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Scenario of Bacterial Leaf Blight of Rice caused by *Xanthomonas oryzae* pv. *oryzae* in Major Rice Growing Regions of Karnataka

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ABSTRACT: Bacterial leaf blight (BLB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the major biotic constraints in rice production. The disease causes 20–40% yield loss at tillering stage and, overall, 50–80% yield loss in case of severity. So there is a need to understand the current occurrence of bacterial blight on rice in the major rice growing regions in Karnataka in order to develop the necessary management measures to successfully combat the disease. Roving survey was carried out in major rice growing districts of Karnataka *viz.*, Belagavi, Davanagere, Dharwad, Haveri, Koppal, Shivamogga and Uttar Kannada during *kharif* 2019 to assess the rice bacterial leaf blight severity (BLB). During survey, Belagavi district recorded the highest BLB severity (45.23 %) and lesser severity of 16.86 per cent was recorded in Haveri district. Among the different varieties observed during survey, maximum mean per cent leaf blight severity was recorded in BPT-5204 (54.18%) and least mean per cent leaf blight was recorded in Navali Sali (7.69%). Among the different growth stages of the crop, the maximum bacterial leaf blight severity was noticed during panicle initiation stage (44.98%) and least severity in tillering stage (21.20%). Three biochemical tests (KOH, Starch hydrolysis and methyl red test) were conducted to characterize isolates of *Xoo*. All the *Xoo* isolates showed positive reaction to KOH and starch hydrolysis test and negative to methyl red test.

Keywords: Rice, Bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae*, Per cent disease index (PDI).

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most widely produced food crops of the world and its productivity is being economically affected by many diseases. Due to various biotic pressures including pests, insects, weeds, and viruses, more than 40% of the world's rice is lost each year. The disease causes 20–40% yield loss at tillering stage and, overall, 50–80% yield loss in case of severity (Ullah *et al.,* 2022). One of the most damaging diseases of rice in both irrigated and rain fed ecosystems is bacterial leaf blight (BLB), also known as bacterial blight (BB), which is caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al*. 1990(Mew, 1987). Rice bacterial leaf blight (BLB) was first identified and characterized in Japan (Fukuoka Prefecture) in 1884 (Ou *et al*., 1973). In India, this disease was first identified and reported in Maharashtra in 1951 (Bhapkar *et al*., 1960). BLB was not considered as major disease until it occurred in an epidemic form in Shahabad district of Bihar state in 1963. Later it got spread to other rice growing areas and started causing significant yield loss particularly in high yielding varieties. During 1979 and 1980 BLB has occurred epiphytotically in Haryana, western Uttar Pradesh and Punjab and the severe kresek caused total crop loss (Mew, 1987) in some places. In Kerala (Palakkad district) BLB was severe during 1998 (Venkatesan and Gnanamanickam 1999) and later on it was likely to appear every year and cause great loss in all rice growing states of India. In tropics two main symptoms are found, withering of leaves or kresek and wilting of young plant; and production of pale yellow leaves at the later stage of the crop (Goto, 1964). The leaf blight symptom is generally severe in flowering stage or panicle initiation stage (Singh *et al*., 2013). Bacterial ooze could be noticed in the severe cases out of hydathodes or cracks. The disease occurs worldwide and can cause 30-50 per cent yield loss under epiphytotic conditions (Adhikari *et al*., 1994). Normally the yield loss due to BLB was 20-30 per cent, but in highly infected area it reach up to 80 per cent (Chaudhary *et al.,* 2012). Bacterial leaf blight is one of the maximum recorded diseases in Basmati rice in India. The yield loss caused by *X. oryzae* pv. *oryzae* ranged from 74-81 per cent in severe condition reliant on varietal susceptibility, crop stage and surrounding weather conditions (Baliyan *et al*., 2018). Bacterial leaf blight occurs at all growth stages of rice crop, causing drastic yield losses ranging between 20 and 30 per cent. The highly infected situation causes a yield loss up to 80 per cent which is influenced by environmental conditions (28 to 34°C), crop phase and degree of susceptibility of the genotypes (Tamilarasan *et al*.,

2018). In general, the production losses due to bacterial leaf blight disease range from 20 to 30 %, but in epidemic years, it reaches up to 50% reduction in yield, varietal susceptibility, crop stage and surrounding weather conditions (Baliyan *et al.*, 2018). The infection chain begins by entering the plant through the hydathodes and continues to the xylem vessels, where it spreads throughout the entire plant and become systemic (Ramlingam *et al.,* 2020). This drew the current study's attention to the need to understand the current occurrence of bacterial blight on rice in the major rice growing regions in Karnataka in order to develop the necessary management measures to successfully combat the disease.

