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Variability Among the Isolates of Sarocladium Oryzae Incitant of Rice Sheath Rot

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ABSTRACT

Ten sympatric isolates were collected, five from each of the two districts and assessed for the variability in radial growth, length of conidia, length of conidiophore and conidiation 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. RLEB was the best liquid medium utilized by S. oryzae (0.82g). Least mycelial dry weight was observed on OMB (0.25g), PDB (0.28g), CDB (0.3g) and PSB (0.3g). Isolate So SKL showed highest dry weight of mycelial mat 0.55g followed by So PTR (0.5g). Isolate So RCP recorded least growth of 0.2g. Among the isolates, maximum dry weight was observed in So SKL on RLEB (1.45g) while the least mycelial dry weight was observed in So RCP (0.04g). The conidiation was highest with isolate So IDP (68.6 X 10^6/sq cm) while it was least with isolate So SKL (24.7 X 10^6/sq cm). Among the media, isolates of S. oryzae utilized OMA (49.2 X 10^6/sq cm) as the best source for conidiation followed by CDA (46.2 X 10^6/sq cm). Conidiation was least on RLEA (22.3 X 10^6/sq cm). Isolate So IDP recorded highest conidiation (174.24 X 10^6/sq cm) on CDA while least was recorded with So PLK (6.4 X 10^6/sq cm) on CDA. Over all the media tested maximum mean length of conidiophore was recorded in isolate So RCP (101.2µm) while the least length of conidiophore was recorded in isolate So IDP (66.6µm). Over all the isolates tested maximum length of conidiophore was recorded on RLEA (95.5µm) while least length of conidiophore was recorded on PSA (81.2µm). 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). Longest length was observed in isolate So DVS on CDA (13.6µm) while the least conidial length was observed in So SKL (4.0µm). 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. Isolate So RCP failed to show any symptoms in vitro indicating possibility of availability of resistance among rice genotypes.

Key words: *Isolates, Sarocladium oryzae (So), radial growth, mycelial dry weight, conidiophore*

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INTRODUCTION

Sheath rot of rice is a serious disease accounting for heavy toll of rice production. The disease was first reported in Taiwan in 1922 and later it has been reported in all countries in South Asia. In India, sheath rot was first reported in 1973 and the losses due to the disease were found to be ranging from 50 to 65 per cent[6].Severe outbreaks of sheath rot causing considerable yield losses were reported in the Indian state of Punjab during 1978-79. In Andhra Pradesh, sheath rot was found to be severe in Godavari, Nellore and Chittoor districts, causing 80 to 85 per cent yield loss[2] necessitating research on sheath rot management. Tasugi and Ikada[8] studied sheath rot disease in detail and gave the conodial measurements of the pathogen as 2.1-3.5 x 0.5- 1.6 µm from the host, 1.8- 13 x 1- 1.6 µm from the culture. Subsequently, Ou[5] who studied the morphology of the pathogen in detail and indicated that the size of main axis of conidiophores was 15 – 22 x 2- 2.5 µm and of terminal branches 23- 45 x 1.5 µm. He further reported that the mycelia of the fungus were hyaline, sparsely branched septate and 1.5- 2 µm in diameter, while the conidia were hyaline, smooth, single celled, cylindrical measuring 4- 9 x 1- 2.5 µm and are borne at tips. Agnihothrudu[1] reported that on potato dextrose agar mycelium of the fungus effuse, white, sparsely branched, up to 2µm in diameter. Conidiophores arising from the mycelium, up to 3µm in diameter, hyaline, smooth, macronematous, mononematous, once or twice branched, with apical conidiogenous cells in groups of 2-5, monophialidic, discrete, elongate, cylindrical. Single, individual, intercalary phialides also observed on the conidiophores. Phialides flask-shaped, elongate, narrow towards the apex, 10-16 X l-1.5µm and in culture some times as long as 20µm. Phialoconida acrogenous, simple, hyaline, produced successively, cylindrial to sub avicular, 4-10 X 0.5-1 µm in nature, but slightly larger in culture measuring 7-10 X 0.6-1 µm, generally with a guttule in the middle, as in the case of species of *Colletotrichum* or *Gloeosporium*.

