



Cultural And Morphological Variability Of *Pyricularia Oryzae* Incitant Neck Blast Of Rice

Ribika¹, Seethiya Mahajan^{1*}

¹Division of Plant Pathology, PG Department of Agriculture, Khalsa university Amritsar

*Seethiyamahajan@gmail.com

ABSTRACT :

Rice (*Oryza sativa*), a member of the Poaceae family, is the most important cereal crop globally and serves as a staple food for nearly half the world's population. However, it is vulnerable to numerous diseases, including Brown Spot, Bacterial Leaf Blight, Sheath Blight, Rice Blast, Bakanae, Stem Rot, Tungro, and False Smut. Among these, Rice Blast, caused by *Pyricularia oryzae*, is the most destructive, leading to 70–80% yield losses. This study focused on the cultural and morphological variability of *P. oryzae* responsible for neck blast in rice. Conducted during Kharif 2023-2024 at the Division of Plant Pathology, P.G. Department of Agriculture, Khalsa College, Amritsar, it analyzed isolates collected from Majha districts of Punjab. Eight isolates were classified into three groups (A, B, and C) based on texture, color, and colony diameter. The colonies ranged from greyish white to greyish black with smooth (A), rough (B), and cottony (C) textures, and diameters of 70–84 mm on PDA media. Virulence studies divided isolates into four groups. Group A (PO5, PO7, PO8) exhibited high virulence, showing symptoms 14 days post-inoculation. Group B (PO3, PO6) showed good virulence (symptoms after 21 days), Group C (PO2, PO4) had fair virulence (symptoms after 28 days), and Group D (PO1) showed poor virulence (symptoms after 32 days). Morphological analysis revealed PO3 had the largest conidia ($10.7 \times 2.3 \mu\text{m}$), while PO2 had the smallest ($7.2 \times 1.5 \mu\text{m}$). This research highlights the diversity in pathogenicity and morphology of *P. oryzae*, aiding better management of Rice Blast disease.

Keywords: Rice, Neck Blast, cultural variability, morphological variability, pathogenicity.

INTRODUCTION :

Rice (*Oryza sativa* L.) is the most important cereal crop of the world. Asia known as rice bowl of the world approximately 90 % or more of the world's rice is grown and consumed in Asia. Among the Asian countries, India is the leading producers of the rice and belongs to family Graminae, and East and South Asia are the main regions for rice production in the world. China (over 210 million metric tons) is the world leading Rice producer followed by India, Indonesia, Bangladesh, Vietnam and rest of the world (FAO 2017). In India, its production is highly concentrated in West Bengal, Punjab, Uttar Pradesh, Andhra Pradesh and Bihar and among them, West Bengal ranked first. Rice grain on an average contains moisture (11.3), fats (0.4), carbohydrates (79.6), proteins (7.0) and crude fibre (0.2) content of percent respectively (Singh et al 2021). The disease was probably first recorded as "rice fever" in China in the year 1637 (Gowrisri et al 2019) and is now present in over 85 countries (Srivastava et al 2014). In Neck blast, initial leaf infection shows brownish lesion with greyish centre and dark brown margin, whereas, size, shape and colour of the lesion are based on the age of lesion, varietal resistance and environmental condition and each lesion from susceptible host can give rise to more than 20,000 conidia, serving as a source for secondary dispersal of the disease infection. The minimum, optimum and maximum temperature for growth and conidial production of *P. oryzae* were 10°C, 25°C and 35°C, respectively (Awoderu et al 1991). The morpho-cultural variability in colony colour, texture, morphological form and margin in conidial characteristics were tested and exist in isolates of *Pyricularia oryzae* (Singh et al 2018).

Material and Methods :

Collection of Samples

The zipper bags were used to collect the disease samples exhibiting characteristics of blast symptoms from different rice growing region of Majha districts in Punjab mentioned in Table 1.

