

## Variability among the Isolates of *Sarocladium oryzae* Isolated from Bihar (Zone I)

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**ABSTRACT:** Sheath rot resistant breeding has been difficult due to a lack of knowledge about the pathogen's genetic variability and virulence pattern. Therefore, better understanding the disease's development, prediction of future disease outbreaks, the current inquiry will be carried out with the following specific aims in mind, limited work done on pathogen diversity. Fourteen isolates were collected from seven districts of Bihar (Zone I) for the study of cultural variability like radial growth, colour of mycelium and type of growth on PDA and morphological variability like length of Conidiophore, length of conidia and conidiation on PDA media. All the fourteen isolates grow fluffy irregular on PDA, Mycelium colour of eight isolates (Sob-1, Sob-2, Sob-4, Sob-5, Sob-6, Sob-8, Sob-9 and Sob-13) were whitish with pale orange colour, two isolates (Sob-3 and Sob-12) were whitish with deep orange and remaining four isolates (Sob-7, Sob-10, Sob-11 and Sob-14) were whitish with orange ochre. Highest radial growth was recorded in the isolate Sob-7 with 3.20cm followed by Sob-3 (2.25cm), Sob-1 (2.10cm) and least growth was recorded in isolate Sob-8 with 0.85cm, conidiophores length was recorded in the ranges between 46.7 to 57.9  $\mu\text{m}$  and Conidia length observed in the ranges between 4.65 to 5.95  $\mu\text{m}$ . highest number of conidia per sq cm was recorded in the isolate Sob-14 ( $52.3 \times 10^6$  /sq cm) followed by Sob-1 ( $50.8 \times 10^6$  /sq cm) and Sob-10 ( $50.3 \times 10^6$  /sq cm) both are on par with each other and least number of conidia was recorded in Sob-6 ( $36.6 \times 10^6$  /sq cm) isolate.

**Key words:** Isolates, variability, Conidia, Conidiophore.

### INTRODUCTION

Rice sheath rot was once thought to be a minor and geographically limited disease, but it has recently gained traction and spread due to changes in rice production systems such as the use of high yielding varieties, increased fertilizer use, seeding methods, and other factors that favors the disease's spread. The disease was originally discovered in Taiwan in 1922, and it has since spread to every country in South Asia. Sheath rot was initially recorded in India in 1973, and losses owing to the disease were determined to be between 50 and 65 percent. The fungus' mycelia were hyaline, sparsely branched septate, and 1.5-2 m in diameter, while the conidia were hyaline, smooth, single celled, cylindrical measuring 4-9  $\times$  1-2.5 m and borne at tips. Mycelium of the fungus effuse, white, sparsely branching, up to 2m in diameter on potato dextrose agar, according to Agnihothrudu (1973). Hyaline, smooth, macronematous, mononematous, once or twice branched, with apical conidiogenous cells in groups of 2-5, monophialidic, distinct, elongate, cylindrical conidiophores developing from the mycelium, up to 3m in diameter, hyaline, smooth, macronematous, mononematous, once or twice branched, On the conidiophores, single, intercalary

phialides were also seen. Phialides are flask-shaped, elongate, and narrow towards the apex, measuring  $10^{-16} \times 11.5\text{m}$  in size and up to 20m on culture media. Tajul *et al.* (2015) reported that Cultural variability of 29 pathogen isolates obtained from 8 different locations was studied on potato dextrose agar (PDA). Mycelial growth of 29 representative isolates was found to vary on PDA and the isolates were divided into 6 groups. The range of the overall size of conidia of the selected isolates was 2.40-7.20  $\times$  1.20-2.40  $\mu\text{m}$ .

### REVIEW OF LITERATURE

Vengadesh kumar *et al.* (2019) revealed that among the media tested both solid and liquid, PDA and PD broth was found to be the best in supporting the mycelial growth (4.55cm) and mycelial dry weight (317.34mg) of the pathogen. Among the isolates SO17 was found as the most virulent which recorded maximum per cent disease incidence (58.33%) with maximum lesion size 39.40 mm length and 5.31 mm width on rice variety ADT 39.

