International Journal of Plant & Soil Science

Volume 35, Issue 19, Page 975-985, 2023; Article no.IJPSS.105332
ISSN: 2320-7035

Isolation and Characterization of Rice Blast and Brown Spot Disease in Different Regions of Tamil Nadu, India

V. Aishwarya [a], S. K. Manoranjitham [a*], C. Gopalakrishnan [a], E. Somasundaram [b] and S. Geethanjali [c]

[a] Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.
[b] Department of Agronomy, Directorate of Agribusiness Development, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.
[c] Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i193633

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105332

Original Research Article

Received: 16/06/2023
Accepted: 22/08/2023
Published: 29/08/2023

ABSTRACT

Rice (*Oryza sativa* L.) is the most important staple food crop cultivated in almost every district of Tamil Nadu. Among the rice fungal diseases, rice blast caused by *Pyricularia oryzae* and brown spot caused by *Bipolaris oryzae* are considered to be most potent threats causing major yield losses in Tamil Nadu. The present study aims in identification and characterization of rice blast and brown spot pathogen from different rice growing regions of Tamil Nadu. Three isolates of blast pathogen and five isolates of brown spot pathogen were collected and characterized based on colony morphology and conidial characters. *P. oryzae* and *B. oryzae* was identified based on cultural and morphological characters. Molecular characterization of blast and brown spot isolates were done with universal ITS primers. Pathogenicity test was carried out for all the isolates of blast

**Corresponding author: E-mail: manoranjitham.k@gmail.com, manoranjitham.sk@tnau.ac.in;*

and brown spot and the isolate showing maximum disease incidence was found as virulent one. The present work of morphological and molecular characterization of blast and brown spot pathogen will therefore be helpful to identify and manage the disease effectively in Tamil Nadu.

Keywords: Bipolaris oryzae; blast; brown spot; characterization; isolates; Pyricularia oryzae; rice.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food crop for more than half of the world's population belonging to the family Poaceae. China is the leading producer of rice followed by India with an area and production of 46.38 million hectares and 130.29 million tonnes respectively during the year 2021-22. Rice is grown in almost all districts of Tamil Nadu. The demand for rice is increasing due to continuous increase in population. There is a need to improve the production strategy of rice by reducing the losses caused by biotic and abiotic stresses [1,2].

Rice diseases are one of the biotic pressures that cause roughly 15.6% of losses annually [3]. Among various diseases of rice, the rice blast and brown spot are the most important fungal diseases causing significant yield loss. The pathogen *Pyricularia oryzae* (T: *Magnoporthe oryzae*) is the causal organism of rice blast. The pathogen infects almost all parts of the plant *viz.,* leaf, collar, neck, panicle etc [4,5]. The symptom appears as minute spots which later become enlarged spindle shaped lesions with grey centre and dark brown margin. The *Bipolaris oryzae* (Syn: *Helminthosporium oryzae*) causes brown spot of rice [6-12]. This pathogen infects the leaves and the panicle, symptom appears as minute brown dots which later become oval shaped resembling sesame seed [13-16].

Various studies on morphological and molecular characterization of rice blast pathogen and brown spot pathogen were carried out [17-19]. Many studies on morphological and molecular characterization of rice blast pathogen *Pyricularia oryzae* [20,21] and rice brown spot pathogen *Bipolaris oryzae* was performed [22,23].

In this study, isolation of the pathogen causing blast and brown spot in rice were done by collecting infected leaf samples from different regions of Tamil Nadu. The isolates were identified and characterized based on morphological characteristics like colony characters, mycelial growth, conidial characters etc. The molecular characterization was done by PCR amplification of ITS regions for all the isolates.

2. MATERIALS AND METHODS

2.1 Isolation of Blast and Brown Spot Pathogen of Rice

Blast and brown spot infected rice leaf samples were collected from different rice growing regions of Tamil Nadu *viz.,* Coimbatore, Perambalur and Thanjavur districts. The infected leaf samples were cut into small pieces along with healthy portion and surface sterilized in 1% sodium hypochlorite for 1 minute followed by subsequent washing with sterile distilled water for three times to remove excess sodium hypochlorite. Then the sterilized leaf bits were blot dried in sterile filter paper and transferred into sterile petri-plate containing Potato Dextrose Agar (PDA) medium. The plates were incubated at 28 ± 2℃ and provided with 12 hours of alternate light and darkness. After 3 days, the actively growing mycelium were sub-cultured and purified by single hyphal tip method. The purified cultures were maintained in PDA slants at 4℃ for further study.