Isolation of pathogen

To verify the presence of the pathogen, both visual and microscopic examinations were conducted on rice samples affected by blast disease. Infected leaves were carefully collected and cut into small pieces, each containing a single blast lesion. These pieces were washed with

tap water and then subjected to surface sterilization using sodium hypochlorite for few seconds, followed by three rinses with sterile distilled water. The surface-sterilized pieces were aseptically transferred to Petri plates containing potato dextrose agar and placed in a BOD incubator at a temperature of $27\pm 10^{\circ}\text{C}$. After three days of incubation, fungal hyphae were observed to have developed in the Petri plates

Immunization

Using a sterile pin, the leaves were punctured, creating small openings. The prepared suspension was then introduced into the punctured leaf using a needle and promptly covered and protected with a cotton swab. Following the inoculation process, the plants were covered with polythene bags. The symptoms appears 5-7 days after inoculation of *P. oryzae*.

Pathogenicity test

To confirm the pathogenic nature of *P. oryzae*, a pathogenicity test was conducted using the isolates of the fungus. Plastic pots were thoroughly cleaned with tap water, disinfected with a 5% sodium hypochlorite solution, and sun-dried. Sterilized soil was then added to the pots. Three healthy seedlings of the rice variety Pusa basmati 1509, aged 21 days, were transplanted into each pot. All recommended agronomic practices were followed to ensure optimal growth of the rice plants in the pots. The inoculum of *P. oryzae* was prepared using PDA culture that had been grown for 15 days. Inoculation was performed by injecting a suspension of the fungus (1×10^8 conidia/ml) onto the rice leaves at the 2-4 leaf stage by using immunization method. The inoculated pots and uninoculated control pots were labelled accordingly and covered with plastic bags to maintain humidity and aseptic conditions. Observations were recorded regarding disease development throughout the crop growth cycle until harvest.

Cultural variability

Five mm mycelial disc of seven day old culture of each isolate was transferred to the centre of sterilized Petri plates containing Potato dextrose Agar (PDA) medium and incubated at $25\pm 1^{\circ}\text{C}$. Colony characters viz., colony diameter, virulence and texture were recorded after 5, 7 and 9 days of inoculation.

Morphological variability

To study the morphological features of the different isolates, five mm mycelial disc from 7 days old cultures were diluted in 5 ml of sterilized distilled water to obtain spore suspension. A drop of spore suspension was put on glass slide and semi permanent slides were prepared and stained with cotton blue in lactophenol. Morphological features viz., spore length (μm) and breadth (μm) of different isolates were recorded and measured with the help of micrometers i.e., ocular micrometer and stage micrometer. Ocular micrometer was placed the tube containing eye piece of 5X magnification and the stage micrometer was placed on the stage of microscope. The objective lens of 40X magnification was focused on ocular division and calculation was made by following formulae:

$$\text{Division of ocular micrometer} = \frac{\text{Reading of stage micrometer}}{\text{Reading of ocular micrometer}} \times 10\mu$$

(Todd *et al* 1979; Mahajan *et al* 2020)

Results and Discussion :

The results based on research investigations entitled “Cultural and Morphological variability of *Pyricularia oryzae* incitant Neck blast of Rice” in Majha region of Punjab. The obtained results are briefly described below:

Isolation of pathogen and its purification

The infected rice blast plants collected from different locations of Majha region of Punjab representing districts viz., Pathankot, Gurdaspur, Amritsar and Tarn taran were brought to laboratory of P.G Department of Plant Pathology, Khalsa College Amritsar for isolation of fungus. The collected disease samples observed under the microscope, to check the presence of the pathogen in infected parts of plant. After confirmation of pathogen in plant's infected parts, it was subjected for isolation. The pathogen *Pyricularia oryzae* was grown on Potato dextrose agar (PDA) medium and were further purified by single spore culture. Sub culturing was done at every 15 days interval to maintain the culture viable. The morphological and cultural characterizations of the cultures grown on PDA medium were studied and on the basis of morphological, cultural and pathogenic characteristics, the isolates were identified as *P. oryzae*.

Symptomatology

The blast symptom caused by the fungus affected both the leaves and the neck of the panicles. Initially, small, water-soaked, greyish dots appeared on the leaves, which later grew into spindle-shaped spots. These spots had a brown margin with a greyish white center. Fully developed lesions reached dimensions of 1-2.5 cm in length and 0.5 cm in width. In severe cases, these spots merged to form large patches of withered tissue. On the neck of the panicle, the fungus caused blackening and shriveling, resulting in a chaffy appearance in the early stages of infection. However, as the grains set, the panicles drooped at the neck. When observed from a distance, the affected field had an overall burnt appearance.