MATERIAL AND METHODS

An intensive roving survey was conducted during *kharif* 2019 in different rice growing areas of Karnataka *viz*., Belagavi, Davanagere, Dharwad, Haveri, Koppal, Shivamogga and Uttar Kannada to record the severity of bacterial leaf blight of rice and to collect BLB infected leaf samples with typical symptoms.

The disease index was assessed by recording severity of BLB in a locality by adopting 0-9 scale (Anon., 1980) (Fig. 1). In each field, three random spots of (1×1 sq.mt.) were selected. In each spots, five plants were randomly selected for diseased leaf area. Per cent disease index (PDI) was calculated by using the formula given by Wheeler (1969). Standard Evaluation System (SES), developed for assessing diseased leaf area (Anon., 1980). The mean percentage of diseased leaf area (% DLA) on the upper 3 leaves of plants was considered.

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

Per cent disease index was calculated by using the formula of Wheeler (1969) as given below

Per cent Disease Index (PDI) =

$$\frac{\text{Sum of the individual rating}}{\text{Number of leaves observed} \times \text{Maximum grade}} \times 100$$

Biochemical tests for *Xanthomonas oryzae* pv. *oryzae*. The colonies of 48 hours old cultures were taken for conducting experiments. Three biochemical tests were conducted for the characterization of the pathogen.

Potassium hydroxide (KOH 3%) test. Potassium hydroxide (3% KOH) test is an excellent validation assay for Gram staining (Sulsow *et al*., 1982). The *Xoo* culture (48 hrs old) was taken with sterilised tooth pick and it was vigorously stirred in the drop of 3 per cent KOH solution on sterilised glass slide under laminar air flow chamber.

Starch hydrolysis. Sterilized starch agar (2 g Starch, 5 g Beef extract, 10 g Peptone, 20 g Agar dissolved in 1000 ml Distilled water) of pH-6.8 medium was poured (10-15 ml) into the Petriplates and inoculated with the individual isolate aseptically, labelled and incubated at 28°C for 3 days for maximum bacterial growth. The inoculated plates were flooded with Lugol's iodine solution (Iodine solution: Iodine 1.0g + Potassium Iodine 20 g in 100 ml of water) after 4-7 days of incubation. Hydrolysis of starch was indicated by opaque zones around the colonies of *Xoo.*

Methyl red test. The representative isolates of every district were taken from the glycerol stocks and cultured on the nutrient agar plates for two days. A loopfull of culture was transferred to the test tubes containing nutrient broth. After two days the inoculated broth was added with methyl red indicator (0.1g methyl red dissolved in 300 ml of ethanol and made up to 500 ml with distilled water) aseptically. Change in colour indicates positive reaction or else negative reaction.

RESULTS

An intensive roving survey was carried out in seven major rice growing districts of Karnataka and a total of seventeen talukas were surveyed from the seven districts. Disease severity scale of 0-9 (Anon, 1980) was used to assess the BLB severity (Fig. 1). BLB was observed in all the places surveyed. The stage of the crop varied from tillering to maturity (Fig. 2).

The study revealed that the per cent disease severity of bacterial leaf blight ranged from 5.06 to 76.04 per cent (Table 1). Among the seven districts, maximum mean per cent disease severity (PDI) of BLB was recorded in Belagavi district (45.23%) followed by Shivamogga district (37.40%) and minimum mean per cent disease severity was recorded from Haveri (16.86%) and followed by Dharwad (22.20%) during *kharif* 2019 (Fig. 3). Among the different varieties observed during survey, maximum mean per cent leaf blight severity was recorded from BPT-5204 (54.18%) followed by Annada (51.74%) and Uduru Sali (50.36%) and least mean per cent leaf blight was recorded in Navali Sali (7.69 %) (Table 2). Among the different growth stages of the crop, the maximum bacterial leaf blight severity was noticed during panicle initiation stage (44.98%) and least severity in tillering stage (21.20%) which is represented graphically in Fig. 4.

