



RESEARCH ARTICLE

Morpho-cultural and pathogenic variability in *Rhizoctonia solani* isolates from rice, maize and green gram

P.K. MISHRA, ROBIN GOGOI*, P.K. SINGH, S.N. RAI, AVINASH SINGODE¹, ARUN KUMAR² and C. MANJUNATHA

Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012, India

¹Directorate of Maize Research, Pusa Campus, New Delhi 110 012, India

²Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar 125 004, Haryana, India

ABSTRACT: Twenty two isolates of *Rhizoctonia solani* collected from rice (10), maize (10) and green gram (2) were studied for their variability with respect to cultural, morphological characters and pathogenicity. Colony appearance of the isolates was sparse, sparse fluffy, cottony and cottony fluffy. Most of the isolates were sparse and light brown in colour. Out of the three patterns of radial growth namely fast, medium and slow, twelve isolates were medium growing where growth completed within 72h, eight isolates were fast growing (48h) and two isolates were slow growing (96h or more). Sclerotia of the isolates were light brown, brown, dark brown and black in colour and formed as central, sub-central ring, peripheral ring, scattered and irregular manner. Majority of the isolates produced high number of sclerotia ranging from 40 to 60 within 3 to 10 days. Cross infectivity of the isolates was positive in all the three hosts with variability in their pathogenicity. Four isolates of rice, two each of maize and green gram isolates were found more aggressive and produced higher incidence of disease which could be utilized in resistant breeding programmes of their respective hosts. Cluster analysis showed four separate groups for the rice and maize isolates of *R. solani*, however the green gram isolates clustered with maize isolates.

Key words: Cross infectivity, cultural characters, morphological characters, *Rhizoctonia solani*, variability

Rhizoctonia solani (Kühn) (Teleomorph: *Thanatephorus cucumeris* (Fr.) Donk) is an ubiquitous soil-borne fungus comprising plant pathogens and saprophytes. It exists in nature as different groups in terms of the number of nuclei in the cells, its cultural features, hosts and virulence. There are three major groups of *Rhizoctonia* from the genus in the anamorphic classification: multinucleate *Rhizoctonia* (teleomorph: *Thanatephorus* and *Waitea*), binucleate *Rhizoctonia* (teleomorph: *Ceratobasidium* and *Tulasnella*) and uninucleate *Rhizoctonia* (teleomorph: *Ceratobasidium*). The most common types of *R. solani* belongs to the multinucleate group (Tangonan and Quebral, 1992) and causes banded leaf and sheath blight, sheath blight, damping-off, aerial blight, stem rot, sheath rot, head rot, black scurf, sprout canker and foliar blights of cultivated crops, wild weed plants and horticultural crops.

Attempts have been made to classify *R. solani* into groups on the basis of various morphological, physiological, pathological characteristics and anastomosis behavior (Ogoshi, 1987). At present, 14 anastomosis groups (AGs), AG 1 to AG 13 and AGB1 are recognized with distinct physiology and genetic composition (Ogoshi, 1987; Yong *et al.*, 2008). Although several AG types had been characterized, some of the isolates of *R. solani* from different AG generally do not anastomose with each other (Carling, 1996). An anastomosis grouping is a convenient but not the ideal method for classification of *R. solani* as misidentification is frequent because of the varying frequencies of hyphal fusion that requires meticulous microscopic experience for their differentiation. However, for any successful

breeding programme, it is very important to know the existence of variability in the population of the pathogen. Also information on cultural, morphological and pathogenic variability helps in selection of virulent strains for identification of host resistance. Although pathogenic variability of *R. solani* had been reported by many workers (Lakshamanan and Nair, 1985; Jones and Belmar, 1989; Khandaker *et al.*, 2008), it needs restudy as the behavior and virulence of the isolates may change through mutation and gene shift over time. Therefore present work was aimed to distinguish cultural and morphological characteristics of *R. solani* isolated from three important food crops of different plant types *viz.* two major cereals rice and maize and one pulse crop green gram and also to ascertain cross infectivity of these isolates.

MATERIALS AND METHODS

The investigations were carried out in laboratory and net house during 2010-2011 at Indian Agricultural Research Institute, New Delhi, India. Isolates of *R. solani* were taken from the maize pathology laboratory of Division of Plant Pathology, IARI which were collected from different parts of India (Table 1). The isolates were passed once through their respective hosts and isolated in water agar (WA) medium. Pure culture was obtained by re-isolation of hyphal tips and maintained on potato dextrose agar (PDA) medium at 27± 2°C in BOD incubator throughout the studies.

