after 48h of incubation compared to other media, the least radial growth was observed in Elliot's Agar of 19.75mm. Modified Potato Dextrose Agar and Potato Dextrose Agar showed creamy white, fluffy and regular growth of mycelia. Rose Bengal Agar, Oat Meal Agar and Richards Agar showed with white colored, fluffy and regular (irregular-Richard's Agar) growth of mycelia. Sabouraud's Agar and Malt Extract Agar showed Light Brown colored mycelia with fluffy and regular pattern. Water Agar and Elliot's Agar showed white transparent with flat and irregular patterns of mycelial growths (Table 3). Sclerotial color was varied from light brown to dark brown including brown on all the culture media. Growth pattern of sclerotia varied from scattered to clustered manner. Shape of sclerotia showed irregular to globose shapes. Texture of sclerotia on different culture media varied from coarse to fine textures (Table 2, Plate 1).

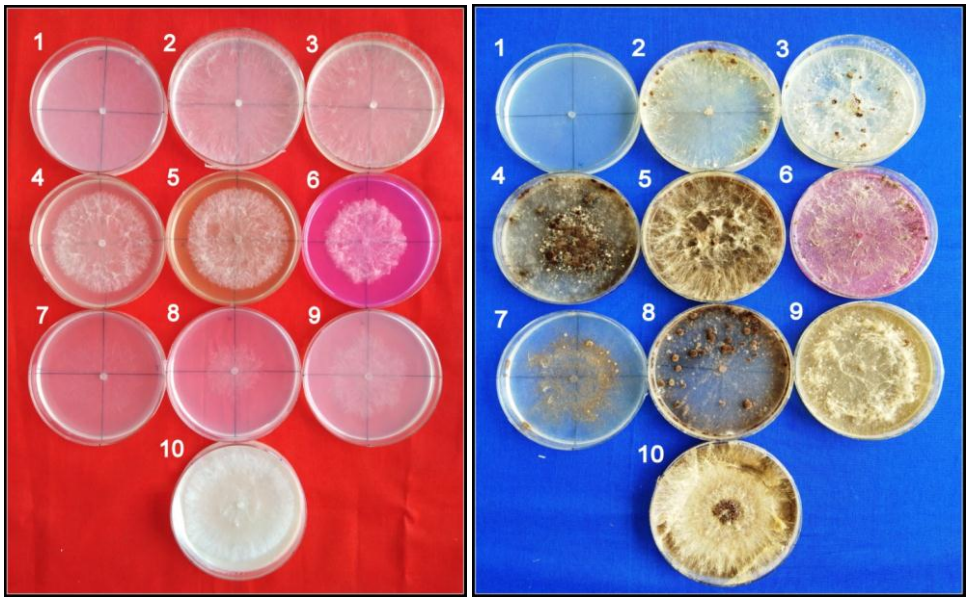

Plate 1: Effect of different culture media on Mycelial and Sclerotial characters of *R.solani*

Table 2: Mycelial characters and sclerotial characters of *R.solani* on different culture media

Name of the Media used	Mean Radial growth (mm)* at different incubation periods.		Mycelial characteristics of *R.solani* isolates			Sclerotial Characters			
	24 hrs	48 hrs	Colony colour	Appearance	Margins	Color	Growth Pattern	Shape	Texture
Modified Potato Dextrose Agar	22.75	44.50	Creamy White	Fluffy	Regular	Brown	Scattered	Irregular	Coarse
Potato Dextrose Agar	21.33	44.50	Creamy White	Fluffy	Regular	Brown	Scattered	Irregular	Coarse
Water Agar	09.00	26.50	Transparent White	Flat	Irregular	Light Brown	Scattered	Globose	Fine
Czapek's Dox Agar	21.25	44.50	Dark Brown	Flat	Irregular	Brown	Scattered	Globose	Fine
Elliot's Agar	08.58	19.75	Transparent White	Flat	Irregular	Light Brown	Scattered	Irregular	Coarse
Rose Bengal Agar	11.66	28.25	White	Fluffy	Regular	Brown	Clustered	Irregular	Fine
Malt Extract Agar	14.25	34.33	Light Brown	Fluffy	Regular	Dark Brown	Scattered	Irregular	Coarse
Sabouraud's Agar	14.66	33.33	Light Brown	Fluffy	Regular	Brown	Scattered	Irregular	Coarse
Richard's Agar	10.41	27.33	White	Flat	Irregular	Dark Brown	Clustered	Globose	Fine
Oat Meal Agar	13.33	34.00	White	Fluffy	Regular	Dark Brown	Clustered	Irregular	Coarse
SE(d)±	0.67	0.61	--	--	--	--	--	--	--
$CD_{(0.05)}$	1.4	1.29	--	--	--	--	--	--	--