In Belagavi district, the disease severity was in the range of 25.36 to 72.85 per cent. The maximum disease severity was recorded from Khanapura taluk (46.11 %) at panicle initiation stage. In Davanagere disease index ranged from 21.02 to 38.25 per cent. The higher disease severity was recorded in Harihara taluk (30.78 %). Among the villages 38.25 per cent at panicle initiation stage. In Dharwad district, the disease severity was in the range of 5.32 to 50.58 per cent. Among the two taluks, the maximum disease severity was recorded from Kalghatagi (28.90 %). Haveri and Hangal taluks were surveyed under Haveri district. The disease severity ranged from 5.06 to 23.39 %. The highest disease severity was noticed at grain filling stage. The two taluks of Koppal, Gangavathi and Koppal were surveyed for BLB severity. The disease severity ranged from 25.036- 40.25 per cent. In Shivamogga the disease severity ranged from 8.26 to 76.04 per cent.

Kadadkatte was recorded with maximum disease severity of 76.04 per cent at panicle initiation stage. In Uttar Kannada district, disease severity ranged from 13.54 to 40.68 per cent. Kathur was recorded with maximum disease severity of 40.68 per cent at booting stage.

DISCUSSION

The results of the current studies showed that the disease, bacterial leaf blight of rice may appear at any stage of the crop. But, the severity of this disease is very high at its maximum tillering and panicle initiation stage of the crop (Fig. 4). In the severe conditions, the disease leads to sterile pollen and poor seed setting. The results of this study are in confirmative with the survey studies of Shivalingaiah and Umesha (2011) where highest disease severity of 37 per cent was recorded in the cultivar Jyothi in Davanagere district. Doddi variety was recorded with least disease incidence of 1.6 per cent in Madikeri district. Similarly, Kumar *et al*. (2013) recorded the maximum disease severity in Kathiramangalam (36.6 %) due to the influence of temperature, rainfall stage of the crop and susceptibility of cultivars. Suresh and Yenjerappa (2014) showed that the disease severity ranged from 25.95-57.25% where the disease severity was correlated with crop stage, varietal susceptibility and high temperature of 35 °C. Temperature and rainfall play an important role in bacterial leaf blight disease development (Lubis *et al.,* 2020). Haque *et al.,* 2022, also concluded that weather parameters pay very important role in making this disease epidemic.

The study regarding biochemical characterization of all 21 *Xoo* isolates showed positive reaction to KOH test and starch hydrolysis and negative to methyl red test (Fig. 5). Shivalingaiah and Umesha (2011) stated that the *Xoo* isolates taken pink-red and exhibited thin viscid mucoid thread like strand representing positive for Gram negative nature and KOH test of the bacteria. Around the bacterial colonies a clear zone was formed when flooded with Lugol's iodine which indicated positive reaction to starch hydrolysis. All the isolates showed negative for methyl red test. Ahmad *et al.* (2015) recorded that all the 35 *Xoo* isolates were Gram negative and rod shaped and positive for KOH test, Starch hydrolysis and Tween- 80 hydrolysis test but negative to methyl red test. All 54 isolates of *Xoo* from different rice growing regions of South India showed positive for 3% KOH test, gelatine liquefaction, catalase test and starch hydrolysis. Negative for oxidase and methyl red test (Shankara *et al.,* 2017). In the similar studies, Padmaja *et al*. (2018) tested 60 *Xoo* isolates from Andhra Pradesh and Telangana which were positive to starch hydrolysis and nitrate reduction and negative to oxidase test, methyl red test, indole and urease production. Rashid *et al.* (2022) showed that all the isolates of *Xanthomonas axonopodis* pv *punicae* were Gram negative and Positive to both starch hydrolysis and KOH test.

Table 1: Survey for the severity of bacterial leaf blight of rice in different rice growing areas of Karnataka.

