Research Article

Pathogenic and Molecular Variability in *Sclerotium Oryzae* Catt. Isolates Collected from Major Rice Growing Areas of Telangana and Andhra Pradhesh States

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Abstract

The present studies on pathogenic and molecular variability in fifteen *Sclerotium oryzae* isolates revealed the existence of significant variation in the virulence pattern of *S. oryzae* isolates and the reaction of rice cultivars. All 15 *S. oryzae* isolates produced average lesion length with a range of 14.0 mm to 32.6 mm on inoculated tillers of different rice cultivars. Based on host pathogen interaction, the isolates of *S. oryzae* were categorized into four different groups as Group-1, Group II, Group III and Group IV. Among these, Group IV consisting of nine isolates SO 3, SO 5, SO 6, SO 7, SO 8, SO 9, SO 12, SO 13 and SO 15 were found to be highly virulent producing susceptible reaction in five rice cultivars of BPT 5204, MTU 3626, MTU 1010, RNR 15048 and JGL 1804 as compared to other groups. The molecular diversity among the 15 isolates of *S. oryzae* using twenty six RAPD primers revealed wide genetic diversity among the 15 isolates. Further, there was no link found between genetic diversity of the isolates and their geographical origin.

Keywords: Pathogenic, Molecular Variability, *Sclerotium oryzae*, Rice, Telangana

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Introduction

Rice is an important cereal crop grown under varied agro-climatic conditions in a variety of soils in Telangana and Andhra Pradhesh states and it is prone to stem rot infection in mild to severe form resulting in significant yield losses. Stem rot of rice caused by *Sclerotium oryzae*. Catt. is present in all rice growing regions worldwide [1]. Primary inoculum of the pathogen consists of sclerotia which float on the surface of the water when the fields are flooded in the spring and germinate to infect the rice at the stem near the water interface [2]. The pathogen has been reported to cause substantial losses in grain yield ranging from 5-80 per cent [3]. Since resistance to stem rot in rice genotypes is rare [4, 5] and screening for resistant varieties is only one source for combating the disease for developing resistant varieties. Keeping this in view the present studies on pathogenic and molecular variability in fifteen *Sclerotium oryzae* local isolates were conducted at Department of Plant Pathology, College of Agriculture, Rajendranagar, Hyderabad.

Materials and Methods

Pathogenic Variability

The inoculum of the pathogen was multiplied in Petriplates containing 15ml of sterilised PDA at 25±2°C for 15 days. Both actively growing mycelium along with agar and sclerotia will be used for inoculation. Fifty rice tillers comprising of lower 2-3 nodes of each genotype were detached at maximum tillering stage and these stem pieces were punctured with needle in the centre of the stem inter node and inoculated with mycelial bit along with sclerotia of each of the fungal isolates in laboratory following cut wound inoculation method [6] for two consecutive seasons. The inoculated stem pieces were placed in the BOD Incubator at 25±2°C for facilitating the pathogen to establish and initiate the disease symptoms. An uninoculated check was also maintained in the experiment in which the tillers were not inoculated with the inoculum.

Molecular Variability

The genetic diversity among fifteen *S. oryzae* isolates collected from different regions of Telangana and Andhra Pradesh were studied based on DNA Polymorphism by using Twenty-six 10-mer RAPD primers of Operon

Technology. The pure fungal mycelial mats of fifteen *S. oryzae* isolates are grown on The Potato Dextrose Broth for 10 days and mycelial mat of the isolates are oven dried and DNA from these mycelial mats are extracted by CTAB method and further amplified through Polymerase Chain Reaction by using 26 RAPD primers. The differences in banding patterns of different isolates are studied.

Salient Results

Pathogenic Variability

Five popular rice cultivars viz., BPT 5204, MTU 3626, MTU 1010, RNR 15048 and JGL 18047 were used to study the pathogenic variability of *S. oryzae* isolates for two consecutive seasons following cut wound inoculation method in the laboratory. In response to the inoculation by isolates fifteen isolates of *S. oryzae*, the tested rice cultivars differed in their reactions against stem rot pathogen. All the 15 *S. oryzae* isolates produced average lesion length with a range of 14.0 mm to 32.6 mm on inoculated rice tillers of different rice cultivars. The maximum lesion length of 32.6 mm was recorded with *S. oryzae* isolate of SO5 and minimum lesion length of 14.0 mm was recorded in SO2 isolate **(Table 1** and **Figure 1)** .

