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Morphological and Pathogenic Variability among Isolates of *Xanthomonas oryzae* pv. *oryzae*, Causal Agent of Bacterial Leaf Blight of Rice from Godavari Zone of Andhra Pradesh

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Abstract

Bacterial leaf blight (BLB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a major biotic constraint in rice cultivation and is widespread across India. Present study was undertaken to assess morphological, biochemical and pathogenic variability among the twenty isolates of *Xanthomonas oryzae* pv. *oryzae* collected from major rice growing areas of Godavari zone of Andhra Pradesh. Variation in colony size, colour, margin, appearance, texture and pigmentation were observed among the isolates. However, all the isolates were gram negative and tested positive for KOH test, catalase test, and starch hydrolysis test while, tested negative for oxidase test. In the pot study, *Xoo* isolates varied in their pathogenic ability in terms on lesion development post artificial inoculation (leaf clip method) on the three rice cultivars *viz*., BPT-5204, TN-1, and MTU-7029 under study. *Xoo* isolates from Konaseema district were found more virulent followed by isolates from Kakinada, West Godavari, Eluru and East Godavari districts.

Key words: BLB, Morphology, biochemical, pathogenic variability, rice, *Xanthomonas oryzae* pv. *oryzae*.

Introduction

Rice (*Oryzae sativa* L) is the most extensively consumed staple food for nearly 2.7 billion people worldwide, cultivated in tropical, subtropical and temperate countries of the world. In India, paddy production and productivity is greatly hampered by various biotic and abiotic factors resulting in yield losses up to 20-30%. Among the diseases, bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a serious problem and threat to rice production in both tropical and temperate rice growing regions due to its high epidemic potential (Mew, 1987). The causal organism of bacterial leaf blight of rice (BLB), is a Gram-negative, rod shaped, non-spore forming bacteria having single polar flagellum. BLB pathogen is known to be dynamic and have high degree of variability within the pathogen population (Chen *et al*., 2019). Morphologically, *Xoo* populations were typically yellow, mucoid, Gram-negative rods with variation in response of *Xoo* to certain biochemical responses *viz*., catalase activity, KOH solubility, starch hydrolysis and gelatin liquefaction (Yugander *et al.,* 2022). The Yield loss due to bacterial blight can be as much as 70% when susceptible varieties are grown and up to 100% in severe conditions (Walters *et al.*, 2013). In Andhra Pradesh, rice is a major crop in Godavari zone of Andhra Pradesh accounting for 27.7% of the total rice cultivation area (ANGRAU Paddy Outlook Report, 2023–2024). Bacterial leaf blight is one of the major diseases of rice in this

region with incidence ranging from 25-80% on different varieties, rice cvs. Prabhat (IET 3626), Samba Mahsuri (BPT 5204) and PL-1100, whereas cv. Swarna (MTU 7029) was found comparatively resistant (Srivastava, 1966; Laha *et al.*, 2017). High degree of race cultivar specificity was recorded among *Xoo* races infecting different rice cultivars in different parts of the world (Salzberg *et al.*, 2008; Quibod *et al*., 2016). Variation in virulence among *Xoo* isolates was observed among resistant and susceptible rice cultivars, with differences in virulence more apparent during advanced crop growth stages. Virulent strains were reported to exhibit faster multiplication rates and higher population densities, which highlights the epidemiological significance of virulent *Xoo* populations in dictating disease progression during the crop season (Noda and Kaku, 1999). Keeping in view the destructive potential of BLB of rice, there is a need to understand region specific characters (morphology and biochemical reactions) of *Xoo* isolates and race cultivar specificity for formulating future disease management and breeding strategies. This study aims at understanding morphological, biochemical and pathogenic variability among *Xoo* isolates of Godavari zone of Andhra Pradesh.