Sr. No.	Districts	Taluks	Villages	Mean PDI of villages	Mean PDI of Talukas
1.	Belagavi	Khanapura	Khanapura	55.36	**46.11**
			Bidi	41.53	
			Gundenatti	43.53	
			Junjwada	30.63	
			Hebbala	35.64	
			Bekwada	69.95	
		Belagavi	Belagavi	48.37	**44.35**
			Bennali	59.32	
			Kakati	25.36	
		Kittur	Kittur	28.3	**45.22**
			Devagav	27.36	
			Degulahalli	72.85	
			Basarkhodu	30.36	
			Honnapura	67.25	
2.	Davanagere	Davanagere	Davanagere	38.25	**29.94**
			Basapura	30.56	
			Kabbla	21.02	
		Harihara	Honnalli	37.2	30.78
			Harihara	25.01	
3.	Dharwad	Dharwad	Mugad	25.26	**20.51**
			Mandihala	5.32	
			Honnapura	30.25	
			Aravatagi	40.05	
			Kumbarakoppa	5.95	
			Alnavara	16.2	
		Kalghatagi	Dummavada	5.6	**28.9**
			Kalghatagi	36.36	
			Hirehonnallii	45.36	
			Dastikoppa	5.94	
			Gudihala	6.36	
			Belavantara	45.65	

			Tavarageri	50.58	
			Machapura	35.36	
4.	Haveri	Haveri	Haveri	5.06	**15.66**
			Hosalli	20.72	
			Aladakatti	15.48	
			Gourapura	21.36	
		Hanagal	Shivalli	23.39	**18.05**
			Akkialur	18.94	
			Hanagal	11.82	
5.	Koppala	Koppala	Kegdal	25.06	**31.78**
			Varadatti	40.25	
			Kamalapua	30.03	
		Gangavathi	Gangavathi	30.12	**31.44**
			Shivapura	38.2	
			Basapura	26.01	
6.	Shivamogga	Shivamogga	Nidige	30.54	**35.1**
			Malavagoppa	45.36	
			Harige	30.56	
			Hadonahalli	13.02	
			Abbalagere	56.04	
		Shikaripura	Amatekoppa	64.02	**43.6**
			Ambaragoppa	25.06	
			Beguru	67.65	
			Annapura	33.06	
			Arasingere	28.2	
		Badravathi	Anaveri	10.35	**33.49**
			Arakere	8.26	
			Kadadakatte	76.04	
			Aralahalli	39.3	
7.	Uttara Kannada	Mundagod	Mundagod	28.53	**27.78**
			Kathur	40.68	
			Hunagunda	20.36	
			Agadi	15.46	
			Indoor	33.85	
		Sirsi	Sirsi	25.74	**21.95**
			Banavasi	18.58	
			Isloore	23.65	
			Kadgod	13.54	
			Yakkumbi	28.25	
		Haliyala	Haliyala	14.2	**25.35**
			Madanalli	29.74	
			Muttalmuri	26.25	
			Havagi	20.68	
			Magwada	35.87	

Table 2: Mean severity of rice bacterial leaf blight in different varieties during kharif 2019.

Sr. No.	Varieties	Mean per cent disease index
1.	Abhilash	31.63
2.	Annada	51.74
3.	BPT-5204	54.18
4.	Dodda saali	32.16
5.	Dodiga	29.24
6.	Indrani	44.95
7.	Intan	30.11
8.	IR-1010	33.85
9.	IR-64	22.3
10.	Jaya	33.46
11.	Kaagi saali	32.66
12.	Mangala	35.64
13.	Navali Saali	7.69
14.	Saali	16.55
15.	Sirsi local	28.5
16	Uduru saali	50.36

Table 3: Biochemical tests for identification *Xanthomonas oryzae* pv. *oryzae* isolates.