*Mean of three replications

Mycelial compatibility studies among *R.solani* isolates

Mycelial compatibility investigations were carried by pairing all the nine isolates in every possible combination. These combinations were analyzed either for their fusion and repulsion, which indicates aversion or non-aversions with various degrees among them. Self pairing was done to check the compatibility nature. Jaccard's similarity coefficient matrix was developed by the data obtained by assuming compatible isolates as 0 and incompatible as 1 and dendrogram was constructed based on the Jaccard's similarity coefficient matrix (Fig.1a)

Mycelial compatibility among the *R.solani* isolates of 45 combinations revealed that 15 combinations showed compatible nature and remaining 30 combinations showed incompatible nature with varied degree of aversion among them. Aversions among the isolates of same geographical

locations and different geographical regions were found. The results obtained in current studies were in support to results obtained by Azhar *et al.*, (2014) [1] who carried out his mycelial compatibility studies on twenty isolates of *R.solani* with one hundred and ninety combinations. The results of one ninety combinations revealed that only 53 combinations had showed in-compatibility and 137 combinations are compatible nature.

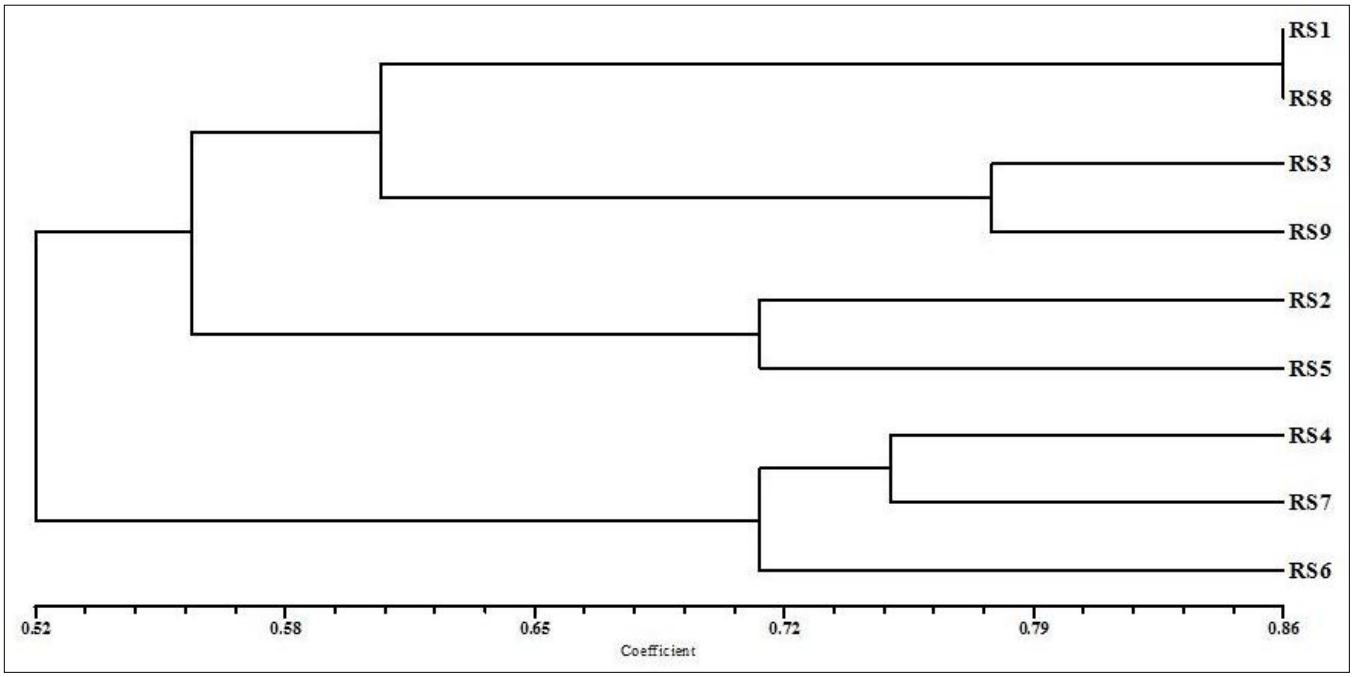

Fig 1a: Dendrogram of *R.solani* isolates showing mycelial compatibility

3.4. Molecular studies of *R.solani* isolates

Molecular variability among *R.solani* isolates was deliberated using 10 RAPD markers. Amplification of isolates was showed by all the RAPD primers used in this study resulted in polymorphic and distinguishable banding pattern. All 9 isolates generated a total of 152 polymorphic bands which are reproducible and scorable within 300-1800bp. Out of 10 primers under this study OPN-05 yielded maximum number of bands i.e., 41 bands. The least bands were generated by OPA-01 which generated, 9 bands respectively. Nine out of ten RAPD primers showed cent percent of polymorphism, where as one primer OPN-05 showed 97.56 percent of polymorphism. Polymorphism Information content (PIC) was calculated for ten RAPD primers used in the study. The highest and lowest PIC value was found in RAPD Primers OPN-05 and OPA-18 with 0.968 and 0.447. In general it is considered as, the higher PIC value the more informative is the primer selected. Hence, primer OPN-5 was observed to be highly informative among all ten primers used (Table 3).