Kumar *et al.* (2017) reported that ten sympatric isolates were assessed for the variability in radial growth, length of conidia, length of conidiophore was observed on five different media. Variability was also assessed on

pathogenic potential (disease causing ability) of all the 10 isolates using detached leaf technique by inoculating 20 day old 5mm culture disc. RLEA and PDA were the best solid media utilized by *S. oryzae* as carbon source. Isolate So DVS had least growth (1.2cm) while isolate So SKL (1.7cm) and So PTR (1.7cm) had maximum radial growth. Among the different isolates tested isolate So PLK recorded longest length of conidiophore (156.9µm) on RLEA while smallest conidiophore length was recorded in So SKL (42.0µm). Over all the media isolate So DVS (9.3µm) recorded longest conidia while So VMP (5.9µm) recorded least conidia length. Over all isolates tested, highest length of conidia was recorded on CDA (8.6µm) while least length of conidia was recorded in RLEA (5.9µm). The pathogen could cause disease only on boot leaf sheath but not on lower leaf sheaths of the culm when detached leaf technique using culture disc placement method was followed on cultivar NLR 3041. Isolate So NDP gave maximum lesion length of 1.5cm in nine days after inoculation. While So PTR gave the least lesion length measuring 0.6cm.

Tasugi and Ikada (1956) reported that conidial length of the pathogen on host plant measured as  $2.1-3.5 \times 0.5-1.6 \mu\text{m}$  and on the culture medium it measure about  $1.8-13 \times 1-1.6 \mu\text{m}$ . Subsequently, the main axis of conidiophores measured  $15-22 \times 2-2.5 \mu\text{m}$ , and terminal branches measured  $23-45 \times 1.5 \mu\text{m}$ , according to Ou (1972).

## MATERIALS AND METHODS

In this study, fourteen distinct isolates were isolated from seven districts in Bihar (Zone I). Table 1 lists the isolates used in this study. Variability was tested in terms of radial growth, mycelium colour, type of growth on PDA, conidia and conidiophores features on PDA after the fungus was isolated.

### A. Radial growth on PDA media

PDA was made separately and aseptically transferred into sterilized Petri dishes at a rate of 20 mL each dish. The media was allowed to cool and solidify in Petri dishes. Individual isolates of *S. oryzae* were placed into each medium in the Petri dish using a sterilised cork borer to transfer mycelial discs of 5 mm size from a 10-day-old fungal culture. At a temperature of  $27 \pm 1^\circ\text{C}$ , the entire inoculated medium was incubated. The diameter of the colony was measured 16 days after inoculation to evaluate the development of the fungus PDA medium. Three replications were maintained, and the experiment was conducted in a completely randomized design. The diameter of the fungal colony on PDA media was used to estimate the growth of *S. oryzae*. Two sets of largest points opposite each other on the colony's edges were picked, and the distance between the broadest points in each set was measured and averaged in order to determine the colony's diameter, which was irregular in shape.

### B. Conidiation per unit area on PDA media

A 10 day old (about 3mm diameter) culture disc was collected, transferred to 4ml of sterile distilled water, macerated, and filtered through muslin cloth to remove the mycelia parts for the assessment of conidiation on solid media. A drop of such spore suspension was inspected using a haemocytometer, and the number of conidia present in each of the smallest squares was counted from ten randomly chosen smallest squares.

### C. Dimensions of conidiophores and conidia on PDA media

The number of divisions of the stage micrometer that coincide with those of the ocular micrometre was counted to calibrate the stage and ocular micrometre. The dimensions (length and width) of conidiophores and conidia were measured using an ocular micrometre attached to the compound microscope's eye piece after calibration.

## RESULTS AND DISCUSSION

### A. Variability among isolates of *S. oryzae* from seven districts of Bihar (zone I)

Fourteen isolates two from each districts viz., Sob-1, Sob-2, Sob-3, Sob-4, Sob-5, Sob-6, Sob7, Sob-8, Sob-9, Sob-10, Sob-11, Sob-12, Sob-13 and Sob-14 were obtained. Variability among the isolates of *S. oryzae* was assessed on PDA.

