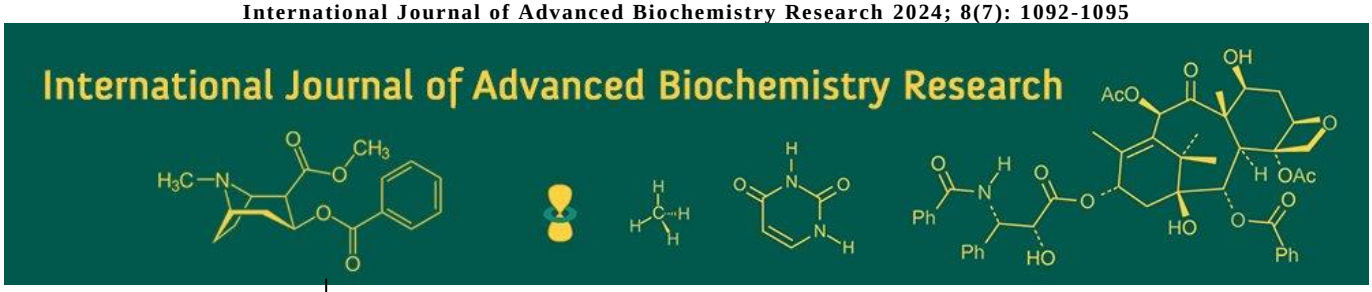

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Aakash
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Ashwani Kumar
College of Agriculture, Kaul, Kaithal, Haryana, India

Anil Kumar Saini
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Mahaveer Singh Bochalya
RRS, Kaul, Kaithal, Haryana, India

Jagdeep Singh
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Lokesh Yadav
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Corresponding Author:
Aakash
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Physiological studies on *Pyricularia oryzae*, inciting rice blast

Aakash, Ashwani Kumar, Anil Kumar Saini, Mahaveer Singh Bochalya, Jagdeep Singh and Lokesh Yadav

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Abstract
Leaf blast, caused by *Pyricularia grisea*, threatens rice cultivation, reducing grain yield by up to 75% (Padmanabhan, 1965). The disease affects various growth stages and causes white to gray lesions, leading to significant crop loss. Physiological studies on *P. oryzae* reveal its optimal growth at 25 °C, 95% humidity, pH 6, and in the presence of maltose and ammonium nitrate. This research aids in developing targeted strategies to manage rice blast disease.

Keywords: Leaf blast, *Pyricularia grisea*, rice cultivation

Introduction
Leaf blast caused by *Pyricularia grisea* poses a significant threat to rice cultivation in both rainfed and irrigated ecosystems. Historical data estimates up to a 75% reduction in grain yield due to this disease (Padmanabhan, 1965) [4]. The disease can manifest at various stages of plant development, including the seedling, tillering, and panicle stages, leading to substantial crop loss during epidemic outbreaks. Early symptoms include white to gray-green lesions or spots with dark green borders. As the disease progresses, these lesions become elliptical or spindle-shaped with whitish to gray centers and red to brownish necrotic borders. The spots are typically diamond-shaped, wider in the center, and tapering towards the ends. These lesions can expand and merge, ultimately leading to the death of entire leaves. Conidiophores of P. oryzae Cav. are simple or rarely branched, 2 - 4 septate, single or in fascicles emerging through stomata, olive to fuliginous, swollen at base, tapering towards lighter colored tip. A single conidium develops at the tip of each conidiophore. Conidia are pyriform to obclavate, light pigmented, mostly 2- septate, rarely 1 - 3 septate and measure 19.2 -27.3 x 8.7 -10.3 µm, with a distinctly protruding basal hilum (Chaube and Pundhir, 2005) [2].

Physiological studies on P. oryzae provide insights into the interactions between the pathogen and its host, the rice plant. Studying the physiological mechanisms of *P. oryzae* infection helps in understanding how the pathogen invades and colonizes the rice plant. This includes the production of appressoria, specialized structures that facilitate host penetration. Investigating how P. oryzae acquires nutrients from the host is vital for understanding its survival and proliferation. The pathogen's ability to efficiently utilize the host's resources directly impacts its virulence and the severity of the disease. *P. oryzae* must cope with various environmental stresses, including temperature fluctuations, pH variations, and limited nutrient availability. Physiological studies reveal the stress response mechanisms of the pathogen, which are essential for its adaptability and persistence. Understanding the physiological aspects of *P. oryzae* is instrumental in developing rice varieties resistant to rice blast disease. Pinpointing physiological vulnerabilities of *P. oryzae* can lead to the development of targeted strategies to disrupt its life cycle and reduce its impact on rice crops. Knowledge of the pathogen's physiological traits enables the breeding of rice varieties with specific resistance genes that can effectively counteract the infection mechanisms of *P. oryzae*.