Shahjahan *et al*.[7] reported that the mycelium was colourless and cottony with light pinkish colour at reverse, septate, 1.5- 3 µm in diameter. Conidiophores were single or branched, 15- 25 µm long with secondary branches in whorls of 2- 5 phialides, 13- 19 µm long. Conidia were borne singly at conidiophores tips, hyaline, smooth, cylindrical, single celled, 3- 17 x 1.2 µm in culture and conidia from infected tissue measured 2.5- 8.0 x 1.0- 2.0 µ m (Av. 5.0 x 1.0 µm). He reported that these measurements were slightly larger than those of Tasugi and Ikeda[8] and very close to Kawamura[3] (5- 22 x 1.5 - 4.5 µm) (Av. 9.6 x 2.8 µm). Manibhushanrao[4] reported that Conidiophores irregularly branched; sometimes with several branches arising in whorls at one level but more commonly with branches arising laterally in dense rows over a considerable length of the supporting cell; secondary branches often exceeding the primary ones; repeatedly branched conidiophores up to 60 µm or more in length and 2-2.5 µm wide at the base. Conidiogenous cells (phialides) arise from conidiophores or directly from undifferentiated vegetative hyphae, 30-40 µm long and 1.5-2 µm wide at the base when formed at the apex of slender conidiophores, 6-20 µm long and 1 -1.5 µm wide when arising in dense broom-like fasicles; tips tapering to 0.6-1 µm in width, lacking any distinct collarette. Conidia formed in slimy masses, cylindrical with rounded ends, sometimes becoming slightly curved, hyaline, thin-walled, smooth, one-celled, 3.5-7 x 0.8 - 1.5 µm. Chlamydospores absent.

MATERIAL AND METHODS

VARIABILITY AMONG SYMPATRIC ISOLATES OF SAROCLADIUM ORYZAE:

Ten different isolates obtained from 10 different regions are used in present investigation. Details of the isolates used are presented in Table 1.After isolating the fungus from different isolates, variability was assessed in terms of radial growth, mycelial dry weight, conidia and conidiophore

characters on five different solid media and also in terms of pathogenicity using detached leaf technique.

RADIAL GROWTH ON DIFFERENT SOLID MEDIA

Five different solid nutrient media *i.e.,* potato dextrose agar, Czapek's Dox agar, potato sucrose agar, oat meal agar and rice leaf extract agar were used for studying the radial growth of *S. oryzae*. All the media were prepared as explained earlier. The different solid media were poured separately into sterilized Petri dishes aseptically at the rate of 20 ml medium per dish. The media in Petri dishes were allowed to cool and solidify. Each medium contained in the Petri dish was inoculated with individual isolates of *S. oryzae* by transferring the mycelial discs of 5 mm size with the help of a sterilized cork borer from 10 day old culture of the fungus. All the inoculated media were incubated at 27 ± 1^0C temperature. The growth of the fungus in different media was estimated by measuring the diameter of the colony when the culture reaches 16 days after inoculation. For each solid medium, three replications were maintained and the experiment was conducted in completely randomized design.

The growth of *S. oryzae* on solid nutrient media was estimated by measuring the diameter of fungal colony. In order to estimate the diameter of colony, which was irregular in shape, two sets of widest points opposite to each other on the margins of colony were selected and the distance between the widest points in each set was measured and averaged.

MYCELIAL DRY WEIGHT IN DIFFERENT LIQUID MEDIA

Five different liquid nutrient media i.e., Potato sucrose broth, Oat meal broth, Rice leaf extract broth, Potato dextrose broth and Czapek's dox broth were used to study the growth of *S. oryzae*. The sterilized liquid media prepared as described earlier were poured into sterilized conical flasks aseptically and allowed to cool. The liquid media in conical flasks were inoculated with individual isolates of *S. oryzae* by transferring the mycelial discs of 5 mm size into each flask with the help of a sterilized cork borer from 10 day old culture of *S. oryzae*. The inoculated media were incubated at 27 ± 1^0C temperature. The growth was estimated by harvesting the mycelial mat of *S. oryzae* from each medium and measuring the dry weight of mycelial mat. For each medium, three replications were maintained and the experiment was conducted in CRD. Growth of *S. oryzae* was measured by estimation of dry weight of the mycelium. For this purpose, the mycelial mat of *S. oryzae* grown in liquid culture media in flasks was harvested carefully and the mycelial mat enclosed in a whatman paper fold was dried in a hot air oven at a constant temperature (60°C) to eliminate the moisture. The weight of the mycelium was calculated by deducting the weight of filter paper from total weight.

