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Morphological, Virulence and Molecular Characterization of *Rhizoctonia solani* Isolates from Rice Belonging to Eastern Uttar Pradesh

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Abstract

Total of 24 isolates of *R. solani* obtained from diseased rice plants showing typical sheath blight symptoms and collected from different areas of eastern UP like Azamgarh, Basti and Faizabad were characterized based on their morphological, pathogenecity and molecular characteristics. Isolates exhibited three types of growth pattern and sclerotial distributions. The virulence of isolates on 10 different rice varieties exhibited varying disease severity. Genetic patterns exhibited some degree of relationship with morphological and virulence characters. Majority of the isolates were macro sclerotia formers showing moderate growth on PDA medium and were moderately virulent on 10 rice varieties tested. Some of the isolates were fast growers and highly virulent whereas micro-sized sclerotia forming isolates were slow growers and less virulent. A comparative analysis of *Rhizoctonia solani* isolates on the basis of morphological, virulence and molecular characteristics revealed some correlations on the basis of RAPD analysis, all the isolates were grouped in two major clusters I and II. The highly virulent isolates grouped in minor cluster II of major cluster II, were macro-sized sclerotia formers and fast growers, whereas less virulent isolates grouped in minor cluster I of major cluster I were slow growers and micro-sized sclerotia formers. The isolate A1 was most virulent, macro-sized sclerotia former in which sclorotia were aggregated near the center of the PDA plates and it was not grouped in any cluster. Out of 10 rice varieties NDR-359 and Ajaya depicted highly resistant disease reaction, whereas variety Swarna depicted highly susceptible disease reaction with most of the isolates tested.

Key words : *Rhizoctonia solani*, Sheath blight, Rice, Morphological and virulence characteristics, RAPD analysis.

Sheath blight caused by *Rhizoctonia solani* Kuhn [Teleomorph : *Thanatephorus cucumeris* (Frank) Donk] is one of the most widely distributed and destructive disease, which has become a major constraint of rice production during last two decades (1). The emergence of *R. solani* as an economically important rice pathogen has been attributed to the identification of the rice-cropping systems with the development of new short-statured, high-tillering, high yielding varieties, high plant densities and increase in nitrogen fertilization (2, 3). These factors promote disease spread by providing a favorable microclimate, due to denser leaf canopy with an increased leaf to leaf and leaf to sheath contacts (4). Diversity within the rice sheath blight isolates has been studied by morphological characterization (5, 6) pathogenicity testing or virulence characterization (7, 8) and various molecular techniques (8—11). The RAPD markers are useful for the detecting the genetic variability among the *R. solani* isolates (6). The present investigation was undertaken to analyze variability among *R. solani* isolates belonging to eastern UP, on the basis of morphological, virulence and molecular characteristics.

Methods

Sheath blight infected samples were collected from different areas of eastern UP like Azamgarh, Basti and Faizabad. A total 24 isolates were isolated by the method of Singh et al. (12), 17 were takes from Crop Research Station Masodha, Faizabad, three from N.D.U.A. & T. Kumarganj, Faizabad ; two from Basti and one from Azamgarh. One isolate D-14 was taken

Table 1. *Rhizoctonia solani* isolates collected from different places in eastern UP.

	Place from where collected	Designation of isolates	Name of rice genotypes used for collection of isolates
1	Faizabad (Masodha)	F1	CRMAS-2232-14
2	Faizabad (Masodha)	F2	CRMAS-2232-17
3	Faizabad (Masodha)	F3	CRMAS-2232-19
4	Faizabad (Masodha)	F4	CRMAS-2232-24
5	Faizabad (Masodha)	F5	CRMAS-2232-46
6	Faizabad (Masodha)	F6	CRMAS-2232-85
7	Faizabad (Masodha)	F7	HPR-2172
8	Faizabad (Masodha)	F8	HPR-2362
9	Faizabad (Masodha)	F9	Chini Kamini
10	Faizabad (Masodha)	F10	Dhusara
11	Faizabad (Masodha)	F11	CR-2027-3
12	Faizabad (Masodha)	F12	CR-2052-3
13	Faizabad (Masodha)	F13	R-1513-806-420-1-1
14	Faizabad (Masodha)	F14	P-2664
15	Faizabad (Masodha)	F15	DJP-19998-11-1-1-1
16	Faizabad (Masodha)	F16	RGL-11226
17	Pant Nagar	D-14	Doft-15
18	Azamgarh Phoolpur	A1	Sambha Mahsuri
19	Basti Harriya	B1	Kalanamak
20	Kumarganj (NDUA &T)	F17	NDR-97
21	Kumarganj (NAUA &T)	F18	NDR-359
22	Faizabad (Masuadha)	F19	CRAC-2223-714
23	Basti (Rudauli)	B2	Tall Mahsuri
24	Kumarganj (NDUA &T)	F20	Swarna

Table 2. Primers used for PCR amplification.

