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VIRULENCE DIVERSITY OF *RHIZOCTONIA SOLANI* CAUSING SHEATH BLIGHT DISEASE IN RICE AND ITS HOST PATHOGEN INTERACTION

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ABSTRACT

The present investigation is carried out for the identification of resistant genotypes of rice against sheath blight disease and virulence diversity among the isolates of pathogen. This disease is caused by *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris*) is one of the most ubiquitous and destructive soil borne disease. The 12 *R. solani* isolates were collected and characterized based on morphological and sclerotial characters and virulence diversity on 10 different rice varieties. The disease progress and severities were analysed using AUDPC value on the basis of lesion length recorded on 4, 8, 12 and 16 DAI. Evaluation on the basis of AUDPC value, out of 10 varieties of rice, Sarju-52 depicted highly resistant (19.91) while Jaya (21.87), UPR-2005-38 (23.97) and IET-15182 (23.16) showed moderately resistant disease reaction with most of the isolates tested; where as the variety Pusa Basmati-1 (46.57) depicted highly susceptible disease reaction. Among these some isolates were highly and moderately virulent whereas most of the isolates were less virulent. The identified resistant (Sarju-52) and susceptible (Pusa Basumati-1) is the potential source for the breeding programmes for further development of resistant varieties in rice.

INTRODUCTION

Sheath blight of rice is caused by *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris*), which is major constraint to rice production during the last two decades (Kobayashi *et al.,* 2002). In rice, sheath blight caused by *R. solani* is the major constraints hampering the rice production (Ou, 1985). Diversity of different rice isolates of *Rhizoctonia solani* collected from sheath blight infected samples has been studied by morphological characterization (Vijayan, 1985), virulence diversity and pathogenicity testing (Banniza, 1996). Each year, the blight causes up to a 50% decrease in the rice yield under favourable conditions around the world (Zheng *et al.,* 2013).

Rhizoctonia solani emerged as an economically important rice pathogen. The disease infection of and spread severely during late tillering, internodes elongation, booting and flag leaf emergence stage. The disease occurrence and spread was severe due to development of high yielding and high nitrogen fertilizer response varieties which become more susceptible. The disease incidence can be reduced by growing resistant varieties to sheath blight. The genetic variability of the pathogen increases the difficulty encountered in developing resistant host genotypes, as well as in effectively deploying available tolerant cultivars. Unfortunately, at present, there is no known rice varieties which is either immune or possess high degree of resistance to sheath blight disease in Uttar Pradesh, India. The varieties grown particularly in Uttar Pradesh do not possess appreciable amount of resistance to the disease, only moderate or low level of resistance is present. Because of lack of varietal resistance against the disease, there is a need to understand more about virulence pattern of the pathogen and to identify resistance genotypes of rice against sheath blight disease. The complex genetic nature of resistance to sheath blight has contributed to a limited success in breeding for sheath blight resistance using traditional approaches. Variability in pathogen population will help the scientist to understand the races present in pathogenic population and would help to choose the parents in crossing programmes. The virulence pattern of the pathogen is helps to identify the evaluation of pathogenic races and to identify disease susceptible and resistant genotypes. From the above fact our main aim is to investigate the virulence pattern of the pathogen and to identify the resistant lines against the sheath blight pathogen in rice.

MATERIALS AND METHODS

Collection and Isolation

The sheath blight infected samples of rice were collected from different parts of Uttar Pradesh *i.e.,* in Azamgarh, Faizabad,

Basti and Varanasi. A total of 12 isolates of *R. solani* were isolated from various germplasm lines of rice showing typical sheath blight symptoms on rice. The isolates were sub-cultured and purified by transferring the hyphal tip of each isolates into the fresh 2 percent PDA medium.

Cultural/Morphological Characteristics

The isolates of *R. solani* were grown on 2 percent PDA medium, in Petri plates, at 28 ± 3ºC until hyphae had almost reached the periphery of the plates, for studying mycelial, hyphal and sclerotial characteristics. The colour of the colony and sclerotia was determined with the help of Munsell's Soil Colour Chart (Munsell's Colour Company Inc., 1954). The systems proposed by (Burpee *et al*., 1980) were followed for the categorization of colony and scelerotial characteristics. The observations of morphological characteristics like angle of branching, septation, presence of moniloid cells etc., were recorded by placing the Petri plates with fungal growth directly under microscope (Singh *et al*., 2002).

