ANALYSIS OF PATHOTYPIC VARIABILITY OF *Xanthomonas oryzae* pv. *oryzae,* THE BACTERIAL BLIGHT PATHOGEN OF RICE AND IDENTIFICATION OF NEW SOURCES OF RESISTANCE

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

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DEPARTMENT OF PLANT PATHOLOGY
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DECLARATION

I, **Aparna V. S. (2017-21-019)** hereby declare that this thesis entitled **"Analysis of pathotypic variability of *Xanthomonas oryzae* pv. *oryzae*, the bacterial blight pathogen of rice and identification of new sources of resistance"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Analysis of pathotypic variability of *Xanthomonas oryzae* pv. *oryzae*, the bacterial blight pathogen of rice and identification of new sources of resistance"** is a record of research work done independently by **Ms. Aparna V. S.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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APARNA V.S.

Dedicated to my family and friends

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LIST OF ABBREVIATIONS

µm	Micrometer
cm	Centimeter
COA	College of Agriculture
DNA	Deoxyribo Nucleic acid
DS	Disease severity
g	Gram
ha	hectare
kg	kilogram
ml	Milliliter
µL	Microlitre
No.	Number
PSA	Peptone Sucrose Agar
RARS	Regional Agricultural Research Station
Xoo	*Xanthomonas oryzae* pv. *oryzae*
Mt	Million tonnes
Mha	Million hectare
Lt	Lakhs tonnes
$CaCl_2$	Calcium Chloride
SES	Standard Evaluation System
h	hour
NILs	Near Isogenic Lines
R	Resistant
MR	Moderately Resistant
MS	Moderately Susceptible
S	Susceptible
HS	Highly Susceptible

Introduction

1. INTRODUCTION

Rice is the most popular staple food grain in the world. Half the world's population has been estimated to subsist entirely or partly on rice. Ninety per cent of the crop is cultivated and consumed inside the Asian continent. India is having an area of 43.79 Mha under rice cultivation with production of 118.43 Mt and productivity of 2705 kg ha^{-1}. In India, Uttar Pradesh is having the largest area under rice (5.68 Mha) followed by West Bengal (5.58 Mha) and Punjab (2.79 Mha). In production, West Bengal is leading with 16.65 Mt followed by Uttar Pradesh (15.66 Mt) and Punjab (12.18 Mt) (GOI, 2021). In Kerala, rice is grown as major crop covering an area of 1.98 Lakh ha with production and productivity of 0.587 Lt and 3058 kg ha^{-1} respectively. In the state, rice is mainly cultivated in Palakkad, Alappuzha, Thrissur and Kottayam district which account for about 81.20 per cent of the total production, their individual shares being 41 per cent, 16 per cent, 14 per cent and 9 per cent respectively (GOK, 2022).

Rice is highly susceptible to different fungal, bacterial and viral pathogens at all stages of growth leading to crop loss. Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most devastating disease of rice in the world causing yield loss depending upon the variety, stage of plant and climatic conditions (Kumar *et al*., 2012). Bacterial blight has high epidemic potential and is a threat in temperate and tropical rice growing regions. It commonly causes an yield loss of 10-20 per cent and in severely affected field's a loss of 50-70 per cent had been reported (Raina *et al.,* 1981; Mew *et al.,* 1993; Lore *et al.,* 2011; Yugander *et al.,* 2017). For the management of the disease, the most effective, economical and environment friendly approach is the development of resistant varieties (Patil *et al.,* 2017; Sirohi, 2019).

In India, it is one of the major devastating diseases in different rice growing regions. In the country bacterial blight of rice was first reported from Maharashtra (Sreenivasan *et al.,* 1959). The disease is major problem in *kharif* season in rice growing areas of Punjab, Haryana, Uttaranchal, Bihar, West Bengal, Tripura, Assam,

Tamil Nadu, eastern Uttar Pradesh, coastal areas of Andhra Pradesh, Andaman Nicobar Islands, Kerala and parts of Maharashtra, Chhattisgarh, Gujarat, Himachal Pradesh, and Karnataka (Laha *et al*., 2009).

In Kerala bacterial blight is one of major production constraints. Rice varieties predominantly cultivated such as Uma and Jyothi are highly susceptible to bacterial blight. Both *kresek* and leaf blight phases of the disease are widely seen in the fields of Kerala. In the state after the floods occurred during the years 2018 and 2019, the spread of the disease has increased in an alarming rate.

Forty seven bacterial blight resistance genes have been identified from diverse sources. Among them, *Xa21*, *Xa4, xa5* and *xa13* are the major genes that provide broad spectrum resistance and are being used in many breeding programs. The effectiveness of individual bacterial blight resistance genes varies from region to region due to the differences in the pathogen population structure. *Xanthomonas oryzae* pv. *oryzae* is highly dynamic in nature and many bacterial blight resistance genes have become ineffective in different regions of India and other countries. Also the durability of the resistant cultivars is short due to evolution of new pathotypes of the pathogen. Hence continuous monitoring of the pathogen virulence structure in a particular area is necessary to make the resistance breeding more successful. This can be done by pathotype analysis of *Xoo* isolates from different regions using a set of differentials /NearIsogenic Lines (NILs) carrying resistance genes.

The genetic variability of bacterial blight pathogen was reported from Kerala by Raji *et al.* (2016a). The pathotypic variability of *Xoo* isolates was also studied during the period 2014 using selected isolates from different lineages (Raji *et al.,* 2016b). However, during the past three years the occurrence of various symptoms *viz.*, *kresek* symptom at early transplanted stage and drying up of plants at active tillering stage in addition to leaf blight has increased in farmer's field. These varying symptoms indicate the chance of evolution of new strains. Detailed analysis of pathotypic variability of *Xoo* is needed for understanding the current population

structure of *Xoo* in Kerala and for the deployment of resistance genes. Use of host plant resistance is considered to be the most effective, economical and environmentally safe option for management of the disease. Identification of germplasm lines carrying effective resistance genes could be useful fordevelopment of resistant varieties. Hence, this study was proposed with the following objectives

1. To study the pathotypic variability of bacterial blight pathogen of rice, *Xanthomonas oryzae* pv. *oryzae* in Kerala.

2. To identify donors with known bacterial blight resistance genes effective against prevailing pathotypes.

Review of literature

2. REVIEW OF LITERATURE

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most important disease of rice. The disease was first noticed by the farmers of Japan in 1884- 1885. From Asian the continent, it has been reported from several countries like Philippines, Bangladesh, Pakistan, Nepal, Indonesia and India (Srivastava, 1967; Ahmed and Singh, 1975; Rangaswami, 1975; Singh *et al.,* 1977; Ou, 1985; Swings *et al.,* 1990). In India, bacterial blight has been reported from major rice-growing states like Andhra Pradesh, Bihar, Haryana, Kerala, Orissa, Punjab, and Uttar Pradesh (Laha *et al*., 2009; Yugander *et al*., 2017; Yugander *et al*., 2022).

2.1 Factors influencing bacterial blight disease

Mizukami and Wakimoto (1969) reported that bacterial blight was influenced by climatic conditions. The disease development was greatly influenced by rainfall, humidity, temperature, flood, and wind. Fujikawa *et al*. (1957) reported that disease development was correlated with temperature. Rainfall and typhoons in the rice growing seasons also affected the disease development.

The development of disease varied according to rice varieties cultivated as well as to the soil conditions. Early transplanting increased disease incidence, while deep ploughing decreased it (Kiryu and Mizuta, 1955; Kojima *et al*., 1959). Manzoor *et al.* (2016) reported that heavy fertilizer application increased the development of bacterial blight in rice. The application of nitrogen fertilizers encouraged disease development and planting density of rice also affected the severity of the disease. Both deficiency and excessive use of nitrogen fertilizer increased bacterial blight disease severity.

2.2 Symptomatology of bacterial blight (BB)

Two distinct types of symptoms were observed in rice due to bacterial blight and they were *kresek* and leaf blight (Ou, 1985; Akhtar *et al.,* 2008). *Kresek* caused either the death of the whole plant or wilting of few leaves. It was the most

destructive phase of the disease, wherein the leaves of the entire plant turned pale yellow and wilted during the seedling to the early tillering stage, which caused partial or total crop failure. The *kresek* symptom appeared during the early stage of the crop, one to two weeks after transplanting and temperatures between 28^0C and 34^0C favoured the development of the disease. Leaf blight symptoms were observed at the tillering stage and the disease incidence increased with plant growth and maximum at the time of panicle initiation. Leaf blight symptom started from the lower portion of the plant and then proceeded gradually towards the upper leaves. The upper half of the leaf or the whole leaf turned pale before drying up (Gnanamanickam *et al.,* 1999).

Reddy and Ou (1976), noticed that leaf blight symptom started with small water- soaked lesions and later turned to yellowish-white colour expanded from the equal sides in a square form and produced elongated circular to quite uneven lesions. The bacterial ooze from infected leaves were observed in warm and humid climates and this also helped in the spread of the disease. Damage due bacterial blight was maximum in *kresek* phase. Post flowering infections had very little effect on grain yield. When infection occurred during panicle initiation or subsequently during stages that precede flowering, severe impairment of grain development and consequent increase in sterility was observed.