Cultural variability

The cultural characteristics were studied when mycelial growth of the fungus seemed to restrict by taking

*Corresponding author: r.gogoi@rediffmail.com

Table 1. Isolates of *Rhizoctonia solani* with their hosts, places and year of collection

Host	Location	Isolate name	Code	Year of collection
Rice	Coimbatore (Tamil Nadu)	RRS1	R-TN	2010
Rice	Gangtok (Sikkim)	RRS2	R-4505	2010
Rice	Dhaulta Kuan (H.P.)	RRS3	RD New	2010
Rice	Dehradun (Uttarakhand)	RRS4	RD-14	2010
Rice	Kapurthala (Punjab)	RRS5	R-KAPR	2010
Rice	Meerut (U.P.)	RRS6	RM-306	2010
Rice	Ghaziabad (U.P.)	RRS7	R-17	2010
Rice	Faizabad (U.P.)	RRS8	R-A2	2010
Rice	Haryana	RRS9	R-4503	2010
Rice	Ludhiana (Punjab)	RRS10	R-4500	2010
Maize	Karnal (Haryana)	MRS11	M6	2005
Maize	IARI (Delhi)	MRS12	M7	2005
Maize	Hoshiyarpur (Punjab)	MRS13	M8	2005
Maize	Bajaura (H.P.)	MRS14	M10	2005
Maize	Eluru (A.P.)	MRS15	M13	2005
Maize	BAU, Ranchi (Jharkhand)	MRS16	M19	2005
Maize	Anandpur (Uttarakhand)	MRS17	M37	2005
Maize	Lohoiingh (Rajasthan)	MRS18	M50	2005
Maize	Varanasi (U.P.)	MRS19	M60	2005
Maize	Nagpur (Maharashtra)	MRS20	M70	2005
Green gram	Khushinagar (U.P.)	PRS21	PKN	2009
Green gram	Kanpur (U.P.)	PRS22	P-KANPUR	2009

observations on colony colour, growth pattern, and radial growth after 10d of incubation. The colour of the colony was observed from the lower side of the culture (plates by comparing with the Munsell' Soil Colour Chart (Munsell' Colour Company, Inc., Baltimore, 1954). Based on the colony pigmentation the cultures were assigned to different groups. The diameter of each isolate (three replications) was measured at the interval of 48h, 72h and 96h. Observations for the colony texture were taken on the 7th day when colony fully occupied the Petri plate. The isolates were designated to different groups based on the nature of the texture of their mycelial growth and appearance.

Morphological variability

The morphological diversity of the isolates of *R. solani* was evaluated by studying various phenotypic features as suggested by Kuninaga *et al.* (1978). Cultural characteristics were studied following the methodologies detailed by Sneh *et al.* (1991). Sclerotial characters *viz.* colour, number, weight, pattern of their development and time needed for initiation of sclerotial bodies were recorded and accordingly different groups/categories were assigned to the isolates of *R. solani*. For sclerotial weight (sclerotia per plate), sclerotia were collected after three weeks and weighed (in mg) using electronic balance. Data of both the cultural and morphological features of *R. solani* (Table 2) were subjected to the software JMP8 for cluster analysis. Prior to analysis, descriptive characters like sclerotial colour, colony

appearance were converted and assigned numerical values and characters like number and weight of sclerotia were used as continuous data.

Cross infectivity test

Seeds of three popular crop varieties *viz.* rice variety Pusa 1121, green gram variety IPM 99-125 and maize variety Vivek Hybrid 9 were used for cross infectivity study using the isolates of *R. solani*. The seeds were surface sterilized and sown in earthen pots (12cm dia) containing a mixture of soil and FYM in 3:1 ratio. Three replications were maintained for each isolate of *R. solani*. The pots were kept in the net house and watered regularly.

Inoculum of *R. solani* was prepared on barley grains following the method prescribed by Ahuja and Payak (1978) for inoculation of maize plants. The mass inoculum was prepared in the typha (water sedge, *Typha angustata*) medium as per the procedure of Bhaktavatsalam *et al.* (1978). Rice plants were inoculated at the maximum tillering stage with *R. solani* colonized typha pieces. Two pieces of typha stem bits were placed between tillers in the central region of rice hills, just above the water level. Water level (5-10 cm) was maintained constantly by regular watering for creating enough humidity to promote disease development. Maize plants were inoculated after 35-40 days old plant during *kharif* season. Three barley grain cultures were inserted between stalk and sheath at second or third internodes level from soil (Ahuja and Payak, 1978). At 5-6 leaves

Table 2. Morphological and cultural characters of rice (RRS), maize (MRS) and green gram (PRS) isolates of *Rhizoctonia solani*

