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Virulence prediction of *Rhizoctonia solani* Kuhn isolates based on pathogenic and cultural variability

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Mycelial colony and sclerotial characteristics and virulence of ten *Rhizoctonia solani* Kühn isolates collected from ten diverse locations under five districts of West Bengal were studied. Based on vertical disease index (VDI) four isolates were graded as virulent under both glass house and field conditions but the virulence was more under the latter. Mycelial dry biomass production was found to have positive correlations with mycelial growth rate on detached leaf sheath and potato dextrose agar medium. Some isolates had thin to dense mycelial growth; others had high to moderately fluffy cottony appearance. Colony colour on upper and lower surfaces for majority of the isolates was creamish, but in a few it was creamish with radiating brownish tints on upper surface and as rays of chocolate brown bands radiating towards periphery at lower surface. Hyphal width for most of the isolates ranged from 7.7 – 15.4 µ. Sclerotial initiation of isolates ranged from 3 -5th day after inoculation and it was formed at or near periphery, at periphery and vertical side of the Petri plate, at periphery but submerged in hyphal growth, in the form of concentric ring or circular pattern surrounding the centre, at a place 3 – 4 cm away from and surrounding the point of inoculation. Sclerotia produced by the isolates were light-, dark-, chocolate - brown with or without exudates and were mostly pitted except one with hairy surface. Sclerotia were mostly solitary but presence of sclerotial aggregation at low level was observed in all isolates. Total number and weight of sclerotia per plate as well as average weight and size of individual sclerotia of isolates varied significantly. Working out of simple correlation matrix based on eight quantitative mycelial and sclerotial parameters revealed that radial growth rate on potato dextrose agar medium (PDA), mycelial growth rate on detached leaf sheath and mycelial dry biomass had negative correlation with average VDI i.e virulence. The average radial growth rate (X_2), average weight (X_5)-, length (X_6)- and breadth of sclerotia (X_7) were the four independent factors identified by step down regression of having significant influence on VDI/ virulence. Of them average radial growth rate (X_2) played a determinative role. X_1 i.e mycelial growth rate on detached leaf sheath though insignificant still influenced the level of significance of factors X_5, X_6 and X_7. So, best prediction model for sheath blight disease severity *vis-à-vis* pathogen virulence (Y) based on some mycelial and sclerotial characteristics would be $Y = 2.228 - 0.249 X_1 - 1.724 X_2^{**} + 21.755 X_5^{*} - 0.010 X_6^{*} - 0.012 X_7^{*}$

Key words: *Rhizoctonia solani*, isolates, variability, sheath blight, virulence, prediction

INTRODUCTION

The sheath blight (C.O: *Rhizoctonia solani* Kühn) is one of the most threatening, devastating and yield limiting diseases of rice worldwide (Rush and Lee, 1992). It is now considered as the second most important disease of rice next to blast (Webster and Gunnel, 1992) and can cause severe crop losses to the extent of 5.9 – 69% (Venkat Rao *et al.*, 1990; Naidu, 1992) especially when semi-dwarf rice cultivars are grown under intensified cultural practices (Xie *et al.*, 1990). The incidence and severity of this disease has been found to vary according to host genotypes, virulence of the pathogenic isolates, climatic factors and cultural practices. Of these, development of virulence of the pathogenic isolates, which may arise due to mutation/ sexual reproduction / heterokaryosis, is considered as one of the most important causes of varying degrees of sheath blight disease intensity (Taheri *et al.*, 2004). It gives advantage to the fungus to attack a vast array of host genotypes, to gain greater survival capacity and differential fungicide sensitivity. As a consequence, the disease occurring on same host in varying intensity even under nearly same set of

environmental conditions in different locations is now becoming increasingly difficult to manage by cultural/ biological/ chemical means or through the manipulation of host resistant genes. Non - availability of suitable resistant donors for the development of sheath blight resistant cultivars makes the situation more severe. Under the circumstances, the study on variability of the fungal population collected from diverse geographical areas using morphological / biochemical / molecular markers/ tools, finding out the existence of any correlation of these parameters with pathogen's virulence and identifying some critical parameters if possible for prediction of pathogen's virulence are essential for the formulation of any effective management strategy. In the present investigation attempts have been made to study the variability of *R. solani* isolates based on some cultural and morphological characteristics and tried to correlate some characters with the virulence of the pathogen. Attempt has also been made to identify critical and contributory parameter(s) for the prediction of pathogen's virulence.