Pathogenicity test

The young, immature 4 days old sclerotia were artificially inoculated in sheath and moisten with sterile water. All the *R.solani* isolates tested were pathogenic to rice. The control plants that were inoculated only with sterile water did not show any symptoms. Isolates of *R. solani* AG-1IA induced typical rice sheath blight symptoms of ellipsoidal shape, with an initially greenish, but later grey, center and dark brown margin.

Collection of different genotypes of rice for testing virulence diversity

The seeds of ten different rice genotypes viz. Narendra-359, Sarju-52, IET-15254, UPRI-2005-38, Pusa Basmati-1, UPR-2760-10-1, IET-15182, UPR 2642-31-1-1, ARC 10539 and Jaya were collected from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradseh. These seeds were grown in pots under green house condition in the Kharif season, in the year 2010-11, for virulence testing of pathogen *R. solani* AG-1 IA anastomosis group.

Sheath inoculation, scoring and disease assessment

Rice (*Oryza sativa* L.) seedlings were raised from 10 different varieties of seeds in 20cm diameter earthen pots by Bi-factorial design of experiment. All plant material was raised in a greenhouse at 25 ± 3ºC. Seedlings that emerged were thinned to five per pot (Singh *et al*., 2000). In green house, second leaf sheath (from the top) at growth stage (GS) 21 in rice were inoculated with a bit (approx. 0.25mg) of four days old immature sclerotia or mycelium of *R. solani* grown on PDA at 28 ± 3ºC. For artificial inoculation, the leaf sheath was opened carefully and inoculum was placed inside the sheath. A few drops of sterilized water were also added to inoculated sheath. Inoculation was done in the evening and inoculated plants were sprayed with water next morning. These plants were maintained in a green house at 28 ± 3ºC. They were examined for appearance of symptoms. The disease severity (lesion length) was assessed 4 days after inoculation. Inoculated plants were re-examined for intra plant spread of the lesion after 40 days of inoculation. All the experiments were carried out in the three replications (Singh *et al*., 2000 and 2001) with Bi-factorial design.

The inoculated plants were regularly examined for appearance of symptoms starting from 48 hours after inoculation and number of lesions and their length on the rice sheath around the inoculation point were recorded from 96 hours after inoculation. The data on disease intensity were recorded on four different dates at four-day intervals i.e. 4^{th}, 8^{th} , 12^{th} and 16^{th} day after inoculation (DAI) (Kumar *et al*., 2008). The Area under Disease Progress Curve (AUDPC) was calculated from disease intensity by using following formula (Chand *et al.,* 2006).

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

Where,

Yi = Disease level at the time ti, {t (i + 1) - ti} = Time days between two disease score. The AUDPC data were analyzed for comparable study of disease resistance of varieties and virulence diversity of pathogens.

Table 1: Analyzed mean AUDPC value of R1, R2 and R3 of different rice genotypes and isolate combination. V_1 to V_{10} stand for different rice varieties *i.e.* Narendra-359, Sarju-52, IET-15254, UPRI-2005-38, Pusa Basmati-1, UPR-2760-10-1-1, IET-15182, UPR 2642-31-1-1, ARC 10539 and Jaya. A-1 to A-11 and D-14 signifies 12 isolates of *R. solani*.

Isolates	Varieties V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	Mean
A-1	46.20	39.93	54.53	38.60	58.26	44.53	27.93	50.73	72.40	39.66	47.28
A-2	8.80	2.66	3.20	0.00	43.46	12.33	25.66	12.66	0.00	0.00	10.88
A-3	36.86	25.86	50.53	42.20	51.86	50.60	29.53	45.13	38.60	39.73	41.09
A-4	11.73	16.06	44.13	25.53	42.26	0.00	0.00	8.93	13.33	5.66	16.77
A-5	38.53	42.93	43.26	41.00	68.26	46.60	28.73	41.13	44.46	29.53	42.45
A-6	26.60	17.73	27.86	28.40	40.46	53.33	19.86	25.60	17.86	15.20	27.29
A-7	17.66	3.13	25.66	10.40	43.93	24.13	12.20	24.93	15.46	27.20	20.45
D-14	57.26	38.00	57.26	39.00	53.66	51.40	46.80	62.00	50.40	36.40	49.22
A-8	15.13	6.00	24.66	9.40	38.13	8.33	19.93	13.06	8.46	10.00	15.31
A-9	31.93	0.00	39.00	36.20	35.26	57.73	31.60	21.33	20.86	17.93	29.19
A-10	10.66	17.80	15.26	10.93	30.00	7.73	0.00	5.66	4.73	0.00	10.28
A-11	14.26	28.80	35.33	5.93	53.20	31.20	35.60	31.80	46.60	41.13	32.39
Mean	29.31	19.91	35.06	23.97	46.57	32.33	23.16	28.58	27.77	21.87	28.55