Materials and Methods

The study was undertaken during the 2024–2025 at RARS, Maruteru (16.63° North, 81.75° West) of Acharya N G Ranga Agricultural University. A total of twenty *Xanthomonas oryzae pv. oryzae* (*Xoo*) isolates were obtained from five districts *viz*., Kakinada, East Godavari, West Godavari, Dr. B. R. Ambedkar Konaseema and Eluru of Godavari zone of Andhra Pradesh. Bacterial leaf blight (BLB)-infected rice leaves were collected during the panicle initiation to heading stages of crop growth. BLB pathogen was isolated from the infected leaves of rice crop as per the procedure described by Shankara *et al.,* (2016). Actively growing bacterial culture (48-72 h old) was used for conducting laboratory assays.

Morphological characterization

The study of colony morphology of twenty *Xoo* isolates was done using the standard procedure described by Bradbury (1970) and Mew *et al.,* (1993) with special consideration on colour, size of colonies, and their outline (circular and entire or indented or wavy or rhizoid). Their elevation was recorded as convex, flat, plate-like, or nodular. The texture and appearance were described as smooth, shiny, slimy, and mucoid, with yellow pigmentation.

Biochemical characterization

Pure culture of the *Xoo* was assayed for their reaction to standard biochemical tests for confirming the identity and understanding variability among the isolates. All the isolates of *X. oryzae* pv. *oryzae* were characterized based on the reaction of isolates in biochemical assays. Five biochemical tests *viz*., Gram staining, KOH test, Catalase, Oxidase test and Starch hydrolysis were performed during the study. Tests were performed as per standard protocols described by Aneja (2003).

Pathogenic Variability

A pot study was conducted at green house facility of Plant Pathology department, RARS, Maruteru. Three rice cultivars *viz*., TN-1 (national susceptible check for BLB), BPT-5204 (local susceptible check) and MTU-7029 (local resistant check) with differential resistance levels were selected for study on pathogenic variability among BLB isolates. Bacterial suspension of each isolate (10^8-10^9cfu/ml) obtained from 3-day old culture was inoculated by leaf clip method of inoculation (Kauffman, 1973). Surface sterilized scissors were dipped in bacterial suspension and used for clipping the top 2-3 cm of healthy leaves during panicle initiation to heading stage of crop growth. Final observations on development of susceptible BLB lesions was recorded at 15 days of inoculation by measuring the diseased lesion length (cm) for confirming pathogenicity of the isolates (Ou, 1985).

Results and Discussion

Cultural characteristics of *Xanthomonas oryzae* pv. *oryzae* isolates were studied at 72 hours after incubation. The pathogenicity of *Xoo* isolates was confirmed by the appearance of typical BLB symptoms 15 days after inoculation. Observations on

the reaction of *Xoo* isolates to biochemical tests were taken as per standard protocol.

Morphological characterization

Colony diameter of twenty *Xoo* isolates collected from Godavari zone of Andhra Pradesh ranged from 1.21 to 2.52 mm. Isolates, *Xoo*10, *Xoo*19, *Xoo*6, *Xoo*11, *Xoo*9, *Xoo*8, and *Xoo*5 produced colonies in the range of 2.04-2.52 mm, whereas, *Xoo*2, *Xoo*12, *Xoo*20, and *Xoo*13 had colonies in the range of 1.24–1.84 mm. Characteristic yellow to dark yellow colonies were observed in twelve *Xoo* isolates, while five isolates recorded light yellow and three isolates creamy yellow colour (**Table 1**). Colony margin was mostly circular, with a few being circular to irregular (*Xoo*3, *Xoo*8, *Xoo*19) or irregular (*Xoo*17). Colony elevation among *Xoo* isolates from Godavari zone was mostly convex, however slightly raised colonies were observed in six *Xoo* isolates and flattened forms were recorded in *Xoo*11 and *Xoo*13. All isolates had a smooth texture and yellow pigmentation (**Table 2**). In terms of colony appearance, all the isolates had slimy and mucoid nature, while ten isolates were observed to be shiny and slimy, and four isolates *Xoo*4, *Xoo*5, *Xoo*10, *Xoo*17 showed shiny and mucoid characteristics. Present findings on variation in *Xoo* colony morphology among the collected isolates were in agreement with Han *et al*., 2005 who summarized *Xoo* morphology as circular, convex, yellow to straw yellow coloured with smooth surface on the nutrient agar medium. Similar findings were also made by Shankara *et al*., (2016) who reported variation in colony size, shape and colour among *Xoo* isolates. Variation in phenotype among BLB isolates of Godavari zone of Andhra Pradesh could likely contribute to differential virulence patterns observed at field level.