MATERIALS AND METHODS

Collection of diseased samples and isolation of pathogen

Sheath blight (C.O. *Rhizoctonia solani*) infected leaf sheaths of *'Swarna Mashuri'* (MTU- 7029) rice cultivar were collected from ten locations under five districts of West Bengal. Ten *R. solani* (RS) isolates namely RS-1 was isolated from Kakdwip and RS – 2 from Basanti under South 24 Parganas, RS - 4 from Majhian under South Dinajpur, RS- 5, RS - 6, RS - 7, RS-8 and RS - 9 from Haringhata, Chakdah - I, Chakdah II, Barasat- I and Barasat- II respectively under Nadia, RS -11 from Usatpur under Midnapore (East) and RS -13 from Kantadanga under Hooghly districts. At the time of isolation diseased plant parts of 1.0 x 0.5 cm size after surface sterilization with 0.1 % mercuric chloride ($HgCl_2$) solution for 45 seconds followed by 5 - 6 times serial washing with sterile distilled water were placed at the centre of a Petri plate containing 20 ml of sterilized water agar medium supplemented with antibiotic chloramphenicol @ 100 mg/ litre. Inoculated plates were incubated at 28 ± 1°C for 48-72 hrs. Mycelial growth that radiated from the diseased tissue into water agar medium was picked up and inoculated on to the fresh potato dextrose agar (PDA) slant. Slants were then incubated at 28 ± 1°C till the formation and maturation of the sclerotia, checked scrupulously for any contamination and kept them in freeze at 5°C for future experimental use.

Determination of virulence

For establishment of virulence, twenty one days old seedlings of *Swarna Mashuri* rice cultivar were transplanted in earthen pots (20 cm height and 15 cm diameter) containing 3 kg alluvial soil (sand- 51.5%, silt – 28.4%, clay – 19.3%, pH – 6.8 – 7.0, organic C(%) – 0.44, N(%) 0.04, available P – 20 ppm, available K – 140 ppm) under glass house condition at Kalyani and under field condition having plot size of 2 m x 2 m at Regional Research Sub-Station, Chakdah , under New Alluvial Zone in Nadia in three replications with recommended spacing, seedlings per hill, fertilizer doses and other agronomic practices as and when required. After 45 days of transplanting, three plants, each separately from a hill of each replication, were inoculated inserting 1.0 cm x 0.5 cm sized colonized leaf sheath at the middle position of the inner side of outer leaf sheath, wrapped the point of inoculation with absorbent cotton soaked with sterilized water and bound the soaked cotton thereafter with 1 cm broad polyethylene strap and small cotton thread. Visible symptom of the disease was first noticed in between 8 – 10 days after inoculation under glass house and 6 - 8 days under field conditions. Final data on the length of lesion and plant height were taken at 20 days after inoculation for working out the vertical disease index (VDI), a ratio of length of lesion and plant height, as a scale for determination of virulence. Virulence of the isolates was graded as very very less virulent to highly virulent as per modified 0 – 9 scale (IRRI, 1980) based on VDI. The description of the 0 – 9 scale was - 0 scale = very very less virulent (VDI range 0 and average VDI = 0), 1 scale = very less virulent (VDI range 0.10 – 0.19 and average VDI = 0.15), 3 scale = less virulent (VDI range 0.20 – 0.37 and average VDI = 0.28), 5 scale = moderately virulent (VDI range 0.38 – 0.60 and average VDI = 0.40), 7 scale = virulent (VDI range 0.61 – 0.89 and average VDI = 0.75) and 9 scale = highly virulent (VDI range 0.90 or more and average VDI = 0.96).