Primer no.	Code used in present study	Primer sequence (5' to 3')
1	OPW-01	CTCAGTGTCC
2	OPW-15	ACACCGGAAC
3	OPR-06	GTCTACGGCA
4	OPAA-09	AGATGGGCAG
5	OPV-07	GAAGCCAGCC
6	OPW-02	ACCCCGCCAA
7	OPW-11	CTGATGCGTG
8	OPR-04	CCCGTAGCAC
9	OPP-05	CCCCGGTAAC
10	OPP-19	GGGAAGGACA
11	OPQ-19	CCCCCTATCA
12	OPR-8	CCCGTTGCCT
13.	OPS-14	AAAGGGGTCC
14	OPS-19	GAGTCAGCAG
15	OPS-04	CACCCCCTTG
16	OPZ-19	GTGCGAGCAA
17	OPAO-18	GTTCTCGAC
18	OPW-16	CAGCCTACCA
19	OPR-04	CCCGTAGCAC
20	OPT-18	GATGCCAGAC
21	OPR-08	CCCGTTGCCT
22	OPS-01	CTACTGCGCT
23	OPZ-13	GACTAAGCCC
24	OPZ-12	TCAACGGGAC
25	OPJ-04	CCGAACACGG
26	OPZ-14	TCGGAGGTTC
27	OPQ-15	GGGTAACGTG

from G. B. Pant University of Agriculture & Technology Pantnagar, Uttarakhand (Table 1).

Morphological Characterization

Morphological characterization was done on the basis of hyphal and sclerotial characteristics depicted by *R. solani* isolates as described by Singh et al. (12).

Virulence Characterization

Virulence characterization was done by artificial inoculation of all the isolates on 10 different rice varieties (like-Sambha Mahsuri, Sarju-52, Kalanamak, NDR-97, NDR-359, Ajaya, PB-1, Swarna, NDR-9930112 and NDR-9830077). The lesion length recorded at day 4, 8, 12 and 16 after inoculation and the

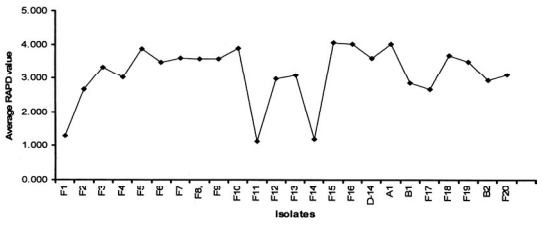

Figure 1. Virulence pattern of 24 *R. solani* isolates on the basis of AUDPC value (the details are given in Table 3).

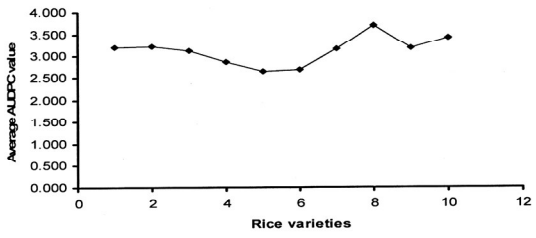

Figure 2. Disease reaction of 10 different rice varieties on the basis of AUDPC value, when inoculated with 24 *R. solani* isolates (the details are given in Table 3).

Table 3. AUDPC values shown by different rice varieties and *R.solani* isolates combinations. Observations are the mean of three replications.V1 to V10 stand for different rice varieties Sambha Mahsuri, Sarju-52, Kalanamak, NDR-97, NDR-359, Ajaya, PB-1, Swarna, NDR-9930112, and NDR-9830077, respectively. F1 to B3 signify 25 *R. Solani* isolates, the details which are given in Table 1.