Goto (1965) reported pale yellow symptom of bacterial blight in rice. This symptom was first reported from Philippines. The causal agent of pale yellowing in rice leaves was identified as *X. oryzae.* Yoshimura (1960) reported that symptom appeared as lesions on leaf blades developed downwards to the basal part and extended further to the sheath through the midribs. Yellowish or greyish streaks appeared according to disease development. On severely affected plants, the whole sheath were discoloured and killed. Discoloured spots surrounded by water-soaked lesions were often appeared on the glumes of plants in paddy fields. These spots were conspicuous while the grains were young and green, later turned to grey or yellowish white in the middle with an indistinct margin during the ripening stage.

2.3 Survey on occurrence of bacterial blight of rice and isolation of the pathogen

Bacterial blight has been reported from several countries. From the Asian countries, it has been reported from Phillippines, Bangladesh, Pakistan, Nepal, Indonesia,India *etc.* (Srivastava, 1967; Ahmed and Singh, 1975; Rangaswami, 1975; Singh *et al.,* 1977; Ou, 1985; Swings *et al.,* 1990). Rafi *et al*. (2013) conducted a survey on bacterial blight of rice in major rice growing zones of Pakistan during 2005-2007. The study revealed that Khyber Pakhtunkhwa (KP) province recorded a disease incidence ranged from 35–80.02 per cent and severity between 3.3 and 7.0 per cent.

In India, Hunjan *et al.* (2012) reported that the prevalence of bacterial blight in thedistricts of Ludhiana, Patiala and Gurdaspur. Disease severity was the highest in Tarn Taran (19.9%) and in Bathinda district, no disease was found. Rice cultivar PAU 201 had the highest disease incidence (12.4%) and severity (10.4%).

Isaka (1970) isolated causal bacterium from leaves of rice with bacterial blight lesion and reported that bacterial exudates from the fresh lesion were better isolation material as compared to infected tissue because of less contamination. Noda *et al*. (1990) collected rice leaves affected by bacterial blight from various districts of Japan during theperiod 1973 to 1987. Leaf segments including the marginal portions of fresh lesions were surface-sterilized by the usual method and then homogenized in 10 ml of sterile distilled water and appropriate dilutions were mixed with melted nutrient agar medium kept at 50^0C in a water bath. The mixture was poured into a Petri plates and incubated at 25^0C for four days. The viscous and yellow bacterial colonies developed were transferred to peptone sucrose agar medium (PSA) and cultured at 25^0C for two days. Di *et al.* (1991) reported that *Xanthomonas oryzae* colonies can easily be isolated from infected leaves sample rather than infected seeds.

Wilson *et al*. (1993) isolated the pathogen from samples with bacterial blight symptoms. The infected leaf pieces of rice (28×7 mm) were excised with a sterile scalpel. The leaf surface was sterilized with one per cent clorox for three minutes

and then washed with sterile distilled water. Leaf pieces (6-7) of rice leaves after drying on sterile blotting paper were transferred to yeast extract dextrose calcium carbonate agar medium and incubated at 25±28^{0}C for 72 h. For short term preservation, the cultures were suspended in sterile distilled water and for the long term in silica gel.

Jabeen (2011) surveyed bacterial leaf blight of rice in various agro ecological zones of Pakistan during 2002-2003 and they collected infected leaf samples of rice having bacterial leaf blight symptoms. Yellow, circular, smooth, convex and viscous bacterial colonies were observed on yeast dextrose calcium carbonate agar medium (YDCA) after 48-72 h of incubation at 28^{0}C. In Wakimoto medium, whitish, mucoid and smooth colonies were observed.

Shanti *et al.* (2010) isolated *Xoo* from infected rice leaf samples from farmer's field of Maruteru. Lore *et al.* (2011) collected *Xoo* isolates from different rice growing districts of Punjab during 1999-2006. Single *Xoo* colonies were multiplied on Wakimoto medium at 27±1^{0}C for 72 h. Mondal *et al*. (2014) collected and isolated rice leaves showing typical bacterial leaf blight symptoms from 13 states of India (Uttar Pradesh, Odisha, Uttarakhand, Punjab, West Bengal, Assam, Meghalaya, Nagaland, Bihar and Jharkhand). Seven hundred and eighty *Xoo* isolates were collected. Kumar *et al*. (2018) isolated *Xoo* from three geographical areas of Chattisgarh.

Yugander *et al.* (2017) collected bacterial blight pathogen from diverse rice growing regions of India during the period 2005–2014. Through random surveys, samples were collected from different farmer's fields and experimental stations. Infected leaves showing typical BB symptoms were collected, wrapped in clean and sterile blotting paper and then put in paper envelopes. Isolations were made from BB infected rice leaves following standard procedures. Four hundred isolates were obtained. Similarly, Padmaja *et al.* (2017) collected sixty *Xoo* isolates from Telangana and Andhra Pradesh.

Ochiai *et al*. (2000) collected bacterial blight infected samples from twenty nine locations of Sri Lanka and sixty isolates were isolated. Singh *et al*. (2003) collected 693 isolates of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) from 10 districts of Punjab during1997. Akhtar *et al.* (2011) collected sixty diseased samples of rice from different rice growing areas of Punjab, Pakistan and isolated *Xoo* isolates from the diseased samples. Mishra *et al.* (2013) collected infected leaf samples of bacterial blight from 20 different rice growing states of India during 2004 – 2012 and isolated 1024 *Xoo* isolates. Hunjan *etal.* (2012) collected 105 isolates of *Xanthomonas oryzae* pv. *oryzae* from different districts of Pakistan.

Samples of bacterial blight were collected from 13 states in India, Uttar Pradesh (50), Odisha (50), Uttarakhand (50), Punjab (80), Haryana (100), Assam (50), Meghalaya (50), Nagaland (50), Bihar (50) and Jharkhand (50) respectively during *kharif* season 2011-2013 (Mondal *et al*., 2014). Yugander *et al.* (2014) surveyed six rice growingdistricts of Andhra Pradesh and samples of bacterial blight were collected. A total of fifty two *Xoo* isolates were collected from Ranga Reddy, Kurnool, West Godavari, Warangal, Mahaboobnagar and Medak districts of Andhra Pradesh.

Shoba *et al.* (2020) collected 247 diseased leaf samples from different districts of Karnataka *viz*., Ramanagara, Chikkaballapura, Kolar, Tumkur, Channapatna, Mandya, and Mysuru. Isolated and purified 72 *Xoo* isolates and studied growth of *Xoo* isolates on 6 different medium. Best growth of *Xoo* isolates was in Yeast extract Dextrose Calcium Carbonate Agar media (YDCA), Nutrient agar (NA) and Tryptone Soya Agar media (TSA), agar medium. Ranjani and Palani, (2020) studied existing population of *Xoo* in southern states of India *viz.,* Tamil Nadu, Andhra Pradesh, Kerala and Karnataka and isolated 96 *Xoo* isolates from plants showing visible symptoms of bacterial blight and reported that *Xoo* isolates exhibited varying level of genetic differences. Ejaz *et al*. (2022) collected and isolated 41 *Xoo* isolates from bacterial blight infected samples from seven districts of Punjab, Pakistan and analysed the existing population dynamics of *Xoo*.

2.4 The pathogen, *Xanthomonas oryzae* pv. *oryzae*

Bacterial blight is caused by the pathogen *Xanthomonas oryzae* pv. o*ryzae* (*Xoo*). Bokura (1911) first isolated the bacterium and based on its morphology and physiology, it was named *Bacillus oryzae* (Mizukami and Wakimoto, 1969). Later Ishiyama (1922) studied the bacterium and renamed the bacterium *Pseudomonas oryzae*. A few years later it was named *Bacterium oryzae* and subsequently as *Xanthomonas oryzae*. According to the revision of the International Code of Nomenclature of Bacteria (ICNB) the committee on taxonomy of phytopathogenic bacteria of the International Society of Plant Pathology proposed the name *Xanthomonas campestris* pv. *oryzae* Dye (Mew *et al.,* 1993; Ishiyama, 1922). Later in 1990, the pathogen was elevated to species status and was named *Xanthomonas oryzae* pv. *oryzae.*

Xanthomonas oryzae pv. *oryzae* colonies are yellow coloured, slime molding, motile, Gram negative rod with a polar flagellum and also reported that the pathogen enters the host through wounds or natural openings. Then it reaches vascular tissue, in thexylem, where it multiplies and spreads throughout the plant. The cells were 1 - 2.5 µm long and 0.4 - 1.0 µm wide, with average dimensions of 0-5 x 1- 4 µm (Ishiyama, 1922; Swings *et al.,* 1990; Furutani *et al*., 2009; Yugander *et al*., 2017)

2.5 Biochemical characterization of *Xanthomonas oryzae* pv. *oryzae*

Biochemical characterization of isolates collected from Telangana region was done by Padmaja *et al.* (2017). They reported that *Xoo* isolates were positive for H_2S production, catalase production, gelatin liquefaction and produced acid from several carbohydrates but not from alanine. The *Xoo* isolates showed a negative reaction for oxidase, urease production and indole production and also showed variable reactions on nitrate reduction, starch hydrolysis and acid production from inositol.

2.6 Spread of the pathogen

The bacteria survived in rice stubble and on weed hosts. Common weed hosts were *Leersia oryzoides, Zizania latifolia, Leptochloa chinensis* and *Cyperus rotundus*. The bacterium remained in the roots of the weed *Leersia oryzoides* wherein the rice growing season it reached the rice nursery beds and further spreads through irrigationwater. Infected straw present in the field or the infected seeds introduced the pathogen into the rice nursery (Mizukami and Wakimoto, 1969).