RAPD binary scores were generated by the use of software Bio-Rad ImageLab™ 3.0. These scores obtained helped to generate Jaccard's similarity coefficient matrix and data was subjected to UPGMA (Unweighted Pair Group Method with Arithmetic Mean). Using the data obtained dendrogram was constructed using NTSYSpc version 2.02g (Fig.1b) A similarity index was computed by using the binary data obtained from polymorphic primers. Graph showing the genetic distance between *R.solani* isolates is shown in the Fig 2a. The similarity coefficients of 10 RAPD markers used ranged from 0.00 to 1.00. Among the 9 isolates of *R.solani*, the highest similarity matrix index 0.791 was observed between RS2 and RS1. The lowest similarity matrix index (0.421) was observed between RS9 and RS5. All the 9 isolates were categorized to two main clusters at 49% similarity co-efficient. Among two clusters, cluster I contained 4 isolates where the cluster was further sub-divided at 52.6% of similarity co-efficient. Cluster II consisted of 5 isolates in it by having sub-division at 54% of similarity co-efficient. Upon the observation of dendrogram the isolates of same fungus *R.solani* collected from different geographical locations of Manipur has revealed genetic diversity by RAPD primers in accordance geographical origin of the isolates.

Considerable genetic variation was observed among 9 isolates of *R.solani* under RAPD-UPGMA analysis. Genetic diversity was revealed among *R.solani* isolates based on different geographical locations. These results were in correspondence with the findings of Banerjee *et al.*, (2012) [2], Sharma *et al.*, (2005) [19], worked on molecular diversity among *R.solani* isolates using RAPD based fingerprinting the study. Sundravadana *et al.*, (2011) [24] and Banerjee *et al.*, (2012) [2], had reported RAPD similarity profiles ranging from 41 to 94% among 22 isolates of *R. solani*.