Isolates	Gram's Staining			KOH test			Starch hydrolysis			Methyl red test		
Belagavi												
KXO-2	–	–	–	+	+	+	+	+	+	–	–	–
KXO-3	–	–	–	+	+	+	+	+	+	–	–	–
KXO-4	–	–	–	+	+	+	+	+	+	–	–	–
Davanagere												
KXo-6	–	–	–	+	+	+	+	+	+	–	–	–
KXo-7	–	–	–	+	+	+	+	+	+	–	–	–
KXo-8	–	–	–	+	+	+	+	+	+	–	–	–
Dharwad												
KXo-9	–	–	–	+	+	+	+	+	+	–	–	–
KXo-14	–	–	–	+	+	+	+	+	+	–	–	–
KXo-15	–	–	–	+	+	+	+	+	+	–	–	–
Haveri												
KXo-16	–	–	–	+	+	+	+	+	+	–	–	–
KXo-18	–	–	–	+	+	+	+	+	+	–	–	–
KXo-19	–	–	–	+	+	+	+	+	+	–	–	–
Koppal												
KXo-20	–	–	–	+	+	+	+	+	+	–	–	–
KXo-22	–	–	–	+	+	+	+	+	+	–	–	–
KXo-23	–	–	–	+	+	+	+	+	+	–	–	–
Shivamogga												
KXo-24	–	–	–	+	+	+	+	+	+	–	–	–
KXo-26	–	–	–	+	+	+	+	+	+	–	–	–
KXo-27	–	–	–	+	+	+	+	+	+	–	–	–
Uttar Kannada												
KXo-28	–	–	–	+	+	+	+	+	+	–	–	–
KXo-30	–	–	–	+	+	+	+	+	+	–	–	–
KXo-31	–	–	–	+	+	+	+	+	+	–	–	–

Fig. 1. Disease rating scale for bacterial leaf blight of rice (0-9).

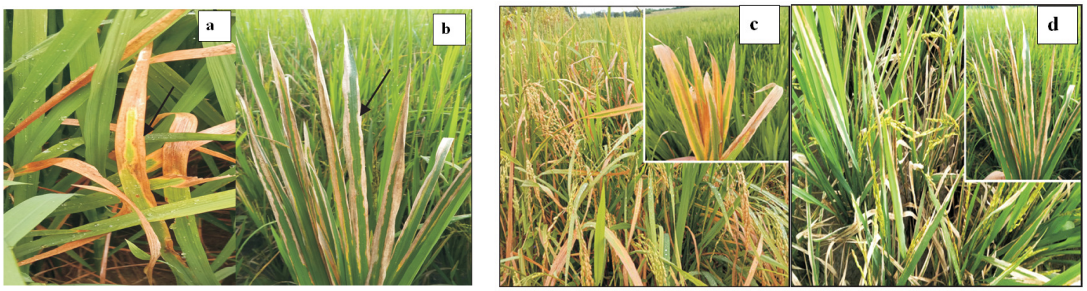

Fig. 2. Symptoms and Severity of bacterial leaf blight of rice in different districts of Karnataka during *kharif* 2019 **(a, b)** Typical wavy margin blighted leaf symptoms **(c)** Belagavi (Degulahalli, Amada) **(d)** Shivamogga (Kadadkatte, Sonamasuri)

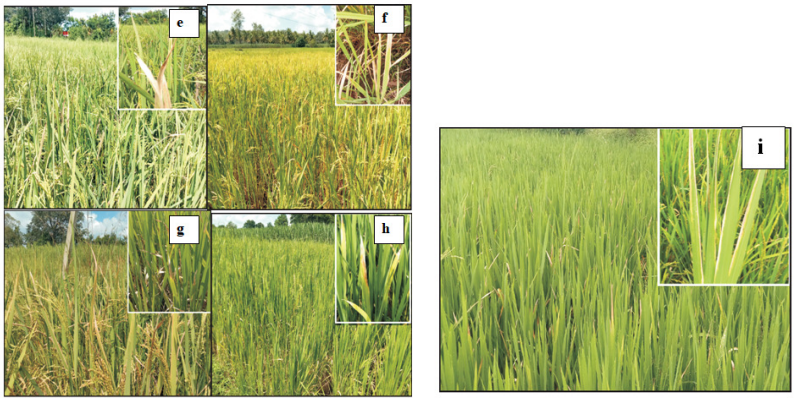

Fig. 2. (e) Koppal (Vadaratti, Sonamasuri) **(f)** Davangere (Basapur, Doddasali) **(g)** Uttara Kannada (Mugavada, Abhilash) **(h)** Dharwad (Kalaghatagi, Intan) **(i)** Haveri (Sivalli, Sona)

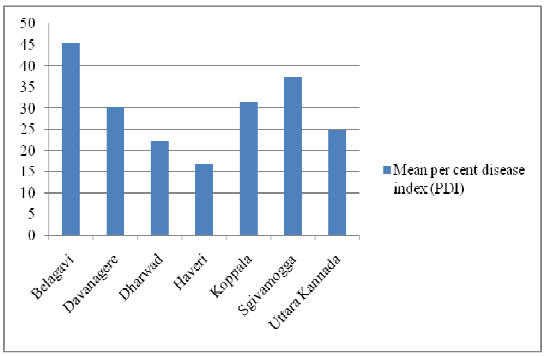

Fig. 3. District wise mean severity of bacterial leaf bight in major rice growing areas of Karnataka.