The pathogen accumulated on the root surface of rice seedlings and further movedupward to the crown (Mizukami, 1961). Dath and Deevadath (1983) reported that the pathogen might be transmitted to basal parts of the leaf sheath through contaminated roots or lower leaves which encounter contaminated water and becomes the source of infection for the rest of the crop period. Tabei (1968) reported that the pathogenic bacterium entered into stomata on coleoptiles and leaf sheath of rice seedling under the moist conditions and multiplied in the intercellular spaces of parenchyma and attacked the vascular system of the plant.

2.7 Pathogenicity test

For proving the pathogenicity of *Xoo* isolates in rice plants, different inoculation methods are used and they are needle prick, clipping, dipping and spraying methods. The needle pricking method is laborious for large scale mass screening in the field. The commonly adopted inoculation method for *Xoo* is clipping method where the leaf tip is clipped off. In dipping method, the root and basal parts of the rice seedlings are dippedin bacterial suspension before transplanting. By this method the resistance of the cultivarsto the *kresek* symptom can be evaluated. In the spraying method, *Xoo* suspension is sprayed directly onto the plants (Mew, 1987).

Kauffman *et al*. (1973) reported that the incubation period for clipping method varied from 3 to 5 days. The incubation period for pin pricking was 4 to 6 days and for spraying the inoculum on the plants was 6 to 8 days. Ghasemie *et al*.

(2008) reported that isolates of *X. oryzae* pv. *oryzae* caused leaf blight symptom on the surface of rice leaves two weeks after inoculation. On young plants, symptoms appeared as pale green to grey green water soaked streaks near the leaf tip and margins. These lesions coalesce and become yellowish-white with wavy edges and leaf sheaths and culms were infected.

Gonzalez *et al.* (2007) tested virulence of 26 *X. oryzae* pv. *oryzae* by leaf clip inoculation method on the rice variety Nipponbare and 16 isolates exhibited bacterial blight symptoms. Muneer and Akhtar (2007) proved the pathogenicity of 49 isolates of Xoo on six rice cultivars including IRRI 6, KS-282, JP 5, Basmati 385, Dilrosh 97 and Kashmir Basmati 385. They reported that clip inoculation method and needle prick methods were equally effective to initiate leaf blight lesion under greenhouse conditions. Akhtar *et al.* (2008) studied different methods of inoculation of *Xoo* in rice plants. Bacterial suspension of *Xoo* was inoculated on one month old plants. After two weeks, a yellow lesion with a wavy margin appeared on the leaf margin and the leave became yellowish and dried. The study showed rice varieties Super Basmati, Bas 370, Bas 385, Bas 2000 inoculated by clipping method exhibited typical BB symptoms, yellow lesions with wavy margin and also observed that clipping method showed the best results as compared to brush and pin prick. The brush and pin pricking method expressed less BB symptoms as compared to the clipping method and brush method was found to be the least effective.

Ali *et al*. (2009) proved the pathogenicity of *X. oryzae* pv. *oryzae* by inoculating the pathogen on susceptible rice varieties IR8 and IR24. Following at flag leaf stage. Top one to three inches of leaves were clipped off using a sterile scissor dipped in bacterial inoculums.

2.8 Pathotyping of *Xanthomonas oryzae* pv. *oryzae*

Vera Cruz *et al.* (1996) reported 10 races of *Xanthomonas oryzae* pv. *oryzae* from Philippines. In India, several studies were conducted on the variability of

Xanthomonas oryzae pv. *oryzae* (*Xoo*). Liu *et al.* (2006) reported more than 30 races of *Xoo.*

Different races of *Xoo* have been identified based on the virulence of *Xoo* strains in particular host genotypes (Mew, 1987). The knowledge of the *Xoo* pathotype diversity is essential for the selection of R genes that could be deployed in future breeding programmes. Studies on *Xoo* pathotype diversity in India revealed 6 to 11 pathogenic races based on their virulence to *Xa/xa* differential lines (Mishra *et al.,* 2013; Mondal *et al.,* 2014). These studies suggested that *Xa4, xa5, Xa7*, *xa8*, *Xa11*, *xa13* and *Xa21* should be targeted as important candidates for resistance breeding against BB races in India.

Adhikari *et al.* (1999) identified five virulence groups of the bacterial blight pathogen based on infection responses on rice lines having a combination of two, three and four major R genes. Dinh *et al.* (2008) identified six pathogenic races of *Xanthomonas oryzae* pv. *oryzae i.e.* A, B, C, D, E and F from Mekong delta of China during 2006-2007. The study showed that distribution dominance varied across provinces. Race A, E and F were dominated and observed in most of the provinces in Mekong Delta.

Ochai *et al*. (2000) collected 60 strains of *Xoo* from different parts of Sri Lanka and evaluated its virulence on 11 near-isogenic lines and one cultivar, Taichung Native 1,each carrying a specific resistance gene*: Xa1*, *Xa2*, *xa3*, *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *Xa11*, *xa13*, *Xa21* and *Xa14* respectively. Fourteen pathotypes were detected based on the virulence analysis. The strains are phylogenetically composed of five different groups. All strains were virulent to resistance genes *Xa1*, *Xa2*, *Xa4*, *Xa10*, *Xa11* and *Xa14*. Only one strain (pathotype 1) was virulent to all major resistance genes including *Xa21*, while strains of the other pathotypes were all avirulent to *Xa21*. A partial relationship was found between the determined phylogenetic groups using the IS1112 probe and pathotypes for all but two clusters.

Suparyono *et al.* (2004) identified three pathotypes of *Xoo*, *ie*. III, IV and VIII from West Java, Central Java and Yogyakarta during the wet season of 2000.

Lore *et al.* (2011) classified *Xoo* population from Punjab into seven distinct pathotypes (PbXo-1 to PbXo-7) by inducing differential reactions on a set of near-isogenic lines in the background of IR24 and some international, national and regional cultivars. They reported that pathogen is highly variable and BB resistance genes (*Xa1*, *xa3*, *Xa10*, *Xa11*, *Xa14*, *Xa18*) were ineffective, whereas *xa13*, *Xa4*+ *xa13*, *xa5* + *xa13*, *xa13* + *Xa21*, *Xa4*+*xa5*+*xa13*, *Xa4*+*xa5*+*Xa21*, *Xa4*+ *xa13* + *Xa21*, *xa5*+ *xa13* + *Xa21* and *Xa4*+*xa5*+*xa13* + *Xa21* and rice line IET8585/Ajaya were effective against all the seven pathotypes analysed. It was observed that *Xa21* was effective against all the pathotypes except PbXo-3 and PbXo-4. The most dominant pathotype was PbXo-7 and produced susceptible / moderately susceptible reaction on 22 of the 40 test genotypes followed by PbXo-1, PbXo-5 and PbXo-6. The least virulent pathotype was PbXo-2.

Six isolates collected from different parts of West Bengal were screened against near isogenic lines to identify variability in virulence. It was found that all the NILs possessed a varying degree of susceptibility to resistance against all the isolates with significant differences in disease development. The pyramided lines showed broad spectra of resistance against all the *Xoo* isolates and most durable resistant monogenes were *xa5, xa13* and *Xa21* (Debnath *et al*., 2013). Padmaja *et al.* (2017) conducted virulence profiling of 60 isolates of *Xoo* on 22 near isogenic lines (IRBB lines) which consisted of different bacterial blight resistance genes and their combination in the background of rice cultivar IR24. The TN-1 (susceptible) and Improved Samba Mahsuri (resistant) were used as checks. The 60 isolates were grouped into 10 pathotypes based on phenotypic response on differentials. The highest number of pathotype was found in East Godavari district (8) followed by Nellore (7) and West Godavari (4).

Four races of *Xanthomonas oryzae* pv. *oryzae* were reported from Guilan province, Iran based on the interactions of 153 *X. oryzae* pv. *oryzae* isolates on 26 near- isogenic rice lines (Khoshkdaman *et al.,* 2012). Mishra *et al*. (2013) isolated 1024 *Xoo* strains from different states in India and subjected to pathotyping and RFLP and 11 pathotypes were identified. Pathotype XI from Tripura was compatible with all the tested *Xa* genes. Gene pyramid lines carrying *Xa21, xa13 and xa5* were resistant to pathotype XI. Genotypic analysis using the IS1112 probe carried out on 50 strains revealed that 20 RFLP haplotypes indicating the genetic diversity of the Indian *Xoo* population. Sakthivel *et al*. (2017) reported the high genetic variability among 12 pathotypes through DNA fingerprinting analysis. The cluster analysis revealed that *Xoo* isolates were grouped irrespective of their origin or virulence potential of pathotype.

Mondal *et al.* (2014) collected 780 *Xoo* isolates from 13 different states in India and based on disease reaction on 12 near isogenic lines grouped into six races. In North Western part of India Race 4 and Race 6 were predominant. Race 1 and Race 2 were less virulent among the races reported and also revealed that co-existence of both the least virulent and highly virulent race in particular location. BB genes *Xa1* and *Xa2* were most susceptible to all the races. *Xa4*, *Xa11*, *xa13* and *Xa21* exhibited resistant to moderately resistant reaction.