Isolate code	Characters									
	Colony appearance	Colony Colour	Time taken for complete radial growth (h)	Time for sclerotia formation (h)	Sclerotia colour	Sclerotia formation pattern	Sclerotia formation on under surface of lid*	No. of sclerotia**	Weight of sclerotia (mg)	
RRS1	Sparse	Brown	72	72	Light brown	Sub-central Ring	-	83 c	72 a	
RRS2	Sparse	Light brown	48	72	Dark black	Sub-central Ring	-	54 f	58 c	
RRS3	Cottony	Light brown	48	72	Dark brown	Sub-central Ring	-	41 g	63 b	
RRS4	Sparse	Light brown	72	72	Light brown	Sub-central Ring	-	41 g	63 b	
RRS5	Sparse Fluffy	Light brown	96	96	Light brown	Sub-central Ring	-	58 e	44 e	
RRS6	Sparse	Brown	72	72	Brown	central	-	26 i	45 e	
RRS7	Sparse Fluffy	Light brown	96	96	Light brown	sub-central	-	27 i	47 d	
RRS8	Sparse	Light brown	72	96	Light brown	central	+	92 b	69 a	
RRS9	Sparse Fluffy	Light brown	72	96	Dark brown	Sub-central Ring	-	45 g	52 d	
RRS10	Sparse Fluffy	Light brown	72	72	Dark brown	Sub-central Ring	-	72 d	61 c	
MRS11	Cottony	Brown	48	72	Brown	PeripheralRing	+	60 e	70 a	
MRS12	CottonyFluffy	LightBrown	72	288	Light Brown	Scattered	-	3 k	5h	
MRS13	Sparse	Light brown	48	72	Light Brown	Scattered	+	113 a	60 b	
MRS14	Sparse	Light Brown	48	96	Light brown	Scattered	+	35 h	60 b	
MRS15	Sparse	Light Brown	72	72	Brown	Peripheral Ring	+	71 d	57 c	
MRS16	Cottony	Light Brown	48	72	Light Brown	Irregular	-	37 h	48 d	
MRS17	Cottony	Light Brown	72	240	Light brown	Scattered	-	2 k	4 h	
MRS18	Sparse	Light Brown	48	96	Light Brown	Scattered	-	55 f	60 c	
MRS19	Sparse	Light Brown	72	96	Light Brown	Central	-	64 e	70 a	
MRS20	Cottony	Light Brown	72	240	Brown	Scattered	+	8 j	15 g	
PRS21	Cottony	LightBrown	72	96	Brown	PeripheralRing	+	30 h	40 f	
PRS22	Sparse	LightBrown	48	72	Light brown	Sub central ring	-	55 f	54 c	
CD(5%)								5.9422	5.0054	
CV								7.04	6.08	

* + Sclerotia formed, - Sclerotia not found

**a-h Mean difference as per DMRT

stage (30-35 days after sowing), green gram plants were inoculated during *kharif* season. Two-three barley grains bearing active mycelia of *R. solani* were placed on the upper surface of leaves and fixed with plastic tape, then covered with transparent poly bag previously sprayed with water for creating humidity. For cross inoculation, isolates of one host were used to inoculate the other hosts in addition to own host. Scoring of the disease was done using the standard scales. For rice (sheath blight), 0-9 scale of Ahn *et al.* (1986) was used. Disease establishment was observed from 3 days after inoculation (DAI) and relative lesion height was recorded at two stages, first after 20 DAI and second after at 35 DAI. For maize (banded leaf and sheath blight), 1-5 scale of Ahuja and Payak (1983) was followed. Disease intensity was recorded at 30-35 DAI. For green gram (aerial blight), CIAT 1-9 scale was adapted and disease intensity was recorded on the inoculated leaves at 10-12 DAI.

RESULTS

Cultural variability

Colony appearance and colour: The appearance of the colonies was sparse, sparse fluffy, cottony and cottony fluffy (Table 2). Out of the 22 isolates, majority (11) of them were sparse in appearance. Four rice isolates *viz.* RRS5, RRS7, RRS9 and RRS10 were sparse fluffy. Cottony appearance was observed in only one rice isolate (RRS3), four maize isolates *viz.* MRS11, MRS16, MRS17 and MRS20 and in green gram PRS21. Only one isolate, MRS12 of maize had cottony fluffy appearance. Based on the colony pigmentation all the 22 isolates were classified into two groups: light brown and brown. Among them RRS1, RRS6 and MRS1 (Table 2) were found brown and rest 19 isolates were light brown in colour.

Radial growth: Isolates wise completion of radial growth was recorded at the interval of 48h, 72h and 96h and then classified into 3 groups: fast (48h), medium (72h), slow growing (96h or more). Eight fast growing isolates included RRS2 and RRS3 of rice, MRS11, MRS13, MRS14, MRS16 and MRS18 of maize and the green gram isolate PRS22. Twelve isolates *viz.* RRS1, RRS4, RRS6, RRS8, RRS9 and RRS10 of rice, MRS12, MRS15, MRS17, MRS19 and MRS20 of maize and PRS21 of green gram were categorized into group two, i.e. medium growing. Remaining two rice isolates RRS5 and RRS7 were placed into the third group, i.e. slow growing (Table 2).

Morphological variability: Diversity in morphology was studied based on the various characters of sclerotia of the isolates of *R. solani* cultured from rice, maize and green gram.

Sclerotial characteristics: In the present study, isolates showed greater variation in the color of sclerotia (Table 2). Based on the pigmentation of sclerotia all the isolates were classified into 4 groups *viz.* light brown, brown, dark brown and dark black. Rice isolates RRS1, RRS4, RRS5,

RRS7 and RRS8, maize isolates MRS12, MRS13, MRS14, MRS16, MRS17, MRS18 and MRS19 and the green gram isolate PRS22 produced light brown sclerotia. Five isolates *viz.* RRS6, MRS11, MRS15, MRS20 and PRS21 produced brown sclerotia while the sclerotia of three rice isolates *viz.* RRS3, RRS9 and RRS10 were dark brown. Another rice isolate RRS2 produced dark black sclerotia.