Studies on mycelial, colony and sclerotial characteristics

Mycelial dry biomass production on potato

dextrose broth (PDB), mycelial growth rate on PDA medium and detached leaf sheath were studied as mycelial characteristics; mycelial growth pattern, colony colour and texture, colour of the medium at the underside of Petri plate were recorded as colony characteristics; colour, initiation and distribution pattern of sclerotia, number and weight of sclerotia per Petri plate, presence or absence of aggregated sclerotia, surface texture and size of sclerotia etc. were examined as sclerotial characteristics. For mycelial, colony and sclerotial characteristics studies 9 millimeter discs of 48 hrs old *R. solani* isolates' cultures were inoculated separately at the centre of 9 cm Petri plate containing 20 ml of PDA and at 250 ml borosil conical flask containing 40 ml PDB. Three replications for each isolate were maintained. Inoculated Petri plates and conical flasks were incubated at 28 ± 1°C for 15 and 6 days respectively. Radial growth of the isolates was measured at 24 hrs interval up to 72 hrs and then the growth rate per hour was determined. Colony morphological characters like mycelial growth pattern, colony colour and texture, colour of the medium at the underside of Petri plate were recorded within 3 – 5 days after inoculation from the same Petri plates used to study mycelial growth rate. Colour, initiation and distribution pattern of sclerotia on the PDA medium were recorded in between 4 - 8 days of growth. The sclerotial number, weight per plate, aggregation presence or absence, surface texture and size were measured from matured sclerotia harvested at 15 days after inoculation. Observation on sclerotial surface texture and measurement of their sizes were made with the help of stereo zoom binocular microscope. However, mycelial mats developed on PDB were harvested on a pre-weighted filter paper and oven dried at 65±1°C to a constant weight for getting dry mycelial biomass. Small bits of actively growing hyphae from 48 hrs old culture of each isolate grown separately for the measurement of hyphal width were taken on the slides, stained with lactophenol - cotton blue. Width of the hyphae was then measured with the standardized ocular micrometer under high power of the compound microscope.

Statistical analysis

The data on VDI under glass house condition, mycelia growth rate on PDA medium and detached leaf sheath and other quantitative sclerotial parameters were analyzed following completely randomized design (CRD) whereas VDI under field condition was analyzed following completely randomized block design (CRBD). Simple correlation matrix and multiple regression (step down) analyses of parameters were done using MSTAT software. Multiple regression analysis for prediction of VDI *vis-à-vis* virulence was done using the equation $Y = b_0 + b_1X_1 + b_2X_2 .... b_nX_n$ where Y = Predicted VDI/ virulence as dependent variable ; b_o = intercept; b_2, b_2 .....b_n = regression co-efficient of independent variables X_1, X_2, ...X_n respectively. Step down multiple regression analyses were done to identify the most critical contributory parameter(s) towards the increment of VDI/ virulence.

RESULTS AND DISCUSSION

Ten *R. solani* isolates collected from different geographical locations were inoculated on highly susceptible *Swarna Mashuri* (MTU – 7029) rice cultivar under both the glass house and field conditions with a view to know whether the isolates differ in their degree of virulence under two sets of environmental conditions. Observation on the first appearance of the disease symptom indicated that the time of onset of disease varied under glass house and field condition. The disease appeared early (i.e within 6 - 8 days after inoculation) in more severe form under field than glass house condi-

Table 1: Determination of degree of virulence of *R. solani* isolates under glass house and field conditions

R. solani isolates	*Degree of virulence based on vertical disease index (VDI) under*		
	Glass house	Field condition	Averag
RS-1	0.74^{aV}	0.89^{aV}	0.81^{aV}
RS-2	$0.35^{de\,LV}$	$0.53^{e\,MV}$	$0.44^{f\,MV}$
RS-4	$0.35^{de\,LV}$	$0.49^{e\,MV}$	$0.42^{f\,MV}$
RS-5	$0.27^{ef\,LV}$	$0.36^{f\,LV}$	$0.31^{g\,LV}$
RS-6	$0.46^{cd\,MV}$	$0.63^{d\,V}$	$0.54^{e\,MV}$
RS-7	$0.64^{ab\,V}$	$0.74^{bc\,V}$	$0.69^{bc\,V}$
RS-8	$0.53^{bc\,MV}$	$0.69^{cd\,V}$	$0.61^{de\,V}$
RS-9	$0.62^{ab\,V}$	$0.73^{bc\,V}$	$0.66^{cd\,V}$
Rs-11	$0.0.69^{a\,V}$	$0.82^{ab\,V}$	$0.76^{av\,V}$
RS-13	$0.20^{f\,VLV}$	$0.29^{f\,LV}$	$0.24^{g\,LV}$
SEm ±	0.044	0.031	0.026
CD 0.05P	0.130	0.091	0.078

V = Virulent, MV = Moderately virulent, LV = Less virulent, VLV = Very less virulent.