Table 1: Isolate code and locations of leaf sample collected for isolation of *Xanthomonas oryzae* pv. *oryzae* isolates (*Xoo*) from Godavari zone of Andhra Pradesh

Sl. No.	Isolate Code	Village	Mandal	District
1	*Xoo* 1	Elakolanu	Rangampeta	East Godavari
2	*Xoo* 2	Gandepalli	Gandepalli	
3	*Xoo* 3	Murari	Gandepalli	
4	*Xoo* 4	Vadisaleru	Rangampeta	
5	*Xoo* 5	Rajupalem	Mummidivaram	Dr. B. R. Ambedkar Konaseema
6	*Xoo* 6	Krapa	Ainavilli	
7	*Xoo* 7	Mummidivaram	Mummidivaram	
8	*Xoo* 8	Magam	Ainavilli	
9	*Xoo* 9	Nadipudi	Penugonda	West Godavari district
10	*Xoo* 10	Chinnamvaripalem	Penugonda	
11	*Xoo* 11	Kodamanchili	Achanta	
12	*Xoo* 12	Pedamallam	Achanta	
13	*Xoo* 13	Gogulapadu	Pedapadu	Eluru district
14	*Xoo* 14	Bhogapuram	Pedavegi	
15	*Xoo* 15	Vatluru	Pedapadu	
16	*Xoo* 16	Koppaka	Pedavegi	
17	*Xoo* 17	Kapavaram	Samalkota	Kakinada district
18	*Xoo* 18	Tatiparthi	Gollaprollu	
19	*Xoo* 19	Gollaprollu	Gollaprollu	
20	*Xoo* 20	Boyanapudi	Samalkota	

Table 2: Morphological characterisation of *Xoo* isolates of Godavari zone of Andhra Pradesh

Sl. No	Isolate	Colony Characters						
		Mean colony diameter (mm)	Colour	Margin	Elevation	Appearance	Texture	Pigmentation
1.	*Xoo*1	1.42	Yellow	Circular	Convex	Mucoid	Smooth	Yellow
2.	*Xoo*2	1.84	Light Yellow	Circular	Slightly Raised	Shiny, Slimy	Smooth	Yellow
3.	*Xoo*3	2.12	Light Yellow	Circular to irregular	Convex	Shiny, Slimy	Smooth	Yellow
4.	*Xoo*4	1.21	Creamy Yellow	Circular	Convex	Shiny, Mucoid	Smooth	Yellow
5.	*Xoo*5	2.52	Dark Yellow	Circular	Convex	Shiny, Mucoid	Glistening	Yellow
6.	*Xoo*6	2.06	Yellow	Circular	Convex	Shiny, Slimy	Smooth	Yellow
7.	*Xoo*7	1.61	Yellow	Circular	Convex	Shiny, Slimy	Smooth	Yellow
8.	*Xoo*8	2.26	Dark Yellow	Circular to irregular	Convex	Slimy	Smooth	Yellow
9.	*Xoo*9	2.14	Dark Yellow	Circular	Convex	Slimy, Shiny	Smooth	Yellow
10.	*Xoo*10	2.04	Dark Yellow	Circular	Slightly Raised	Shiny, Mucoid	Smooth	Yellow
11.	*Xoo*11	2.13	Dark Yellow	Circular	Flattened	Slimy, Shiny	Smooth	Yellow
12.	*Xoo*12	1.24	Dark Yellow	Circular	Slightly Raised	Shiny, Slimy	Smooth	Yellow
13.	*Xoo*13	1.25	Light Yellow	Circular	Flattened	Slimy	Smooth	Yellow
14.	*Xoo*14	1.34	Light Yellow	Circular	Slightly Raised	Shiny, Slimy	Smooth	Yellow
15.	*Xoo*15	1.74	Yellow	Circular	Convex	Slimy	Smooth	Yellow
16.	*Xoo*16	1.64	Creamy Yellow	Circular	Convex	Slimy	Smooth	Yellow
17.	*Xoo*17	1.84	Dark Yellow	Irregular	Slightly, Raised	Shiny, mucoid	Glistening	Yellow
18.	*Xoo*18	1.52	Light Yellow	Circular	Slightly Raised	Lightly Mucoid	Smooth	Yellow
19.	*Xoo*19	2.06	Creamy Yellow	Circular to irregular	Convex	Shiny, Slimy	Smooth	Yellow
20	*Xoo*20	1.24	Yellow	Circular	Convex	Shiny, Slimy	Smooth	Yellow