Total number of sclerotia produced by the individual isolate ranged from 0 to >60 per plate. Also, based on the sclerotia number per plate, isolates were categorized as poor (no sclerotia), fair (1-10), moderate (11-20), good (21-40), very good (41-60) and excellent (>60). Very less number of sclerotia (fair) were produced by the maize isolates MRS12 (Fig. 2B), MRS17 and MRS20. Isolates RRS6, RRS7, MRS11, MRS14, MRS16 and PRS21 were categorized under good. Isolates RRS1, RRS2, RRS3, RRS4, RRS5, RRS8, RRS9 (Fig. 2A), RRS10, MRS13, MRS15, MRS18, MRS19 and PRS22 were excellent. Maximum weight of the sclerotia was recorded in the rice isolate RRS1 (72mg), followed by the isolates MRS11 and MRS19 (70mg), RRS8 (69mg), RRS3 and RRS4 (63mg), RRS10 (61mg) and MRS13, MRS14 and MRS18 (60mg) green gram isolates PRS21 (40mg) and PRS22 (54mg). Minimum sclerotial weight was noted in the least sclerotia producing maize isolates MRS12 (5mg), MRS17 (4mg) and MRS20 (15mg).

The formation patterns of sclerotia were categorized into 5 groups *viz.* central, sub-central ring, peripheral ring, scattered and irregular. The first group producing the sclerotia at the centre of the Petri plates included the isolates RRS6, RRS8 and MRS19 (Table 2). Majority of the isolates *viz.* RRS1, RRS2, RRS3, RRS4, RRS5, RRS7, RRS9, RRS10 and PRS22 were placed in the second group where sclerotia formation pattern was sub-central ring. The third group (peripheral ring) included three isolates MRS11, MRS15 and PRS21 and the fourth group (scattered) included the isolates MRS12, MRS13, MRS14, MRS17, MRS18 and MRS20. The sole maize isolate MRS16 was placed under fifth group (irregular).

There was also variation in the time required for sclerotia formation/initiation which ranged from 3 to 12 days. Eleven isolates *viz.* RRS1, RRS2, RRS3, RRS4, RRS6, RRS10, MRS13, MRS15, MRS16, MRS18 and PRS22 required 3 days only for initiation of sclerotia. It was followed by the isolates RRS5, RRS7, RRS8, RRS9, MRS14, MRS18, MRS19 and PRS21 where 4 days were needed to form sclerotia. Two maize isolates MRS17 and MRS20 took 10 days for sclerotia formation, MRS12 required a maximum of 12 days.

Cross infectivity

Rice isolates could infect maize and green gram apart from infecting their own host (Table 3). The disease incidence produced by the rice isolates on the rice plants ranged from the grade 1.0 (RRS3 and RRS10) to 5.0 (RRS4, RRS5, RRS7 and RRS9). The disease incidence produced by the rest four isolates (RRS1, RRS2, RRS6 and RRS8) was graded 3.0. In maize, highest incidence

Table 3. Disease scores resulted from cross infectivity test of *Rhizoctonia solani* isolates on rice, maize and green gram

Isolate	Disease score		
	Rice	Maize	Green gram
RRS1	3	3	3
RRS2	3	1.5	3
RRS3	1	3	3
RRS4	5	3.5	5
RRS5	5	3.5	5
RRS6	3	1.5	3
RRS7	5	3	5
RRS8	3	1.5	3
RRS9	5	3	5
RRS10	1	1.5	3
MRS11	5	3.5	5
MRS12	3	4.0	3
MRS13	1	1.0	3
MRS14	5	3.5	5
MRS15	1	1.5	3
MRS16	5	3.5	5
MRS17	1	1.5	3
MRS18	3	3.5	3
MRS19	3	1.5	3
MRS20	1	1.5	1
PRS21	3	3.5	5
PRS22	5	3.5	5

Disease rating as per the scale of Ahuja and Payak, (1983) for maize, Ahn *et al.* (1986) for rice and CIAT (1987) for green gram Data of three replications.

(scale 3.5) was produced by the rice isolates RRS4 and RRS5. It was followed by the lesser incidence (3.0) caused by four rice isolates RRS1 (Fig. 1A), RRS3, RRS7 and RRS9 and minimum incidence (1.5) was produced by RRS2, RRS6, RRS8 and RRS10. In green gram, the rice isolates RRS1, RRS2, RRS3, RRS6, RRS8 and RRS10 produced low disease incidence (3.0) and rest of the four isolates produced slightly higher disease incidence (5.0). Out of the ten rice isolates, four isolates namely RRS4 (Fig. 1B), RRS5, RRS7 and RRS9 had shown more aggressiveness by causing higher disease incidence in all the three host plants.