CONIDIATION PER UNIT AREA *IN VITRO* ON DIFFERENT SOLID MEDIA

For the assessment of conidiation on solid media, 40 day old culture on PDA (approximately 3mm diameter) culture disc was taken, transferred to 4ml of sterile distilled water, macerated in waring blender and strained through muslin cloth to exclude the mycelia bits. A drop of such spore suspension was examined under higher power (400X) of microscope for counting the number of conidia. By using haemocytometer number of conidia present in each smallest square is counted from 10 randomly selected smallest squares.

DIMENSIONS OF CONIDIOPHORES AND CONIDIA ON DIFFERENT SOLID MEDIA

For the estimation of dimensions of conidiophores and conidia on different solid media, calibration of stage and ocular micrometer was done by counting the number of divisions of stage micrometer that are coinciding with that of ocular micrometer. After calibration the dimensions (length and Width) of conidiophores and conidia were measured using ocular micrometer fitted into the eye piece of compound microscope.

ASSESSMENT OF METHOD OF INOCULATION

1. Inoculation without puncturing on boot leaf sheath

In this method healthy boot leaf sheath bits of 4cm length were injected by one ml of spore suspension of *S. oryzae* into boot leaf sheath bits without any injuries. The inoculated leaf bits were incubated in moist chamber.

2. Spray inoculation after puncturing on boot leaf sheath

In this method boot leaf sheath bits were punctured with the help of surgical syringe and the spore suspension was sprayed on the leaf bits and they are incubated in moist chamber.

3. Placing mycelial disc in the boot leaf sheath

In this method 5mm size culture discs were placed in the centre of boot leaf sheath bits and incubated in moist chamber.

4. Placing mycelial disc on panicle

In this method 5mm size culture discs were placed over the panicles bits of 6cm length and incubated in moist chamber.

5. Placing mycelial disc on lower leaf sheath (other than boot leaf)

In this method 5mm size culture discs were placed over the healthy green leaf bits and incubated in moist chamber.

Observations were recorded on number of days to initiate symptoms and extent of damage measured as linear lesions, yellowing on leaf sheath or panicle. Symptoms are visualized as yellowing or necrosis extended along the leaf sheath.

One method was chosen based on the results obtained for assessing variability among *Sarocladium oryzae* isolates.

ASSESSMENT OF VARIABILITY AMONG SYMPATRIC ISOLATES OF *SAROCLADIUM ORYZAE*

The method standardized based on the result obtained from 3.3.5 was used to assess variability among sympatric isolates.

In the present investigation placing mycelial disc on boot leaf sheath is followed for assessment of variability among sympatric isolates. In this method boot leaf sheaths were made into small bits of 6cm length. Culture discs of 5mm size were inoculated on the centre of boot leaf sheath and the leaf sheaths are incubated in moist chamber. In each petri dish 3 boot leaf sheaths were placed and 3 such plates of each isolate were maintained. Observations were made regularly for the appearance and development of symptoms.

RESULTS AND DISCUSSION

VARIABILITY AMONG SYMPATRIC ISOLATES OF *S .ORYZAE* FROM NELLORE AND CHITTOOR DISTRICTS OF ANDHRA PRADESH

Ten isolates five from Nellore, viz., So NDP, So IDP, So PLK, So DVS, So NLR and five from Chittoor, viz., So SKL, So PTR, So VMP, So NGR, So RCP were obtained. Variability among the isolates of *S. oryzae* in utilizing various carbon sources was assessed using five different media, Viz., Rice Leaf Extract, Potato Dextrose, Potato Sucrose, Oat Meal and Czapek's Dox media. Radial growth on solid media, mycelial dry weight on liquid media, conidia and conidiophores characters on solid media. Variability was also assessed among the isolates in their pathogenic potential using detached leaf technique

RADIAL GROWTH OF *SAROCLADIUM ORYZAE* ON DIFFERENT SOLID NUTRIENT MEDIA

Five different solid nutrient media, *viz*., RLEA, PDA, PSA, OMA and CDA were tested for their ability to support the optimal growth of *S. oryzae*.