	Varieties										
Isolates	VI	V2	V3	V4	V5	V6	V7	V8	V9	V10	Average
F1	2.701	1.650	1.531	1.531	1.360	1.267	0.707	0.707	0.707	0.707	1.287
F2	3.798	3.769	3.454	3.420	3.279	2.629	1.794	1.650	1.531	1.347	2.667
F3	4.644	4.617	3.768	3.758	3.734	3.340	3.060	3.010	2.699	0.707	3.334
F4	4.949	3.399	3.289	3.174	3.169	2.793	2.477	2.403	2.305	2.276	3.023
F5	4.909	4.881	4.323	4.048	3.826	3.676	3.470	3.379	3.084	3.000	3.860
F6	4.117	3.851	3.735	3.610	3.518	3.506	3.206	3.142	3.097	2.975	3.476
F7	4.840	4.457	4.424	3.884	3.714	3.624	3.193	2.925	2.743	2.273	3.608
F8	4.989	4.310	4.252	3.948	3.764	3.551	3.176	2.957	2.523	2.406	3.588
F9	4.967	4.817	4.142	3.810	3.565	3.368	3.301	3.202	3.146	1.499	3.582
F10	5.802	4.706	4.281	4.062	4.028	3.767	3.387	3.302	3.189	2.383	3.891
F11	3.304	1.408	1.224	1.105	0.707	0.707	0.707	0.707	0.707	0.707	1.128
F12	4.137	3.906	3.481	3.472	3.463	3.297	2.479	2.351	2.034	1.259	2.988
F13	4.958	4.455	4.130	4.128	3.497	2.958	2.499	1.732	1.408	1.113	3.088
F14	2.336	2.114	1.455	1.267	1.209	0.707	0.707	0.707	0.707	0.707	1.192
F15	5.246	4.589	4.507	4.382	4.074	4.051	3.949	3.833	3.695	2.249	4.058
F16	4.888	4.791	4.589	4.553	4.352	3.928	3.721	3.645	3.255	2.331	4.005
D-14	4.601	4.411	4.158	3.504	3.495	3.333	3.316	3.270	3.038	2.830	3.596
A1	5.911	5.236	4.920	4.369	4.368	4.071	3.585	2.702	2.555	2.323	4.004
B1	4.143	3.557	3.274	3.037	2.865	2.807	2.635	2.515	2.148	1.552	2.853
F17	4.939	3.436	3.370	3.344	3.015	2.743	1.991	1.649	1.541	0.707	2.674
F18	4.918	4.857	4.445	4.270	3.993	3.762	3.149	3.111	2.576	1.687	3.677
F19	4.847	4.481	4.391	3.999	3.902	3.834	3.234	2.638	1.976	1.698	3.500
B2	4.194	4.037	3.956	3.943	3.672	2.362	2.080	1.831	1.723	1.616	2.941
F20	4.882	4.563	4.222	4.004	3.876	3.304	2.505	2.073	0.977	0.707	3.111
Average	4.542	4.012	3.722	3.526	3.352	3.058	2.680	2.477	2.223	1.711	3.130

ANOVA for sheath blight disease severity on 10 different rice varieties when inoculated with *R. solani* isolates.

Source	df	MSS	*F* value	CD at 5%
Replication	2	0.25	3.02	
Variety	9	7.7115	1.9	0.44785
Isolate	24	20.897	1.54	0.44785
Variety × Isolate	216	2.5057	1.22	0.89569
Error	498	0.3133		

disease intensity was calculated by area under disease progress curve (AUDPC) value by following formula (13).

$$AUDPC = \sum_{i=1}^{a} [\{(Y_i + Y_{(i+1)})/2\} X (t_{(i+1)} - t_i)]$$

Where Y_i = disease level at the time t_i,

$\{t_{(ti+1)}$-ti $\}$=time (days) between two disease scores.

Molecular Characterization

DNA Extraction. The mycelialmat of different isolates of *R. solani* was prepared on PDA broth in five replications. Seven day after inoculation the mats were collected by filtering or pressing the mats between blotting sheets (Whattman no. 3) and freeze dried at -20 to— 80 C for long term storage. The procedure for extraction of DNA from *R. solani* isolates was adopted by CTAB method described by Singh et al . (12).

Table 4. Disease reaction of *R. solani* isolates on 10 different rice varieties. HR = Highly resistant, MR = Moderately resistant, HS = Highly susceptible, MS = Moderately susceptible, V= Varieties.