tions. Besides, *R. solani* isolates exhibited differential virulence toward the susceptible *Swarna Mashuri* cultivar (Table 1). On the basis of VDI - scoring of field grown plants, six isolates *viz.* RS – 1, RS – 6, RS – 7, RS – 8, RS – 9 and RS – 11 were graded as virulent; two isolates *viz.* RS – 2 and RS – 4 as moderately virulent and two isolates *viz.* RS – 5 and RS – 13 as less virulent. Under glass house grown plants, four isolates *viz.* RS – 1, RS – 7, RS – 9 and RS – 11 were graded as virulent, two isolates *viz.* RS – 6 and RS – 8 as moderately virulent, three isolates *viz* RS – 2, RS – 4 and RS – 5 as less virulent and one *viz.* RS – 13 as very very less virulent. With a few exceptions, the isolates tested for virulence under field condition exhibited parallel trend with those obtained from glass house grown condition. The isolates RS – 6 and RS – 8 graded moderately virulent under glass house condition became virulent under field situation as exception. In the same way isolates RS – 2 and RS – 4 marked less virulent and RS – 13 marked very less virulent under glass house condition became moderately virulent and less virulent respectively under field condition. It was indicative that the virulence of some isolates was enhanced under field than glass house conditions.

Differential mycelial growth rate on PDA medium and detached leaf sheath, and biomass production ability of *R. solani* isolates on PDB were observed during experimentation (Table 2). Some isolates exhibited higher radial growth rate in between 0 – 24 hrs whereas others in between 24 – 48 hrs. However, radial growth rate averaged over 0 – 48 hrs indicated that RS – 13 had higher value of this parameter than RS – 2, RS – 5, RS – 6 and others whereas RS – 11 exhibited lower. Mycelial growth rate on detached leaf sheath was scaled at higher range in RS – 6 followed by RS – 13, RS – 5 and others whereas the same was measured at lower range in RS – 7, RS – 9 and RS – 4. The growth behavior of isolates on broth culture with respect to dry biomass production indicated that RS – 2 had the highest dry biomass yield followed by RS – 13 and others whereas RS - 8 had the lowest and at par with RS – 4, RS – 9 and RS – 11. There were positive correlations of mycelial growth rate on PDA medium with mycelial growth rate on detached leaf sheath and mycelial dry biomass yield on PDB (Table 5).

There were variations in colony characteristics and hyphal width of *R. solani* isolates grown on PDA medium (Table 3). Dense, highly fluffy cottony growth as colony morphology distinguished RS – 8 from rest of the isolates even from closely re-

Table 2: Mycelial dry biomass yield and mycelial growth rate of different *R. solani* isolates

R. solani isolates	Radial growth rate (mm/hr) of mycelia on PDA between			Mycelial growth rate (mm/hr) on detached leaf sheath	Dry biomass production (mg) on 60 ml potato dextrose broth after 6 days
	0 – 24 hrs	24 – 48 hrs	0 – 48 hrs (Average)		
RS - 1	1.34 ^{d}	1.55 ^{bc}	1.45 ^{c}	1.72 ^{d}	387.7 ^{b}
RS – 2	1.59 ^{ab}	1.46 ^{cd}	1.53 ^{bc}	2.00 ^{c}	462.3 ^{a}
RS – 4	1.38 ^{cd}	1.33 ^{d}	1.36 ^{d}	1.52 ^{ef}	325.3 ^{c}
RS – 5	1.48 ^{bc}	1.69 ^{ab}	1.59 ^{b}	2.10 ^{b}	409.0 ^{b}
RS – 6	1.60 ^{ab}	1.45 ^{cd}	1.53 ^{bc}	2.25 ^{a}	402.3 ^{b}
RS – 7	0.99 ^{f}	1.41 ^{cd}	1.20 ^{e}	1.43 ^{f}	393.0 ^{b}
RS – 8	1.19 ^{e}	1.48 ^{cd}	1.34 ^{d}	1.60 ^{e}	314.7 ^{c}
RS – 9	1.28 ^{de}	1.10 ^{e}	1.19 ^{e}	1.48 ^{f}	344.3 ^{c}
RS – 11	1.07 ^{f}	1.39 ^{cd}	1.23 ^{e}	2.03 ^{bc}	329.0 ^{c}
RS – 13	1.69 ^{a}	1.77 ^{a}	1.73 ^{a}	2.12 ^{b}	431.7 ^{ab}
SEm ±	0.039	0.057	0.033	0.030	14.6
CD 0.05P	0.116	0.169	0.097	0.088	43.0

Table 3 : Colony characteristics and hyphal diameter of *R. solani* isolates on potato dextrose agar medium