It is evident from the table 3 that maize isolates infected rice and green gram as well as their own host maize. On maize, disease incidence produced by the isolates ranged from the scale 1.0 (MRS13), 1.5 (MRS15, MRS17, MRS19 and MRS20), 3.5 (MRS11, MRS14, MRS16 and MRS18) and 4.0 (MRS20). In rice, highest incidence (grade 5.0) was produced by the maize isolates MRS11, MRS14, and MRS16. It was followed by the lesser incidence (grade 3.0) caused by three maize isolates MRS12, MRS18 (Fig. 1C) and MRS19 and minimum incidence (grade 1.0) was produced by MRS13, MRS15, MRS17 and MRS20. In green gram, the maize

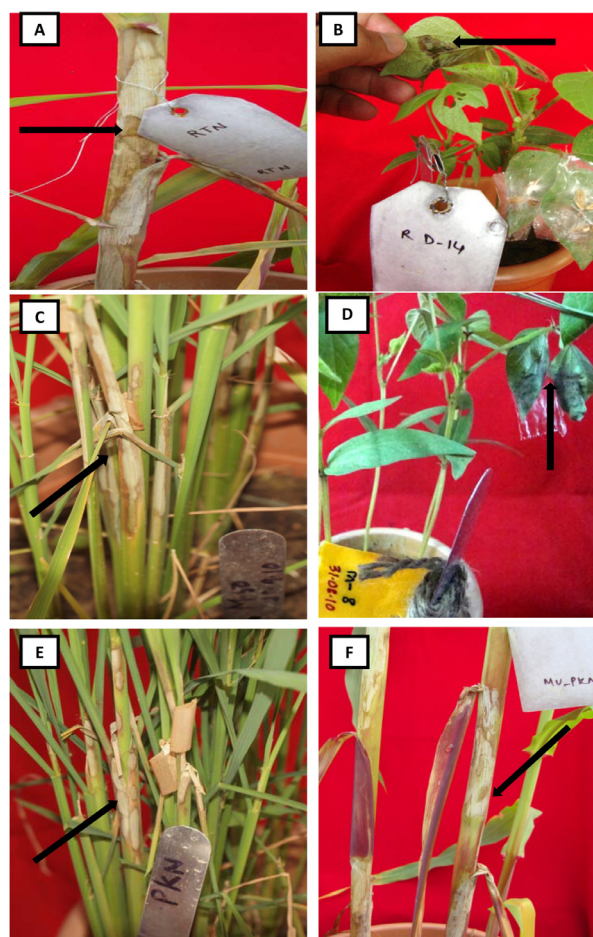


Fig. 1. Cross infectivity of the isolates of *Rhizoctonia solani* on rice, maize and green gram. Rice isolates R-TN (RRS1) on maize (A) and RD-14 (RRS4) on green gram (B); maize isolates M-50 (MRS18) on rice (C) and M-8 (MRS13) on green gram (D); Green gram isolate PKN (PRS21) on rice (E) and maize (F)

isolates MRS12, MRS13 (Fig. 1D), MRS15, MRS17, MRS18 and MRS19 produced low disease incidence (3.0) and three isolates namely MRS11, MRS14 and MRS16 produced slightly higher disease incidence (5.0). But the isolates MRS20 could not cause any disease in green gram. Two isolates namely MRS11 and MRS16 were found to be more aggressive as they resulted into high level of blight incidence in all the hosts.

The green gram isolates (PRS21 and PRS22) caused same level of disease incidence (scale 5.0) on the green gram plants. Isolate PRS22 produced highest disease incidence (grade 5) on rice plant where as PRS21 (Fig. 1E) caused lower incidence (grade 3). On maize (Fig. 1F), both the isolates produced severe blight incidence (scale 3.5) and also showed higher aggressive nature against the tested host plants.

The UPGMA analysis of morpho-cultural characters of *R. solani* revealed four major clusters where cluster I included three isolates of maize (MRS16, MRS18 and MRS19), two rice isolates (RRS1 and RRS6) and green gram isolate PRS22. Cluster II was comprised of four

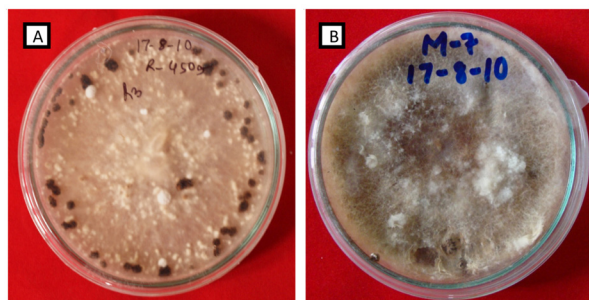


Fig. 2. Pattern of sclerotia formation in the fast growing [A, excellent, -4503(RRS9)] and slow growing [B, fair, M-7(MRS12)] isolates of *Rhizoctonia solani* on PDA at 72h and 288h, respectively

maize isolates (MRS11, MRS13, MRS14 and MRS15) and green gram isolate PRS21. The cluster III included 8 isolates solely from rice (RRS2, RRS3, RRS4, RRS5, RRS7, RRS8, RRS9 and RRS10), whereas cluster IV was formed exclusively with three isolates (MRS12, MRS17 and MRS20) (Fig. 3).

DISCUSSION

Fungi possess a variety of mechanism for introducing genetic variation in their life cycle either during sexual reproduction or independently (Kistler and Miao, 1992). *Rhizoctonia solani* (Kühn) shows tremendous diversity in morphology, pathogenicity and physiology (Ogoshi, 1987; Sneh *et al.*, 1991). Keeping this concept, variability

among the 22 isolates of *R. solani* of three hosts origin viz., rice, maize and green gram was investigated and also cross infectivity on all the three hosts was conducted.

Cultural diversity of *R. solani* was studied based on the phenotypic appearance of the isolates. Sunder *et al.* (2003) noted various colony colour of *R. solani* which ranged from brown, light brown, dark brown and yellowish brown along with changes in the colour of the media. The discolouration of growth media was mainly attributed due to production of pigments by the pathogens.