2.2 Morphological Characterization

Morphological characterization of all the isolates of *Pyricularia oryzae* and *Bipolaris oryzae* were carried out in PDA medium and incubated at room temperature for 7 days. After incubation, colony growth, colony characters, conidium shape, colour, and septations were observed for all the isolates. For the sporulation of *P. oryzae* and *B. oryzae*, a 9mm mycelial disc were placed at centre of a sterile glass slide under aseptic condition kept on sterile petri-plate with moist sterile cotton. The petri-plates were sealed and incubated at 25 ± 2℃ for 3 days with alternate 12 hours light and dark period. After 3 days of incubation, the spores were collected using sterile distilled water and observed under compound microscope. The stress condition was given to the pathogen for inducing sporulation by growing in plain agar medium.

2.3 Pathogenicity Test

To prove the pathogenicity of the pathogen, variety CO39 susceptible to both blast and brown spot were raised in pots under glasshouse condition. 21 days old seedlings were inoculated with spore suspension of 5 × 10^5 conidia/ml of each isolate of *Pyricularia oryzae* and *Bipolaris oryzae.* The inoculated plants were given frequent watering and covered with polythene bags to maintain humid condition favourable for disease development. For each pathogen 5 pots were maintained. A healthy plant without inoculation of pathogen served as control. After symptom expression, the pathogen was re-isolated from blast and brown spot infected leaves. The re-isolated culture was confirmed with original culture and confirmed based on cultural and morphological characters.

2.4 Molecular Characterization

The total genomic DNA from all the isolates of *Pyricularia oryzae* and *Bipolaris oryzae* was extracted by the CTAB method [24]. The isolates were grown in Potato Dextrose broth for 15 days. The mycelial mats were collected through filter paper, dried at room temperature for 24 hours and then used for DNA extraction. The genomic DNA extracted was electrophoresed on 0.8% agarose gel and confirmed the presence of genomic DNA by documentation under image analyser.

2.4.1 PCR amplification of ITS region

The universal primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG -3') and ITS- 4 (5'-TCCTCCGCTTATTGATATGC -5') were used for identification of all the isolates of *P. oryzae* and *B. oryzae.* The PCR amplification reaction was carried out for each isolate with a final volume of 10µl, which consists of 5µl Master mix, 1µl of forward primer (ITS-1), 1µl of reverse primer (ITS-4), 2µl of sterile distilled water and 1µl of DNA. The PCR amplification was carried out under the following condition in Thermocycler: initial denaturation at 95℃ for 2 minutes, followed by 40 cycles of denaturation at 95℃ for 1 minute, annealing at 58℃ for 1 minute, extension at 72℃ for 1 minute and final extension at 72℃ for 5 minutes, and then hold at 10℃. Then the PCR products were electrophoresed in 1% agarose gel with ethidium bromide for 45 minutes in 1X TAE buffer and then visualized under UV light in gel documentation unit.

2.4.2 Sequencing of PCR products

A PCR reaction was performed for a total volume of 40µl, using Emerald Amp® GT PCR master mix, forward primer (ITS-1), reverse primer (ITS-4) for genomic DNA of virulent *P. oryzae* isolate (PO CBE1) and *B. oryzae* isolate (BO CBE1). The PCR amplification was carried out in thermocycler. The PCR products were resolved in 1% agarose gel electrophoresis. The PCR products was then sequenced. The obtained nucleotide sequence was used as input sequence in nucleotide blast analysis program at NCBI database. The output data obtained were analysed and the organism showing major score was considered as the closely related species to the test fungus (*P. oryzae* and *B. oryzae*). A phylogenetic tree was constructed using Mega11 software for the virulent isolate and ten closely related isolates obtained from NCBI database.

3. RESULTS

3.1 Isolation of *P. oryzae* and *B. oryzae*

Blast infected leaf samples showing characteristic spindle shaped lesions and brown spot infected leaf samples showing oval shaped lesions were collected from different regions of Tamil Nadu. The pathogen *P. oryzae* and *B. oryzae* were isolated from blast and brown spot infected leaf samples respectively. A total of three isolates of *Pyricularia oryzae* and five isolates of *Bipolaris oryzae* were obtained from different regions of Tamil Nadu (Table 1).

3.2 Morphological Characterization of *P. oryzae* and *B. oryzae*

The colony morphology, growth pattern, radial mycelial growth, conidial characters of all the isolates of *P. oryzae* and *B. oryzae* were studied on PDA medium and presented in Table 2 and Table 3 respectively. The colony characters varied for different isolates of *P. oryzae* (Fig. 1) and *B. oryzae* (Fig. 2). The diameter of the mycelial growth was measured at 7^{th} day after incubation for all the isolates. Among the three isolates of *Pyricularia oryzae,* the PO CBE1 isolate grew well on PDA medium with mycelial growth of 6.72 cm. The isolate BO CBE1 showed maximum mycelial growth of 8.92 cm compared to other isolates of *Bipolaris oryzae*.