Significant variability was observed among the different isolates tested in the radial growth on different solid media used.

Among the five solid nutrient media, RLEA and PDA supported significantly the largest colony growth (1.7cm) followed by PSA and OMA (1.5cm), while the least growth was observed on CDA (1.3cm) after 16 days after inoculation at 27 ± 1^{o}C.

Among all the isolates tested, isolate So SKL and So PTR recorded largest colony growth (1.7cm) followed by isolate So NDP, So PLK and So NGR (1.6cm), isolate So NLR, So VMP and So RCP (1.5cm) and isolate So

IDP (1.3cm). Least growth was recorded in isolate So DVS (1.2cm).

Interactions between isolate and media were found to be significant. On PDA 100% of the isolates recorded more than 1.5cm radial growth (mean radial growth), 90% isolates on RLRA, 80% isolates on PSA, 70% isolates on OMA. On CDA only 40% of the isolates had more than mean radial growth. Least radial growth eas observed with So DVS (0.7cm) on CDA.

Data from the present investigation was in contradictory to Revathy who reported CDA as the best carbon source.

From the results it is clear that RLEA and PDA were the best solid nutrient media utilized by *S. oryzae* as carbon source. Isolate So DVS had least growth and So SKL and So PTR had maximum radial growth.

MYCELIAL DRY WEIGHT OF *S. ORYZAE* IN DIFFERENT LIQUID MEDIA

Significant variability in terms of mycelial dry weight was observed among the different isolates of *S. oryzae* indicating variability in utilizing available carbon sources.

Of all the liquid media tested Rice leaf extract broth recorded highest dry weight of mycelial mat (0.82g) that differed significantly with other media tested. Potato sucrose broth (0.30g) and Czapek's dox broth (0.30g), Potato dextrose broth (0.28g) and Oat meal broth (0.25g) had least growth with insignificant difference among them.

Among all the isolates tested, isolate So SKL showed the highest dry weight of mycelial mat (0.55g), followed by isolate So PTR (0.50g) with significant difference between them. Isolate So RCP recorded significantly least growth (0.2g) among all the liquid media tested. Other isolates in descending order of mean mycelial dry weight (over all the media tested) with So VMP (0.48g), So NLR (0.47g), So NGR (0.45g), So DVS (0.38g), So PLK (0.34g), So NDP (0.26g) and So IDP (0.25g).

For finding suitability of the medium, overall mean mycelial dry weight, *i.e.,* 0.39g was taken in to consideration.

Interaction between isolates and different liquid media were found to be significant. All the isolates recorded less than 0.39g mycelial dry weight from PSB, PDB OMB and CDB.

Among all the liquid media tested RLEB supported highest dry weight of mycelial mat (0.82g) because, 70% of the isolates recorded more than 0.39g mycelial dry weight (mean mycelial dry weight). On RLEB So NDP (0.18g), So IDP (0.14g) and So RCP (0.04g) had least growth and recorded less than 0.39 mean mycelial dry weight. All other media tested did not support the growth of *S. oryzae* in comparison to RLEB as 95% of the isolates tested showed less than 0.39g dry weight (mean mycelial dry weight). In case of So SKL on PDB (0.44g) and So NLR (0.41g) on CDB, more than 0.39g mean mycelial dry weight was recorded.

Urmila also reported that PDB did not support the growth of *S. oryzae*. Further, in the present investigation least growth was obtained with CDB which was in contradictory to the report published by Revathi.

From the results it is clear that RLEB was the best liquid medium utilized by *S. oryzae* as carbon source. Isolate So SKL (0.55g mean mycelial dry weight) best utilized the available carbon sources among all the isolates tested.

CONIDIATION PER UNIT AREA IN DIFFERENT SOLID NUTRIENT MEDIA

Significant differences were observed in conidiation per unit area on different solid nutrient media indicating variability among the isolates tested.

Irrespective of the media, number of conidia per square centimetre was highest (68.6 x 10^6/sq cm) with isolate So IDP which differed significantly with all other isolates, isolate So VMP (50.8 x 10^6/sq cm), So NGR (41.8 x 10^6/sq cm), So NDP (38.3 x 10^6/sq cm), So PLK (38.1 x 10^6/sq cm) and So RCP (34.6 x 10^6/sq cm) were in between maximum and minimum. Least number of conidia per square centimetre (24.7 x 10^6/sq cm) was recorded with isolate So SKL which differed insignificantly with So PTR (27.7 x 10^6/sq cm), So DVS (30.3 x 10^6/sq cm) and So NLR (31.3 x 10^6/sq cm).