Table 1 Reaction of *S. oryzae* isolates on different rice cultivars grown in Telangana and Andhra Pradesh states

ISOLATES	Rice cultivars (Lesion length in mm*)					
	MTU 3626	BPT 5204	MTU 1010	RNR 15048	JGL 18047	MEAN
SO1	13.8 (S)**	18.9 (S)	8.9 (R)	12.9 (S)	19.5 (S)	14.8
SO2	12.2 (S)	24.2 (S)	8.1 (R)	8.8 (R)	16.8 (S)	14.0
SO3	33.2 (S)	42.2 (S)	13.1 (S)	22.5 (S)	16.0 (S)	25.4
SO4	20.0 (S)	35.7 (S)	12.0 (S)	17.8 (S)	7.5 (R)	18.6
SO5	51.2 (S)	44.7 (S)	19.2 (S)	24.0 (S)	23.8 (S)	32.6
SO6	38.1 (S)	43.5 (S)	17.1 (S)	21.2 (S)	21.5 (S)	28.3
SO7	45.2 (S)	41.5 (S)	17.1 (S)	22.0 (S)	21.1 (S)	29.4
SO8	21.7 (S)	37.8 (S)	10.3 (S)	17.8 (S)	20.8 (S)	21.7
SO9	26.5 (S)	41.4 (S)	13.9 (S)	19.9 (S)	16.8 (S)	23.7
SO10	34.0 (S)	32.6 (S)	15.7 (S)	18.5 (S)	8.4 (R)	21.9
SO11	20.3 (S)	35.8 (S)	8.9 (R)	9.8 (R)	18.9 (S)	18.7
SO12	44.9 (S)	32.7 (S)	14.3 (S)	18.9 (S)	16.7 (S)	25.5
SO13	27.4 (S)	23.0 (S)	14.0 (S)	15.0 (S)	17.7 (S)	19.4
SO14	23.6 (S)	28.2 (S)	8.6 (R)	15.9 (S)	15.6 (S)	18.4
SO15	22.2 (S)	34.0 (S)	15.0 (S)	14.0 (S)	19.5 (S)	20.9
Mean	28.9	34.4	13.5	17.3	16.9	

*Based on average lesion length on 50 rice tillers

** R - Resistant (lesion length < 10 mm); S - Susceptible (lesion length > 10 mm)

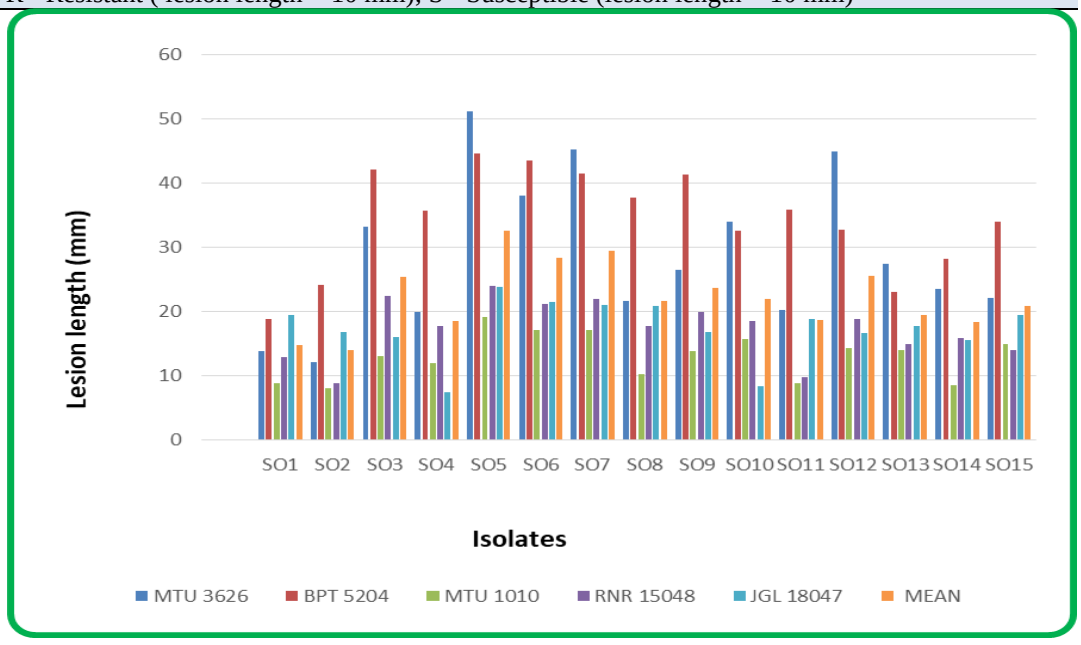

Figure 1 Virulence of 15 S. *oryzae* isolates on five rice cultivars

Rice cultivars showed significant differences in their reaction to *S. oryzae* isolates. The average lesion length of 13.5 mm to 34.4 mm was recorded with the tested *S. oryzae* isolates. Rice cultivars viz., MTU 1010, RNR 15048 and JGL 18047 showed resistant reaction while the other two rice cultivars MTU 3626, BPT 5204 were susceptible in their reaction and recorded an average lesion length of 28.9 mm and 34.4 mm, respectively (**Table 2**).