### B. Radial growth of *Sarocladium oryzae* on PDA media

In the radial growth on PDA media, there was significant variation among the isolates that were studied. Among all the isolates tested, isolate Sob-7 recorded largest colony growth (3.20cm) followed by Sob-3 (2.25cm), Sob-1 (2.10cm), Sob-6 (1.90cm), Sob-10 (1.80cm), Sob-4 and Sob-13 (1.70cm), Sob-14 (1.65cm), Sob-5 and Sob-12 (1.60cm), Sob-9 (1.45cm), Sob-11 (0.95cm) and least was recorded in Sob-8 (0.85cm). The results of this experiment which is contradictory to the work of and Sunil kumar *et al.* (2017) revealed that ten sympatric isolates were assessed for the variability in radial growth recorded in the ranges between 1.2 to 1.7cm.

### C. Colour of mycelia and type of growth on PDA media

All the fourteen isolates grow fluffy irregular on PDA media. Mycelial colour of eight isolates (Sob-1, Sob-2, Sob-4, Sob-5, Sob-6, Sob-8, Sob-9 and Sob-13) were whitish with pale orange, two isolates (Sob-3 and Sob-12) were whitish with deep orange and remaining four isolates (Sob-7, Sob-10, Sob-11 and Sob-14) were whitish with orange ochre. Earlier in 1996 Manibhushan Rao reported the same result that is type of growth and color of mycelium on PDA.

### D. Conidiation per unit area on PDA media

On PDA media, substantial differences in conidiation per unit area were found. The isolate Sob-14 ( $52.3 \times 10^6$  /sq cm) had the largest number of conidia per square centimeter, which differed considerably from all

other isolates, followed by isolate Sob-1 ( $50.8 \times 10^6$  /sq cm), Sob-10 ( $50.3 \times 10^6$  /sq cm), Sob-7 ( $48.5 \times 10^6$  /sq cm), Sob-12 ( $47.6 \times 10^6$  /sq cm). Sob-4 ( $44.6 \times 10^6$  /sq cm), Sob-3 ( $44.3 \times 10^6$  /sq cm), Sob-5 ( $44.1 \times 10^6$  /sq cm), Sob-9 ( $43.5 \times 10^6$  /sq cm), Sob-2 and Sob-11 ( $41.9 \times 10^6$  /sq cm), Sob-8 ( $40.3 \times 10^6$  /sq cm) and least number of conidia recorded in Sob-13 isolate ( $38.6 \times 10^6$  /sq cm). There is no information on the number of conidia produced per square centimeter on PDA media or among different *Sarocladium oryzae* isolates. Urmila (2013) stated that the optimal temperature and relative humidity for conidiation of *S. oryzae* were  $28 \times 10^7$  conidia/ml at 25°C and  $14.5 \times 10^7$  conidia/ml at 90 percent relative humidity.

#### E. Length of conidiophores on PDA media

On PDA, the length of conidiophores from various isolates varied significantly. In the Sob-4 isolate, the maximum mean length was 57.9  $\mu$ m, followed by Sob-14 (54.4  $\mu$ m), Sob-13 (54.2  $\mu$ m), Sob-7 (51  $\mu$ m), Sob-1, Sob-6, Sob-9, Sob-10, and Sob-11 with ranges from 50.1 to 50.8  $\mu$ m and Sob-2, Sob-3, Sob-12 with ranges of 48.1 to 49.2  $\mu$ m and least was recorded in the isolate

Sob-8 with 46.7  $\mu$ m. The findings of this study revealed that conidiophore length varied significantly among isolates, and that conidiophore size was more dependent on isolate type. The current analysis contradicts Manibhushan rao, 1996; Ou, 1972; Reddy, 1974; Gams, and Hawksworth's, 1975, Sunil kumar, 2015 report, which stated that conidiophore length varied between 14 and 43 meters.