Pathogenicity test

A week after a susceptible host plant (Pusa Basmati 1509) was artificially inoculated by using immunization method, typical disease symptoms started to manifest on the healthy plant. Following that, the pathogen was extracted from the infected lesions once more and compared to the initial culture and symptoms of diseased plant, which validated Koch's Postulates. The re-isolated pathogen's cultural traits corresponded with those of the initial isolate. The pathogen was recognised as *pyricularia oryzae*.

Table 1: Collection of Isolates from different districts of Majha region, Punjab (Kharif2023-2024)

Sr.No.	Districts	Locations	Isolates
1.	Pathankot	Narrot Jaimal Singh	PO ₁
2.		Sarna	PO ₂
3.	Gurdaspur	Hayat Nagar	PO ₃
4.		Jaura chittran	PO ₄
5.	Amritsar	Khalsa College	PO ₅
6.		Sialka	PO ₆
7.	Tarn Taran	Mari Megha	PO ₇
8.		Sarhali Kalan	PO ₈

Table 2: Variability of *Pyricularia oryzae* isolates on the basis of cultural characteristics on PDA medium

Sr.No.	Isolates	Colony Diameter* (mm)	Virulence Pattern**	Colony Characters	
				Colour	Texture
1.	PO ₁	71.6	+	Greyish with white periphery	Smooth
2.	PO ₂	70.9	++	Greyish black	Smooth
3.	PO ₃	75.0	+++	Greyish white	Smooth
4.	PO ₄	75.6	++	Greyish black	Rough
5.	PO ₅	82.3	++++	Greyish white	Cottony
6.	PO ₆	72.3	+++	Light greyish white	Rough
7.	PO ₇	84.6	++++	Greyish white	Smooth
8.	PO ₈	82.3	++++	Greyish white	Rough

*Average of three replication

**Virulence pattern

+ = Poor (32 DAS)

++ = Fair (28 DAS)

+++ = Good (21 DAS)

++++ = Excellent (14 DAS)

Cultural Variability in different isolates of *P. oryzae* on PDA medium

Eight isolates of *P. oryzae* were obtained from different locations of Majha districts of Punjab and were coded as PO₁-PO₈. All the isolates of pathogen, when grown in sterilized petri plates on autoclaved PDA medium, exhibited variability in respect of colony diameter, virulence, texture and colour after 5, 7 and 9 days of incubation at 25±1°C (Table 2). On the basis of their colony diameter, texture, virulence and colour, the isolates were divided into three groups (A, B and C). Group A comprised four isolates PO₁, PO₂, PO₃ and PO₇ that were smooth in texture and showed greyish white to greyish black colonies while in case of virulence PO₁ showed poor virulence (+), PO₂ showed fair virulence (++), PO₃ showed good virulence (+++) and PO₇ showed excellent virulence (++++). The series of group A isolates were the least to highly virulent along with mycelial diameter 71.6mm, 70.9mm, 75.0mm and 84.6mm respectively. Group B comprised three isolates PO₄, PO₆ and PO₈ that were rough in texture and showed greyish black to greyish white colonies and in this group PO₄ showed fair virulence (++), PO₆ showed good virulence (+++) and PO₈ showed excellent virulence (++++) and mycelial diameter of these isolates were 75.6mm, 72.3mm and 82.3mm respectively, whereas, Group C comprised one isolate PO₅ that had cottony texture and was showing greyish white colony and PO₅ showed excellent virulence (++++) with mycelial diameter 82.3mm. The maximum mycelial diameter was observed in isolate PO₇ (86.4mm) among all the isolates and the minimum mycelial diameter was observed in PO₂ (70.9mm). Similar findings were reported by Rahila *et al* (2020) that *P. oryzae* isolates produced greyish white colonies, smooth, rough and cottony texture along with diameter 80-90mm on PDA medium. Our results are consonance with Panda *et al* (2017) who reported that isolates of *P. oryzae* based on colony colour were greyish black to greyish white. Similarities were found in results of Tann *et al* (2012) also reported whitish grey colonies produced by *P. oryzae*. Similarly, Singh *et al* (2018) reported that most of the isolates of *P. oryzae* have blackish grey to whitish grey colonies. Similar results were depicted by Aruna *et al* (2016) concluded that the isolates of *P. oryzae* have rough and smooth surface, grey colony colour with colony diameter ranged from 78 to 90mm.