Among the different media number of conidia per square centimetre recorded highest (49.2 x 10^6/sq cm) on OMA followed by CDA (46.2 x 10^6/sq cm) which are on par with each other, followed by PDA (40.2 x 10^6/sq cm) and PSA (35.2 x 10^6/sq cm). Least number of conidia per square centimetre was recorded on RLEA (22.3 x 10^6/sq cm).

Interaction between isolates and media were found to be significant. Isolate So IDP recorded highest number of conidia per square centimetre (174.2 x 10^6/sq cm) on CDA followed by isolate So VMP (97.8 x 10^6/sq cm) on OMA. Isolate So PLK recorded least number of conidia per square centimetre (6.4 x 10^6/sq cm) on CDA followed by isolate So NDP (8.8 x 10^6/sq cm) on RLEA.

Number of conidia per square centimetre recorded highest on OMA as 70% of the isolates was recorded more than 38.6 x 10^6 conidia per square centimetre (*i.e.,* grand mean). RLEA recorded least number of conidia per square centimetre because 100% of the isolates were recorded less than the grand mean. Isolate So IDP, PLK, NGR recorded more than grand mean on at least four media which was not the same with other isolates.

The results of the present investigation revealed variability in number of conidia per square centimetre. Further, the conidiation depended on both media and the isolates tested. Further, OMA favoured higher number of conidia per square centimetre followed by CDA, PDA, PSA and RLEA.

It is interesting to note that RLEA supported maximum vegetative growth but conidiation was very poor. On OMA and CDA that did not support vegetative growth, highest conidia per square was recorded.

No report is available on the variation in the number of conidia per square centimetre produced on different solid media and among different *Sarocladium oryzae* isolates. Urmila reported that Sarocladium oryzae produced 28 X 10^7 conidia/ml at 25^{o}C and 14.5 X 10^7 conidia per ml at 90% relative humidity, found to be the best temperature and relative humidity suitable for *S. oryzae* conidiation.

LENGTH OF CONIDIOPHORES IN DIFFERENT SOLID NUTRIENT MEDIA

Significant variation was observed in the length of conidiophores of different isolates tested on different solid media.

Over all the media tested, maximum mean length of conidiophores was recorded in isolate So RCP (101.2 µm) followed by So PLK (100.9µm), So VMP (92.0µm), So PTR (88.8 µm), So DVS (86.7µm) and So NGR (84.7µm) with insignificant differences among them. Isolate So NLR (81.7µm), So SKL (81.6µm) and So NDP (80.7µm) were found to be in between maximum and minimum. The least length of conidiophores recorded in isolate So IDP (66.9µm).

Among different solid media maximum length of conidiophores recorded on RLEA (95.5µm) followed by CDA (90.3µm), PDA (84.3µm) and OMA (81.3µm). Least length of conidiophores was recorded on PSA (81.2µm). However, insignificant difference existed among them. This indicated that type of medium (carbon source) had no effect on conidiophores length.

Interaction between isolates and media were found to be significant. Longest conidiophores were obtained on RLEA because 70% of the isolates recorded more than 86.5µm length of conidiophores (*i.e.,* grand mean).

Among the isolates tested isolate So PLK recorded longest length of conidiophores (156.9µm) on RLEA. Isolate So SKL recorded smallest conidiophores (42.0µm). Least mean length of conidiophores was recorded on PSA because 60% of the isolates recorded less than the grand mean.

The results of the present investigation revealed significant variation in the length of conidiophores among the isolates and size of conidiophores depended more on the type of isolate than on the type of media used.

It may noted here that conidiation was least on RLEA while the length of conidiophore, though insignificant, was maximum on RLEA. This indicated that conidiophores had more sterile part than that of conidiation on RLEA.

Manibhushanrao[4] reported that length of conidiophores in *Sarocladium oryzae* is above 60µm. Further in the present investigation, mean conidiophores length of 86.5µm is in contradictory to the report published by Ou[5] , Reddy and Gams and Hawksworth who reported that conidiophore length varies between 14-43µm.