Yugander *et al.* (2014) collected 52 *Xoo* isolates from major rice growing areas of Andhra Pradesh. the virulence characterization of these *Xoo* isolates on rice differentials revealed that NILs with single genes were susceptible to 53 - 84 per cent of the isolates except IRBB 13 which exhibited susceptible reaction to 23 per cent of the isolates. Gene combination of *Xa4* and *xa5* were susceptible to many pathotypes. Ten pathotypes were reported. Yugander *et al*. (2017) revealed that the *Xoo* population in different rice growing regions of India are highly diverse. Four hundred *Xoo* isolates were collected from diverse rice growing regions of India and virulence analysis was conducted on set of 22 rice differentials along with checks. They reported that NILs possessing single BB resistance genes were susceptible to 59

- 94 per cent of *Xoo* isolates except IRBB 13 (35%). None of the single BB resistance genes gave wide spectrum resistance to BB in India and gene combinations like *xa5+xa13, Xa4+xa5+xa13, Xa4+xa13+Xa21, xa5+xa13+Xa21* and *Xa4+xa5+xa13+Xa21* provided broad spectrum durable resistance.*Xoo* isolates were categorized into 22 pathotypes and pathotypes IXoPt-1 and IXoPt-2 were the least virulent also XoPt-18 to XoPt-22 were highly virulent. Padmaja *et al*. (2017) categorized 60 *Xoo* isolates collected from different rice growing regions of Telangana and Andhra Pradesh into ten pathotypes based on their reaction on 22 rice differentials. Among the ten pathotypes pathotype 1 and 2 were the least virulent and pathotype 21 and 22 were highly virulent.

Kumar *et al.* (2018) classified *Xoo* population into virulence groups/ races based on interaction on differentials/ near isogenic lines. Sahu, (2018) collected 60 *Xoo* isolates from different rice growing areas of Chhattisgarh and analyzed for their pathogenic and genetic variability. The isolates were grouped into nine pathotypes. Some of the pathotypes showed resistance to major BB resistance genes, *xa13* and *Xa21*.

One hundred and eighteen *Xoo* isolates were reported from major rice growing areas of Bangladesh. All the isolates were susceptible to rice varieties IR 24, Purbachi and BR11. The virulence analysis of *Xoo* isolates were done on 113 NILs and 12 pathotypes / race were identified. Most predominant pathotypes were Race 1 (48%), Race 2 (14%) and Race 3 (11%). Race 5 exhibited most virulent reaction on all tested genes. BB genes *xa5, xa8, xa13, Xa21* and *Xa23* were effective against bacterial blight. The highest resistant reaction was shown by *Xa21* gene against 94.91 per cent of tested isolates (Rashid *et al*., 2021).

Sudir and Yuliani (2016) collected 2,658 isolates *Xoo* from 10 provinces of Indonesia and reported three pathotypes III, IV, and VIII based on reaction on five differential varieties. Pathotype VIII was predominant in four provinces. Pathotypes III and IV were predominant in three provinces.

The virulence analysis of 300 isolates of *Xoo* from 17 districts of Punjab, Pakistan was conducted by inoculation on a set of six rice IRRI-differentials. The isolates were grouped into 29 pathotypes Pt1- Pt29. Highly virulent pathotype (Pt-1) consisted of 39 *Xoo* isolates spread across 12 districts. The reaction on differentials shown that most of *Xoo* isolates were moderately resistant to least virulent (21.7% – 43%) (Arshad *et al*., 2017).

Suryadi *et al*. (2016) categorized 15 *Xoo* isolates into eight pathotypes based on differentvirulence patterns on the NILs with a single resistance gene and reported rice genotypes having high resistance against *Xoo* were IRBB 50 (*Xa4*+*xa5*), IRBB 51 (*Xa4*+*xa13*), IRBB 52 (*Xa4*+*Xa21*), IRBB 53 (*Xa4*+*Xa21*), IRBB 56 (*Xa4*+*xa5*+*xa13*), IRBB 57 (*Xa4*+*xa5*+*Xa21*), IRBB 59 (*Xa4*+*xa13*+*Xa21),* IRBB 64 (*Xa4*+*xa5*+*Xa7*+*Xa21*), IRBB 66 (*Xa4*+*xa5*+*Xa7*+*xa13*+*Xa21*), IRBB 7 (*Xa7*), Angke (*Xa4*+*xa5*) and Code (*Xa4*+*Xa7*).

The virulence analysis of 52 isolates of *Xanthomonas oryzae* pv. *oryzae*, was doneon 41 rice genotypes including five Japanese and five Philippines differentials. Highly susceptible reactions was observed in Japanese differentials Kinmaze and Rantai Emas and two Philippines differentials IR 8 and IR 20 against all the 52 bacterial isolates. Based on reaction pattern on five new Indian differentials, *Xoo* isolates categorized into six pathotypes, pathotype -1, 4, 7, 14, 15 and 16. Pathotype-1 was present in four states of India *viz*., Andhra Pradesh, Orissa, West Bengal and Bihar (Suparyono *et al.,* 2004).

Noer *et al*. (2018) reported six pathotypes in North Sumatra by studying reaction pattern of 10 *Xoo* isolates. Most frequent pathotype were pathotype IV. Pathotype I were virulent on all the *Xa* genes. Based on the interaction of resistant genes in combination of *Xa2*, *Xa4,* and *Xa21* genes were suitable for development of rice cultivars in North Sumatra.

2.9 Phenotypic screening for BB resistance

The effectiveness of resistance genes varies over locations due to the geographical structuring of the pathogen. There is a huge potential of utilizing the untapped sources of resistance from the germplasm for development of disease resistant rice varieties with other quality parameters. Utilizing different sources of resistance for introgression of the traits will be useful for broadening the genetic base rather than utilizing the same source. Several researchers have studied the local germplasm for the identification of multiple bacterial blight resistance. Several researchers located landraces/cultivars/wild rice accessions with resistance / tolerance to bacterial blight (Thimmegowda *et al*., 2011; Singh *et al*., 2015; Banerjee *et al*., 2018; Majumder *et al*., 2020; Zhao *et al*., 2022).

Sharma and Pandey (2012) tested 30 germplasm accessions for resistance to bacterial blight. The line UPR 2869-98-121 showed reaction to immune to BB infection and four lines AC-19-1-1, BJ 1, CAMOR and IR 22082-41-2 showed resistant reaction. Singh *et al*. (2015) studied resistance of 35 wild rice accessions by inoculation with BX043 wild type strain of *Xoo.* The results showed that 11 accessions were moderately resistant, 21 were moderately susceptible and three accessions were susceptible to *Xoo* strain BX043. Fordjour *et al*. (2020) phenotypically screened six rice cultivars for resistance to *Xoo* strain K1 along with controls and assessed that rice cultivar Popa was the most resistant Ghanaian phenotype.

Awoderv *et al*. (1991) screened 14 rice varieties for BB resistance and revealed that rice cultivars 52-37, IR 1529-6803-3 and BR 51-46-5 were highly resistant to bacterial blight. Kumar *et al.* (2016) reported that none of the accessions screened for bacterial blight resistance were resistant. Eleven accessions exhibited moderate resistance, 21 were moderately susceptible, and three accessions were susceptible to the BX043 wild-type strainof *Xoo.*

Screening of 15 Pakistani rice genotypes revealed Kashmir Basmati as a highly resistant genotype, followed by IR-6, Basmati-370, JP-5 and KSK-370, while the remaining genotypes were susceptible to all the strains/isolates of *Xanthomonas oryzae* pv. *oryzae* tested (Ali *et al*., 2009).

Khan *et al.* (2009) reported that out of 50 varieties/lines screened during the year 2003-2004 and 26 varieties/lines in 2007-2008, none of the varieties were resistant against bacterial blight. Lines IR-72102-3-107-1-1-2, and P-52-9-2 were moderately resistant during 2003 and DM-1-30-3-99 were moderately resistant in 2004.

Abbasi *et al*. (2011) conducted phenotypic screening of 60 rice varieties along with 10 Pakistan Basmati varieties and reported that disease reactions of varieties carrying *xa5* gene ranged from resistant to moderately resistant. The varieties without *xa5* gene produced moderately resistant to susceptible reactions and 13 varieties carrying *xa5* gene exhibited moderately resistant reaction and 19 varieties showed resistant reaction.

Banerjee *et al*. (2018) screened 40 irrigated and 55 rainfed rice varieties for bacterial blight resistance. Among the irrigated varieties 12 were resistant, 17 moderately resistant and 11 susceptible. Resistant varieties were Pyari, CR-Dhan 204, Saket-4, Jaya, Khitish, Naveen, Tapaswini-MAS, CR 2983–4, CR-Dhan 300, Satyakrishna, IR8 and CR-Dhan 601. In rainfed varieties, 17 were resistant, 25 moderately resistant, and 13 susceptible. Out of 115 rice landraces from Assam (75), Odisha (21), West Bengal (17) and Bihar (2) assessed for response to bacterial blight seven per cent genotypes were resistant, while 60 per cent of the landraces were susceptible to bacterial blight. Kalajeera, Kasalath (ARC6000), Rudra ahu (ARC5801), ARC5774, ARC5791, Pani Kekoa and Murgi Badam were resistant landraces.