Isolation of the pathogen: The methodology described by (Tuite, 1969) [6] was adopted for

isolation of pathogen. Briefly, small pieces of diseased leaves, along with some healthy tissue, were cut carefully using a sterile scalpel. These sections were surface sterilized in a 0.1% mercuric chloride solution for 30 seconds, followed by three consecutive rinses in sterilized distilled water under aseptic conditions within a laminar airflow cabinet. The sterilized samples were then transferred aseptically into sterilized Petri dishes containing solidified oatmeal agar. These dishes were incubated at 27±1 °C for two weeks in a BOD incubator. After the incubation period, fungal growth was observed under a microscope.

Identification and maintenance of the pathogen

P. oryzae was identified by its morphological, cultural (texture, colony color, and appearance), and conidial (pear-shaped or pyriform, three-celled and bi-septate with a protruding basal hilum) characteristics. After identification, the pathogen was maintained on OMA slants. To ensure genetic purity, the pathogen was periodically transferred to new slants every 15-20 days and stored at 4°±2 °C. Careful attention was given throughout the study to maintain the genetic integrity of the pathogen.

Effect of different temperature regimes on disease development: To study the effect of various temperature regimes on the mycelial growth of the test pathogen, Petri plates containing PDA were inoculated with 5 mm culture bits of the pathogen and incubated under different temperatures: 20±2 °C, 25±2 °C, 30±2 °C and 35±2 °C for up to 144 hours. Each treatment was replicated three times and data were recorded for average diametric growth (mm), colony characteristics and sporulation. The optimal temperature for further experiments was determined based on these observations.

Effect of different pH levels on disease development

To investigate the effect of various pH levels on the mycelial growth of the test pathogen, PDA media was adjusted to pH levels of 5.5, 6.0, 6.5, 7.0 and 7.5 using 1N HCl and 1N NaOH. The adjusted media was poured into Petri plates and inoculated with 5 mm culture bits. These plates were then incubated at 28±2 °C for up to 144 hours. Each treatment was replicated three times and data were recorded for average diametric growth (mm), colony characteristics, and sporulation.

Effect of different relative humidity levels on disease development: To study the effect of different RH levels on the mycelial growth of test pathogen, Petri plates containing PDA media were inoculated with 5 mm bit of the test pathogen and subjected to different RH levels *viz*., 80%, 85%, 90% and 95% in growth chamber and incubated for 144 hours. Each treatment was replicated thrice and data were recorded in terms of average diametric growth (mm), colony characters and sporulation.

Results

The experiment was designed to assess the effect of temperature on mycelial growth. Fig. 1 shows the results, indicating that the fungus exhibited the most significant growth variation at 25 °C (67.10) after 144 hours of incubation, followed by 20 °C (31.40). Growth and sporulation were inhibited at temperatures below 20 °C and above 30 °C. The optimal temperature for sporulation on potato dextrose agar medium was found to be 25 °C, where *Pyricularia oryzae* demonstrated maximum growth and sporulation. Similar observations were reported by Awoderu *et al*. (1991) [1] and Okeke *et al*. (1992) [3] in their studies on *Pyricularia grisea*. They identified the optimal temperature range for the growth and sporulation of *P. grisea* to be between 20 °C and 30 °C. Their research indicated that deviations from the optimal temperature of 30 °C, whether an increase or a decrease, led to a noticeable and steady decline in the fungal growth. This decline underscores the sensitivity of *P. grisea* to temperature variations, reaffirming the importance of maintaining specific environmental conditions for managing the spread and impact of the pathogen. Fig. 2 shows that *Pyricularia oryzae* exhibited the highest mycelial growth (61.23 mm) at 95% relative humidity, followed by 90% relative humidity (53.25 mm). The least mycelial growth was observed at 80% and 85% relative humidity, with measurements of 32.40 mm and 46.52 mm, respectively. These results indicate that mycelial growth increases with higher relative humidity. The growth of *Pyricularia oryzae* mycelium was significantly influenced by pH, as shown in Fig. 3. The maximum mycelial growth occurred at pH 6 (59.45 mm), which was also accompanied by sporulation, followed closely by growth at pH 5.5 (48.5 mm). In contrast, pH 7.5 exhibited the least mycelial growth (20.40 mm) with no sporulation observed. Optimal conditions for both growth and sporulation were observed at pH 6 and pH 6.5. On potato dextrose agar medium, P. oryzae exhibited maximum growth with traces of sporulation under pH conditions of 5.5, 6, and 6.5, highlighting the fungus's preference for a slightly acidic to neutral pH environment for optimal growth and sporulation. The results regarding carbon sources (Table 1) show that five different carbon sources were tested for their effect on the mycelial growth and sporulation of *Pyricularia oryzae* over various incubation periods. Maltose led to the highest mycelial growth (32.20 mm) after 144 hours of incubation, followed by dextrose (26.20 mm) and glucose (24.10 mm). In contrast, sucrose and fructose resulted in minimal mycelial growth, with measurements of 20.30 mm and 21.40 mm, respectively, compared to the control (17.70 mm). Notably, none of the carbon sources induced sporulation in *Pyricularia oryzae*. Table 2 shows that ammonium nitrate supported the highest mycelial growth of *Pyricularia oryzae* (45.70 mm), followed by potassium nitrate (41.40 mm). In contrast, calcium nitrate resulted in the least mycelial growth (24.26 mm), followed by sodium nitrate (25.50 mm), compared to the control (29.20 mm) after 144 hours. None of the nitrogen sources induced sporulation in *Pyricularia oryzae*. These results are consistent with the findings of Suryanarayanan (1958), who reported similar optimal conditions for the growth of *Pyricularia oryzae.*