LENGTH OF CONIDIA IN DIFFERENT SOLID NUTRIENT MEDIA

Significant variation was observed in the length of conidia on different solid media. Irrespective of the media longest conidia were recorded with isolate So DVS (9.3µm) followed by isolate So PLK (9.1µm) and isolate So SKL (8.3µm) which are on par with each other. Smallest conidia were recorded in isolate So VMP (5.9µm) followed by isolate So PTR (6.5µm) which are on par with each other.

Among different solid nutrient media highest length of conidia was recorded on CDA (8.6µm) which differed significantly with all other media. This was followed by OMA (7.8µm), PSA (7.6µm) and PDA (7.2µm) with insignificant differences among them. Least length of conidia was recorded on RLEA. In CDA maximum length of conidia was recorded because 60% of the isolates recorded more than 7.4µm length of the conidia (*i.e.,* grand mean). In RLEA least length of conidia was recorded because 70% of the isolates recorded less than grand mean.

Interaction between isolates and media were found to be significant. From the interaction between isolates and media longest conidia recorded in isolate So DVS on CDA which is 13.6µm. Isolate So PLK recorded more than 7.4µm length of conidia (*i.e.,* grand mean) on at least four media which was not same with the other isolates.

The results of the present investigation revealed significant variability among the isolates and media tested in terms of conidia length. The length of the conidia was highest on CDA (8.6µm) followed by OMA (7.8µm), PSA (7.6µm), PDA (7.2µm) and RLEA (5.9µm). This result was similar to the number of conidia per square centimetre which was found better on CDA and least on RLEA.

Shahjahan *et al.*[7] reported that the conidial length of *S. oryzae* varied between 3-17µm. Joe and Manibhushanrao[4] and Manibhushanrao[4] reported that the conidial length varied between 3.5-7µm. No reports are available so far on the variation in conidial length associated with type of media or isolate tested.

ASSESSMENT OF VARIABILITY AMONG THE ISOLATES OF *SAROCLADIUM ORYZAE* IN PATHOGENIC POTENTIAL USING DETACHED LEAF TECHNIQUE

In order to assess the pathogenic potential of *S. oryzae* on rice, different methods were assessed using detached leaf technique described in chapter 3.3.5 of material and methods.

Of the different methods tested, placing 5mm disc of 20 day old PDA culture on boot leaf sheath found to be better in terms of measurable lesion length within 9 days after inoculation.

Methods using injecting conidial suspension without injecting and spraying conidial suspension after puncturing boot leaf with syringe needle did not yield measurable lesions. Further, inoculation of mycelial disc on lower leaf sheath (other than boot leaf) did not yield any symptoms indicating innate resistance of lower leaves and leaf sheaths to the pathogen. Inoculation of panicles (at flowering stage) with mycelial disc resulted in blackening of grains. However, disease cannot be quantified. Hence, in the present investigation, inoculation of 5mm culture disc from 20 day old PDA culture on boot leaf sheath was followed for finding variability among different of *S. oryzae*.

Previous reports of Estrada, Raina and Singh and Raju and Singh also indicated that grain inoculation (grain covered with *S. oryzae* culture) or culture disc inoculation was better than spray inoculation.

Cut pieces of uninoculated boot leaf sheaths remains green up to 9 days in moist chamber. The fungus *S. oryzae* was re isolated on the culture medium from the infected plant. Microscopic examination confirmed the

identity of the fungus as *S. oryzae* and the characters were comparable with those of original pathogen obtained from diseased rice plants. When the cut pieces (6cm) of boot leaf sheath of rice cultivar NLR 3041 was inoculated with individual isolates of *S. oryzae*, variation observed on lesion length.

Among all the isolates isolate So NDP with lesion length of 1.5cm was on par with isolate So SKL (1.3cm). Isolate So SKL, So DVS, So IDP, So NLR were on par with each other with lesion lengths of 1.3cm, 1.2cm, 1.2cm, and 1.1cm respectively. The least lesion length recorded with isolate So PTR was 0.6cm, while the isolate So RCP did not show any symptoms (lesion length '0' cm). Thus the present investigation revealed highly pathogenic nature of So NDP and least pathogenic nature of So RCP on rice cultivar NLR 304. Thus the variation observed signified the presence of resistance in rice (no disease with So RCP) and occurrence of races within geographical area.