Table 3: Polymorphism and PIC of *R.solani* isolates revealed by RAPD primers

Sl. No.	Primer	Sequence (5'- 3')	Total number of bands (a)	Total number of polymorphic bands (b)	Number of monomorphic bands	Polymorphism % (b/a X 100)	PIC Value
RAPD Primers							
1	OPA-01	CAGGCCCTTC	9	9	0	100.00	0.649
2	OPA-02	TGCCGAGCTG	10	10	0	100.00	0.685
3	OPA-03	AGTCAGCCAC	11	11	0	100.00	0.528

4	OPA-04	AATCGGGCTG	12	12	0	100.00	0.733
5	OPA-18	AGGTGACCGT	15	15	0	100.00	0.447
6	OPK-14	CCCGCTACAC	14	14	0	100.00	0.612
7	OPK-20	GTGTCGCGAG	12	12	0	100.00	0.637
8	OPN-05	ACTGAACGCC	41	40	1	97.56	0.968
9	OPU-06	ACCTTTGCGG	13	13	0	100.00	0.673
10	OPV-12	ACCCCCCACT	15	15	0	100.00	0.528

PIC-polymorphic information content

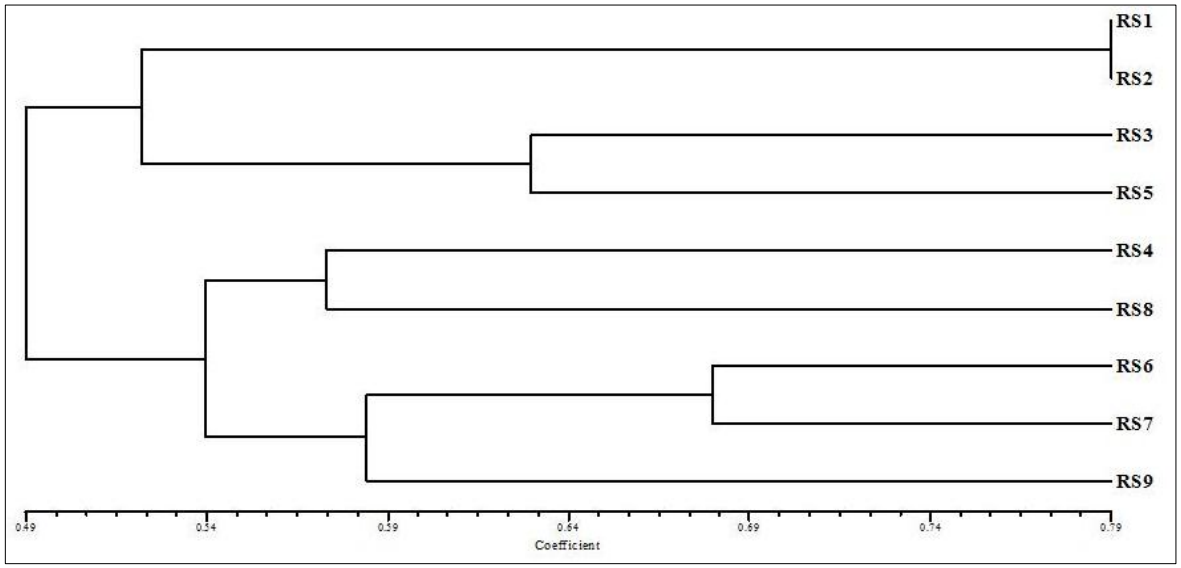

Fig 1b: Dendrogram showing variability in *R.solani* isolates by using RAPD primers