#### F. Length of conidia on PDA media

Significant variation was observed in the length of conidia on PDA. Among the isolates highest length of conidia was recorded in Sob-10 with 5.95  $\mu$ m followed by Sob-4 (5.50  $\mu$ m), Sob-2 (5.45  $\mu$ m), Sob-1 (5.40  $\mu$ m), Sob-14, Sob-9, Sob-6, Sob-12 with in the ranges between 5 to 5.25  $\mu$ m, Sob-3, Sob-Sob-7, Sob-11, Sob-13, Sob-8 with ranges from 4.80 to 4.95  $\mu$ m and least conidia length was recorded in Sob-5 isolate with 4.65  $\mu$ m. the present investigation is contradictory to Shajahan *et al.*, (1977) reported that conidia length of *S. oryzae* varies from 3-17  $\mu$ m. conidia length of *S. oryzae* ranges from 3.5-7  $\mu$ m reported by Manibhushanrao (1996).

**Table 1: Designation of *Sarocladium oryzae* isolates from different districts of Bihar (Zone I).**

Sr. No.	Districts	Designation
1	Samastipur	Srb-1
		Srb-2
2	Muzaffarpur	Srb-3
		Srb-4
3	Begusarai	Srb-5
		Srb-6
4	Darbhanga	Srb-7
		Srb-8
5	Vaishali	Srb-9
		Srb-10
6	Madhubani	Srb-11
		Srb-12
7	Sitamarhi	Srb-13
		Srb-14

**Table 2: Cultural variability of different isolates of *S. oryzae* on PDA.**

Isolates	Radial growth (cm)	Mycelium colour	Type of growth
Sob-1	2.10	Whitish with pale orange	Fluffy irregular
Sob-2	1.40	Whitish with pale orange	Fluffy irregular
Sob-3	2.25	Whitish with deep orange	Fluffy irregular
Sob-4	1.70	Whitish with pale orange	Fluffy irregular
Sob-5	1.60	Whitish with pale orange	Fluffy irregular
Sob-6	1.90	Whitish with pale orange	Fluffy irregular
Sob-7	3.20	Whitish with orange ochre	Fluffy irregular
Sob-8	0.85	Whitish with pale orange	Fluffy irregular
Sob-9	1.45	Whitish with pale orange	Fluffy irregular
Sob-10	1.80	Whitish with orange ochre	Fluffy irregular
Sob-11	0.95	Whitish with orange ochre	Fluffy irregular
Sob-12	1.60	Whitish with deep orange	Fluffy irregular
Sob-13	1.70	Whitish with pale orange	Fluffy irregular
CD (P=0.05)	0.17		
SEm( $\pm$ )	0.06		
CV %	6.50		

**Table 3: Morphological variability of different isolates of *S. oryzae* on PDA.**

Isolates	Number of <i>S. oryzae</i> conidia per square cm (X10) <sup>6</sup>	Conidiophore length (µm)	Conidia length (µm)
Sob-1	50.8	50.8	5.40
Sob-2	41.9	49.2	5.45
Sob-3	44.3	48.4	4.95
Sob-4	44.6	57.9	5.50
Sob-5	44.1	48.2	4.65
Sob-6	36.6	50.7	5.05
Sob-7	48.5	51.0	4.95
Sob-8	40.3	46.7	4.80
Sob-9	43.5	50.5	5.10
Sob-10	50.3	50.8	5.95
Sob-11	41.9	50.1	4.90
Sob-12	47.6	48.1	5.00
Sob-13	38.6	54.2	4.85
Sob-14	52.3	54.4	5.25

**CONCLUSION**

Among the isolates, significant differences were observed in the length of conidiophores of different isolates tested on PDA media. Over all the isolates tested isolate Sob-8 (0.85cm) and Sob-11 (0.95cm) had least growth while isolate Sob-7 (3.20cm) had maximum radial growth on PDA, maximum length of Conidiophore was recorded in isolate Sob-4 (57.9µm) while the least length of Conidiophore was recorded in isolate Sob-8 (46.7µm). Longest length of conidia was observed in isolate Sob-10 (5.95µm) while the least conidial length was observed in Sob-5 (4.65µm).

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