Table 2 Disease Reaction of rice cultivars against isolates of *S. oryzae*

S. No.	Rice Cultivar	Group – 1	Group - II	Group- III	Group- IV
1	MTU 3626	S	S	S	S
2	BPT 5204	S	S	S	S
3	MTU 1010	R	R	S	S
4	RNR 15048	R	S	S	S
5	JGL 18047	S	R	R	S

Based on host pathogen interaction, the isolates of *S. oryzae* were categorized into four different groups (**Table 3**). Isolates SO 2 and SO11 were categorized into Group- I which were less virulent in reaction and producing an average lesion length of 8.1 mm to 9.8 mm in the rice cvs. MTU 1010 and RNR 15048. Group – II comprising of SO 1 and SO 14 isolates which showed similar virulence pattern in rice cvs. MTU 1010. While Group –III consists of two isolates SO 4 and SO10 which showed similar virulence in the rice cv. JGL 18047. The remaining nine isolates of SO 3, SO 5, SO 6, SO 7, SO 8, SO 9, SO 12, SO 13 and SO 15 of Group –IV were found to be highly virulent producing susceptible reaction in five rice cultivars of BPT 5204, MTU 3626, MTU 1010, RNR 15048 and JGL 18047, respectively.

Fifteen *S. oryzae* isolates collected from different locations showed variation in the virulence pattern on five rice cultivars viz. BPT 5204, MTU 3626, MTU 1010, RNR 15048 and JGL 18047 confirming variation in pathogenicity of different *S. oryzae* isolates. The variation among *S. oryzae* isolates while screening rice germplasm and cultivars was also reported earlier [7].

Table 3 Grouping of *S. oryzae* isolates based on virulence exhibited on five rice cultivars

GROUP	Isolates
I	SO 2 and SO 11
II	SO 1 and SO 14
III	SO 4 and SO 10
IV	SO 3, SO 5, SO 6, SO 7, SO 8, SO 9, SO 12, SO 13 and SO 15

Molecular Variability

The genomic DNA among fifteen isolates of *S. oryzae* was amplified by using Twenty six 10-mer RAPD primers of Operon Technology and the extent of intraspecific variation was recorded among the isolates of stem rot pathogen. The ten random primers which produced consistent and reproducible RAPD patterns were used for analysis. Ten RAPD primers generated 102 polymorphic bands. The binary data, in the form of one (1) or zero (0) based on the presence or absence of a particular band, was used for the estimation of similarity matrix to calculate genetic divergence and relatedness among *S. oryzae* isolates tested using NTSYS pc version 2.02i. The similarity matrix revealed that, the isolates SO 5 and SO 7 were similar followed by SO 1 and SO 6. On the other hand, the isolates SO 2 and SO 13 were distant followed by SO 2, SO 15 and SO 2 and SO 14 (**Table 4**).

Further, the cluster analysis (UPGMA analysis) grouped the isolates into two clusters (**Figure 2**). The cluster I comprised of 13 isolates viz., SO 10, SO 3, SO 8, SO 11, SO 9, SO 12, SO 13, SO 14, SO 15 and SO 2 while the cluster II contained the two isolates SO 5 and SO 7. The grouping of the isolates was not correlated with geographic origin of the isolates. However, few isolates were grouped together in a cluster based on their geographical origin viz., SO 13 and SO 14 collected from Ranga Reddy district were grouped in close proximity in cluster I. The results indicated that there was no definite correlation between the genetic diversity of the isolates and their geographical origin.

Table 4. Similarity index values of *S. oryzae* isolates based on RAPD analysis

	SO 1	SO 2	SO 3	SO 4	SO 5	SO 6	SO 7	SO 8	SO 9	SO 10	SO 11	SO 12	SO 13	SO 14	SO 15
SO 1	1.00														
SO 2	0.23	1.00													
SO 3	0.42	0.23	1.00												
SO 4	0.41	0.15	0.27	1.00											
SO 5	0.37	0.21	0.48	0.42	1.00										
SO 6	0.62	0.21	0.43	0.56	0.45	1.00									
SO 7	0.46	0.22	0.43	0.53	0.63	0.59	1.00								
SO 8	0.42	0.23	0.47	0.37	0.44	0.43	0.43	1.00							
SO 9	0.31	0.22	0.45	0.36	0.34	0.38	0.38	0.41	1.00						
SO 10	0.39	0.15	0.42	0.37	0.47	0.43	0.43	0.42	0.33	1.00					
SO 11	0.32	0.22	0.44	0.21	0.32	0.33	0.32	0.44	0.38	0.31	1.00				
SO 12	0.43	0.16	0.32	0.32	0.41	0.40	0.37	0.43	0.42	0.36	0.32	1.00			
SO 13	0.30	0.06	0.14	0.22	0.22	0.28	0.26	0.17	0.10	0.32	0.19	0.26	1.00		
SO 1	0.37	0.09	0.20	0.19	0.13	0.31	0.20	0.24	0.19	0.29	0.23	0.26	0.43	1.00	
SO 15	0.17	0.08	0.13	0.23	0.24	0.20	0.24	0.25	0.18	0.21	0.21	0.34	0.23	0.26	1.00