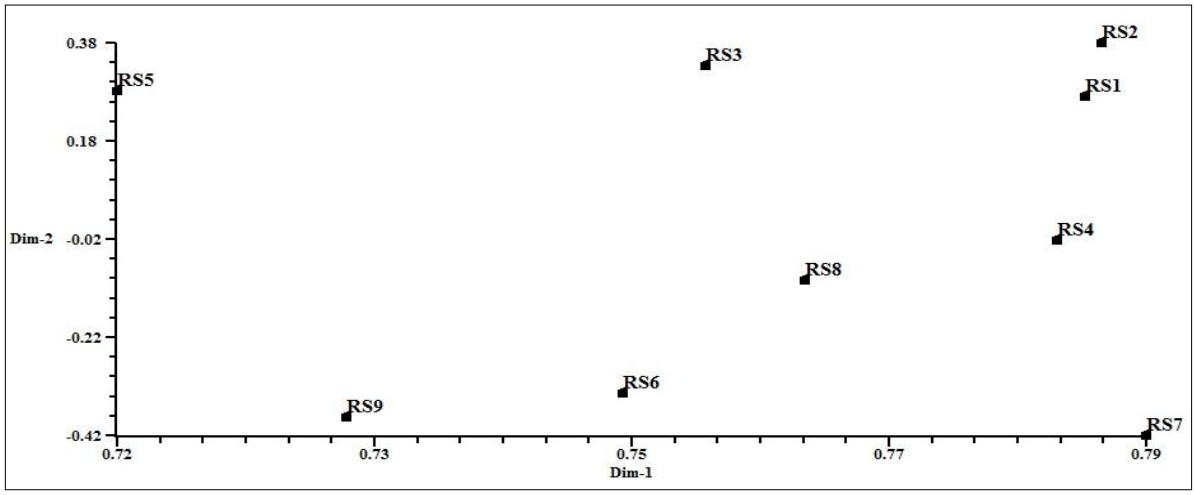

Fig 2a: 2D graph showing the genetic distance between *R.solani* isolates by using RAPD primers

Molecular variability among *R. solani* isolates was deliberated using 10 ISSR markers. Amplification of isolates was showed by 8 primers used under this study which resulted in polymorphic and distinguishable banding pattern. Amplification was not showed by 2 primers i.e., R-19 and R-31. All 9 isolates generated a total of 84 polymorphic bands which are reproducible and scorable within 200-2000bp. Among 10 primers used, UBC-807 yielded maximum number of bands i.e., 17 bands. The least number of bands were generated by R-30 and UBC-808 which generated 5 bands each respectively. All ISSR primers which sowed amplification showed cent percent of polymorphism. Polymorphism Information content (PIC) was calculated for eight ISSR primers used in the study. The highest and lowest PIC values were found in ISSR Primers R-30 and UBC-809 with 0.9153 and 0.4313 respectively. In general it is considered as, the higher PIC value the more informative is the primer selected. Hence, primer R-30 was found to be more highly informative among all ten primers used (Table 4).

ISSR binary scores were generated by the use of software Bio-Rad ImageLab^{TM} 3.0. These scores obtained were utilized to generate Jaccard's similarity coefficient matrix and data was subjected to UPGMA (Unweighted Pair Group Method with Arithmetic Mean). Using the data obtained dendrogram was constructed using NTSYSpc version 2.02g (Fig.1c). A similarity index was computed by using the binary data obtained from polymorphic primers. Graph showing the genetic distance between *R. solani* isolates is shown in the Fig 2b. The similarity coefficient values obtained for comparison of each pair among the 9 isolates were presented in the table

6. The similarity coefficients of 8 ISSR markers used ranged from 0.00 to 1.00. Among the 9 isolates of *R.solani*, the highest similarity matrix index 0.720 was observed between RS10 and RS7. The lowest similarity matrix index 0.269 was observed between RS6 and RS1. Among 9 isolates of *R.solani* under ISSR-UPGMA analysis the isolates were clustered to two main clusters at 39% degree of similarity where cluster I consists of 2 isolates and cluster II consists of 7 isolates under it. Zhou *et al.,* (2002) [30], Yugander *et al.,* (2015) [29] studied genetic variability of *R.solani* by ISSR primers stating that diversity among isolates was based on geographical origin. Shu *et al.,* (2014) analyzed variability among 72 isolates of *R. solani* by ISSR primers and found a total of 116 amplified bands, among them 110 were polymorphic bands and the rest 6 were monomorphic bands. The results grouped 72 isolates into six major clusters at 73% of similarity coefficient by UPGMA with Dice's distance matrices.

All the 9 isolates were categorized to two main clusters at 39% similarity co-efficient. Among two clusters, cluster I contained 2 isolates. Cluster II consisted of 7 isolates which had further sub-division at 40% of similarity co-efficient. Upon the observation of dendrogram the isolates of same fungus *R.solani* collected from different geographical locations has revealed genetic diversity by ISSR primers in accordance geographical origin of the isolates.

