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CULTURAL AND MORPHOLOGICAL VARIABILITY IN ISOLATES OF *Sclerotium oryzae*, THE RICE STEM ROT PATHOGEN

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ABSTRACT

Twelve isolates of *Sclerotium oryzae* (Catt). causing stem rot of rice were collected from major rice growing areas in Andhra Pradesh, India. Variability among these isolates of *S. oryzae* was assessed based on utilizing various carbon sources using three different growth media and growth rate on PDA. Among the different media tested, PDA was well supported for growth of all isolates and there was sparse growth on OMA and very sparse growth on CZA of all the isolates of *S. oryzae*. was noticed. Depending on the growth rate on PDA medium, isolates of *S. oryzae* categorised into four groups very fast growing, fast growing, medium growing and slow growing. Mostly reddish brown, dark brown and light brown sclerotia were observed in the isolates.Hence this study clearly indicated the cultural and morphological variability among *Sclerotium oryzae* isolates

KEYWORDS : Growth media, morphology, mycelium, sclerotia

INTRODUCTION:

Stem rot of rice caused by *Sclerotium oryzae* Catt. is one of the major constraints for rice production in the Indian subcontinent especially in Haryana (Singh et al., 2002).

The rice stem rot fungus was first described in its sclerotial state from Italy and was named *Sclerotium oryzae* Catt. (Cattaneo, 1876). The fungus associated with the stem rot disease was identified as *S.oryzae* Catt based on the morphological and cultural characteristics and by formation of distinct white colonies and abundant globose, dark coloured sclerotia from the fourth day onwards, sclerotia were white in colour, later turned to reddish brown and then dark brown to almost black with spherical shape (Gopika *et al*., 2016). The pathogen infects leaf sheath and causes rotting of stem and death of the leaf sheath. The fungus perpetuates by producing hard sclerotial bodies. Profusely branched white fluffy to greyish hyphae are seen in the mycelium in the sclerotial stage (Tullis, 1953). Sclerotia are extremely hard and relatively survival structures (Singh *et al*., 2003) and considered as principle means of dispersal (Okabe *et al*., 2000). Isolates grouped into fastest growing, fast growing, intermediate growing and slow growing based on the growth of mycelial diameter at different time intervals on PDA medium (Ranga Rani, 2018). Potato dextrose medium was best supported medium for the mycelial growth and sclerotial production compared with other growth media and there was no growth observed in Czapeck dox agar (Kumar *et al.,* 2011; Ranga Rani, 2018). The objective of the present investigation was to study the cultural and morphological relationship among the collected isolates of *Sclerotium oryzae* from different hosts and loactions of Andhra Pradesh, India.

MATERIAL AND METHODS

Cultural and morphological variability among the *S. oryzae* isolates

The isolates were cultured from the stem rot infected samples collected from various localities of Andhra Pradesh such as Kadapa, Kurnool and West Godavari districts and were designated as SOP1 to SOP12 and incubated at 27 ± 1°C. In the present investigation three different types of media i.e., Potato Dextrose Agar (PDA), Czapek –Dox Agar (CDA), and Oat Meal Agar (OMA) were used for selecting the best medium suitable for the growth of the pathogen. The radial growth and production of sclerotia of *Sclerotium oryzae* were studied on PDA, OMA and CZA media.

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Twenty millilitre of melted sterilized medium was poured asceptically into each petridish. For each tested medium, three replications were maintained and inoculated by placing a culture disc of 5.0 mm. diameter taken from margin of the freshly grown colony of *S. oryzae* (Kumar *et al*., 2010; Ranga rani, 2018).

The colour of culture , mycelial characters and no. of days to form sclerotial bodies and sclerotial colour were recorded at regular time intervals (Rasu *et al*., 2013).

RESULTS AND DISCUSSION

Cultural and morphological variability of *the Sclerotium oryzae* isolates on different growth Media

Ten days after incubation, growth pattern of *S. oryzae* isolates incubated at 27± 1^{oC} showed the significant differences in the nature of culture growth, surface of the colony colour and pattern of sclerotia in three growth media i.e. Potato dextrose agar medium (PDA), Czapek-dox agar medium (CDA) and Oat meal Agar medium, (OMA).

The differences in the cultural and morphological characters of mycelia and sclerotia were studied to observe the influence of different nutrient media on the isolates of *Sclerotium oryzae*.

On PDA medium, the isolates of *S. oryzae* SOP-3, SOP-6, SOP-11 showed abundant white and fluffy mycelium and sclerotia were intermixed within the mycelium. Isolates of SOP-1, SOP-5, and SOP-7 with white sparse mycelium and not fluffy and sclerotia were scattered just above the mycelium and grouped as concentric rings. Reverse colony was white in colour. Isolates of SOP-4, SOP-8 and SOP-12 are with sparse mycelium and sclerotia was intermixed in mycelium. Isolates of SOP-2, SOP-9 and SOP-10 showed mycelium with zonations and sclerotia were submerged with the mycelium in SOP-9 and SOP-10 whereas in SOA-2 sclerotia with intermixed mycelium was observed. Colony reverse was dark brown in colour in SOP-2, SOP-3, SOP-6, SOP-9, SOP-10 SOP-11 isolates.