All the isolates produced spores on PDA medium. The spores were observed under phase contrast microscope and size, shape and number of septations were studied. The conidia of

B. oryzae isolates varied from oval to cylindrical in shape, slightly curved with a bulge in the middle and tapering towards the ends (Fig. 3). The size of conidia and number of septations also varied among different isolates of *B. oryzae*. The conidia of *P. oryzae* isolates were typically pyriform with a narrow apex, round base and a short hilum, 2 – 3 septate (Fig. 4).

Table 1. Isolates of *Pyricularia oryzae* and *Bipolaris oryzae*

S. No.	Isolates	Location	District
Isolates of *Pyricularia oryzae*			
1.	PO CBE1	Paddy Breeding Station, TNAU	Coimbatore
2.	PO CBE2	Wetland, TNAU	Coimbatore
3.	PO ECK1	AC & RI, Eachangkottai	Thanjavur
Isolates of *Bipolaris oryzae*			
1.	BO BSR1	Research station	Bhavani Sagar
2.	BO CBE1	Paddy Breeding Station, TNAU	Coimbatore
3.	BO CBE2	Wetland, TNAU	Coimbatore
4.	BO PBR1	Farmer's field	Perambalur
5.	BO ECK1	AC & RI, Eachangkottai	Thanjavur

Table 2. Morphological characteristics of *P. oryzae*

S.No.	Isolate	Radial mycelial growth (in cm)*	Colony characters
1.	PO CBE1	6.72 (2.59)	Greyish brown, flat mycelium with concentric growth
2.	PO CBE2	6.08 (2.47)	Light grey to white, flat mycelium with concentric growth
3.	PO ECK1	6.24 (2.50)	Greyish white, slightly raised mycelium with concentric growth
	SE(d)	0.03	
	CD (0.05)	0.065	

** - Mean of five replications*

Table 3. Morphological characteristics of *B. oryzae*

S.No.	Isolate	Radial mycelial growth (in cm)*	Colony characters
1.	BO BSR1	6.68 (2.59)	Mixture of grey and white, fluffy mycelium
2.	BO CBE1	8.92 (2.99)	Greyish black, fluffy mycelium with grey cottony mycelium in centre
3.	BO CBE2	7.68 (2.77)	Greyish, fluffy mycelium
4.	BO PBR1	8.64 (2.94)	Greyish brown, fluffy mycelium
5.	BO ECK1	7.52 (2.74)	Greyish brown, fluffy mycelium with white dots
	SE(d)	0.022	
	CD (0.05)	0.045	

** - Mean of five replications*

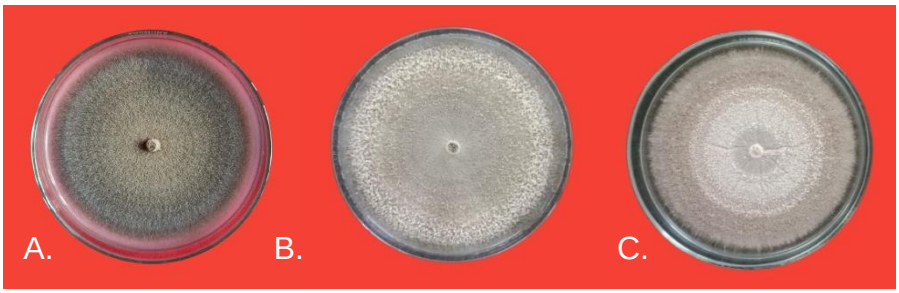

Fig 1. Colony characters of *P. oryzae* isolates
A. PO CBE1; B. PO CBE2; C. PO ECK1

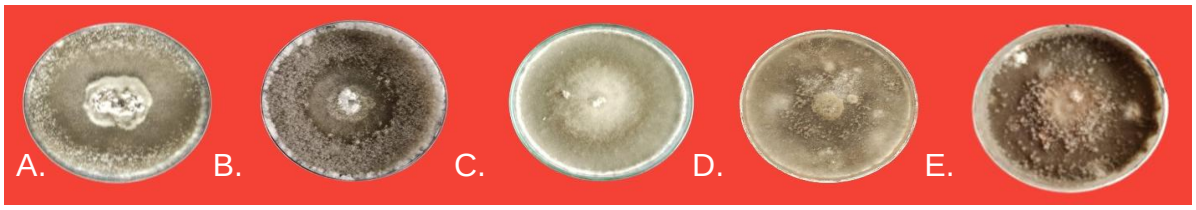

Fig. 2. Colony characters of *B. oryzae* isolates
A. BO BSR1; B. BO CBE1; C. BO CBE2; D. BO PBR1; E. BO ECK1