Colonies of most of the isolates of *R. solani* were sparse and cottony in nature. The rate of radial growth was fast in seven isolates where the growth completed within 48h, whereas twelve isolates were medium growing and required 72h to cover the Petri plates (90mm dia). Two isolates exhibited a sluggish nature by requiring 96h to complete the growth. On the basis of growth pattern of the aerial mycelium, Lal (1980) categorized the isolates of *R. solani* into three groups, viz. abundant growth: aerial mycelium obscures surface mycelium and touch the cover of the Petri dish; moderate growth: aerial obscures surface mycelium, but does not touch the cover of Petri dish and slight growth: aerial mycelia do not obscure surface mycelia. Most of the isolates examined by Lal (1980) exhibited slight growth of the mycelia as compared to the other two groups.

Morphological diversity of *R. solani* was determined by studying various characteristics of sclerotia like colour, formation pattern, duration of initiation, their number and

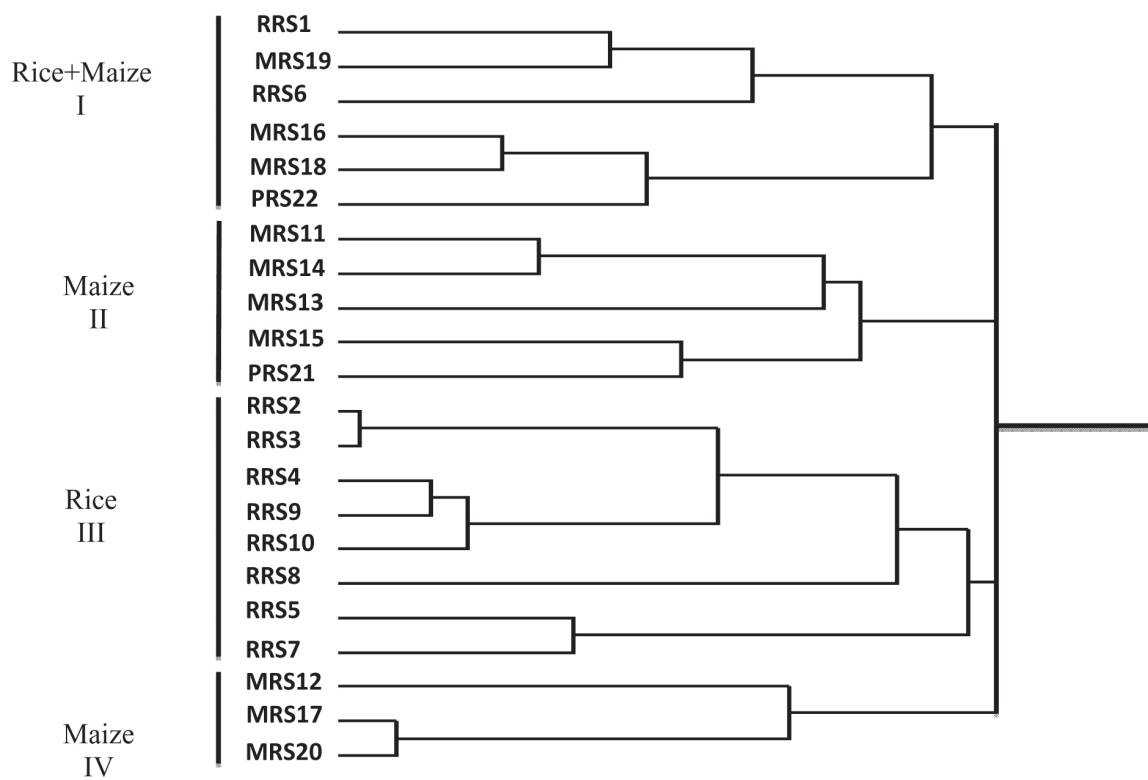


Fig. 3. Dendrogram generated from the morphological and cultural characteristics of 22 isolates of *Rhizoctonia solani* originated from rice (RRS1-10), maize (MRS11-20) and green gram (PRS21-22)

weight. Light brown sclerotia were produced by majority of the isolates (13) and only three isolates of maize (RRS3, RRS9 and RRS10) produced dark brown sclerotia. Among the rice isolates maximum were highly sclerotia producer while the maize isolates were placed almost equally under each group. Green gram isolates were either moderately or higher sclerotia producer. Interestingly, a very wide variation in the sclerotia formation was observed among the maize isolates, such as poor (MRS12, MRS17) to highest (113 per plate) sclerotia producer (MRS13). The total weight of sclerotia in case of most of the rice isolates was higher than those of maize and green gram isolates of *R. solani*. There was no correlation between the number and weight of the sclerotia produced by the isolates since the lesser number of sclerotia of an isolate weighed more than the isolates producing higher number of sclerotia. It was also observed that the total weight of sclerotia is dependent on their size and texture but not on the sclerotial count. Variation in the type and colour of mycelium and size, colour, number and type of sclerotia among the isolates of *R. solani* were studied earlier (Hoa, 1994).