R. solani isolates	Colony morphology	Colour of colony	Colour at the under side of medium	Hyphal width (μ)
RS-1	Moderately fluffy, aerial hyphae over grown on to the lid of Petri plate mostly from periphery as also from the middle of plate	Creamish	Creamish with rays of chocolate band at periphery	11.6 - 15.4
RS-2	Moderately fluffy, aerial hyphae over grown like RS-1	Creamish	Creamish	3.9 - 15.4
RS-4	Thin to slightly fluffy, scanty powdery appearance of hyphae on upper lid of Petri plate	Whitish to creamish	Creamish	7.7 - 15.4
RS-5	Thin mycelial growth, aerial hyphae fluffy and grow on lid of Petri plate	Creamish with brownish tints on radial growth	Creamish with brownish rays of band at periphery	7.7 - 15.4
RS-6	Moderately fluffy, aerial hyphae over grown on lid of Petri plate	Creamish	Creamish	7.7 – 13.5
RS-7	Thin mycelial growth, aerial hyphae thin	Creamish	Creamish	7.2 – 7.7
RS-8	Dense, fluffy cottony growth mostly at the periphery, aerial mycelium fluffy, powdery appearance of aerial mycelium on upper lid of Petri plate	Whitish	Creamish yellow with faint chocolate rays	7.7 - 9.7
RS-9	Dense, fluffy mycelial growth but less than RS-8, white concentric zone present, mycelium clouded at the periphery	Creamish	Creamish yellow	3.9 - 7.7
RS-11	Thin mycelia spreading with thin aerial mycelium	Creamish	Creamish yellow	7.7 - 11.6
RS-13	Moderately fluffy mycelium	Dark creamish	Creamish with chocolate bands at periphery	3.9 - 15.4

sembled RS – 9. Other isolates had either moderately fluffy or thin mycelial growth. Colours of the colony of most of the isolates on upper and lower surfaces appeared creamish. But in case of RS - 5 the upper surface exhibited creamish with radiating brownish tints and lower surface viewed as rays of chocolate brown bands radiating towards periphery. The same type of banding pattern at the lower surface was also observed in RS – 1, RS – 8 and RS – 13 though they differed in colony colour on the upper surface. Besides colony morphology, hyphal width was measured as one of the mycelial parameters. For most of the isolates it ranged from 7.7 – 15.4 μ but it was measured at the lower range, 3.9 – 7.7μ in RS – 9 and at higher range, 11.6 – 15.4 μ, in RS – 1.

Besides colony characteristics, *R. solani* isolates exhibited significant and differential variations on sclerotial characteristics (Table 4). Sclerotial initiation date, formation pattern, colour, surface textures, aggregation, total number, total and average weight of sclerotia, size of the sclerotia were the parameters recorded as sclerotial characteristics. Sclerotial initiation of five isolates started on 3rd day, four on 4th day and one on 5th day after inoculation. There were variations in sclerotial formation of isolates. Sclerotia formed at (RS – 1) or near (RS – 9) periphery, at periphery and vertical side of the Petri plate (RS – 5 and RS – 13), at periphery but submerged in hyphal growth (RS - 8), in the form of concentric ring (RS - 4) or circular pattern (RS – 7) surrounding the centre, at a

Table 4: Sclerotial characteristics of *R. solani* isolates on potato dextrose agar medium