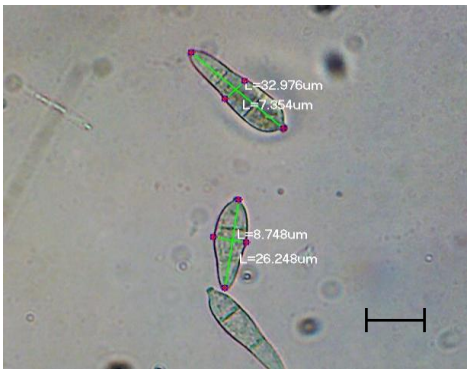

Fig. 3. Conidia of *P. oryzae*

Fig. 4. Conidia of *B. oryzae*

Table 4. Percent disease incidence of isolates of *P. oryzae* and *B. oryzae*

S. No.	Isolates	Percent disease incidence (%)
Isolates of *P. oryzae*		
1.	PO CBE1	85.00
2.	PO CBE2	60.00
3.	PO ECK1	70.00
Isolates of *B. oryzae*		
1.	BO BSR1	50.00
2.	BO CBE1	75.00
3.	BO CBE2	55.00
s4.	BO PBR1	65.00
5.	BO ECK1	50.00

3.3 Pathogenicity Test

The pathogenicity test was performed using variety CO39 susceptible to brown spot and blast. The symptom of brown spot started to appear 3rd day after inoculation. The pathogen initially produced minute, brown spots, which gradually enlarged to oval shaped spots. The blast symptom initiated after 4 days of spraying. The symptom initially appeared as minute spots which later turned into characteristic spindle shaped, brown lesions. The pathogens were re-isolated from the leaf showing typical symptoms. The re-isolated pathogen was confirmed as *P. oryzae* and *B. oryzae* based on colony characters and conidial morphology. The colony characters and conidial morphology of the re-isolated pathogen was observed under compound microscope and confirmed as *P. oryzae* and *B. oryzae*.

From the pathogenicity test, the isolate PO CBE1 and BO CBE1 taken from Paddy Breeding station, Coimbatore were found to be virulent showing highest percent disease incidence when compared to other isolates (Table 4) The plants infected with different isolates are given in Figs. 5 and 6.

3.4 Molecular Characterization of *P. oryzae* and *B. oryzae*

PCR amplification of ITS-1 and ITS-4 regions using universal primers showed an amplicon of

size 560bp for all the isolates of *P. oryzae* (Fig. 7) and *B. oryzae* (Fig. 8). The unpurified PCR product of the virulent isolate of *P. oryzae* and *B. oryzae* were sequenced. The newly obtained sequences of PO CBE1 and BO CBE1 were then deposited in GenBank (NCBI database). The accession number obtained for PO CBE1 was OR304310 and for BO CBE1 was OR143391. The phylogenetic tree for *P.oryzae* showed 100% similarity and *B. oryzae* showed 99% similarity with the other closely related species obtained from NCBI database (Fig. 9. and Fig. 10.)

4. DISCUSSION

4.1 Isolation of *P. oryzae* and *B. oryzae*

The pathogen *P. oryzae* causing rice blast and *B. oryzae* causing brown spot of rice were isolated from infected leaves showing typical symptoms using Potato dextrose agar medium and sub-cultured using single hyphal tip method. Different media like Oat meal agar medium, Rice straw extract agar medium, etc can be used for culturing of the pathogen. The pathogen was identified as *P. oryzae* and *B. oryzae* based on descriptions of colony characters and conidial characters given by Ou [25]. Six isolates of *P. oryzae* was isolated from different regions of Tamil Nadu [21]. Five isolates of *B. oryzae* were collected from different villages in Cuddalore district, Tamil Nadu and characterized on PDA medium [22].

4.2 Morphological Characterization of *P. oryzae* and *B. oryzae*

In the present study, all the isolates of *P. oryzae* and *B. oryzae* showed variations in cultural and morphological characteristics on PDA medium. The mycelium of *P. oryzae* varied from flat to slight raised, greyish white to greyish brown, showing concentric growth pattern. The results were in accordance with the prior work of Gowrisri et al. [20] on the morphological and molecular characterization of *Magnoporthe oryzae,* causing rice blast. They have grown 6 isolates of *Magnoporthe oryzae* on PDA medium and studied the variation in colony characters and conidial size. The mycelia growth showed variation in colour from brownish grey to pure white and both flat and aerial. The spore size also varied between the isolates. Kumar et al. [21] grouped 71 isolates collected from three states of eastern India into five groups based on colony characters. Sahu et al. [26] identified and characterized the rice blast pathogen from different rice growing regions of Odisha and categorized 20 isolates into three groups.