On CZA medium, very sparse mycelial growth were observed in isolates of SOP-3, SOP-5, SOP-6 and SOP-11. Among these, only five sclerotial bodies were noticed in SOP-6. Isolates of SOP-1, SOP-7, SOP-8, SOP-10 and SOP-12 only mycelial growth was observed at centre elevated and no sclerotial bodies was produced. In SOP-2, SOP-4 and SOP-9 isolates very sparse or no mycelia and sclerotia were observed.

Table No.1 Morphological Variability among the isolates of *S.oryzae* in terms of Mycelial type and sclerotial colour on different media
(LB:light brown,RB:reddish brown,B:black)

Isolate No.	Mycelial characters		Colour of sclerotia			CZA
	PDA	OMA	CZA	PDA	OMA	
SOP-1	Sparse	Sparse	Sparse	RB	LB	Nil
	Concentric rings		Elevated			
			centre			
SOP-2	Zonations	Sparse	Very sparse	RB to B	LB	Nil
		Concentric rings				
SOP-3	Fluffy	Sparse	Very sparse	Black	RB	Nil
		Zonation				
SOP-4	Sparse	Sparse	Very sparse	Black	LB	Nil
		Concentric rings				
SOP-5	Sparse	Sparse	Very sparse	RB to B	RB	Nil
	Concentric rings	Elevated centre				
SOP-6	Fluffy	Fluffy	Sparse	RB	RB	White
		Concentric rings				
SOP-7	Sparse	Sparse	Sparse	RB to B	RB	Nil
	Concentric rings	Elevated centre	Elevated			
			centre			
SOP-8	Sparse	Sparse	Sparse	LB	Creamy to	Nil
		Elevated centre	Elevated		brown	
			centre			
SOP-9	Zonations	Sparse	Very sparse	RB to B	LB	Nil
SOP-10	Zonations	Sparse	Sparse	RB to B	LB	Nil
		Concentric rings	Elevated			
			centre			
SOP-11	Fluffy	Sparse zonation	Very sparse	RB	RB	Nil

SOP- *Sclerotium oryzae* Pathogen

On OMA medium, SOP-2, SOP-4, SOP-6, SOP-10 and SOP-12 isolates showed sparse mycelium and concentric rings were noticed. Sclerotia were scattered in the mycelium, when compared with other isolates SOP-6 produced more sclerotia. Isolates of SOP-1, SOP-3, SOP-9 and SOP-11with sparse mycelium but not fluffy and sclerotia were scattered and zonations were observed in SOP-3 and SOP-11. Isolate of SOP-5 showed sparse mycelium and elevated at centre and sclerotia were scattered at periphery. Isolates of SOP-7 and SOP-8 very-sparse mycelium and elevated at centre. Production of sclerotia was delayed. Colony reverse was brownish to black colour in SOP-2, SOP-4, SOP-6, SOP-10 and SOP-