Table 4: Polymorphism and PIC of *R.solani* isolates revealed by ISSR primers

Sl. No.	Primer	Sequence (5'- 3')	Total number of bands (a)	Total number of polymorphic bands (b)	Number of monomorphic bands	Polymorphism % (b/a X 100)	PIC Value
ISSR Primers							
1	R-15	AGAGAGAGAGAGAGAGC	11	11	0	100	0.552
2	R-18	GAGAGAGAGAGAGAGAC	12	12	0	100	0.661
3	R-19	GAGAGAGAGAGGAGAA	0	0	0	0	0.000
4	R-25	TGTGTGTGTGTGTGTGAG	9	9	0	100	0.818
5	R-30	HVHTGTGTGTGTGTGTG	5	5	0	100	0.915
6	R-31	AGAGAGAGAGAGAGAGVC	0	0	0	0	0.000
7	UBC-807	AGAGAGAGAGAGAGAGT	17	17	0	100	0.661
8	UBC-808	AGAGAGAGAGAGAGAGC	5	5	0	100	0.891
9	UBC-809	AGAGAGAGAGAGAGAGG	11	11	0	100	0.431
10	UBC-827	ACACACACACACACACG	14	14	0	100	0.721

PIC-polymorphic information content

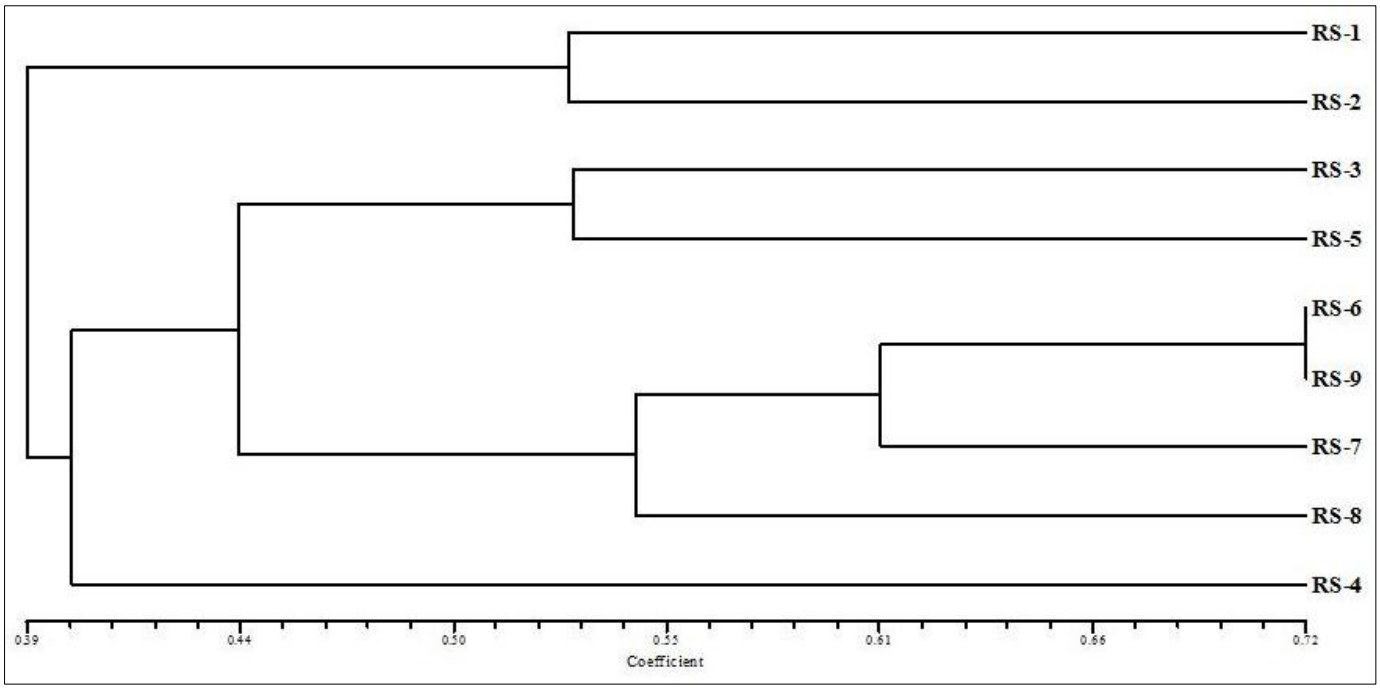

Fig 1c: Dendrogram showing variability in *R.solani* isolates by using ISSR primer