R. solani isolates	Day of initiation	Pattern of development	Colour	Total no./ Petri plate	Total weight (g)	Average weight (g)	Surface texture	Aggre-gation	Average length (µ) ± Std. deviation	Average breadth (µ) ± Std. deviation
RS – 1	3rd	Form at the periphery	Dark chocolate with exudates	37.8 cd	18.9 cde	0.50 g	Pitted	+	550 ± 64 (432-618)	422 ± 74 (382 – 576)
RS – 2	3rd	Scattered throughout plate	Light brown with exudates	26.7 de	17.4 de	0.65 f	Hairy surface but pitted	+	713 ± 125 (520 – 912)	577 ± 102 (467 – 739)
RS – 4	3rd	Concentric ring surrounding centre	Dark brown with exudates	56.7 a	28.8 bc	0.51 g	Pitted	+	576 ± 116 (396 – 766)	445 ± 79 (255 – 667)
RS – 5	5h	Form near periphery, mostly at rim, in a pattern of ring	Dark brown with exudates	34.3 cd	9.4 e	0.27 i	Pitted	+	315 ± 65 (255 – 405)	220 ± 39 (198-312)
RS – 6	3rd	Scattered throughout plate	Light to dark brown without exudates	52.2 ab	21.4 cd	0.41 h	Pitted	+	463 ± 60 (358 – 517)	351 ± 56 (286 – 409)
RS – 7	4th	Form circular pattern surrounding centre	Light to dark brown with exudates	42.3 bc	60.1a	1.42 d	Pitted	+	1575 ± 227 (1375 – 1886)	1250 ± 123 (1089 – 1324)
RS – 8	4th	Form near periphery, submerged in hyphal growth	Dark brown with exudates	16.3 e	34.9 b	2.14 c	Pitted	+	2403 ± 215 (2335 – 2718)	1864 ± 136 (1762 – 2015)
RS – 9	3rd	Form near the periphery	Light to dark brown with exudates	5.3 f	11.6 de	2.19 b	Pitted	+	2352 ± 189 (2250 – 2673)	2021 ± 276 (1825 – 2322)
RS – 11	4th	Form at 3-4 cm away from and surrounding centre	Dark brown with exudates	36.3 cd	32.2 b	0.89 e	Pitted	+	924 ± 174 (778 – 1162)	833 ± 127 (650 – 1056)
RS - 13	4th	Form at periphery and vertical side of plate	Dark brown with exudates	16.7 e	37.3 b	2.23 a	Pitted	+	2320 ± 186 (2134 – 2558)	2092 ± 238 (1786 – 2253)
SEm ±				3.6	3.4	0.01				
CD 0.05 P				10.8	9.9	0.03				

Table 5: Correlation matrix of mycelial and sclerotial parameters with average vertical disease index i.e virulence

	Mycelial growth rate (mm/ hr) on detached leaf sheath (x_1)	Average radial growth rate (mm/ hr) on PDA (x_2)	Mycelial dry biomass (mg) on PDB (x_3)	Total no. of sclerotia/ Petri plate (x_4)	Average weight of sclerotia (mg) (x_5)	Length of sclerotia in µ (x_6)	Breadth of sclerotia in µ (x_7)	Average vertical disease index (Y) i.e virulence
x_1	1.000							
x_2	0..727 *	1.000						
x_3	0.544	0.702*	1.000					
x_4	0.115	-0.001	-0.017	1.000				
x_5	-0.364	-0.192	-0.231	-0.781**	1.000			
x_6	-0.391	-0.214	-0.250	-0.775**	0.998**	1.000		
x_7	-0.339	-0.176	-0.214	-0.786**	0.999**	0.994**	1.000	
Y	-0.444	-0.752*	-0.466	0.044	-0.011	0.026	-0.006	1.000

PDA = Potato dextrose agar; PDB = Potato dextrose broth; **P> 0.01 r = 0.765 for 8 df; *P> 0.05 r = 0.632 for 8 df;

Table 6: Determination of critical independent parameter(s) for virulence prediction of *R. solani*

Multiple regression equation	Co-efficient of determination (R^2)	Adjusted R^2	Multiple correlation (R)
$Y = 2.180 + 0.1005\ X_1 - 1.238\ X_2^{*} + 0.00011X_3 - 0.0034\ X_4 + 19.371X_5 - 0.009X_6 - 0.0107X_7$	0.858	0.362	0.926
$Y = 2.203 + 0.099\ X_1 - 1.2195\ X_2^{**} - 0.0036\ X_4 + 19.431X_5^{*} - 0.009X_6 - 0.0107X_7^{*}$	0.858	0.573	0.926
$Y = 2.284 - 1.1283\ X_2^{**} - 0.0038\ X_4 + 19.595X_5^{*} - 0.0092X_6^{*} - 0.0107X_7^{*}$	0.849	0.661	0.921
$Y = 2.002 - 1.0619\ X_2^{**} + 18.320X_5^{*} - 0.0086X_6^{*} - 0.0099X_7^{*}$	0.811	0..659	0.900

