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Isolation and identification of various isolates of *Xanthomonas oryzae pv. oryzae (Xoo)* (Ishiyama) from different parts of Tamil Nadu and Evaluate its virulence under pot trial

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ABSTRACT

Rice, a staple crop and lifeline for millions, is India's second most important crop after wheat, with the country being one of Asia's leading producers. However, bacterial leaf blight (BLB) caused by Xanthomonas oryzae pv. oryzae (Xoo) poses a severe threat to rice production across both tropical and temperate regions due to its high epidemic potential. This study focused on isolating and evaluating the virulence of various Xoo isolates from different rice-growing areas in Tamil Nadu under controlled pot trials. Among the isolates, Xoo3 was identified as the most virulent, causing a BLB severity of 43.9%, while Xoo6 showed the lowest virulence with a 29.8% severity rate. These findings highlight the variability in pathogenicity among Xoo isolates and underscore the importance of targeted disease management practices to mitigate BLB impact on rice yields in affected regions.

Keywords: *Xanthomonas Oryzae, Rice cultivation, Temperature, Tamil Nadu, Isolation, Pathogens, Bacterial Leaf, BLB Symptoms and Identification*

Introduction

Rice (*Oryza sativa* L.) holds a vital place in global agriculture as a primary food source for more than half of the world's population. In India, rice is not only a staple but also a significant contributor to the economy, positioning the country among the top producers of rice in Asia. However, rice cultivation faces considerable challenges due to various biotic stresses, among which bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is particularly destructive [1]. BLB has emerged as a major constraint on rice production across both tropical and temperate rice-growing regions, due to its capacity to cause extensive crop damage and severe yield losses under epidemic conditions.

Xanthomonas oryzae pv. *oryzae* infects rice plants through natural openings and wounds, progressing from leaf blight symptoms to more extensive tissue necrosis and yield reduction. Its high epidemic potential and adaptability have made BLB an increasingly serious problem, especially under favorable environmental conditions that can accelerate disease spread. Despite extensive research and management practices, the control of BLB remains challenging, mainly due to the pathogen's high genetic variability and adaptability, which lead to frequent outbreaks in rice-growing areas [2].

This study investigates the virulence levels of various *Xoo* isolates collected from different regions in Tamil Nadu to assess the diversity in pathogenicity among these isolates. Pot trials were conducted to evaluate the severity of BLB symptoms associated with each isolate, with the goal of identifying highly virulent strains that pose a heightened risk to rice cultivation. The findings contribute to a better understanding of *Xoo* population diversity and provide insights into the development of region-specific management strategies to control bacterial leaf blight in rice.

Materials and Methods

A field survey was carried out to assess the incidence of bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) across major rice-growing areas in Tamil Nadu, specifically in the districts of Thanjavur, Thiruvarur, and Nagapattinam, during 2013. In each district, three villages were selected for sampling, with five fields surveyed in each village. Within each field, five plots of one square meter were randomly chosen to estimate disease incidence. BLB severity was assessed using the Standard Evaluation System (SES) for rice, as outlined by the International Rice Research Institute (IRRI, 1980). This methodology provided a detailed overview of BLB occurrence and helped identify regions with the highest disease pressure in these districts.

Grade	Description
0	No lesions
1	1% diseased leaf area (DLA)
3	1-5% diseased leaf area (DLA)
5	6-25% diseased leaf area (DLA)
7	26-50% diseased leaf area (DLA)
9	51-100% diseased leaf area (DLA)

The per cent Disease Index (PDI) was then calculated using the following formula (McKinney,1923)

$$PDI = \frac{\text{Sum of numerical rating}}{\text{Total number of leaves graded}} \times \frac{100}{\text{Maximum disease grade}}$$

Isolation and identification of pathogen

To isolate and identify the pathogen responsible for bacterial leaf blight (BLB), symptomatic rice leaves were carefully collected, washed thoroughly with tap water, and allowed to air-dry. Small segments of the infected leaf tissue, approximately 5×5 mm in size, were cut and immersed in sterilized distilled

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water within Petri dishes for 5 minutes, allowing bacterial cells to stream out from the infected tissue. Using a sterilized needle, the bacterial suspension was streaked onto nutrient agar plates as per the procedure of [3]. The plates were then incubated at room temperature for 48-72 hours. After incubation, light yellow colonies characteristic of *Xanthomonas oryzae* pv. *oryzae* were observed. These colonies were then transferred to nutrient agar slants to establish pure cultures, which were preserved for subsequent pathogenicity testing and further biochemical and molecular analysis

Evalution of virulance of Xoo isolates in Pot culture condition

Preparation of bacterial inoculum: To prepare the bacterial inoculum for pathogenicity testing, pure cultures of *Xanthomonas oryzae* pv. *oryzae* were first grown on nutrient agar slants. The slants were incubated at room temperature for 48 hours to ensure robust bacterial growth. After incubation, a sterile loop was used to collect a small amount of the bacterial culture from the slant.