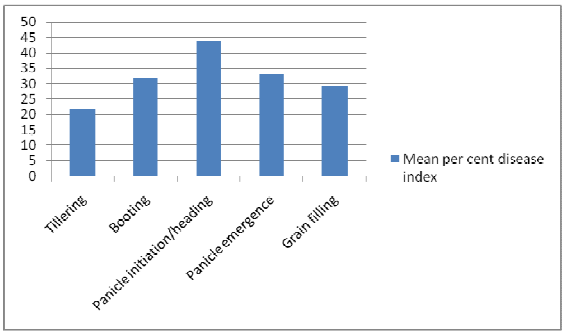

Fig. 4. Mean severity rice bacterial leaf blight in different stages of crop growth during kharif 2019.

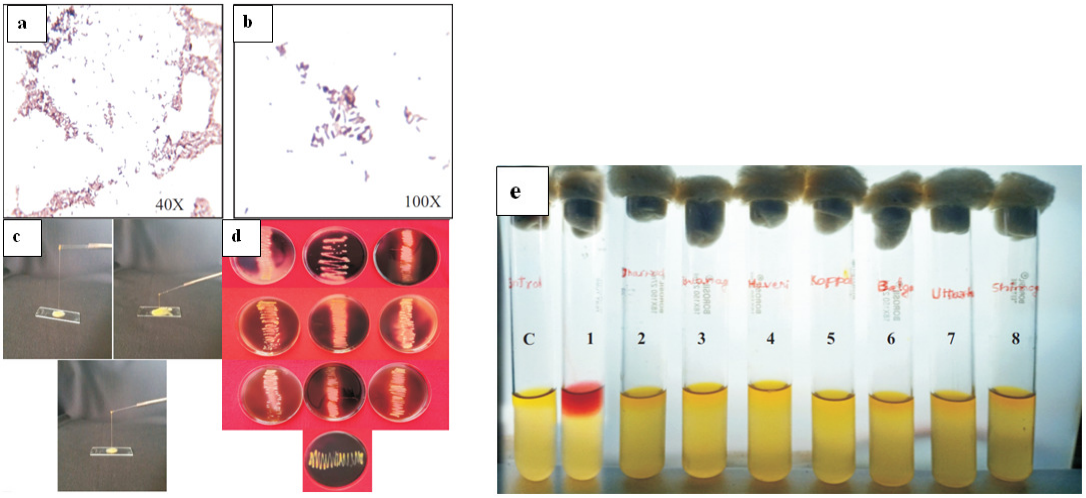

Fig. 5. Biochemical characterization of *Xanthomonas oryzae* pv. *oryzae* **(a and b)** Gram's reaction of *Xoo* **(c)** KOH test (3%) **(d)** Starch hydrolysis **(e)** Methyl red test (C-Control, 1-+ve reaction of *E. coli* and 2-8 -ve reaction of *Xoo*).

CONCLUSIONS

An intensive roving survey was carried out in major rice growing districts of Karnataka *viz.,* Belagavi, Davanagere, Dharwad, Haveri, Koppal, Shivamogga and Uttar Kannada during *kharif* 2019. Belagavi district recorded the highest BLB severity (45.23 %) and least severity of 16.86 per cent was recorded in Haveri district. The maximum mean per cent leaf blight severity was recorded in BPT-5204 (54.18%) and least recorded in Navali Sali (7.69%). The maximum bacterial leaf blight severity was noticed during panicle initiation stage (44.98%) and least in tillering stage (21.20%). Three biochemical tests that were conducted to characterize 21 isolates of *Xoo* showed positive reaction to KOH test, starch hydrolysis and negative to methyl red test.

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