		Disease reaction on different 10 rice varieties									
	Isolates	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
1.	F1	HR	MR	HR	MR	HR	HR	MR	MR	MS	MR
2.	F2	MS	MS	HR	MS	MR	HR	MR	MR	HR	MS
3.	F3	MR	MS	MR	MR	MS	MR	MS	MS	MS	HR
4.	F4	MR	MR	MR	HR	MR	MR	MR	MS	MR	HR
5.	F5	MR	MS	MR	MS	HR	HR	MS	MS	MR	MR
6.	F6	HS	HS	HS	MR	MR	MR	HS	HS	HS	HS
7.	F7	MR	HR	MR	HR	MR	MR	MS	MS	MS	MR
8.	F8	MR	HR	HR	MS	MS	MR	MS	MR	HR	MR
9.	F9	MR	MR	MS	MS	HR	MR	MR	MR	MR	MS
10.	F10	MS	MR	MS	HR	MR	MR	HS	MR	MS	MS
11.	F11	HR	HR	MR	MR	MR	MR	MR	MR	HS	MR
12.	F12	MS	MS	MS	HR	MR	MR	MS	MS	MR	MS
13.	F13	HS	MS	HS	MR	HR	MR	HR	MS	HR	HS
14.	F14	MR	HR	MR	HS	HR	HS	HR	HS	MR	MR
15.	F15	MS	MS	MR	HR	MS	MR	MR	MS	MR	MR
16.	F16	MS	MS	MS	MS	HR	MR	MR	MS	MR	MS
17.	D-14	HS	HS	MR	MR	HR	HS	HR	MR	MR	MR
18.	A1	HR	HR	MR	MR	HR	HR	MR	MR	MS	MS
19.	B1	MR	MR	MS	MS	MR	MR	MS	MR	MR	HR
20.	F17	MR	MS	MR	MR	MS	HR	MS	HS	MS	MS
21.	F18	MR	MR	MS	MR	HR	HR	MR	MS	MS	MS
22.	F19	HR	HR	MR	HR	MS	MS	MS	MS	MR	MS
23.	B2	MR	MR	MS	MR	MR	HR	HR	MS	MS	HR
24.	F20	HS	HS	MR	MS	HS	MR	HR	MS	HR	HS

RAPD Analysis. RAPD analysis of all the 24 isolates was performed as described by Singh et al. (12). The genomic DNA of 24 isolates was amplified with 27 primers out of 27, 9 primers generating reproducible banding patterns and were selected for RAPD analysis (Table 2). All the gels were scored twice manually and independently, the band presence was denoted by 1 and absence denoted by 0. The presence or absence of each fragment was scored for generating similarity coefficients which were used to construct the dendogram by UPGMA computer analysis using computer programme NTSYSPC version 2.1 (14).

Results and Discussion

Morphological Characterization

All the macro-sized sclerotia forming isolates were fast growers (F2, F4, F5, F6, F8, F10, F12, F13, F14, F16. D-14 and F20). Some of the isolates were moderate growers (F7, F15, F19, F17, F18, A1 and B1) while others were slow growers (F1, F3, F9, F11 and B2). The mean colony diameter recorded at 72 h after inoculation and were used for characterization of isolates as fast,moderate or slow growers. The isolates showing >40 mm, 35-40 mm and 30-35 mm mean colony diameter were designated as fast, moderate and slow growers, respectively.

Virulence Characterization of R. solani Isolates on Different Rice Varieties

Results of experiment on the artificial inoculation of 10 rice varieties with all the 24 isolates of *R. solani* belonging to AG-1 1A revealed that disease severity varied among different isolates as well as different rice varieties. Data of the lesion length was analyzed by using AUDPC value (13) (Table 3). The lesion length recorded on 10 different rice varieties depicted four types of disease reactions like highly resistant, moderately resistant, highly susceptible and moderately susceptible (Table 4).