The time requirement for initiation of sclerotial bodies ranged from 3 to 12 days. Out of the total 22 isolates, half (11) of them produced sclerotia within three days. As compared to the maize isolates, rice isolates were fast sclerotia producer. In addition, very late sclerotia producers were found among the maize isolates (MRS12, MRS17 and MRS20). Meena *et al.* (2001) also experienced 3 to 11 days time required to form sclerotia by *R. solani*. In another *in vitro* study, five different types of sclerotia formation pattern namely central, sub-central, peripheral ring, scattered and irregular were noticed. Similar patterns of sclerotia formation was documented earlier (Singh *et al.*, 2002). In the present study, majority of the rice isolates (8) produced sclerotia sub-centrally whereas most of the maize isolates produced sclerotia at the peripheral ring and as scattered manner in the Petri plates. However, there was no host wise definite trend of sclerotia formation because the two green gram isolates (PRS21 and PRS22) also formed sclerotia in scattered as well as sub-central ring manner.

Pathogenicity of all 22 isolates of *R. solani* on three tested hosts namely rice, maize and green gram showed that all the isolates could cause infection in their hosts of origin as well as other hosts. Usually disease incidence was rated high in the own hosts, however, some of the isolates of *R. solani* could produce very conspicuous symptoms of disease along with higher incidence across the hosts. Thus four rice isolates namely RRS4, RRS5, RRS7 and RRS9, three maize isolates namely MRS11, MRS14 and MRS16 and both the green gram isolates (PRS21 and PRS22) were found highly aggressive and caused higher disease incidence in all the test hosts. This might be due to the ability of those isolates to become more adaptive towards the various hosts under different geographical conditions despite of their differences in anastomosis group (AG). Earlier, Lakshmanan and Nair (1985) used four isolates of *R. solani* obtained from four hosts *viz.*, rice, cowpea, jack

and cotton and inoculated 14 plant species. They found the rice isolates as pathogenic to all 14 plant species whereas other isolates differed in their pathogenicity. Like present study, pathogenicity of *Rhizoctonia* spp. obtained from rice, soybean and other crops was characterized by Jones and Belmar (1989) and found variation among AG groups, with isolates of *R. solani* AG-1 IA and *R. solani* AG-UNK more virulent on rice and isolates of AG-1 IA, AG-1 IB, and AG-UNK more virulent on soybean. Hosts and AG group wise pathogenic variability was evidenced among 428 isolates of *R. solani* from different crops (Tewoldemedhin *et al.*, 2006).

A correlation between the aerial mycelial growth of the isolates/strain of *R. solani* and their virulence pattern was reported by Tu (1967). The strain with less aerial mycelium was more virulent and very poor mycelium was less pathogenic. Wamishe *et al.* (2007) opined that as compared to the slow growing *R. solani* isolates fast growing isolates were more aggressive and produced more disease lesions. Contrary to this, the isolates showing higher aggressiveness in our study were categorized under the medium (72h) and slow (96h) growing groups. The entire fact is in agreement with the findings of Basu *et al.* (2004) who reported that there was no correlation between the mycelium growth of the isolate and its virulence on the hosts. According to Butranu (1988) also, the number, viability, size and weight of sclerotia of *R. solani* could not be correlated with sheath blight intensity in rice. However, in the present studies a correlation was observed between the size of sclerotia and virulence of the pathogen. Since the isolates of *R. solani* bearing bigger size sclerotia could produce higher disease incidence irrespective of any host. The size of sclerotia was reported to be directly related to the infection potential and bigger sclerotia caused more disease in rice (Hoa, 1994).

The UPGMA analysis showed four major clusters partially separated maize isolates from the rice isolates. However both the green gram isolates were fallen in the cluster of maize isolates. This may probably due to their collection done from the maize dominating areas. Although there was a common origin of location, these two isolates might have co-evolved and undergone recombination within the isolates which in turn separated the green gram isolates from maize. It is also likely that the relative roles of these factors might have changed markedly the pathogen-host associations in different agricultural and natural ecosystem. Similar behavior was also observed in case of two rice isolates RRS1 and RRS6 that grouped with maize isolates, but exhibiting clear identity by forming a separate group within the maize cluster. Further rest of the eight isolates of rice also grouped in single which included a very close relationship with each other.

Present study has generated a set of information on morpho-cultural and pathogenic variability among the isolates of *R. solani* originated from three different hosts rice, maize and green gram. Yet there is a need to investigate if any genotypic variability existed among the

isolates so as to know the identity of *R. solani* with respect to the respective hosts in a better way.

ACKNOWLEDGMENT

Authors are thankful to the Head, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India for providing necessary facilities during the course of study.