Biochemical characterization

All the twenty *Xoo* isolates of Godavari zone of Andhra Pradesh uniformly exhibited a Gram-negative reaction and tested positive for KOH solubility indicating Gram -ve nature of *Xanthomonas oryzae* pv *oryzae* (**Table 3**). Further, all isolates were found aerobic as evidenced by bubble formation on exposure to H_2O_2 (catalase test). All *Xoo* isolates were found to have ability to hydrolyse starch (starch hydrolysis test) and the reaction for oxidase test was found negative for all the isolates as evidenced by lack of development of characteristic blue-purple colour when tested

with Kovacs reagent. Although previous findings suggested variation among isolates to catalase, starch hydrolysis and oxidase tests, except being Gram negative and positive reaction to KOH solubility test, (Shankara *et al.*, 2016, Padmaja, 2017 and Sreeramulu and Nayudu, 1987, Jabeen *et al.,* 2012, Patil *et al.,* 2023). However, uniform reaction of *Xoo* isolates to catalase activity, gelatin liquefaction, KOH solubility and protein digestion, with variation mainly confined to starch hydrolysis was observed by Chatterjee (2023). This study observed uniform reaction of *Xoo* isolates to biochemical tests, *viz*., starch hydrolysis, oxidase and catalase tests.

Table 3. Biochemical characterisation of different *Xoo* isolates of Godavari zone of Andhra Pradesh

Sl. No	Isolate	Gram staining	KOH Test (3%)	Catalase Test	Oxidase Test	Starch Hydrolysis
1.	*Xoo*1	Negative	Positive	Positive	Negative	Positive
2.	*Xoo*2	Negative	Positive	Positive	Negative	Positive
3.	*Xoo*3	Negative	Positive	Positive	Negative	Positive
4.	*Xoo*4	Negative	Positive	Positive	Negative	Positive
5.	*Xoo*5	Negative	Positive	Positive	Negative	Positive
6.	*Xoo*6	Negative	Positive	Positive	Negative	Positive
7.	*Xoo*7	Negative	Positive	Positive	Negative	Positive
8.	*Xoo*8	Negative	Positive	Positive	Negative	Positive
9.	*Xoo*9	Negative	Positive	Positive	Negative	Positive
10.	*Xoo*10	Negative	Positive	Positive	Negative	Positive
11.	*Xoo*11	Negative	Positive	Positive	Negative	Positive
12.	*Xoo*12	Negative	Positive	Positive	Negative	Positive
13.	*Xoo*13	Negative	Positive	Positive	Negative	Positive
14.	*Xoo*14	Negative	Positive	Positive	Negative	Positive
15.	*Xoo*15	Negative	Positive	Positive	Negative	Positive
16.	*Xoo*16	Negative	Positive	Positive	Negative	Positive
17.	*Xoo*17	Negative	Positive	Positive	Negative	Positive
18.	*Xoo*18	Negative	Positive	Positive	Negative	Positive
19.	*Xoo*19	Negative	Positive	Positive	Negative	Positive
20.	*Xoo*20	Negative	Positive	Positive	Negative	Positive