The isolate A1 was most virulent on all the 10 rice varieties, some *R. solani* isolates were highly virulent (F10, F15 and F16) ; most of them were moder-

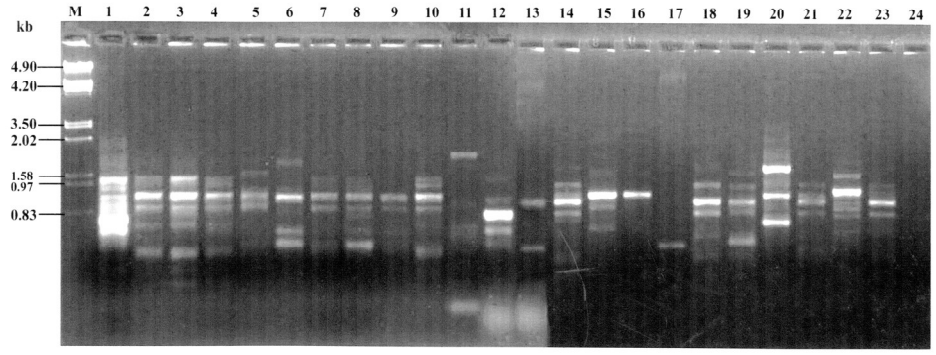

Figure 3. Agarose gel sowing PCR amplification products of *Rhizoctonia solani* isolates obtained with RAPD primer OPP5. M = molecular weight marker, Lanes 1 to 24 correspond to *R. solani* isolates F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12, F13, F14, F15, F16, D14, A1, B1, F17, F18, F19, B2 and F20, respectively.

ately virulent (F12, F13, F4, B1, F17, F3, B2, F20, F6, F9, D14, F7, F8, F19, F18 and F5) and few of them (F1, F11 and F14) were less virulent on the basis of AUDPC value. The varieties NDR-359 and Ajaya depicted highly resistant disease reaction with most of isolates tested whereas the variety Swarna depicted highly susceptible disease reaction (Figs. 1 and 2).

Molecular Characterization Based on RAPD Analysis

The size of RAPD bands ranged from 4.9 kb to 0.83 kb compared with molecular marker lemda DNA/Eco-R1 (Fig. 3). The dendogram constructed from RAPD data using Jaccord's coefficient of similarity and UPGMA clustering was divided into two major clusters. Jaccard's similarity coefficient ranged from 0.13 to 0.82 (Fig. 4). The major cluster 1 was divided in to two minor clusters and the minor cluster I further sub-divided into two sub clusters. Sub cluster I was composed of 4 isolates (F1, F11, F14 and F2) which were micro-sized sclerotia forming, slow growers and less virulent while sub-cluster II was made up of two

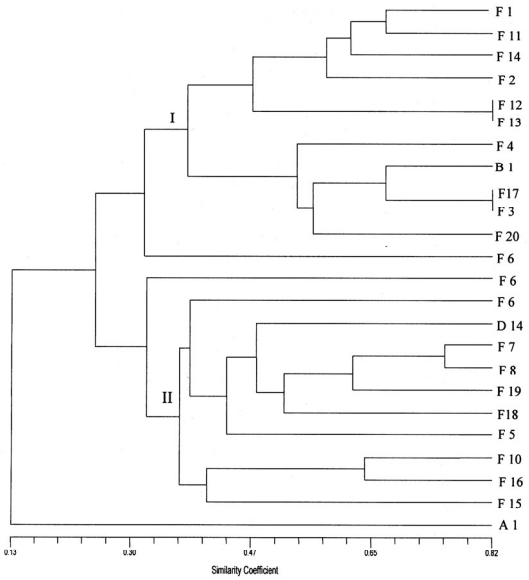

Figure 4. Dendogram of 24 isolates of *R. solani* constructed by UPGMA culture analysis of genetic similarities in RAPD data amplified with 9 primers.

genetically close isolates (F12, and F13) which were micro-sized sclerotia forming, slow growers and less virulent. Minor cluster II was made up of 5 isolates (F4, B1, F17, F3, and F20) which were macro-sized sclerotia forming, moderate growers were showing moderate virulence. The isolates F6 and F9 were closely related with major cluster I and II.

Major cluster II was composed of 12 isolates (F9, B2, D14, F7, F8, F5, F10, F15 and A1) and was divided into two minor clusters. The minor cluster I was composed of 7 isolates (B2, D14, F7, F8, F19, F18, and F5) which were macro-sized sclerotia forming, moderate growers and moderate virulent. Out of 7 isolates, F18 and F19 were micro-sized sclerotia forming, moderate growers and moderate virulent. The minor cluster II was made up of 4 isolates (F10, F16, F15, and A1) in which three isolates (F10, F16 and F15) were micro-sized sclerotia forming, fast growers and highly virulent whereas isolate A1 was macro-sized sclerotia forming in which sclerotia were mostly aggregated near the point of inoculation and it was most highly virulent isolate.

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