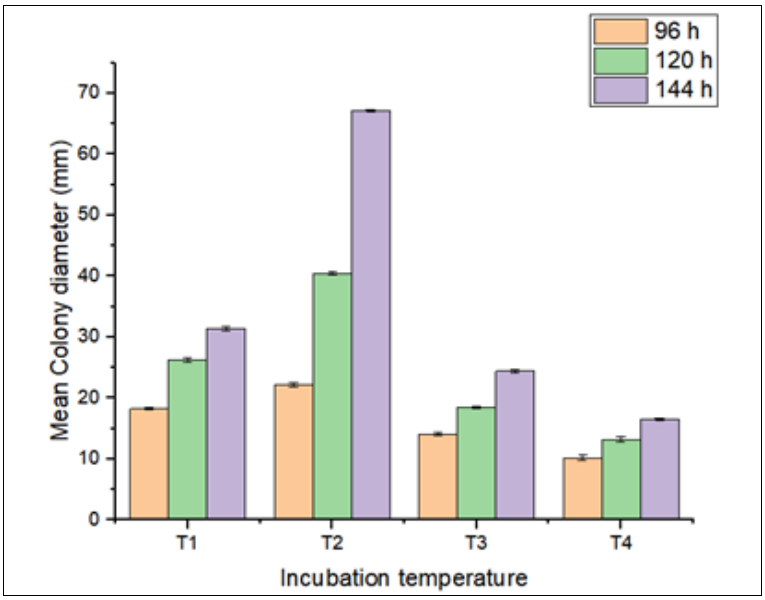

Fig 1: Effect of different temperatures on growth of *P. oryzae*.T_1: 20 ℃ ; T_2: 25 ℃ ; T_3: 30 ℃; T_4: 35 ℃

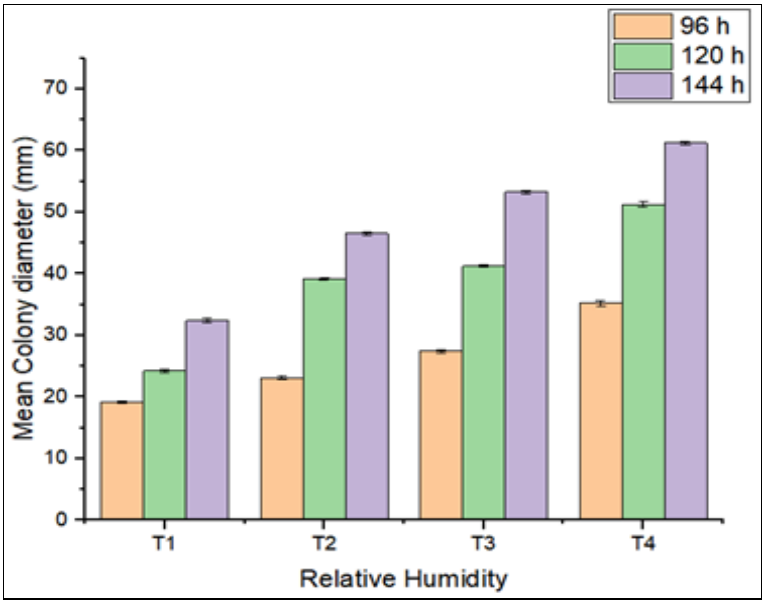

Fig 2: Effect of relative humidity on growth of *P. oryzae*. T_1: 80% ; T_2: 85% ; T_3: 90%; T_4: 95%