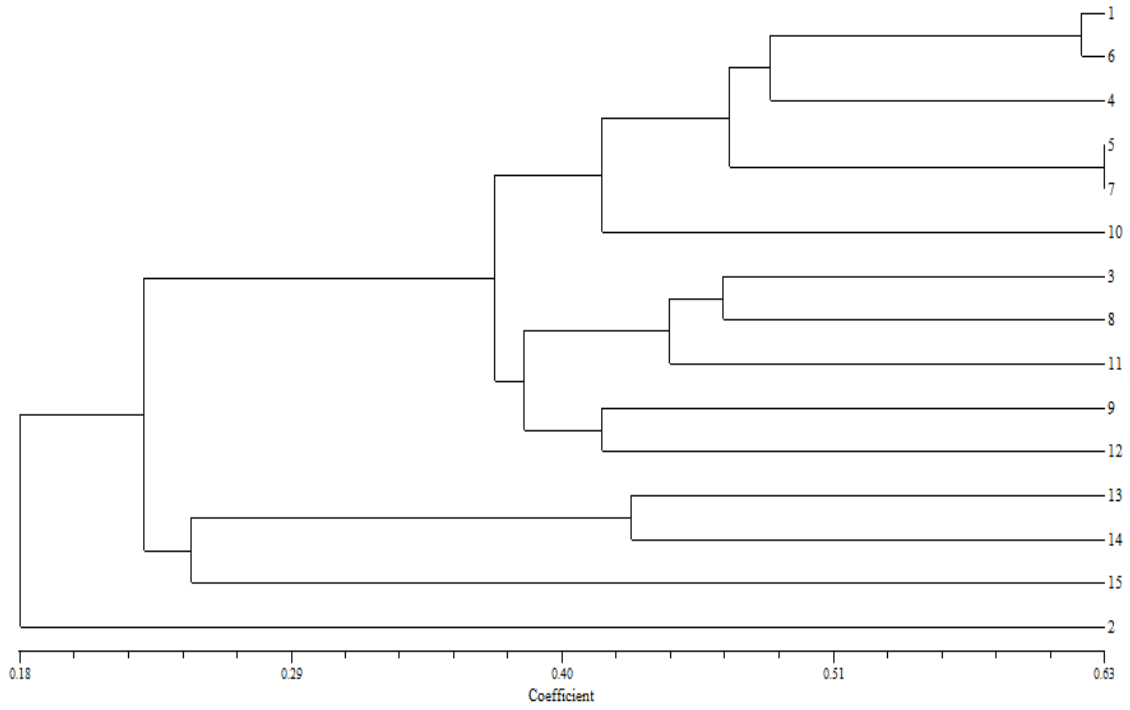

Figure 2. UPGMA generated dendrogram of RAPD analysis representing genetic distance among isolates of *S. oryzae*

A high level of intraspecific genetic diversity among the fifteen isolates of *S. oryzae* using nine random amplified polymorphic (RAPD) primers was reported earlier [8] which were confirming the results obtained in the present investigation. They investigated fifteen isolates of stem rot fungus from major rice growing areas of Haryana by DNA finger printing by using 22 RAPD primers which resolved 251 bands across 15 isolates with a maximum resolution of 15 bands were observed in nine primers. Highest similarity value was recorded between isolates of SO 14 and SO 15

collected from the varieties HKR 126 and Pusa Basmati. Maximum genetic variation was observed in isolates of SO2 from rice cv. Govind followed by SO 9 from rice cv.IR 64.

The variability studies among 15 isolates of banded leaf and sheath blight (SLSB) by using RAPD revealed a high magnitude of genetic diversity among the isolates [9]. Similar diversity studies conducted using 30 RAPD primers, reported wide genetic diversity among the isolates of S. *rolfsii* inciting groundnut stem rot which is not linked with their geographical origin [10]. Phylogenetic analyses and morphological characteristics were used to assess the taxonomic placement of eight plant-pathogenic *Sclerotium* species [11].

Conclusion

Hence, looking to the above results and available literature it was concluded from the studies, that the intraspecific diversity of *S. oryzae* isolates may not always have direct correlation with virulence or geographical origin of isolates.

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