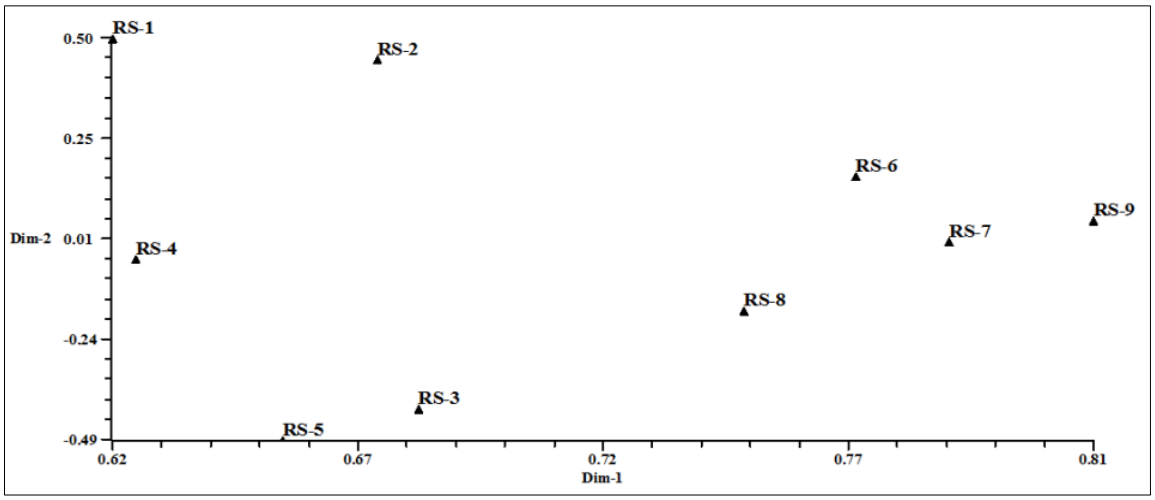

Fig 2b: 2D graph showing the genetic distance between *R.solani* isolates by using ISSR primers

Discussions

The radial growth of *R.solani* isolates at 48 hours period of successive incubation, were range from 45.0 to 18.95mm. Thind and Aggarwal (2008) [25], Khodaryari *et al*, (2009) [10] and Guleria *et al*, (2007) [8], reported similar results of growth rate of *R.solani* isolates. The data on colony radius revealed significant difference among *R.solani* isolates incubated on PDA medium for 48 hours of time duration. Based upon the results all the 9 isolates were grouped into 4 groups *viz*., Group I (RS1) specifying rapid growth, Group II (RS7 and RS8) specifying fast growing, Group III (RS3, RS2, RS4, RS5, and RS6) specifying medium growth and the last Group IV RS9 specifying slowest growth. Mycelial color of the isolates appreciably varied from creamy white to dark brown, mycelial appearance from flat to fluffy and margins from regular to irregular, which had marked difference among the isolates of *R. solani*. Fresh and dry weights of mycelia had shown remarkable difference in their growth.

The variability among the sclerotia of *R. solani* isolates different formation patterns (scattered, centre, in concentric), color (Light brown to dark brown), texture (fine to coarse), shape globose to irregular) and size. Similar findings on sclerotial weight range from 4.6- 6.3mg on PDA medium recorded by Sharma *et al.*, (2013) [20]. Variation among color, light brown to dark brown, growth patterns from scattered to clustered, shape resembling from globose to irregular, texture varying from fine to coarse were observed among the isolates. The results showed an abridgement with the results generated by Upadhyay *et al.*, (2013) [27] who categorized 45 *R.solani* isolates into 3 groups based on the above stated characters.