Pradhan *et al.* (2015) reported that the high yielding rice variety Jalmagna was improved by incorporating three resistance genes *xa5, xa13* and *Xa21.* Four

resistance genes *Xa4, xa5, xa13* and *Xa21* were pyramided to a popular variety Ranidhan and developed a variety with broad spectrum resistance to bacterial blight (Pradhan *et al.,* 2022). Improvement of rice variety Basmati 385 was done by pyramiding of *Xa4, xa5, xa13* and *Xa21* genes by Ullah *et al*. (2022).

2.10 Bacterial blight resistance in rice

Host plant resistance was the most efficient, cost-effective, and environmentally friendly method of controlling BB. The use of resistant varieties were the best method for control of bacterial blight in rice (Bhasin *et al.,* 2012; Natrajkumar *et al.,* 2012).

Until now 47 bacterial blight (BB) resistant genes (R) conferring resistance to *Xoo* were identified from various rice cultivars, wild relatives of rice, and mutation populations (Lin *et al.,* 1996; Nagato and Yoshimura, 1998; Zhang *et al.,* 1998; Khush and Angeles, 1999; Chen *et al.,* 2002; Lee *et al.*, 2003; Tan *et al.,* 2004; Xiang *et al.,*2006; Busungu *et al*., 2016; Chen *et al.,* 2020). Cultivation of rice varieties carrying a single resistance gene for long term resulted in significant shift in pathogen-race frequency and consequent breakdown of resistance (Mew *et al*., 1992). The effectiveness of resistance genes varied over locations due to the geographical structuring of thepathogen (Shanti *et al.*, 2009).

Based on different morphological and molecular markers 12 R-genes (*Xa4, Xa7, Xa22, Xa30, Xa31, Xa33, xa34, Xa35, Xa39, Xa40, xa42* and *Xa42*) were fine-mapped (Busungu *et al.,* 2016; Zhang *et al*., 2015; Liang *et al*., 2017). Sixteen recessive genes *viz*., *xa5, xa8, xa9, xa13, xa15, xa19, xa20, xa24, xa25, xa26b, xa28, xa31, xa32, xa33, xa34* and *xa42* (Chen *et al*., 2011; Vikal and Bhatia, 2017; Liang *et al*., 2017). Major classes of *R*-genes were nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes and the cell surface pattern recognition receptors (Song *et al*., 1995; Liu *et al*., 2010).

The resistance gene *Xa4* was widely used in rice breeding for imparting resistanceagainst bacterial blight disease of rice. *Xa4* was first identified by Petpisit *et al*. (1977). Yoshimura *et al.* (1995) mapped the gene roughly to the terminal region of chromosome 11. Li *et al.* (2001) further mapped the *Xa4* locus between two RFLP (restriction fragment length polymorphism) markers, RZ536 and L457b. Wang *et al*. (2001) localizedthe *Xa4* locus between G181 and L1044 at a distance of 4.4 and 3.8 cm from the flankingmarkers, respectively. Sidhu *et al*. (1978) conducted experiment on 74 cultivars of rice. Bacterial blight isolate PX061 from the Philippines was used for the inoculation of parental and hybrid populations and reported that *Xa4* locus conveyed resistance in 38 cultivars. Of these, 18 were resistant at all stages of plant growth. At the maximum tillering stage, 20 cultivars were susceptible but were resistant at booting and flowering stages.

The *xa5* is one of the major BB resistance gene which provided race specific resistance to bacterial blight. This gene was located in chromosome 5. It provided broad resistance against many strains of *X. oryzae* pv. *oryzae* (McCouch, 1990; Yoshimura *et al.,* 1995; Blair and McCouch, 1997; Huang *et al.,* 1997; Sanchez *et al.,* 2000; Singh *et al.,* 2001; Garris *et al.,* 2003; Iyer and McCouch, 2004; Jiang *et al*., 2006; Mishra *et al*., 2013)

Wang *et al.* (1996) reported that bacterial blight resistance gene *Xa21* was coming under fifth class of disease resistance. *Xa21* encoded a receptor-like kinase consisted of LRRs in the putative extracellular domain were similar to the tomato CF-9 protein and a serine khreonine kinase in the putative intracellular domain were similar to the PTO kinase.

2.11 Genotypic screening of germplasm accessions for bacterial blight resistance

The use of host plant resistance considered as the most effective, economical and environmentally safe option for the management of bacterial blight of rice. Identification of germplasm lines carrying effective resistance genes were useful for the development of resistant varieties. Several works were carried out in India and

abroad to locate donors forbacterial blight resistance with known resistance genes. To identify germplasm containing BB resistance gene polymerase chain reaction (PCR) based DNA markers were used (Blair and McCouch, 1997).

Singh *et al.* (2015) screened 35 accessions of wild rice using PCR based markers pTA 248, *xa13*prom, RM-13, RM-224 and RM-317 for BB resistance genes *Xa21, xa13*, *xa5, Xa4* and *Xa2* respectively. BB resistance genes were determined by visualization of amplicons near 982 bp (*Xa21*), 498 bp (*xa13*), 139 bp (*xa5*), 160 bp (*Xa4*), and 154 bp (*Xa2*) of positive fragments. BB resistance genes *Xa21* and *xa13* were not found in any of the accessions and 12 accessions showed amplicons specific to *xa5* and nine accessions showed amplicons specific to *Xa4* and *Xa2* were present in 16 accessions. Amgai *et al.* (2015) identified germplasm accessions carrying *Xa10, xa13, Xa7, Xa3, Xa4* and *xa5* genes using SSR and STS markers.

Ullah *et al*. (2012) reported landrace Basmati-122 possessed homozygous resistance alleles for *xa5* and *Xa7* and a heterozygous status for *Xa4*. *Xa4* and *Xa7* were found in cultivar Basmati-2000 and Basmati-385 and landraces Basmati-106, Basmati-189 and Basmati-208. Landraces Basmati-427 and Basmati-433 possessed the combination of *xa5* and *Xa7* genes. The combination of *Xa7 and xa13* resistance genes were found in landraces Basmati-48, Basmati-51A, Basmati-334, and Basmati-370A. Banerjee *et al.* (2018) carried out genotypic screening for BB resistance in 210 rice germplasms comprised of released varieties and landraces from eastern and north eastern India. Seventy genotypes were used for molecular screening for 10 BB resistance genes. The frequency of R genes in genotypes varied from zero to five. The most frequent gene was *Xa1* followed by *Xa7, Xa4, Xa10* and *Xa11*. Entries such as Nua Kalajeera, Kalinga III, Naveen, CR Dhan 701, Swarna Sub1, Kalajeera, and ARC5791 possessed three to five genes. *Xa11* was detected using two tightly linked SSR markers, RM347 and RM1350 and reported that only RM1350 was polymorphic between the controls. Amplicons corresponding to *Xa11* (160 bp) was present in eight resistant and two moderately resistant entries. Only two released varieties, IR64-MAS and Tapaswini- MAS had *xa5, xa13,* and *Xa21*.

Arif *et al*. (2008) conducted molecular survey to identify the rice germplasm / lines for the presence of bacterial blight resistance gene *Xa4*. PCR with primers specific for the *Xa4* resistances gene was used in the study. Out of 100 rice germplasm lines, 49 lines showed the presence of *Xa4* gene. Singh *et al*. (2012) screened 42 landraces of rice using sequence tagged site (STS) markers MP specific for *Xa4, xa13* gene and pTA248 specific to *Xa21* and revealed that 69 per cent of landraces carried *Xa4* gene while none of them had *xa13* and *Xa21.* Abbasi *et al*. (2011) screened 60 rice varieties for BB resistance genes and reported that *xa5* gene was present in 31 rice varieties and also in Pakistani Basmati varieties, Kashmir Basmati, Basmati Pak, Shahley Basmati and Basmati-622.

Panwar *et al*. (2018) conducted genotypic screening of 36 landraces and local cultivars of Gujarat for the presence of major BB resistance genes *Xa4, xa5* and *Xa21.* Ten genotypes (IR20, IR64, IR72, NWGR1095, NWGR2014, IET14726, GR7, GR102, Pankhali 203 and Ratna) possessed *Xa4* gene. *Xa21* was reported only in IET18483 whilenone of the genotype contained *xa5* BB gene.

A study was conducted to reveal the bacterial blight resistance of sixty rice genotypes by using molecular markers MP, RM122, M5, and pTA248. The plants were tested for the presence of resistance genes *Xa4, xa5, Xa7,* and *Xa21*. IRBB 60 used as resistant check while Jumli Marshi used as susceptible checks. *Xa4* gene was present in twenty five genotypes, *xa5* in 24 genotypes and *Xa7* in 14 genotypes. None of these genes tested were present in 24 genotypes. More than one gene was found in 24 genotypes (Acharya *et al*., 2018).

Amgai *et al.* (2015), screened Nepalese rice germplasm accessions and identified BB resistance gene *Xa10* in two accessions, *xa13* in six accessions, *Xa7* in 23 accessions,*Xa10* in five accessions, *Xa3* and *Xa4* in 52 rice accessions, *xa5* in 25 accessions, x*a8* in 30 accessions. BB resistance gene *Xa21* was not found in any of the accessions screened.