CD at 5%: For Isolates: 4.56; for Varieties: 4.16; for Varieties × Isolates: 14.43; SEM: For Isolates: 2.32; for Varieties: 2.12; for Varieties × Isolates: 7.36

Table 2: Host pathogen interaction and disease reaction on 10 different rice varieties and 12 *R. solani* isolate in combinations

Isolates	Varieties V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
A-1	S	MS	S	MS	HS	S	MR	S	HS	MS
A-2	R	R	R	R	S	R	MR	R	R	R
A-3	MS	MR	S	MS	S	S	MS	S	MS	MS
A-4	R	MR	S	MR	MS	R	R	R	R	R
A-5	MS	MS	S	MS	HS	S	MR	MS	S	MS
A-6	MR	MR	MR	MR	MS	S	MR	MR	MR	MR
A-7	MR	R	MR	R	S	MR	R	MR	MR	MR
D-14	S	MS	HS	MS	S	S	S	HS	S	MR
A-8	MR	R	MR	R	MS	R	MR	R	R	MS
A-9	MS	R	MS	MS	MS	HS	MS	MR	MR	MR
A-10	R	MR	MR	R	MS	R	R	R	R	R
A-11	R	MS	MS	R	S	MS	MS	MS	S	MS

R = Resistant MR = moderately resistant S = Susceptible; MS = moderately susceptible HS = Highly susceptible

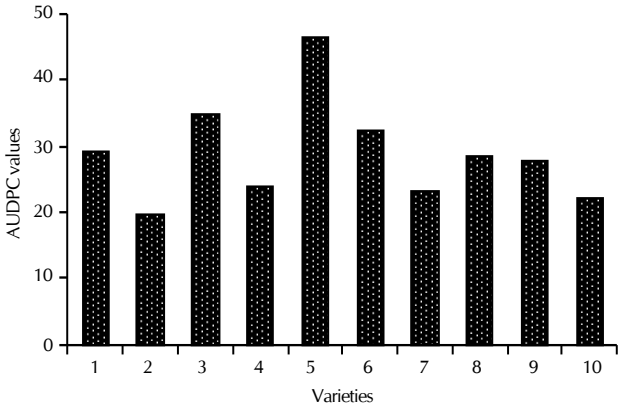

Figure 1: The mean AUDPC values on 10 different rice varieties with respect to all the 12 *R .solani* isolates. The numbers 1 to 10 stand for different rice varieties (Narendra-359, Sarju-52, IET-15254, UPRI-2005-38, Pusa Basmati-1, UPR-2760-10-1-1, IET-15182, UPR 2642-31-1-1, ARC 10539 and Jaya).

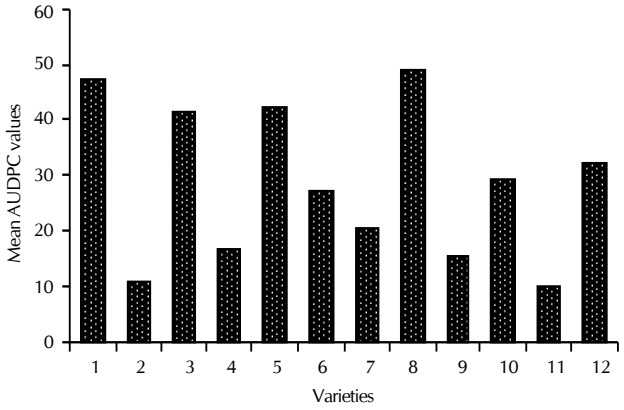

Figure 2: The mean AUDPC value of 12 *R. solani* isolates with respect to all the 10 different rice varieties. The numbers 1 to 12 stands for different *R. solani* isolates (A-1, A-2, A-3, A-4, A-5, A-6, A-7, D-14, A-8, A-9, A-10 and A-11)

Statistical analysis

The bi-factorial design was followed in this whole experiment and the data analysed by two factor involving virulence of the pathogen and resistant in the plant. The data analysed was the replicated AUDPC values observed on genotypes by incitation of pathogen.