The collected culture was then transferred to a sterile test tube containing 10 mL of sterilized nutrient broth. The suspension was mixed thoroughly to achieve a uniform bacterial distribution. The optical density (OD) of the bacterial suspension was measured at 600 nm using a spectrophotometer, and the concentration was adjusted to reach a final OD of approximately 0.5, corresponding to approximately 10^8 colony-forming units (CFU)/mL.

Once prepared, the inoculum was used immediately for inoculating rice plants or stored at 4°C for short-term use. Prior to inoculation, the bacterial suspension was gently agitated to ensure homogeneity. This standardized inoculum allowed for consistent and reliable assessments of the virulence of different *Xanthomonas oryzae* isolates in subsequent experiments.

Inoculation

For the inoculation of rice plants, the susceptible cultivar ADT 38 was cultivated from surface-sterilized seeds in cement pots, with six hills maintained in each pot. At 45 days after transplanting (DAT), inoculation was performed using the clipping method as described by [4]. A pair of scissors was sterilized by dipping it in the bacterial suspension of *Xanthomonas oryzae* pv. *oryzae* before clipping the top 2.5 to 7.5 cm of the leaves. Control plants were treated with scissors that had been dipped in sterile water to prevent any bacterial infection.

Immediately following the inoculation, the plants were covered with polyethylene bags to create a humid environment conducive to infection. After 24 hours, the bags were removed, and the plants were monitored for disease symptoms. The length of the lesions that developed on the leaves was measured in centimeters after a 14-day observation period.

The Percent Disease Index (PDI) was calculated to quantify the severity of the disease using the following formula as per [5]

$$PDI = (\text{Total number of plants} \times \text{Maximum disease severity} \sum (\text{Disease severity}) \times (\text{Number of plants in each category})) \times 100$$

This method allowed for a comprehensive assessment of the virulence of different *Xanthomonas oryzae* isolates on the susceptible rice cultivar.

Result and Discussion

Survey on the Incidence of Bacterial Leaf Blight in Different Localities of Tamil Nadu

A comprehensive field survey was conducted in 2013 across major rice-growing areas of Tamil Nadu, including Thiruvaiyaru, Thiruvidaimaruthur, Orathanadu, and Papanasam in Thanjavur District, as well as Kuthalam, Sembanarkovil, and Kathiramangalam in Nagapattinam District, and Poondi, Mannargudi, and Nannilam in Thiruvarur District. The data collected during the survey are summarized in Table 1.

The findings revealed that the highest mean percent disease incidence was observed in Orathanadu at 34.7% during the tillering stage, followed closely by Nannilam at 32.3%. In contrast, Poondi exhibited the lowest percent disease index at 10.4%. The variability in disease incidence across these regions may be attributed to differences in the virulence of pathogen isolates and the susceptibility of the rice cultivars grown in these localities.

These results align with previous findings by [6] which indicated that bacterial leaf blight can affect host plants at various growth stages, including seedling, vegetative, and reproductive stages. However, infection during the tillering stage is particularly severe, potentially leading to yield losses of up to 75%, influenced by environmental conditions, geographic location, and the specific rice cultivar. Bacterial leaf blight is recognized as a highly destructive and widespread disease, posing a significant threat to rice production in both temperate and tropical regions due to its high epidemic potential, especially during the tillering stage (Mew, 1987).

Isolation and Identification of Various Isolates of Xanthomonas oryzae pv. oryzae from Different Parts of Tamil Nadu

The isolation of Xanthomonas oryzae pv. oryzae (Xoo) from infected rice plants revealed notable variations in colony characteristics when cultured on nutrient agar medium (Table 2). The ten isolates of Xoo exhibited distinct differences in colony color and morphology. Specifically, the colonies appeared circular, flattened, slightly raised, or convex, displaying a color range from yellow to bright yellow.

Among the isolates, Xoo3, obtained from Orathanadu, exhibited a bright yellow color with a circular, raised, and glittering appearance. In contrast, isolate Xoo8, sourced from Poondi, presented a light yellow color with a more flattened morphology. Additionally, when infected leaf samples were plated on yeast extract dextrose calcium carbonate agar medium, they produced yellow, circular, smooth, convex, and viscous bacterial colonies, corroborating the findings of [7]. This consistency in colony characteristics across different isolates reinforces the identification of Xoo and highlights the phenotypic diversity present in isolates from various geographic locations in Tamil Nadu.