The colony characters of *B. oryzae* varied from greyish brown to greyish black in colour, slightly raised or aerial, fluffy mycelium showing white dots. The conidial size, shape and number of septations also varied among the different isolates. The similar results were obtained by Jaiganesh and Kannan [22]. They studied the cultural characters and pathogenicity of *Helminthosporium oryzae* causing brown spot of rice. The colony morphology of the isolates showed light brown to black, septate, aerial or submerged, branched mycelium. The conidia were light brown to brown in colour, slightly curved with bulge in the middle and tapering toward the end. Monisha et al. [23] collected five isolates of *B. oryzae* from different locations of Tamil Nadu and characterized them based on morphological and cultural characters. Meghana et al (2019) studied the cultural, morphological and molecular variability of *B. oryzae* causing brown spot of rice in Northern Karnataka. Among different media test, Potato dextrose agar medium showed better growth of *B. oryzae.*

4.3 Pathogenicity Test

The pathogenicity of *P. oryzae* and *B. oryzae* were proved by artificial inoculation of spore suspension on the susceptible rice variety CO39 and humid condition was provided. The symptom development was observed 7 days after inoculation and the virulent isolate was obtained based on maximum percent disease incidence. The similar method was followed for proving the pathogenicity of *Magnoporthe oryzae* [20,21,27] and *Bipolaris oryzae* on rice [22,23]. The pathogenicity test was performed by Gowrisri et al. [20] for the virulent isolate of *M. oryzae* and symptom was expressed after seven days of inoculation as typical spindle shaped lesions. Similarly, Kumar et al. [21] performed pathogenicity test for *M. oryzae* isolates on susceptible cultivar and grouped them based on their virulence. Monisha et al. [23] used moderately susceptible variety (CO50) towards brown spot for proving pathogenicity of *B. oryzae* and the symptom initiated 3 days after inoculation.

4.4 Molecular Characterization of *P. oryzae* and *B. oryzae*

The PCR amplification of ITS regions were done using universal primers ITS-1 and ITS-4 for molecular identification of *P. oryzae* and *B.*

oryzae. An amplicon size of ~560bp were obtained from all the isolates of *P. oryzae* and *B. oryzae*. The similar method was used for molecular confirmation of isolates of *P. oryzae* with the amplicon size of ~550 bp for ITS region and ~680 bp for Pot2 transposon region [20]. An amplicon size of ~590 bp was obtained for all the isolates identified by Mohammadpourlima et al. [28]. Monisha et al. [23] examined the isolates for amplification of ITS region and an amplicon size of ~570 bp was observed for all the isolates. The confirmation of the pathogen at species level can be done using gene specific primers. Molecular characterization of *Magnoporthe oryzae* was done by PCR amplification of Pot2 transposon region using specific primers [20].

PO CBE1 **PO CBE2** **PO ECK1**

Fig 5. Pathogenicity test for isolates of *P. oryzae*

BO BSR1 **BO CBE1** **BO CBE2**

BO PBR1 **BO ECK1**

Fig. 6. Pathogenicity test for isolates of *B. oryzae*

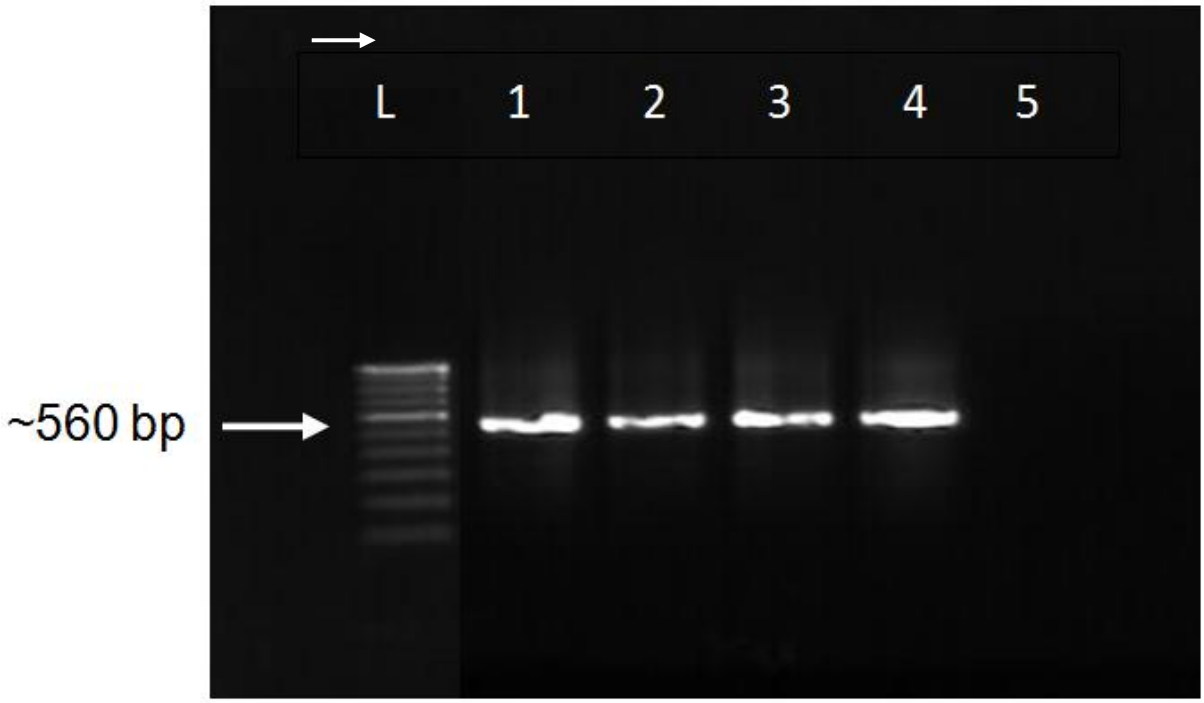

Fig. 7. PCR amplification of ITS region of *P. oryzae*

L – Ladder (100bp), Lane 1 – PO CBE1, Lane 2 – PO CBE2, Lane 3 – PO ECK1, Lane 4 – Positive Control (PC), Lane 5 – Negative control (NC)