RESULTS AND DISCUSSION

Morphological characterization of *R. solani* isolates were observed on the basis of hyphae colour in Petri plates on the 2 percent PDA medium and found that 12 isolates showed differential hyphae colour of very pale brown to light yellowish brown. We observed that among the 12 isolates, some of them formed macro-sclerotia (A-1, A-3, A-5, A-11 and D-14) which are dark brown but varied among their size and weight of sclerotia differed majority of isolates forms surface sclerotia are fast growers (A-1, A-3, A-5 and D-14) then embedded sclerotia are slows growers (A-2, A-4, A-7, A-8 and A-10) on the PDA medium. The criteria for fast growing isolates fixed on the basis of >45mm, moderate growers 35-45mm and slow growers <35mm were mean colony diameter 48h after inoculation on PDA medium at 28 ± 2ºC. The size and weight of the sclerotia were low (< 0.35 mg/ sclerotia) of A-2, A-6, A-10, Medium (0.35-0.70mg/ sclerotia) of A-4, A-7, A-8, A-9, A-11 and High (>0.70 mg/sclerotia) A-1, A-3, A-5 and D-14. Similarly, morphological characterization of *R. solani* isolates were done on the basis of mycelial colour, size and position of sclerotia in Petri-plate on the PDA medium (Banniza *et al.,* 1996; Sherwood *et al.,* 1969; Vijayan, *et al.,* 1985; Vilgalys and Cubeta, 1994).

Virulence characterization of *R. solani* isolates on different varieties of rice

Results obtained on the artificial inoculation of 12 isolates on 10 different germplasm of rice showed different virulence pattern, disease severity and disease progress among different isolates. The incubation period of sheath blight 48h after inoculation varied in different rice varieties and *R. solani* isolates combinations. Depending on the rice variety × isolates combinations the 96h after artificial inoculation of 12 isolates showed variable lesions length and number of lesions produced on the rice. Data of lesion length were analyzed using the AUDPC (Chand *et al.,* 2006). Virulence characterization of 12 isolates of *R. solani* was done on the basis of AUPDC values of disease on rice (Singh *et al.,* 2002; Kumar *et al.,* 2008). The isolate D-14 (49.22) was most virulent on all the 10 varieties. Some isolates were highly virulent, A-1

(47.28), A-3 (41.09), A-5 (42.45) and D-14 (47.28). Some of the isolates were moderately virulent, A-6(27.29), A-9 (29.19), A-11 (32.39) and few of them A-2 (10.88), A-4 (16.77), A-7 (20.45), A-8 (15.31) and A-10 (10.28) were less virulent (Table 1 and Fig. 1)

Degree of resistance of rice against *R. solani*

Out of ten varieties were tested for sheath blight disease resistance. The variety Sarju-52 showed less AUDPC value of 19.91 has depicted significantly resistant among all the varieties; followed by Jaya (21.87), IET-15182 (23.16) and UPR-2005-38 (23.97) exhibited moderately resistant; Narendra-359 (29.31), UPR-2642-31-1-1 (28.58) and ARC 10539 (27.77) showed moderately susceptible and IET-15254 (35.06), UPR-2760-10-1-1 (32.33) depicted susceptible, Pusa Basmati-1 (46.57) showed highly susceptible disease reaction (Table.1, 2 and Fig.1). Similarly an experiment was conducted with some varieties of rice for resistance to sheath blight disease and observed that 17 varieties were moderately resistance, 12 were susceptible and one variety showed resistance (Biswas, 2011).

Our results indicate that multinucleate *R. solani* AG-1 IA isolates showed different morphological, virulence, disease severity and disease interaction among different isolates. The isolate D-14 was most virulent and isolates A-4, A-7 and A-10 were less virulent. The similar kind of virulence pattern of *R.solani* isolates were observed and reported by Singh *et al.* (2001) and Kumar *et al*. (2008) causing sheath blight disease in rice. The correlations between morphological and virulence characterization of the *R. solani* isolates were found that, all the macro sclerotia forming isolates were fast growers and highly virulent, while micro sclerotia formers were slow growers and less virulent (Singh *et al*., 2000). The disease progress and disease interaction based on Area under Disease Progress curve scores and values results was similar coincidence with Chand *et al*., 2006 and Taheri *et al*., 2007. Our results showing that the sheath blight resistance genotypes and host pathogen interaction were clearly investigated based on IRRI Standard Evaluation Scale for sheath blight resistance in rice. Out of 10 varieties the Sarju-52 depicted highly resistant to sheath blight, similar kind of investigation and results were obtained in breeding lines of rice by Jia *et al*., 2012. The rice varieties grown in India do not possess an appreciable amount of resistance sheath blight disease. The identified resistant lines may be a potential source for further development of resistant genotypes against rice sheath blight disease.

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