Table 1. List of identified BB resistance genes (Khan *et al.,* 2014; Fiyaz *et al*., 2022)

Gene identified	Resistance source	Origin	Reference
Xa1	Temperate Japonica	Japan	Sakaguchi,1967; Yoshimura *et al.,*1998
Xa2	Indica	Vietnam	Kurata and Yamazaki, 2006
xa3/Xa26	Japonica	Japan	Sun *et al*., 2004; Xiang *et al.,* 2006
Xa4	Indica	India	Wang *et al*., 2001
xa5	Aus	Bangladesh	Petpisit *et al.,* 1977
Xa6/Xa3	–	USA	Sidhu *et al*.,1978
Xa7	Aus	Bangladesh	Sidhu *et al*., 1978; Lee and Khush, 2000
xa8		USA	Sidhu *et al.,* 1978; Singh *et al*., 2002
xa9	–	Laos	Singh *et al.,*1983; Ogawa *et al.,* 1991
Xa10	–	Senegal	Yoshimura *et al*., 1983; Kurata and Yamazaki, 2006
Xa11	Indica	Philippines	Kurata and Yamazaki, 2006
Xa12	Japonica	Japan	Ogawa, 1987
xa13	–	India	Ogawa *et al*., 1987; Kurata and Yamazaki, 2006
Xa14	Japonica	Taiwan	Sidhu *et al*., 1978; Kurata and Yamazaki, 2006
xa15	–	–	Nakai *et al.,* 1988; Ogawa, 1996
Xa16	Indica	Vietnam	Kurata and Yamazaki, 2006
Xa17	Japonica	South Korea	Kurata and Yamazaki, 2006
Xa18	Indica, Japonica	Philippines, Japan	Liu *et al*., 2004; Kurata and Yamazaki, 2006
xa19	–	–	Taura *et al.,* 1992; Kurata and Yamazaki , 2006
xa20			Taura *et al.,*1992; Kurata and Yamazaki, 2006
Xa21	Wild spps of *Oryza*	Africa, Mali	Song *et al*., 1995
Xa22(t)	–	China	Sun *et al.,* 2004; Kurata and Yamazaki 2006
Xa23	Wild spps of *Oryza*	China/Cambodia	Zhang *et al.,* 1998

Table 1. (Cont.) List of identified BB resistance genes (Khan *et al.,* 2014; Fiyaz *et al*., 2022)

Gene identified	Resistance source	Origin	Reference
xa24	–	Bangladesh	Khush and Angeles,1999
xa25(t)	Indica	China	Liu *et al*., 2011
xa26(t)	Indica	China	Lee *et al*., 2003
Xa27(t)	Wild spps of *Oryza*	Philippines	Lee *et al*., 2003; Gu *et al*., 2004
xa28(t)	Indica	Bangladesh	Lee *et al.,* 2003
Xa29(t)	Wild spps of *Oryza*	–	Tan *et al.,* 2004
Xa30(t)	Wild spps of *Oryza*	India	Cheema *et al.,* 2008
Xa31(t)	Japonica	China	Wang *et al*., 2009
Xa32(t)	Wild spps of *Oryza*	–	Ruan *et al.,* 2008; Zheng *et al.,* 2009
Xa33	Wild spps of *Oryza*	–	Natrajkumar *et al.,* 2012
xa33(t)	–	Thailand	Korinsak *et al*., 2009
xa34(t)	Indicia	Sri Lanka	Chen *et al*., 2011
Xa35(t)	Wild spps of *Oryza*	Philippines	Guo *et al*., 2010
Xa36(t)	–	China	Miao *et al*., 2010
Xa38	Wild spps of *Oryza*	–	Bhasin *et al.,* 2012
Xa39	FF329	China, Philippines	Zhang *et al*., 2015
Xa40(t)	IR65482-7-216-1-2	Korea	Kim *et al*., 2015
xa41(t)	Rice germplasm		Hutin *et al.,* 2015
xa42	XM14, a mutant of IR24	Japan	Busungu *et al*., 2016
Xa43(t)	P8	Japan	Kim and Reinke, 2019
xa44(t)	IR73571-3B-11-3-K3	Japan	Kim, 2018
xa45(t)	O. glaberrima IRGC102600B	Philippines	Neelam *et al*., 2020
Xa46(t)	Mutant H120	Japan	Chen *et al*., 2020

Materials & Methods

3. MATERIALS AND METHODS

The present study on "Analysis of pathotypic variability of *Xanthomonas oryzae* pv. *oryzae,* the bacterial blight pathogen of rice and identification of new sources of resistance" was conducted at the Department of Plant Pathology, College of Agriculture, Vellanikkara and Regional Agricultural Research Station, Pattambi, Palakkad during2017 to 2021. The details of the materials used and the techniques adopted for the investigation are described below.

3.1 Survey and occurrence of bacterial blight of rice

Survey was conducted in major rice growing areas of Palakkad, Thrissur, Malappuram, Alappuzha and Kottayam districts during the period 2018 to 2019 to study the occurrence of bacterial blight of rice and to collect diseased samples. The fields showing typical symptoms of bacterial blight such as *kresek* or leaf blight were surveyed. The symptoms noticed and severity were recorded. In Palakkad district 24 panchayats were surveyed covering 83 fields. In Malappuram district five panchayats were surveyed covering 19 fields. Thirteen panchayats were surveyed in Thrissur district covering 44 fields. In Alappuzha, five panchayats were surveyed covering 11 fields. In Kottayam district 4 panchayats surveyed covering 11 fields (Table 2). The disease in the field was confirmed by ooze test (Plate 1). The information on stage of crop, variety and location were recorded. The plants showing typical symptoms as well as leaf samples, were collected from the field. The samples were collected in polythene bags and brought to the laboratory for isolation of the pathogen. Small pieces of infected leaf samples were stored in plastic vials with caps. After putting small quantity of cotton in the tube, leaf pieces were kept and again cotton was placed on which little quantity of $CaCl_2$ was added and closed tightly and stored at $^{-}20^{0}$C.

3.2 Isolation and purification of the pathogen

Freshly collected samples showing typical bacterial blight symptoms were used for isolation of the pathogen. Diseased samples were washed in tap water for removing adhering external materials. The leaf samples were cut into small pieces of size 1cm along

with some healthy tissue using sterilized razors. The media used for isolation of pathogen was pepton sucrose agar (PSA). The leaf bits were placed in 0.1 per cent mercuric chloride for 30 seconds, then washed twice thoroughly with sterilized distilled water. The leaf bits were transferred to a sterilized Petri plate and added 0.5 ml of sterile water. The leaf bits were crushed using forceps until the water turns turbid by using a heat sterilized inoculation loop, a loopful of the water containing the bacterial ooze was streaked on Peptone Sucrose Agar (PSA) in Petri plates. The plates were incubated at 28 ± 2^0C for three to four days. After the development of bacterial colonies typical yellow coloured single colonies were picked up and and subcultured on to fresh PSA plates.

3.3 Pathogenicity studies

Pathogenicity studies were carried out in susceptible variety Jyothi. Seeds were sown in pots and grown in glass house. Twenty days old seedlings were transplanted to pots filled with potting mixture. Three plants were transplanted per pot. Other cultural practices as per package of practices recommendations were followed. Plants were inoculated 40 days after transplanting with each isolate of *Xoo* separately adopting clip inoculation method (Kauffman *et al*., 1973). The leaf tips were cut off by using sterilized scissors dipped in bacterial suspension containing 10^8 cfu/ml. Forty eight hours old culture of *Xoo* isolates were used for preparing culture suspension. The observations wererecorded 15 days after inoculation as per Standard Evaluation System scale of IRRI (2014) (Table 3).

3.4 Morphological and biochemical characterization of *Xoo* isolates

The colony characters of each isolate of *Xoo* was recorded (Bradbury, 1986; Schaad, 1992). The isolates were then labeled as Xoo1 onwards. The purified isolates were maintained in PSA slants in refrigerator for future studies. For long term storage bacterial cultures were also stored in 15 per cent glycerol at $^{-}20^0$C.

Plate 1. Ooze test for confirming bacterial blight in rice

Table 2. Details of districts and panchayats surveyed for bacterial blight of rice

District	Panchayat
Palakkad	Kuthannur
	Kuzhalmannam
	Peringottukurissy
	Kottayi
	Kollengode
	Pirayiri
	Chittur
	Pattanchery
	Perumatty
	Muthalamada
	Vallappuzha
	Alathur
	Thenkurissi
	Kannambra
	Anakkara
	Kappur
	Pattithara
	Chalissery
	Nagalassery
	Koppam
	Vilayur
	Pattambi
	Ongallur
	Kuamaramputhur
Malappuram	Angadippuram
	Perumpadappa
	Alamkode

District	Panchayat
Malappuram	Vettathur
	Thazhekode
Thrissur	Nadathara
	Thrissur
	Tholur
	Kodakara
	Varandarappally
	Vengittangu
	Elavally
	Mundathikkode
	Thekkumkara
	Wadakkanchery
	Pazhayannur
	Thiruvillamala
	Chelakkara
Alappuzha	Thakazhy
	Karuvatta
	Chambakkulam
	Edathua
	Mannar
Kottayam	Vaikom
	Vechoor
	Thalayazham
	Arpookkara

Table 3. SES scale for bacterial leaf blight disease severity (IRRI, 2014)

Score	Diseased leaf area (%)	Response	Description
1	1-5	Resistant	R
3	6-12	Moderately Resistant	MR
5	13-25	Moderately Susceptible	MS
7	26-50	Susceptible	S
9	>50	Highly Susceptible	HS

3.4.1 Biochemical characterization of *Xoo* isolates

Biochemical characterization of 100 *Xoo* isolates selected for further studies was carried out by following various tests (Fahy and Persley, 1983; Schaad, 1988; Mew and Mishra, 1994).