REFERENCES

- Ahn, S.W., DenaDela, R.C., Candole, B.L and Mew, T.W.** (1986). A new scale for rice sheath blight disease assessment. *Int. Rice Res. Newsl.* **11**: 17.
- Ahuja, S.C. and Payak, M.M.** (1978). A field inoculation technique for evaluating maize germplasm to banded leaf and sheath blight. *Indian Phytopath.* **34**: 34-37.
- Ahuja, S.C. and Payak, M.M.** (1983). A rating scale for banded leaf and sheath blight of maize. *Indian Phytopath.* **36**: 338-340.
- Basu, A., Podder, M. and Sengupta, P.K.** (2004). Variability and anastomosis among the rice isolates of *Rhizoctonia solani*. *Indian Phytopath.* **57**: 70-72.
- Bhaktavatsalam, G., Satyanarayana, K., Reddy, A.P.K and John, V.T.** (1978). Evaluation of sheath blight resistance in rice. *Int. Rice Res. Newsl.* **3**: 9-10.
- Butranu, W.** (1988). Carrying capacity of component crops on *Rhizoctonia solani* Kuhn inoculum in relation to multiple cropping. A Ph. D. thesis, University of the Philippines at Los Baños (Philippines) UPLB College, Laguna 4031. p. 124.
- Carling, D.E.** (1996). Grouping *Rhizoctonia solani* by hyphal anastomosis reaction. In. *Rhizoctonia species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (Sneh B, Jabaji-Hare S, Neate S, Dijkstra G, Eds.). Kluwer Academic Publishers, The Netherlands. pp 37-47.
- CIAT (Centro Internacional de Agricultura Tropical)** (1987). Standard system for the evaluation of bean germplasm. Compilers: Van Schoonhoven, A. and Pastor-Corrales, M.A. Cali, Colombia 54 p.
- Hoa, T.T.C.** (1994). Characterization and pathogenicity of *Rhizoctonia solani* Kuhn isolates from different rice zones and management of sheath blight of rice Ph. D Thesis, IARI. New Delhi-12. pp122.
- Jones, R.K. and Belmar, S.B.** (1989). Characterization and pathogenicity of *Rhizoctonia spp.* isolated from rice, soybean and other crops grown in rotation in Texas. *Plant Dis.* **73**: 1004-1010.
- Khandaker, M., Khair, A. and Bhuiyan, K.A.** (2008). Disease reaction of different crops against virulent potato isolates of *Rhizoctonia solani* Kuhn. *Bangladesh J. Botany* **37**: 75-80.
- Kistler, H.C. and Miao, V.P.W.** (1992). New modes of genetic changes filamentous fungi. *Annu. Rev. Phytopathol.* **30**:131-152.
- Kuninaga, S., Yokosawa, R. and Ogoshi, A.** (1978). Anastomosis grouping of *Rhizoctonia solani* Kuhn, isolated from non-cultivated soil. *Ann. Phytopathol. Soc. Japan* **44**: 591-598.
- Lakshamanan, P. and Nair, M.C.** (1985). Comparative studies on four isolates of *Rhizoctonia solani*. *Madras Agric. J.* **72**: 653-655.
- Lal, S., Baruah, P. and Butchaiah, K.** (1980). Assessment of yield losses in maize cultivars due to banded sclerotial diseases. *Indian Phytopath.* **33**: 440-443.
- Meena, B., Ramamoorthy, V. and Muthuswamy, M.** (2001). Morphological and pathological variation in isolates of *Rhizoctonia solani* causing sheath blight of rice. *Pl. Dis. Res.* **16**: 166-172.
- Ogoshi, A.** (1987). Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. *Annu. Rev. Phytopath.* **25**: 125-143.
- Singh, V., Singh, U.S., Singh, K.P., Singh, M. and Kumar, A.** (2002). Genetic diversity of *Rhizoctonia solani* isolates from rice: differentiation by morphological characteristics, pathogenicity, anastomosis behavior and RAPD fingerprinting. *J. Mycol. Pl. Pathol.* **32**: 332-344.
- Sneh, B., Burpee, L. and Ogoshi, A.** (1991). Identification of *Rhizoctonia* species. 1st Edn., APS Press, St. Paul, Minnesota, USA. pp: 133.
- Sunder, S., Singh, R. and Dodan, D.S.** (2003). Standardization of inoculation methods and management of sheath blight of rice. *Indian J. Plant Pathol.* **21**: 92-96.
- Tanganon, N.G. and Quebral, F.C.** (1992). Host Index of Plant Diseases in the Philippines, 2nd edn. Los Banos, Philippines: University of the Philippines at Los Banos.
- Tewoldemedhin, Y.T., Lamprecht, S.C., McLeod, A. and Mazzola, M.** (2006). Characterization of *Rhizoctonia* spp. recovered from crop plants used in rotational cropping systems in the Western Cape province of South Africa. *Plant Dis.* **90**: 1399-1406.
- Tu, J.C.** (1967). Strains of *Pellicularia sasakii* isolates from rice in Taiwan. *Plant Dis. Repr.* **51**: 682-684.
- Wamish, Y.A., Yulin, J.I.A., Singh, P. and Cartwright, R.D.** (2007). Identification of field isolates of *Rhizoctonia solani* to detect quantitative resistance in rice under green house condition. *Front. Agric. China* **1**: 361-367.
- Yong, X., Ming-wei, L., Gang, L., Er-xun, Z., Ling-xia, W., Jie, T., Fu-Rong, T., Ai-Ping, Z. and Ping, L.** (2008). Genetic diversity and pathogenic variation in *Rhizoctonia solani* isolates from rice in Sichuan Province, China. *Rice Sci.* **15**(2): 137-144.

Received for publication: February 11, 2014

Accepted for publication: April 25, 2014