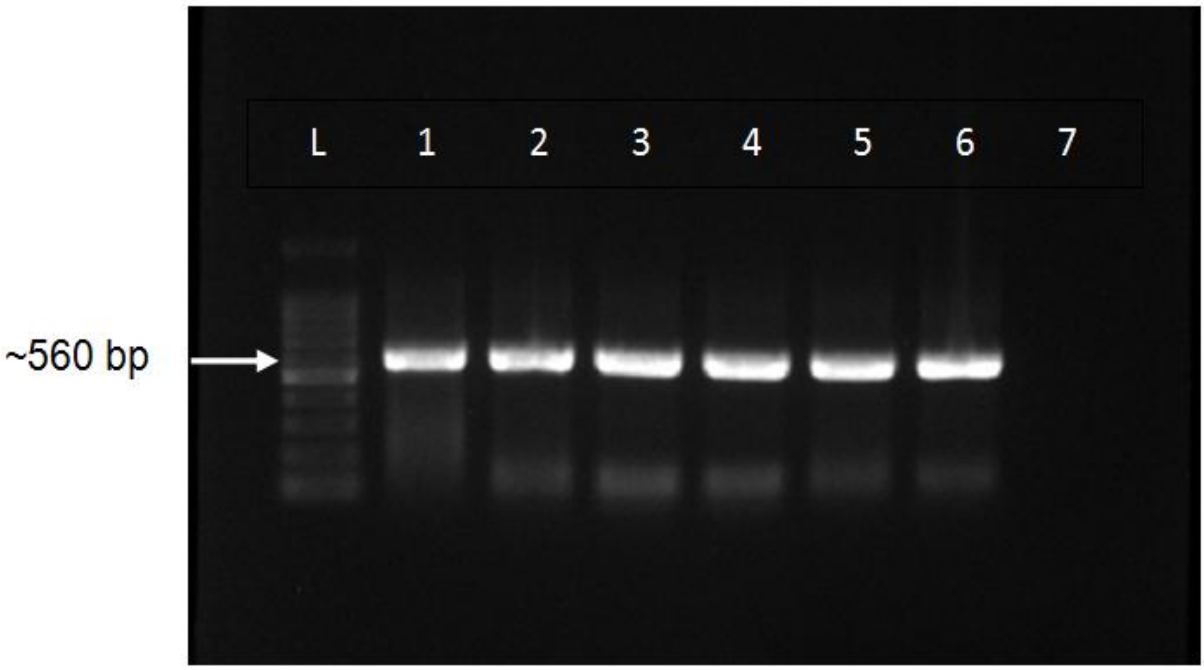

Fig 8. PCR amplification of ITS region of *B. oryzae*

L – Ladder (100bp), Lane 1 – BO BSR1, Lane 2 – BO CBE1, Lane 3 – BO CBE2, Lane 4 – BO PBR1, Lane 5 – BO ECK1, Lane 6 – Positive control (PC), Lane 7 – Negative control (NC)