Evaluation of Virulence of Xanthomonas oryzae pv. oryzae Isolates in Pot Culture Conditions

The assessment of the virulence of various Xanthomonas oryzae pv. oryzae (Xoo) isolates under pot culture conditions demonstrated that all isolates were capable of infecting rice plants and eliciting typical bacterial leaf blight (BLB) symptoms, thereby confirming their pathogenic potential (Table 3). Pathogenicity tests indicated a significant variation in virulence among the ten isolates.

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Specifically, isolates Xoo3, Xoo10, and Xoo5 exhibited high levels of virulence, with BLB incidences of 43.9%, 37.2%, and 36.7%, respectively. Conversely, isolate Xoo6 displayed the lowest virulence, with a BLB incidence of 29.8%.

These findings align with the observations of [3] who noted that the virulence of Xoo can vary significantly depending on local environmental factors such as temperature, humidity, and rainfall. The results underscore the importance of understanding the pathogenicity of different isolates, as this knowledge can guide effective disease management strategies in rice cultivation.

Table 1. Survey of disease incidence of BLB incited by Xooin different locality of Tamil Nadu.

S.No	District	Locality	Crop stage	Variety	Disease index (%)
1.	Thanjavur	Thiruvaiaru	Panicle intiation stage	ADT 36	20.3
2.		Thiruvidaimaruthur	Boot leaf stage	ASD 16	19.7
3.		**Orathanadu**	Maximum tillering stage	**ADT 38**	**34.7**
4.		Papanasam	Boot leaf stage	CR 1009	18.4
5.	Nagappattinam	**Kuthalam**	Panicle intiation stage	**ADT 38**	**30.1**
6.		Sembanarkovil	Maximum tillering stage	White ponni	17.8
7.		Kathiramangalam	Boot leaf stage	TKM 9	14.7
8.	Thiruvarur	Poondi	Panicle intiation stage	Co 43	10.4
9.		Mannargudi	Maximum tillering stage	BPT 5204	19.8
10.		**Nannilam**	Panicle intiation stage	**ADT38**	**32.3**

Table.2 Isolation and identification of various isolates of Xoo from different parts of Tamil Nadu

S.No	Isolates	Locality	Colonies of the bacterium (10^5cfu/ml)	Colour	Shape	Appearance
1.	Xoo 1	Thiruvaiaru	78.50	Light yellow	Circular	Slightly raised
2.	Xoo 2	Thiruvidaimaruthur	80.00	Light yellow	Circular	Flattened
3.	Xoo 3	**Orathanadu**	**128.50**	**Bright yellow**	**Circular**	**Raised, glistering**
4.	Xoo 4	Papanasam	47.50	Dull white	Irregular	Slightly raised
5.	Xoo 5	kuthalam	90.50	Light yellow	Circular	Slightly raised
6.	Xoo 6	Sembanarkovil	66.25	Light yellow	Circular	Slightly raised
7.	Xoo 7	Kathiramangalam	57.00	Light yellow	Circular	Slightly raised
8.	Xoo 8	Poondi	49.25	Light yellow	Circular to Irregular	Flattened
9.	Xoo 9	Mannargudi	62.50	Light yellow	Circular	Slightly raised
10.	Xoo 10	Nannilam	88.75	Light yellow	Circular	Slightly raised

Table 3. Evaluation of virulence of Xoo isolates in pot culture condition

S.No	Isolates	Per cent disease index
1.	Xoo 1	32.6
2.	Xoo 2	34.8
3.	**Xoo 3**	**43.9**
4.	Xoo 4	30.1
5.	Xoo 5	36.7
6.	Xoo 6	29.8
7.	Xoo 7	31.7
8.	Xoo 8	34.0
9.	Xoo 9	33.2
10.	Xoo 10	37.2

Conclusion

This study successfully isolated and identified various isolates of *Xanthomonas oryzae* pv. *oryzae* (Xoo) from different rice-growing regions of Tamil Nadu, revealing significant diversity in both colony characteristics and virulence among the isolates. The pathogenicity tests confirmed that all isolates were capable of causing bacterial leaf blight (BLB) in susceptible rice cultivars, with isolates Xoo3, Xoo10, and Xoo5 demonstrating the highest virulence. These isolates exhibited BLB incidence rates of 43.9%, 37.2%, and 36.7%, respectively, indicating their potential threat to rice production.

The findings of this research highlight the critical need for continuous monitoring of Xoo isolates and their virulence in various rice cultivation areas, as environmental factors significantly influence their pathogenicity. Understanding the virulence profiles of different isolates will be instrumental in developing targeted management strategies to mitigate the impact of BLB on rice yield. Future studies should focus on exploring the genetic basis of virulence among these isolates and evaluating the effectiveness of resistant rice varieties against the most virulent strains. Overall, this work contributes valuable insights into the dynamics of bacterial leaf blight in Tamil Nadu and sets the stage for further research on disease management in rice production.

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