Table3.2. Designation of *S. oryzae* isolates collected from Nellore and Chittoor districts of Andhra Pradesh

Isolate	District	Locality	Designation assigned to the isolate	Pigmentation of colony growth
1	Nellore	Naidupeta	So NDP	Yellow ochre
2		Indukurpeta	So IDP	Golden deep
3		Pellakur	So PLK	Orange lake
4		Doravarisatram	So DVS	Golden deep
5		Nellore	So NLR	Golden deep
6	Chittoor	Srikalahasthi	So SKL	Golden deep
7		Puttur	So PTR	Golden ochre
8		Vadamalapeta	So VMP	Orange lake
9		Nagari	So NGR	Golden ochre
10		Ramachandrapuram	So RCP	Orange lake

Pigmentation on the under surface of *S. oryzae* culture in PDA plate
Pigmentation based on Munsell colour chart

Table 4.7 Length of the conidia on different solid nutrient media

	Conidia length (µ m)					
Isolate	**PSA**	**OMA**	**PDA**	**RLEA**	**CDA**	**Mean for isolates**
So NLR	7.4	7.7	5.6	5.3	7.9	6.7
So IDP	6.5	6.8	5.6	5.2	8.8	6.6
So PLK	9.1	11.1	4.7	10.2	10.4	9.1
So DVS	7.7	7.3	10.0	8.1	13.6	9.3
So NLR	7.3	9.2	6.7	5.3	10.6	7.8
So SKL	4.6	8.3	12.4	4.0	12.0	8.3
So PTR	9.9	8.7	4.3	4.2	5.2	6.5
So VMP	7.4	6.1	5.3	4.2	6.6	5.9
So NGR	6.9	5.3	8.6	7.8	6.6	7.0
So RCP	9.0	7.1	9.2	5.0	4.7	7.0
Mean for media	7.6	7.8	7.2	5.9	8.6	
	SEm ±	**CD(P =0.01)**	**CV**			
Isolate	0.23	0.65	12.12			
Media	0.16	0.45	12.12			
Interaction	0.51	1.45	12.12			

PSA – Potato Sucrose Agar
OMA – Oat Meal Agar
PDA – Potato Dextrose Agar
RLEA – Rice Leaf Extract Agar
CDA – Czapek Dox Agar

Table 4.5 Conidiation per unit area on different solid nutrient media

Number of conidia per square cm (X 10^6)						
Isolate	**CDA**	**OMA**	**PDA**	**RLEA**	**PSA**	**Mean for isolates**
So NLR	26.3	56.0	65.3	8.8	35.3	38.3
So IDP	174.2	43.4	57.6	14.9	53.1	68.6
So PLK	6.4	62.8	46.3	26.0	49.3	38.1
So DVS	20.1	44.7	43.1	24.9	18.9	30.3
So NLR	36.2	28.7	25.2	36.6	30.0	31.3
So SKL	28.7	18.1	22.2	34.7	19.7	24.7
So PTR	54.7	25.1	21.3	23.6	13.9	27.7
So VMP	48.5	97.8	39.4	16.4	51.8	50.8
So NGR	53.7	40.0	45.8	22.3	47.1	41.8
So RCP	13.1	76.0	36.0	15.1	32.7	34.6
Mean for media	46.2	49.2	40.2	22.3	35.2	
	SEm±	**CD (P= 0.01)**	**CV**			
Isolate	1.9	5.5	19.78			
Media	1.4	3.9	19.78			
Interaction	4.4	12.4	19.78			

PSA – Potato Sucrose Agar OMA – Oat Meal Agar
PDA – Potato Dextrose Agar RLEA – Rice Leaf Extract Agar
CDA – Czapek Dox Agar