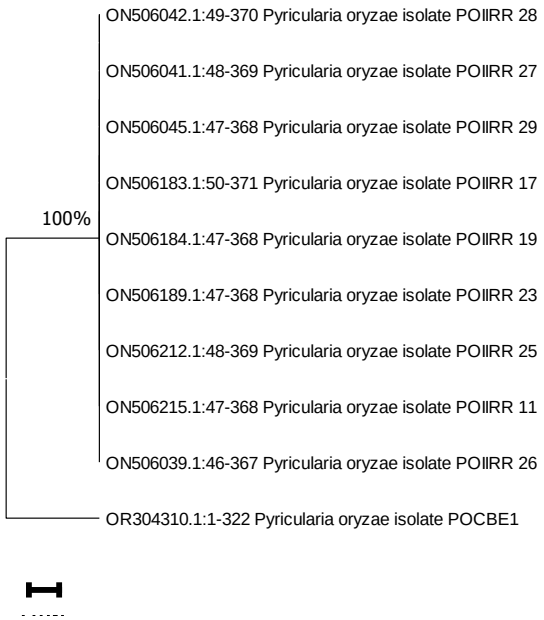

Fig. 9. Phylogenetic relationship for *Pyricularia oryzae*

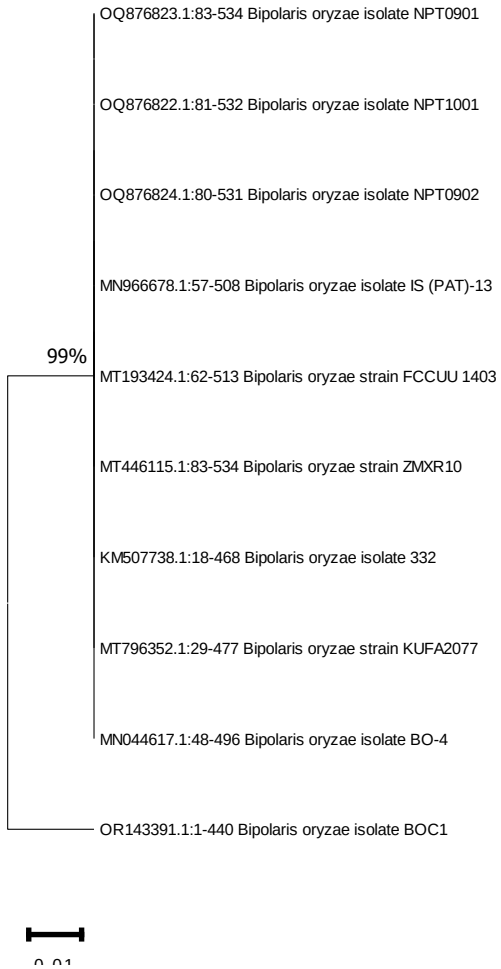

Fig. 10. Phylogenetic relationship for *Bipolaris oryzae*

5. CONCLUSION

Three isolates of *Pyricularia oryzae* causing rice blast and five isolates of *Bipolaris oryzae* causing brown spot of rice were collected from different locations of Tamil Nadu. The isolates were characterized based on morphological and molecular characteristics. The isolates were confirmed as *P. oryzae* and *B. oryzae* based on morphological and cultural characters. All the isolates showed variations in colony growth, growth pattern, mycelial characters, conidia size, shape etc. The cultural variability can also be studied by growing the pathogen in different media.

From the pathogenicity test for both the pathogen, the virulent isolate was found and can be used for further screening. Molecular confirmation of *P. oryzae* and *B. oryzae* was done using PCR amplification of ITS regions using primers ITS-1 and ITS-4. Further confirmation of the pathogen at species level can be done using gene specific primers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. El-Shafey RA, Elamawi RM, El-Wahsh SM, Abdlkhalek AF, Badr EA, El-Refaee YZ. Morpho-pathological and molecular variation studies among different pathotypes of Helminthosporium fungus causing brown spot disease of rice. Egypt. J. Phytopathol. 2011;39(1):135-53.
2. Jagadeesh D, Devaki NS. Morphological and biochemical variation of rice blast fungus Magnaporthe oryzae in Karnataka, India. International Journal of Agricultural Technology. 2020;16(4):799-818.
3. Mondal D, Ghosh A, Roy D, Kumar A, Shamurailatpam D, Bera S, Ghosh R, Bandopadhyay P, Majumder A. Yield loss assessment of rice (*Oryza Sativa* L.) due to different biotic stresses under system of rice intensification (SRI). Journal of Entomology and Zoology Studies. 2017;5(4):1974-80.
4. Priyanka P, Kumar YM, Prabhukarthikeyan SR, Raghu S, Keerthana U, Umakanta N, Pramesh D. Morpho-molecular characterization of Indian population of Magnaporthe oryzae, an incitant of blast disease in rice. Research Journal of Biotechnology. 2021;16:1.
5. Shaw S, Prabhukarthikeyan SR, Keerthana U, Aravindan S, Yadav MK, Raghu S, Baite MS, Naveenkumar R, Parida S, et al. Morphological and molecular characterization of Magnaporthe grisea and bio-efficacy of Bacillus strains against M. grisea. Int. J. Curr. Microbiol. App. Sci. 2019;8(6):1900-8.
6. Dubina E, Nartymov D, Ruban M, Aniskina Y. Molecular and morphological traits of Pyricularia oryzae Cav. in the South of Russia. InBIO Web of Conferences. EDP Sciences. 2022;43:01002
7. Waktola TG. Isolation and morphological characterization of rice pathogens Pyricularia and *Bipolaris oryzae* in metekal zone, North West Ethiopia. population; 3:4.
8. Sudhasha S, Balabaskar P, Kumar KS, Sivakumar T. Prevalence Of Rice Brown Spot Disease Incidence In Northern Districts Of Tamilnadu, India And Observations On Morpho Pathogenic Variability Among Isolates Of Bipolaris Oryzae. Plant Archives. 2020;20(2):3819-25.
9. Aravind K, Rajeswari B, Babu TK, Pushpavalli SN. Pathogenic and molecular variability of Magnaporthe oryzae isolates collected from major rice growing locations of Telangana State, India. Indian Phytopathology. 2022;75(3):661-71.
10. Nazifa Z, Aminuzzaman FM, Akhter K, Rehena MK, Laila L. Survey on rice blast and morphological characterization of Magnaporthe oryzae oryzae. Journal of Advances in Microbiology. 2021 Feb 14;21(1):8-21.
11. Chadha S. Molecular detection of Magnaporthe oryzae from rice seeds. Magnaporthe oryzae: Methods and Protocols. 2021:187-97.
12. Abrol S, Singh SK, Singh VB, Singh AK, Singh R. Morpho-Cultural Variability Characterization of Bipolaris oryzae Causing Brown Leaf Spot of Rice in Jammu Sub-Tropics. International Journal of Environment and Climate Change. 2022;12(11):3378-84.
13. Sobanbabu G, Sabarinathan KG, Parthiban VK, Ramamoorthy V. Isolation, screening and identification of virulent isolates of Bipolaris oryzae causing rice brown spot and Sarocladium oryzae causing sheath rot disease. International Journal of Current Microbiology and Applied Sciences. 2018;7(09):930-9.