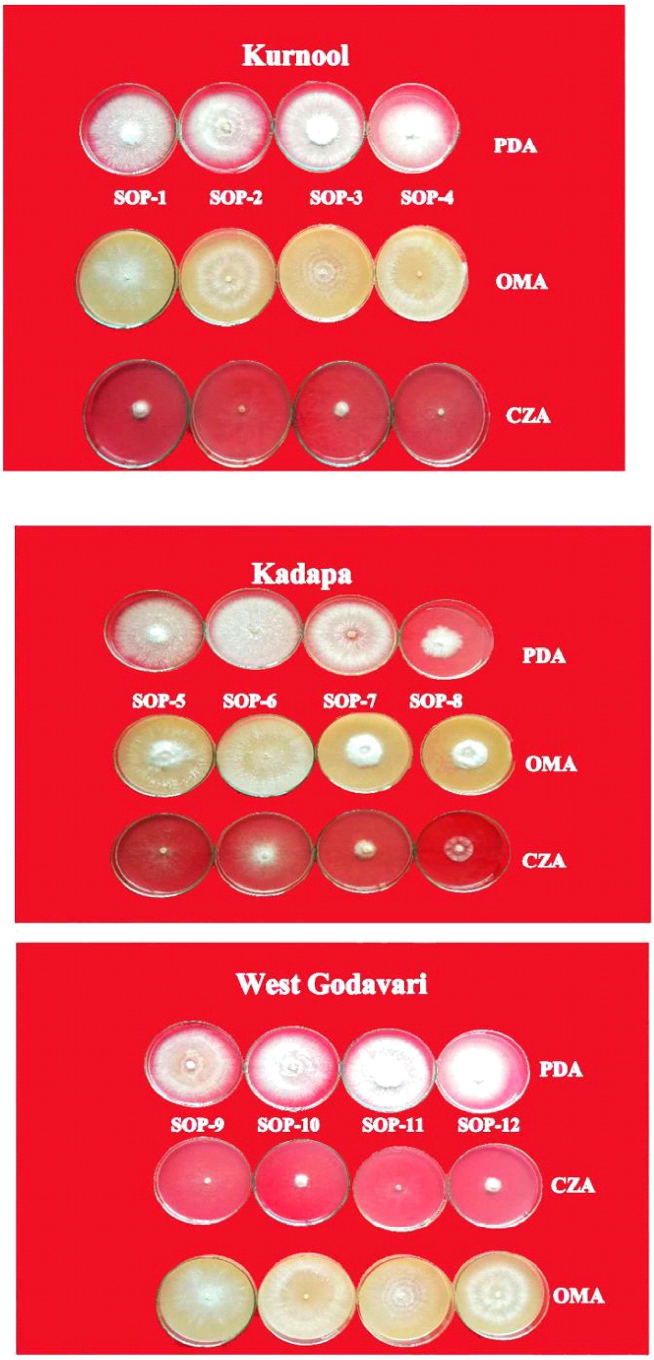

Plate no. 1 . Morphological variability among the isolates of *Sclerotium oryzae* on different growth media

12 isolates. Isolate SOP-1, SOP-3, SOP-5, SOP-7, SOP-9 and SOP-11 showed reddish brown colour on colony reverse. The sclerotial production was delayed compared with PDA and the sclerotia are light brown and reddish brown in colour.

Based on the above reasons, PDA was considered to be good growth supporting medium for the mycelial growth and sclerotial production of *Sclerotium oryzae* whereas sparse growth on OMA and very sparse growth or no growth was recorded on CZA media

Grouping of isolates of *S. oryzae* based on the growth rate on PDA medium

Isolates of *S. oryzae* were categorised into three groups based on the growth rate on Potato dextrose agar (PDA) medium. Group 1 was categorised with the fastest growing isolate of SOP-6 with mycelial diameter of 90 mm after 4 days of incubation, Group 2 with fast growing isolates of SOP-7, SOP-11 and SOP-3 having a diameter of 90 mm of mycelial growth after 5 days of incubation and produced sclerotia intermixed in the mycelium.

Group 3 comprised with five isolates of SOP-1, SOP-2, SOP-5, SOP-9 and SOP-10 were medium growing of 90 mm diameter after 6 days of incubation with sclerotia scattered above the mycelium where zonation was observed in SOP-9 and SOP-10 isolates.

Group 4 consisted with isolates of SOP-4 and SOP-8 and SOP-12 were slow growing with 90 mm diameter of mycelial growth after 7 days of incubation with submerged and scattered sclerotia intermixed in mycelium.Similar study found, (Rasu *et al.* 2013) categorized *Sclerotium spp*. isolates into three groups based on their growth rate on PDA medium.

Table 2 Growth of mycelium of *Sclerotium oryzae* isolates on Potato dextrose agar medium (PDA)

S. No	Isolates	Colony diameter (mm) in no. of days								Sclerotial characters		
		1	2	3	4	5	6	7	8	Initiation	Maturation	Pattern
												Scattered
1	SOP-1	17	28	42	55	64	76	90		5 days	7 days	above
												mycelium
												Intermixed
2	SOP -2	22	31	44	69	74	81	90		5 days	7 days	in mycelium
												Intermixed
3	SOP -3	23	37	58	69	85	90			5 days	7 days	in mycelium
	SOP -4											Intermixed
4		13	26	39	45	56	62	80	90	7 days	9 days	in mycelium
												Scattered
5	SOP -5	24	36	44	57	69	77	90		5 days	7 days	above
												mycelium
												Intermixed
6	SOP -6	32	53	65	78	90				4 days	6 days	in mycelium
												Scattered
7	SOP -7	28	41	59	68	79	90			5 days	7 days	above
												mycelium
												Intermixed
8	SOP -8	10	21	32	48	57	63	74	90	8 days	10 days	in mycelium
												Submerged
9	SOP -9	23	35	42	56	63	78	90		6 days	9 days	
												Submerged
10	SOP-10	22	38	47	59	74	82	90		6 days	9 days	
												Intermixed
11	SOP -11	26	43	64	77	82	90			5 days	7 days	in mycelium
												Intermixed
12	SOP -12	14	24	38	54	63	74	82	90	7 days	10 days	in mycelium

In Group 1 after 3 days and in group 2 after 4 days of incubation, the mycelium started to congregate and developed as round full-fledgedly and turned from white to reddish brown and black sclerotia. Sclerotial initiation and maturation were recorded from 4 days to 7 days respectively. In group 3, the isolates have taken 5 days to 8 days to form sclerotia which are scattered, submerged and intermixed within the mycelium.Whereas in isolates of fourth category taken 8 days to 10 days of incubation for the sclerotial initiation and maturation. Sclerotia were intermixed within the mycelium. Similar differences among different isolates of *S. oryzae* were observed by (Ali and Singh, 1994) ; (Ali, 1997) ; (Kumar *et al*. 2011) and (Ranga Rani, 2018) for their variability on potato dextrose agar medium.

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