3.4.1.1 Indole production test

Test tubes with 5 ml of tryptone broth were inoculated with the *Xoo* cultures and were incubated in the shaker at 28 ± 2^0C for two to five days. After incubation, 0.5 ml of Kovac's Indole reagent was added to all the culture tubes and shaken well. Color change if any, was observed. The development of dark red color at the surface of the culture indicates a positive result.

3.4.1.2 MR-VP test

Test tubes with MR-VP broth were inoculated with *Xoo* cultures and incubated in shaker at 35^0C for 48 h. After incubation, the Methyl Red indicator was added to the MR test tubes and shaken well. The development of red color indicates a positive result. For VP test, 12 drops of VP reagent I (naphthol solution) and two to three drops of VP reagent II (40% KOH) were added. Tubes were shaken well, and color change was observed.

3.4.1.3 Starch hydrolysis

The *Xoo* cultures were spot inoculated onto starch agar medium. The plates were incubated at 28 ± 2^0C for four to seven days. After incubation, the plates were flooded with Lugol's Iodine. The plates were observed for any opaque zones around the colonies, indicating positive results.

3.4.1.4 Tween 80 hydrolysis

The tween 80 hydrolysis test was done in Sierra's medium. After adding sterilized tween 80 to the Petri plate, media was added. The *Xoo* cultures were spot inoculated onto

Sierra's medium and incubated at 28 ± 2^0C for seven days. The formation of opaque zones around the colonies indicated the production of esterase.

3.4.1.5 Urease test

The urea agar medium was prepared and 200 ml of sterilized urea solution was added. The media was transferred to sterilized test tubes and slants were prepared. The bacterial cultures were inoculated into the tubes and kept at 28 ± 2^0C for five to seven days.The color change of media to dark pink is a positive result.

3.4.1.6 KOH test

48 h old *Xoo* cultures were taken for the KOH test. The culture was taken with a sterilized inoculation loop and mixed with a drop of 3 per cent KOH solution on a glass slide. Thread like formation indicates positive result.

3.4.1.7 Catalase test

The *Xoo* cultures were taken and mixed with a drop of hydrogen peroxide on a sterilized glass slide. Formation of bubbles indicates the presence of catalase enzyme.

3.4.1.8 Oxidase test

The 48 hours old *Xoo* culture were rubbed on to the oxidase disc impregnated with 1 per cent tetra-methyl-p-phenylenediamine dihydrochloride. Dark purple colour change of positive result indicates the presence of cytochrome oxidase in the bacteria.

3.4.1.9 Nitrate reduction test

Nitrate broth in test tubes were inoculated with the *Xoo* cultures, incubated at 28±2^0C for three to seven days. After incubation, six to eight drops of Nitrite reagent A (sulfanilic acid) and Nitrite reagent B (naphthylamine) were added and observed for colour change. Pink colour change indicated positive result.

3.4.1.10 Carbohydrate utilization test

The Dye's medium containing bromocresol purple and filter sterilized 0.5 per cent carbohydrate were prepared and transferred to sterilized test tubes for making slants. The *Xoo* cultures were inoculated to the tubes and kept at 28 ± 2^0C for three to five days. Yellow colour change of the medium indicates the acid production.

3.5 Pathotyping of *Xoo* isolates on differentials/ NILs

Pathotyping of *Xoo* isolates was done on differentials carrying bacterial blight resistance genes.

3.5.1 Differentials/ NILs

Thirty one differentials including 28 NILs in the background of IR24 carrying single bacterial blight resistance genes or their combinations (IRBB lines) were used for the study. Improved Samba Mashuri a high yielding bacterial blight resistant variety released from IIRR, Hyderabad and two susceptible variety TN-1 and Jyothi were also used for the study (Table 4). NILs obtained from IIRR and maintained at the Regional Agricultural Rresearch Station, Pattambi were used. Nursery was raised in pots and maintained in glass house (Plate 2-3). Fourteen days old seedlings were transplanted in GI trays filled with a mixture of soil and farm yard manure. Differentials were transplanted in lines comprising of 10 plants. Sixteen differentials were accommodated per tray. Recommended dose of fertilizers were applied as basal and top dressing. The trays were irrigated and maintained well to keep the plants vigorous.

3.5.2 *Xoo* isolates

Out of the 168 isolates of *Xoo* collected from different locations of Palakkad, Malappuram, Thrissur, Alappuzha and Kottayam districts, 100 isolates were selected representing all surveyed locations and based on their virulence on susceptible variety Jyothi for pathotyping on differential / NILs.

3.5.3 Inoculation of *Xoo* isolates

NILs were inoculated 40 days after transplanting. *Xoo* isolates were multiplied in peptone sucrose agar media. Bacterial suspensions were prepared from 48 hours old culture of each isolate in sterile water so as to get 10^8 cfu/ml. Artificial inoculation was done by clip inoculation method (Kauffmann *et al.,* 1973). Fully expanded leaves were clip inoculated using sterilized scissors dipped in freshly prepared bacterial suspension.

3.5.4 Analysis of virulence of *Xoo* isolates

Observation on disease reaction was recorded 15 days after inoculation. Lesion lengths of top four different inoculated leaves from three plants were measured for each *Xoo* isolates from 31 differentials including susceptible checks. Average lesion length upto 5 cm was taken as resistant (R), 5-10 cm as moderately resistant (MR), 10-15 cm as moderately susceptible (MS) and more than 15 cm as susceptible (S). Average lesion length were converted in to disease reactions as per the scheme developed by IRRI for screening for bacterial blight resistance of breeding lines.
(http://wwwknowledgebank.irri.org/ricebreedingcourse/documents/Disease_Resistance.doc).

Table 4. Differentials used for pathotyping of *Xoo* isolates

Sl. No.	Differentials / NILs	BB resistance genes
1	IRBB 1	*Xa1*
2	IRBB 3	*xa3*
3	IRBB 4	*Xa4*
4	IRBB 5	*xa5*
5	IRBB 7	*Xa7*
6	IRBB 8	*xa8*
7	IRBB 10	*Xa10*
8	IRBB 11	*Xa11*
9	IRBB 13	*xa13*
10	IRBB 14	*Xa14*
11	IRBB 21	*Xa21*
12	IRBB 50	*Xa4 + xa5*
13	IRBB 51	*Xa4 + xa13*
14	IRBB 52	*Xa4 + Xa21*
15	IRBB 53	*xa5 + xa13*
16	IRBB 54	*Xa7 + Xa21*
17	IRBB 55	*xa13 + Xa21*
18	IRBB 56	*Xa4 +xa5 + xa13*
19	IRBB 57	*Xa4 + xa5 + Xa21*
20	IRBB 58	*Xa5 + xa13 + Xa21*
21	IRBB 59	*xa5 + xa13 + Xa21*
22	IRBB 60	*Xa4 + xa5 + xa13 + Xa21*
23	IRBB 61	*Xa4 + xa5 + Xa7*
24	IRBB 62	*Xa4 + Xa7 + Xa21*
25	IRBB 63	*xa5+Xa7+xa13*
26	IRBB 64	*Xa4+xa5+Xa7+Xa21*
27	IRBB 65	*Xa4+Xa7+xa13+Xa21*
28	IRBB 66	*Xa4+xa5+Xa7+xa13+Xa21*
29	Improved Samba Mahsuri	-
30	TN-1	-
31	Jyothi	-

3.5.5 Grouping of *Xoo* isolates into pathotypes

The average lesion length of each differential against each *Xoo* isolates were converted to reaction categories R, MR, MS and S. Based on the disease reaction on differentials a pathotype scheme was developed with modification of scheme of IIRR (Yugander *et al.,* 2017). Major emphasis was given on the reaction on single genes such as *Xa4, xa5, xa8, xa13* and *Xa21.* According to this scheme *Xoo* isolates were grouped into 15 pathotypes. The pathotypes were arranged in the order of increasing virulence starting from least virulent to highly virulent and given codes as XoPt1 onwards.

3.6 Phenotypic screening of germplasm accessions for bacterial blight resistance

Phenotypic screening was carried out at the Regional Agricultural Research Station, Pattambi. The germplasm accessions included in the study is given in table 5 (Plate 4.1).

Seeds of 50 germplasm accessions, susceptible check IR24, susceptible variety, Jyothi were sown in pots and grown in glass house. Twenty days old seedlings were transplanted to pots filled with potting mixture. Three plants were planted in each pot and three replications were maintained. Plants were maintained in open condition. Other cultural practices as per recommendations were followed. Plants were inoculated 40 days after transplanting with individual isolates of *Xoo* separately adopting clip inoculation (Kauffman *et al.,* 1973). The leaf tips were cut off by using sterilized scissors dipped in bacterial suspension containing 10^8 cfu/ml (Plate 4.2). The plants were covered using moistened polythene cover overnight and removed next day (Plate 4.3). The observations on top four diseased leaves were recorded 15 days after inoculation as per Standard Evaluation System scale of IRRI (2014). Based on the disease reaction, germplasm accessions were categorised as Resistant with score 1 (1-5%), Moderately Resistant with score 3 (6-12%), Moderately Susceptible with score 5 (13-25%), Susceptible with score 7 (26-51%) and Highly Susceptible with score 9 (51-100%). The virulent isolates of *Xanthomonas oryzae* pv. *oryzae viz.,* Xoo 13, Xoo 57, Xoo 63 selected from pathogenicity studies were used for artificial inoculation.