Table 3: Variability of *Pyricularia oryzae* isolates on the basis of morphological characteristics (length and breadth) on PDA medium

S.No.	Isolates	Locations	Length		Breadth	
			Ranges	Mean*	Ranges	Mean*
1	PO ₁	Narrot Jaimal Singh	9.7-10.7	10.1*	1.7-2.7	2.3*
2	PO ₂	Sarna	6.7-8.1	7.2*	1.1-1.9	1.5*
3	PO ₃	Hayat Nagar	9.9-11.6	10.7*	1.7-2.7	2.3*
4	PO ₄	Jaura Chittran	8.9-11.3	9.9*	1.5-2.4	1.9*
5	PO ₅	Sialka	7.8-8.9	8.2*	1.4-2.2	1.7*
6	PO ₆	Khalsa College	8.7-9.5	9.1*	1.5-2.4	1.9*
7	PO ₇	Mari Megha	7.7-8.9	8.1*	1.4-2.2	1.7*
8	PO ₈	Sarhali Kalan	9.6-11.5	10.3*	1.8-2.5	2.2*

*Average Length & breadth of three replication

PO₁PO₂PO₃PO₄PO₅PO₆PO₇PO₈

Plate 1: Collection of different isolates from Majha districts**Plate 2: Morphological variability of P.oryzae under stereo microscope at 40X magnification****Morphological variability in different isolates of P. oryzae on PDA medium**

The isolates of *P. oryzae*, were grown on PDA culture medium and observations were recorded in length, breadth of spores and number of septa in different isolates (Table 3). The maximum length and breadth were observed in spores of isolate PO₃ (10.7×2.3), followed by PO₈ (10.3×2.2), PO₁ (10.1×2.3), PO₄ (9.9×1.9) and PO₆ (9.1×1.9) and minimum length and breadth was observed in spores of isolate PO₂ (7.2×1.5), followed by PO₇ (8.1×1.7) and PO₅ (8.2×1.7). The conidial spores of all the isolates were pyriform (pear shaped) in shape along with 2septations. Similar results were depicted by Srivastava *et al* (2014) that the shape of conidia of the different isolates was pyriform (pear shaped) with rounded base. Our results are in line with Atlatet *et al* (2023) who also concluded that the conidia of *P. oryzae* were in pyriform shape with two septa and size ranged from 20.76-27.28 ×8.66-10.92 μm. Similarly, Yashaswini *et al* (2023) delineated that conidial size of isolates of *P. oryzae* were ranged from 8-9×3-4 μm to 8-12 ×3-4 μm among all the different isolates. Similar findings were reported by Singh *et al* (2018) reported that the conidial size of *P.oryzae* were ranged from 20.74-24.91×7.53-10.23 μm.

REFERENCES :