14. Sonah H, Deshmukh RK, Parida SK, Chand S, Kotasthane A. Morphological and genetic variation among different isolates of Magnaporthe grisea collected from Chhattisgarh. Indian Phytopathology. 2009;62(4):469.
15. Srivastava D, Shamim MD, Kumar D, Pandey P, et al. Morphological and molecular characterization of Pyricularia oryzae causing blast disease in rice (*Oryza sativa*) from North India. International Journal of Scientific and Research Publications. 2014;4(7):2250-3153.
16. Terensan S, Fernando HN, Silva JN, Perera SC, Kottearachchi NS, Weerasena OJ. Morphological and molecular analysis of fungal species associated with blast and brown spot diseases of Oryza sativa. Plant Disease. 2022;106(6):1617-25.
17. Kulkarni K, Peshwe S. Screening, isolation and molecular identification of rice pathogen Magnoporthe oryzae. Int. J. Adv. Res. 2019;7:428-33.
18. Manjunatha B, Krishnappa M. Morphological characterization of Pyricularia oryzae causing blast disease in rice (*Oryza sativa* L.) from different zones of Karnataka. Journal of Pharmacognosy and Phytochemistry. 2019;8(3):3749-53.
19. Nayak MS, Hiremath SV. Cultural, morphological and molecular characterization of Bipolaris oryzae causing brown leaf spot of rice in Northern Karnataka. Journal of Pharmacognosy and Phytochemistry. 2019;8(2):1235-9.
20. Gowrisri N, Kamalakannan A, Malathi VG, Rajendran L, Rajesh S. Morphological and molecular characterization of Magnaporthe oryzae B. Couch, inciting agent of rice blast disease. Madras Agricultural Journal. 2019;106.
21. Kumar YM, Aravindan S, Prabhukarthikeyan SR, Keerthana U, Raghu S, Archana B, Pankajini S, Motilal B, Kumari KM, PC R, Pramesh D. Characterization and molecular phylogeny of Magnaporthe oryzae causing rice blast disease in eastern India. Research Journal of Biotechnology. 2019;14:6.
22. Jaiganesh V, Kannan C. Studies on the cultural characters and pathogenicity studies of brown leaf spot of rice caused by *Helminthosporium oryzae* (Syn: *Bipolaris oryzae*). Plant Archives. 2019;19: 585-7.
23. Monisha S, Praveen NM, Ramanathan A. Isolation, characterization and management of brown spot disease of rice. Journal of Pharmacognosy and Phytochemistry. 2019;8(3):4539-45.
24. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical bulletin. 1987.
25. Ou SH. Rice diseases. IRRI; 1985.
26. Sahu C, Yadav MK, Panda G, Aravindan S, Umakanta N, Raghu S, Prabhukarthikeyan SR, Keerthana U, Adak T, Sharma V, Mohanty MR. Morphological and molecular characterization of *Magnaporthe oryzae* causing rice blast disease in Odisha. ORYZA-An International Journal on Rice. 2018;55(3):467-72.
27. Panda G, Sahu C, Yadav MK, Aravindan S, Umakanta N, Raghu S, Prabhukarthikeyan SR, Lenka S, Tiwari JK, Kar S, Jena M. Morphological and molecular characterization of *Magnaporthe oryzae* from Chhattisgarh. ORYZA-An International Journal on Rice. 2017;54(3): 330-6.
28. Mohammadpourlima M, Yassoralipour A, Tong PE, Ahmad ZA, Yun WM. Morphological and molecular characterizations of rice blast fungus, Magnaporthe oryzae. Pakistan Journal of Agricultural Sciences. 2017;54(4).

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/105332