**P> 0.01; *P > 0.05

place 3 – 4 cm away from and surrounding the point of inoculation. Formation of sclerotia on the lid of the Petri plate was noticed only in RS – 8. There were variations in the colour of sclerotia. Based on pigmentation, sclerotia of isolates could be grouped into dark brown with exudates (RS – 4, RS – 5, RS – 8, RS – 11 and RS – 13), light to dark brown with (RS – 7 and RS – 9) or without (RS – 6) exudates, dark chocolate - and light brown- exudates on its surface. Sclerotia were mostly solitary but presence of sclerotial aggregation was observed in all isolates. Surface textures of sclerotia of most of the isolates were pitted but RS – 2 had hairy surface. Total number of sclerotia per plate were found to be significantly higher in RS – 4 (56.7) and RS – 6 (52.2) and significantly lower in RS – 9 (5.3) than all other isolates. Total weight of sclerotia per plate was higher in RS – 7 (60.1 mg) and lower in RS – 5 (9.4 mg) as compared to other isolates. Average weight and size of individual sclerotia remained at the higher range in RS – 13, RS – 9 and RS – 8 and the same was recorded lower range in RS – 5, RS – 1 and RS – 4.

From the calculation of simple correlation matrix using eight quantitative mycelial and sclerotial parameters *viz*. average VDI, mycelial growth rate on detached leaf sheath, average radial growth rate

on PDA medium, mycelial dry biomass, total no. of sclerotia per plate, average weight, length and breadth of sclerotia it was evident that average radial growth rate on PDA medium had significant negative correlation with average VDI followed by mycelial growth rate on detached leaf sheath and mycelial dry biomass on PDB (Table 5). But average radial growth rate on PDA medium and mycelial dry biomass yield on PDB had significant positive correlation with average mycelial growth rate on detached leaf. The former had also significant positive correlation with mycelial dry biomass yield. Of these three mycelial parameters, mycelial growth rate showed stronger negative correlation with average VDI than others. This means that the *R .solani* isolates having slow growth rate on PDA medium or detached leaf sheath or low biomass on PDB medium exhibited higher VDI i.e virulence than fast growing isolates. The result of this experiment contradicts with the findings of Akai *et al.* (1960) and Shahjahan *et al.* (1987) wherein they observed strains with poor mycelial growth were less pathogenic and the strains with high mycelial growth rate were virulent respectively. Mycelial growth rate though some workers considered to be an important factor governing pathogenicity or virulence but it may not be considered as sole determinant always. Sometimes, pathogenicity of the isolates depends on or could be correlated with higher production of enzymes like pectic enzymes/polygalacturonase and cellulase (Basu and Sengupta, 1992; Benniza and Rutherford, 2001), toxin (Sriram *et al.*, 1997) and oxalic acid production (Nagarajkumar *et al.*, 2005). The *R. solani* isolates considered for our experiment probably are much dependent on enzymatic, toxin and other biochemical weapons rather than mycelial growth rate only. It was also evident from the result that total number of sclerotia per plate, average weight, length and breadth of sclerotia did not show any significant relation with VDI. Some workers found relationship between the sizes of the sclerotia produced by different isolates with their virulence (Basu and Sengupta, 1992). Total number of sclerotial production/ plate had significant negative correlation with average weight-, length- and breadth of sclerotia. However, average weight of sclerotia had predictable significant positive correlation with length- and breadth of sclerotia.

For prediction of sheath blight disease severity *vis-à-vis* pathogen virulence mycelial growth rate on detached leaf sheath, average radial growth rate on PDA medium, mycelial dry biomass weight, total no. of sclerotia per plate, average weight-, length- and breadth of sclerotia was considered as independent variables and average vertical disease index (VDI) as dependent variables (Table 6). The stepwise multiple regression, however, predicted that the average radial growth rate on PDA medium, average weight-, length- and breadth of sclerotia i.e size of the sclerotia were the four independent factors having significant influence on VDI/ virulence. Out of these four factors, average radial growth rate on PDA medium was considered the most critical factor because its elimination during step down regression process had demonstrable effect on the reduction of values of coefficient of multiple determinant and multiple correlation (multiple regression equation no. 4 and 5). Besides, the factor X_1 i.e mycelial growth rate on detached leaf sheath though insignificant still its omission from the regression equation not only influenced the values of coefficient of multiple determinant and multiple correlation but also influenced the level of significance of factors X_5 i.e average weight, X_6 i.e length of sclerotia and X_7 i.e breadth of sclerotia (multiple regression equation no. 3 and 4). So, the best prediction model for sheath blight disease severity *vis-à-vis* pathogen virulence based on some mycelial and sclerotial characteristics would be $Y = 2.228 - 0.249\,X_1 - 1.724\,X_2^{**} + 21.755X_5^{*} - 0.010X_6^{*} - 0.012X_7^{*}$.

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