Pathogenicity Variability

All the *Xoo* isolates under study collected from Godavari zone of Andhra Pradesh were individually inoculated on three cultivars by leaf clip method of inoculation. After 3 days of inoculation, small water-soaked lesions with pale yellow discoloration appeared at the cut ends, which gradually expanded into yellow lesions with wavy margins, progressing to leaf blighting towards the base. Bacteria from the infected leaf was reisolated and compared with the original culture to prove Koch's postulate. Differences in lesion development indicated relative virulence among BLB isolates in addition to confirming pathogenicity of the isolates. BLB isolates inducing more lesion length in susceptible checks were considered more virulent in comparison in less lesion length producing isolates (Liu *et al.,* 2022). Among the three varieties, local susceptible check, BPT-5204 was found more susceptible to all the *Xoo* isolates recording mean lesion length of 3.66 cm in comparison to 3.25 cm recorded TN1 (**Table 4**). Local resistant check, MTU-7029 recorded mean lesion length of 0.32 cm, confirming its stable resistance to bacterial leaf blight (BLB). These findings are consistent with

earlier reports which indicated TN-1 and BPT-5204 as highly susceptible varieties, while MTU-7029 was recognized for its resistance to *Xoo* strains (Sundaram *et al.,* 2011).

Table 4: Variation in pathogenic ability (lesion length) of *Xoo* isolates on three standard check varieties of rice (Pot study)

Sl. No	BLB Isolate	Mean lesion length (cm) - 15 Days after inoculation			
		BPT-5204 Local Susceptible check	TN-1 National Susceptible check	MTU-7029 Local Resistant check	Mean
1.	*Xoo*1	3.04* (1.88)^{j**}	1.91 (1.55)^{p}	0.28 (0.88)^{vwx}	1.74 (1.43)^{j}
2.	*Xoo*2	3.44 (1.98)^{h}	1.83 (1.52)^{p}	0.27(0.87)^{vwxyz}	1.84 (1.46)^{ij}
3.	*Xoo*3	2.62 (1.76)^{klm}	1.52 (1.41)^{q}	0.47 (0.98)st	1.53 (1.38)^{k}
4.	*Xoo*4	2.01 (1.58)^{p}	4.25 (2.18)^{f}	0.41 (0.94)^{stu}	2.22 (1.57)^{h}
5.	*Xoo*5	4.58 (2.25)^{e}	2.60 (1.76)^{klm}	0.26(0.87)^{vwxyz}	2.48 (1.62)^{g}
6.	*Xoo*6	4.14 (2.15)^{fg}	3.47 (1.99)^{h}	0.33 (0.91)^{uv}	2.64 (1.68)^{f}
7.	*Xoo*7	5.51 (2.45)^{a}	5.35 (2.42)^{ab}	0.68 (1.08)^{r}	3.84 (1.98)^{a}
8.	*Xoo*8	5.28 (2.40)^{ab}	3.05 (1.88)^{j}	0.32 (0.90)^{uvw}	2.88 (1.73)^{e}
9.	*Xoo*9	5.12 (2.37)^{bc}	4.75 (2.29)^{de}	0.51 (0.99)^{s}	3.46 (1.86)^{b}
10.	*Xoo*10	3.59 (2.02)^{h}	3.17 (1.91)^{ij}	0.29 (0.89)^{uvwx}	2.35 (1.60)^{g}
11.	*Xoo*11	2.42 (1.71)^{mno}	2.29 (1.67)^{no}	0.36 (0.92)^{tuv}	1.69 (1.43)^{j}
12.	*Xoo*12	2.21 (1.64)^{o}	3.41 (1.97)^{hi}	0.21 (0.83)^{xyz}	1.94 (1.48)^{i}
13.	*Xoo*13	2.50 (1.73)^{lmn}	2.78 (1.81)^{k}	0.23 (0.85)^{wxyz}	1.83 (1.46)^{ij}
14.	*Xoo*14	5.21 (2.39)^{b}	3.46 (1.99)^{h}	0.34 (0.91)^{uv}	3.01 (1.76)^{de}
15.	*Xoo*15	4.86 (2.31)^{cde}	2.67 (1.78)^{kl}	0.33 (0.91)^{uvw}	2.62 (1.66)^{f}
16.	*Xoo*16	1.41 (1.38)^{q}	4.01 (2.12)^{fg}	0.17 (0.82)^{z}	1.86 (1.44)^{j}
17.	*Xoo*17	2.33 (1.68)^{no}	2.37 (1.69)^{no}	0.17 (0.82)^{yz}	1.62 (1.39)^{k}
18.	*Xoo*18	3.42 (1.98)^{h}	3.41 (1.97)^{hi}	0.27 (0.88)^{vwxy}	2.36 (1.61)^{g}
19.	*Xoo*19	4.71 (2.28)^{de}	3.95 (2.11)^{g}	0.34 (0.92)^{uv}	3.01 (1.77)^{d}
20.	*Xoo*20	4.91 (2.32)^{cd}	4.91 (2.32)^{cd}	0.26 (0.87)^{vwxyz}	3.36 (1.83)^{c}
Mean	3.66 (2.02)^{a}	3.25 (1.92)^{b}	0.32 (0.91)^{c}		
	Variety (A)	**Isolates (B)**	**Interaction: VxI (AxB)**		
SEm ±	0.005	0.013	0.022		
C.D. (p≤0.05)	0.014	0.036	0.063		
CV (%)	2.39				