Based on the growth results of the fungus at different pH levels, it is classified Maximum growth was supported by pH6, Moderate by pH4, pH5 pH7, pH8 and Least by pH3,pH10, pH11, and pH12. pH2 did don't support the growth of the fungus. This is due to incapabilyity of the fungus to absorb nutrients. All the results acquired were in manner of corresponding with findings of Ritchie *et al.*, (2009) [17], who revealed that growth *of R.solani* was highest at pH 5.6. The results obtained were inaccordance with the results obtained by Singh *et al*., (2014) [21] who reported radial growth and growth rate at different pH levels of 2.0, 3.0, 4.0 5.0, 6.0, 7.0, 8.0 9.0, 10, 11 and 12.

Potato Dextrose Agar medium showed best among all ten different media used. The results from this study are supported by the results recorded by (Imran et *al.*, 2016) [9] whose study results reveals among four different media i.e., PDA, CMA, WA, PDA had shown highest growth. The other characters of mycelial color, appearance and margins and other sclerotial characters were showed a notable change in the formation color, texture and shape. This is due to provision of some additional nutrients in PDA media than other media used as reported by different workers (Sahi *et al.*, 1992; Devi and Singh, 1998 and Meena *et al.*, 2001) [18, 4, 13]. Townsend (1957) [26] reported that high concentration of sucrose might initiate sclerotial formation but they do not mature until sugar concentration is appreciably reduced. Singh and Kaiser (1994) [22] reported that PDA supported the highest growth of the fungal isolates. However, glucose proved to be the best carbon source for linear growth of the fungus (El-Wakil *et al.*, 1985) [5].

The in-compatibility/antagonistic reactions of in-compatible combinations had showed thin band or dead mycelia at the zone of contact. Mycelial compatibility in *R.solani* can be termed as Somatic compatibility. The evidence of aversion line were refers to incompatible nature of isolates paired. The results acquired were in a manner of conforming with results generated by Ping Qu *et al.*, (2013) [15], who stated through his studies on field isolates of *Thanatephorus cucumeris* (*Rhizoctonia solani*) AG-1 IC and AG-2-2 IV. Incompatibility nature among isolates were clearly depicts binary trait, but additional genetic criteria are needed to determine whether mycelial compatibility groups (MCGs) are clones. The underlying phenomena have to be observed by genetic and molecular level investigations. The actual mechanisms of somatic incompatibility in *R.solani* as in basidiomycetes and other ascomycetes are not known.

The main concern of this particular study was to access genetic variability of *R.solani* isolates by the use of nuclear markers *viz*., RAPD and ISSR and also to validate the efficacy of these primers. Percent of polymorphism, monomorphism, polymorphic information content (PIC) and effective multiplication ratio of primers are the parameters to be considered to have better results of efficiency if markers. A total of 152 bands in RAPD analysis and 84 bands in ISSR analysis were produced (Table 5 and 6). Whereas, the polymorphism percentage among all the primers both RAPD and ISSR was almost same and some exceptions were found among the primers like OPN-05 among RAPD primers and of R-19 and R-31 among ISSR markers. There was a varied significance among PIC values both the markers. RAPD

markers had an average PIC of 0.64 and ISSR markers had an average PIC of 0.56 Thus, considering all parameters it was concluded that RAPD markers are suited well for diversity studies compared to ISSR.

Conclusion

Knowledge on morphological and genetic diversity among *R. solani* isolates of Manipur collected from the same and different agro-ecological regions has an ideal role in disease management practices. Resistant cultivars to minimize losses incurred by pathogen could be developed by having the knowledge of genetic variation, its nature and identification of particular races of the pathogen. These studies are ultimately used in implementing and efficient disease management practices. The results obtained by the use of molecular tools enhance future to develop region specific resistant varieties. Ultimately the genetic variability studies on *R.solani* clearly indicate the existence of varied population among the isolates irrespective of geographical origin and ecological regions. For better understanding of the pathogen population and the existence of its races, further study is needed with more number of isolates covering all the agro -ecological regions of the country.

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