Table 4.6 Length of the conidiophore on different solid nutrient media

Conidiophore length (µm)						
Isolate	**PSA**	**OMA**	**PDA**	**RLEA**	**CDA**	**Mean for isolates**
So NLR	92.9	61.7	52.7	91.0	105.2	80.7
So IDP	84.2	57.6	60.9	88.2	43.7	66.9
So PLK	80.6	88.7	98.4	156.9	79.8	100.9
So DVS	82.3	86.4	107.5	77.3	79.8	86.7
So NLR	65.9	77.3	80.9	98.6	85.9	81.7
So SKL	42.0	76.3	94.8	88.0	107.1	81.6
So PTR	104.8	88.8	47.7	95.4	107.4	88.8
So VMP	93.5	52.0	134.3	64.8	115.5	92.0
So NGR	107.6	100.4	60.93	73.1	81.8	84.7
So RCP	58.4	124.1	104.7	122.4	96.5	101.2
Mean for media	81.2	81.3	84.3	95.5	90.3	
	SEm±	**CD (P =0.01)**	**CV**			
Isolate	6.4	18.0	28.76			
Media	4.5	N.S	28.76			
Interaction	14.4	40.4	28.76			

PSA – Potato Sucrose Agar OMA – Oat Meal Agar
PDA – Potato Dextrose Agar RLEA – Rice Leaf Extract Agar
CDA – Czapek Dox Agar

Table 4.3 Radial growth of *S. oryzae* on different solid nutrient media

Isolate	**Radial growth (cm)***					**Mean for isolates**
	RLEA	**PSA**	**PDA**	**OMA**	**CDA**	
So NDP	1.9	1.4	1.5	1.5	1.5	1.6
So IDP	1.9	1.5	1.6	1.6	1.1	1.3
So PLK	1.9	1.4	1.6	1.6	1.4	1.6
So DVS	1.0	1.5	1.7	0.9	0.7	1.2
So NLR	1.7	1.5	1.7	1.6	1.1	1.5
So SKL	1.7	1.9	1.9	1.6	1.3	1.7
So PTR	1.8	1.7	1.9	1.7	1.4	1.7
So VMP	1.7	1.6	1.7	1.4	1.2	1.5
So NGR	1.8	1.5	1.7	1.5	1.5	1.6
So RCP	1.7	1.6	1.6	1.3	1.5	1.5
Mean for media	1.7	1.5	1.7	1.5	1.3	
		SEm ±	**CD (P= 0.01)**	**CV**		
	Isolate	0.02	0.05	0.00		
	Media	0.01	0.03	0.00		
	Interaction	0.03	0.10	0.00		

*Mean of three replications Observations were recorded 16days after inoculation
RLEA – Rice Leaf Extract Agar PSA – Potato Sucrose Agar
PDA – Potato Dextrose Agar OMA – Oat Meal Agar
CDA – Czapek Dox Agar

Table 4.4 Mycelial dry weight of *S. oryzae* on different liquid media

Isolate	Mycelial dry weight (g)*					Mean for isolates
	RLEB	PSB	PDB	OMB	CDB	
So NDP	0.18	0.19	0.32	0.30	0.31	0.26
So IDP	0.14	0.35	0.28	0.22	0.27	0.25
So PLK	0.67	0.21	0.37	0.21	0.29	0.34
So DVS	1.01	0.23	0.17	0.26	0.23	0.38
So NLR	1.08	0.32	0.31	0.23	0.41	0.47
So SKL	1.45	0.28	0.44	0.23	0.38	0.55
So PTR	1.23	0.34	0.38	0.28	0.29	0.50
So VMP	1.30	0.37	0.21	0.23	0.30	0.48
So NGR	1.16	0.30	0.27	0.25	0.27	0.45
So RCP	0.04	0.37	0.16	0.26	0.28	0.22
Mean for media	0.82	0.30	0.28	0.25	0.30	
		SEm ±	**CD (P= 0.01)**	**CV**		
	Isolate	0.03	0.08	25.64		
	Media	0.02	0.05	25.64		
	Interaction	0.06	0.17	25.64		

*Mean of three replications Observations were recorded 16days after inoculation
RLEB – Rice Leaf Extract Broth PSB – Potato Sucrose Broth
PDB – Potato Dextrose Broth OMB – Oat Meal Broth
CDA – Czapek Dox Broth

Table 4.8 Variation in lesion length due to different isolates in detached leaf technique

Isolate	Lesion length* (cm)
S. o NDP	1.5
So IDP	1.2
So PLK	1.0
So DVS	1.2
So NLR	1.1
So SKL	1.3
So PTR	0.6
So VMP	1.0
So NGR	1.0
So RCP	0.0
S.Em ±	0.1
CD (P = 0.01)	0.2
CV	9.5

*Mean of three replications Observations were recorded 9 days after inoculation

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