1. Anonymous (2017) FAOSTATDatabase.Rome:FoodandAgriculturalOrganization[FAO].
2. Anonymous (2022) Ministry of Agriculture and Farmer Welfare. *Department of AgricultureandFarmersWelfare*:4-6.
3. Altaf H, Mohiddin FA, Ahanger MA, Shikari AB, Jeeani F, Bhat MA, Manzoor T, Nisa N, Khan RS, Khan GH, Dar MS and Amin Z (2023) Cultural and Morphological characterization of rice blast pathogen (*Magnaporthe oryzae*) isolated from temperate region of India. *The Pharma Innovation Journal* **12(12)**: 2705-2708.
4. Awoderu VA, Esuruoso OF and Adeosun OO (1991) Growth and conidia production in race NG-5/IA-65 of *Pyricularia oryzae* Cav. *in vitro*. *Journal of basic microbiology* **31(3)**: 163-168.
5. ArunaJ, KumarSV, RambabuR, RameshS, YashaswiniCH, BhaskarB, MadhaviKR, BalachndranSM, RavindrababuVandPrasadMS(2016)Morphologicalcharacterizationoffivedifferentisolatesof*Pyriculariaoryzae*causingriceblastdisease.*ProgressiveResearch***11** :3377-3380.
6. Gowrisri NA, Kamalakannan AA, Malathi VG, Rajendra LA and Rajesh SB (2019) Morphologicaland molecular characterization of *Magnaporthe oryzae* B. couch, inciting agent of rice blastdisease.*MadrasAgricultural Journal***106**:255-260.
7. GuleriaS, AggarwalR, ThindTSand SharmaTR(2007)Morphologicalandpathologicalvariabilityinriceisolatesof*Rhizoctoniasolani*andmolecularanalysisoftheirgeneticvariability.*JournalofPhytopathology***155**:654-661.
8. KoutroubasDS, KatsantonisD, NtanosADandLupottoE(2009)Blastdiseaseinfluenceonagronomicandqualitytraitsof ricevarietiesunderMediterraneanconditions.*TurkishjournalofAgricultureand forestry***33**:487-494.
9. Kariaga MG, JW and Were KH (2016) Identification of Rice Blast (*Pyricularia oryzae* Cav.) races from Kenyan rice growing regions using culture and classical characterization. *Journal of Research in Agriculture and Animal Science* **4**:16-24.
10. Mahajan S, Kumar D, Singh SK, Mahajan D, Kumar D and Paswal S (2020) Evaluation of different fungicides and bio-agents for the management of chickpea wilt (*Fusarium oxysporum* f. sp. *Ciceri*). *Current Journal of Applied Science and Technology* **39(14)**: 19-30.
11. Miah G, Raffii MY, Ismail MR, Puteh AB, Rahim HA and Asfaliza R and Latif MA (2013) Blastresistance in rice: a review of conventional breeding to molecular approaches. *MolecularBiologyreports* **40**:2369-2388.
12. Nasruddin A and Amin N (2013) effects of cultivar, planting period and fungicide usage on rice blastinfectionlevels andcropyield.*Journal ofAgricultural Science***5**:160-167.
13. Panda G, Sahu C, Yadav KM, Aravindan S, Umakanta N, Raghu S, Prabhukarthikeyan SR, LenkaS, Tiwari KJ, Kar S and Jena M (2017) Morphological and molecular characterization of*Magnaportheoryzae*from chattisgarh. *Oryza An International Journal on Rice***54(3)**: 330-336.
14. Padmanabhan SY (1965) Breeding for blast resistance in India. In 'The rice blast disease'.Ed. Johns Hopkins Press, Baltimore and Maryland, USApp: 203-221.

15. Rahila R, Harish S, Kalpana K and Anand G (2020) Morphological and Pathogenic Variability of *Magnaporthe oryzae* the Incitant of rice blast. *International Journal of Current Microbiology Applied Sciences* **9(11)**:231-238.
16. Sharma OP and Bambawale OM (2008) Integrated management of Key diseases of cotton and rice. *Integrated Management of Plant Pest and Diseases* **4**:271-302.
17. Srivastava, Deepti, Shamim MD, Kumar D, Pandey P, Khan NA and Singh KN (2014) Morphological and molecular characterization of *Pyricularia oryzae* causing blast disease in rice (*Oryza sativa*) from North India. *International Journal of Scientific and Research Publications* **4(7)**: 2250-3153.
18. Seebold, DJ, Correa V, K, Snyder and G (2004) effects of silicon and fungicides on the control of leaf and neck blast in upland rice. *Plant Disease* **88**: 253-258.
19. Singh J, Jain J, Jain S and Lore JS (2018) Morpho-cultural variability among neck blast isolates of *Pyricularia oryzae* from basmati rice in Punjab. *Plant Disease Research* **33**:69-75.
20. Singh P, Hausila, Raigar PM, Basandrai D (2021) Screening for blast resistant in aromatic and basmati rice under northwestern Himalayas. *Plant Disease Resistance* **36**:55-61.
21. Tann H, Makhonpas C, Utthajadee A and Soyong K (2012) Effect of good agricultural practice and organic methods on rice cultivation under the system of rice intensification in Cambodia. *Journal of Agricultural Technology* **8(1)**: 289-903.
22. Todd JC (1979) *Clinical Diagnosis by Laboratory Methods*, Philadelphia, PA, W.B. Saunders Company.
23. Talbot and Nicholas J (2003) On the trail of a cereal's killer: exploring the biology of *Magnaporthe oryzae*. *Annual Reviews in Microbiology* **57**:177-202.
24. Yashaswini CH, Gopika K and Ashwini D (2023) Morphological variability of rice blast pathogen *Magnaporthe oryzae*. *International Journal of Environment and Climate Change* **13**: 4168-4174.