**Figures in parentheses are square root transformed values; * Values are mean of three replications

Among the BLB isolates, *Xoo*7 from Konaseema was the most virulent, with a mean lesion length of 3.84 cm. Isolates *Xoo*7, *Xoo*9, *Xoo*14, *Xoo*19, and *Xoo*20 were virulent on both susceptible checks, while *Xoo*8 and *Xoo*14 were more virulent on BPT-5204, and *Xoo*4 and *Xoo*16 on TN-1. *Xoo*11 and *Xoo*3 showed moderate reactions on the susceptible checks but higher virulence on MTU-7029 summarised

in **Table 3**. Mean lesion length recorded on susceptible cultivars (**Figure 1**) indicated that *Xoo* isolates from Konaseema district showed more virulence followed by Kakinada, West Godavari, Eluru and East Godavari districts.

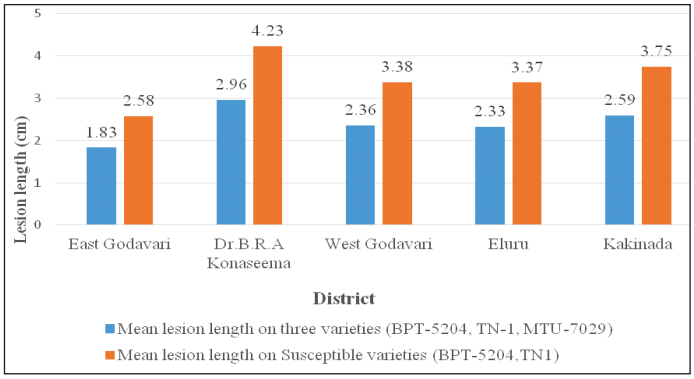

Figure 1: Variation in virulence (mean lesion length on identified check varieties) among *Xoo* isolates of Godavari zone of Andhra Pradesh (Pot study)

These results indicate variability in pathogenicity among *Xoo* isolates from the Godavari zone, consistent with findings by Adhikari *et al.,* (1995) and Hajira *et al*., (2016). Diversity among the *Xoo* genotypes and races could also be due to genomic variation and strain-specific adaptations (Nelson *et al*., 1994, Salzberg *et al.*, 2008). Similarly, Yugander *et al.,* (2022) reported extensive variability among *Xoo* isolates in Andhra Pradesh classifying based on virulence and pathotypes were grouped in three genetic clusters, with several highly aggressive types predominating in the region. Morphological and biochemical variations among the *Xoo* isolates could have induced changes in pathogenic ability among *Xoo* isolates. Further, pathogenic diversity could be attributed to selection pressure on *Xoo* pathogen by the rice cultivar in the location, crop management practices and weather conditions.

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