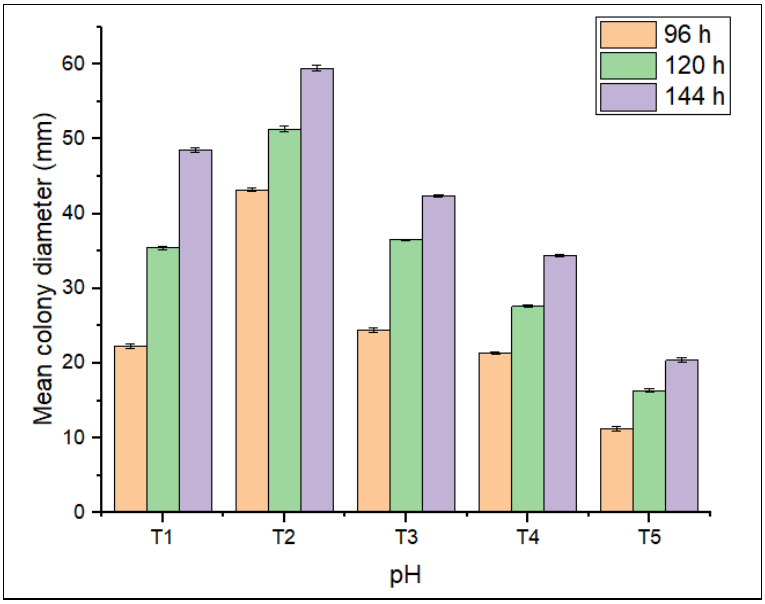

Fig 3: Effect of pH on growth of *P. oryzae*. T_1: 5.5; T_2: 6.0; T_3: 6.5; T_4: 7.0; T_5: 7.5

Table 1: Influence of carbon and nitrogen sources on mycelial growth and sporulation of *P. oryzae*

Sr. No.	Carbon source	Mean colony diameter during incubation (mm)*			Sporulation
		96 hr.	120 hr.	144hr.	
1	Glucose	14.10	22.40	24.20	NIL
2	Dextrose	16.50	24.60	26.20	NIL
3	Sucrose	12.40	19.20	20.30	NIL
4	Fructose	13.20	20.25	21.40	NIL
5	Maltose	18.50	26.40	32.30	NIL
	Control	10.20	15.30	17.70	
		C.D.	SE(m)		
	Factor(A)	0.55	0.19		
	Factor (B)	0.43	0.14		
	FACTOR(AXB)	0.96	0.33		

Table 2: Influence of nitrogen sources on mycelial growth and sporulation of *P. oryzae*

Sr No.	Nitrogen source	Mean colony diameter during incubation (mm)*			Sporulation
		96 hr.	120 hr.	144hr.	No. of conidia/cm^2 (X 10 ^{6})
1	Ammonium nitrate	24.10	41.30	45.70	NIL
2	potassium nitrate	23.40	38.35	41.40	NIL
3	calcium nitrate	18.25	21.40	24.26	NIL
4	sodium nitrate	20.80	22.40	25.50	NIL
	Control	20.25	25.40	29.20	
		C.D.	SE (m)		
	Factor (A)	0.63	0.21		
	Factor (B)	0.54	0.18		
	FACATOR (AXB)	1.09	0.37		

Conclusion

The experimental results highlight the significance of physical factors impact on the mycelial growth and sporulation of *Pyricularia oryzae*. Optimal mycelial growth was observed at a temperature of 25 °C and 95% relative humidity, indicating that *P. oryzae* thrives under warm and highly humid conditions. Among the tested carbon sources, maltose promoted the greatest mycelial growth, whereas sucrose and fructose were less effective. In terms of nitrogen sources, ammonium nitrate and potassium nitrate facilitated the highest mycelial growth, whereas calcium nitrate and sodium nitrate resulted in lower growth. None of the carbon or nitrogen sources tested induced sporulation in *P. oryzae*, suggesting that factors other than the type of carbon or nitrogen source may be critical for sporulation. These findings provide valuable insights into the optimal conditions for the growth of *P. oryzae* and can inform the development of targeted strategies to manage rice blast disease effectively.

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