Plate 2. Nursery of differentials in glass house for pathotyping

Plate 3. Pathotyping of *Xoo* isolates on differentials

Plate 4.1. Germplasm accessions before inoculation

Plate 4.2. Clip inoculation of *Xoo* culture

Plate 4.3. Plants covered with moist polythene cover to retain humidity

Plate 4. Phenotypic screening of germplasm accessions for bacterial blight resistance

3.7 Genotypic screening for bacterial blight resistance

Genotypic screening for bacterial blight resistance genes in germplasm accessions was conducted at the Regional Agricultural Research Station, Pattambi. Seeds of 50 germplasm accessions along with positive resistant checks IRBB 4 (*Xa4*), IRBB 5 (*xa5*), IRBB13 (*xa13*), IRBB 21 (*Xa21*) and susceptible check IR24 were sown in pots. Plants were maintained in glass house.

3.7.1 Extraction of genomic DNA

Leaf samples were collected from 21 days old seedlings. DNA was extracted following CTAB method (Doyle and Doyle, 1990). The quantity of DNA was checked performing electrophoresis using 0.8 per cent agarose gel. DNA was quantified using spectrophotometer by measuring A260/A280. The total genomic DNA was dissolved in 100 µL nuclease free water and stored at $^{-}20^{0}$C for further use.

3.7.1.1 DNA extraction protocol

1. Pre heated the extraction buffer containing 100 mM Tris–HCl (pH 8), 1.4 M NaCl, 20 mM EDTA (pH 8), 2% (w/v) CTAB in water bath at 60^{0}C for about30 minutes. After completely dissolving the contents the extraction buffer was taken out and kept for cooling.

2. 1 g of fresh plant sample was taken in pre chilled sterilized mortar and pestle andwere ground well with chilled liquid nitrogen. Then added 1 per cent PVP (Polyvinylpyrrolidone) and 1 ml of pre-warmed extraction buffer at room temperature.

3. Transfered the ground material into 2 ml centrifuge tubes and incubated in waterbath at 60^{0}C for 30 minutes and vortexed occasionally.

4. After cooling at room temperature centrifuged at 10,000 rpm for 10 minutes at4^{0}C and supernatant was collected in 1.5 ml centrifuge tube.

5. Added equal volume of chloroform: isoamyl alcohol (24:1) and mixed byinversion for 15 minutes.

6. Kept for centrifugation at 10,000 rpm for 10 minutes at 4^{0}C and collected the supernatant in 1.5 ml centrifuge tube. The same steps was repeated once again.

7. Supernatant was collected in 1.5 ml centrifuge. For precipitating the DNA, addedtwice the volume of chilled isopropanol and incubated at $^{-}20^{0}$C for 30 minutes.

8. Centrifuged at 10,000 rpm for 10 minutes at 4^{0}C and supernatant was discarded and collected the pellet.

9. Added 1 ml of 70 per cent ethanol and kept for centrifugation at 5000 rpm for 5 minutes. This step was repeated once again. Then the pellet obtained was air dried.

10. After complete removal of ethanol, added 50 µl of nuclease free water to dissolvethe DNA and stored at $^{-}$20°C for further use.

3.7.1.2 Quality and quantity of DNA

After DNA isolation the quality and quantity of DNA were checked. The quantity of DNA was measured at an optical density (O.D.) of A260 and A280 using Spectrophotometer. Quality of DNA was checked performing electrophoresis using 0.8 per cent agarose gel.

3.7.1.3 Agarose gel electrophoresis

For preparing the loading gel, 0.8 g of agarose was dissolved in 100 ml of 1X TAE buffer. Heated the solution until agarose fully dissolved in it. After cooling, to the molten gel added 2 µl ethidium bromide. Mixed thoroughly by gentle swirling. The solution was poured to the gel loading tray, after placing the comb gel was allowed to set completely and the comb was removed carefully. The gel was placed in the electrophoresis tank and electrophoresis buffer was added so as to cover the gel. DNA sample were prepared by mixing 5 µl of DNA and 1 µl of 6X gel loading dye. The samples were loaded to the wells using micropipette. After closing the lid of the gel tankit was connected to the power pack. The voltage was set at 80 V for running the DNA. Once the blue dye in the DNA samples has migrated to the bottom of the gel, the power supply was turned off and the gel was removed. The image was captured under UV light using a Gel documentation system (Bio-Rad, USA).

3.7.2 PCR amplification

PCR amplification was carried out using PCR based SSR/STS markerssynthesised by IDT USA *viz*., MP for *Xa4,* RM 390 and RM122 for *xa5*, RM 230 and *xa13* prom for *xa13* and pTA 248 for *Xa21* (Table 6). Amplification was carried out in a reaction mixture of 20µL containing 30 ng of genomic DNA, 0.25 mM PCR buffer (GeNei^{TM}), 2.5µM dNTPs (GeNei^{TM}), 3U of Taq DNA polymerase (GeNei^{TM}) and 100 µM primer using a thermal cycler (Mastercycler Gradient, Eppendorf) (Table 7). The thermal cycling program for respective primers are mentioned in Table 8-13. The amplified PCR products with a 100 bp DNA marker ladder (GeNei^{TM}) were size fractioned by electrophoresis in 2 per cent agarose gel prepared in TAE buffer and visualized by staining with ethidium bromide (0.5 µg/mL) in a gel documentation system (Bio-Rad, USA).

Table 5. Germplasm accessions used for phenotypic and genotypic screening

Sl. No.	Germplasm accessions
1	Eruvakkali (2050)
2	Mandupakki (2053)
3	Mangalapuram (2071)
4	Chenkayama (Ambalapara) (2085)
5	Ponmani (2095)
6	Chettivirippu (2097)
7	Vellapokkali (2098)
8	Virippu (2105)
9	Bolamgittikayama (2112)
10	Vellakkayama (2113)
11	Mundakan (2117)
12	Anakkodan (2118)
13	Cheriya orpandy (2122)
14	Gandhasala (1) (2151)
15	Parambuvattan (2153)
16	Champan (2157)
17	Gandhasala (2) (2159)
18	Jeerakasala (2161)
19	Kokkankoli (2162)
20	Pandi Champan (2164)
21	Kalladiaryan (Red rice) (2168)
22	Kothambalarikayama (1) (2171)
23	Njavara (Black) (2201)
24	Mundon (Cheruli) (2219)
25	Veliyan (1) (2221)
26	Basmati (2228)

Sl. No.	Germplasm accessions
27	Krishnakamod (2229)
28	Karutha njavara (2230)
29	Kalluruli upland (2231)
30	Chitteni (Alathur) (3040)
31	Wayanad 2 (3049)
32	Black Chitteni (Thavanur) (3075)
33	Chembavu
34	Cheruvellari
35	Ithikandan
36	Kariyadukkan
37	Kokkan
38	Kothambalarikayama (2)
39	Kunnamkulamban
40	Kuruva
41	Mallimatta
42	Mannuveliyan
43	Marathondi
44	Mullankayama
45	Mundon
46	Odiyan
47	Ottadi
48	Thondi
49	Veliyan (2)
50	Vellari
51	Jyothi (Susceptible check)
52	IR24 (Susceptible check)

Table 6. Markers used for identification of BB genes

Sl. No	*Xa* gene	Chromosome	Marker	Type	Sequence (5' – 3')	Reference
1	*Xa4*	11	MP	SSR	ATCGATCGATCTTCACGAGG TCGTATAAAAGGCATTCGGG	Ma *et al.,*1999
2	*xa5*	5	RM 390	SSR	CGTCAATGGGGTAGGTCTTG GGAGGCCAAGGAAGAGGTAG	Blair and McCouch, 1997
			RM 122	SSR	CCCTTGTTTCAGTGGCTCAG CCAAGATCAAGAACAGCAGGAATC	Patel *et al.,* 2015
3	*xa13*	8	RM 230	SSR	GCCAGACCGTGGATGTTC CACCGCAGTCACTTTTCAAG	Ashiba *et al*., 2020
			xa13 prom	SSR	GCCAGACCGTGGATGTTC CACCGCAGTCACTTTTCAAG	Amgai *et al.,* 2015
4	*Xa21*	11	pTA 248	STS	AGACGCGGAAGGGTGGTTCCCGGA AGACGCGGTAATCGAAGATGAAA	Ronald *et al.,* 1992

Table 7. Components of PCR reaction mixture

Components	Concentration
Genomic DNA	30 ng
PCR buffer (GeNei^{TM})	0.25 mM
$MgCl_2$	25 mM
dNTPs (GeNei^{TM})	2.5 mM
Forward primer	100 µM
Reverse primer	100 µM
Taq DNA polymerase	3U

Table 8. Thermal profiling conditions for *Xa4* (MP)

No.	Step	Temperature	Duration	
1	Initialization	94^0C	5 min	35 cycles
2	Denaturation	94^0C	1 min	
3	Annealing	56^0C	1 min	
4	Extension	72^0C	2 min	
5	Final extension	72^0C	10 min	
6	Hold	4^0C		

Table 9. Thermal profiling conditions for *xa5* (RM 390)

No.	Step	Temperature	Duration	
1	Initialization	94^0C	4min	35 cycles
2	Denaturation	94^0C	1 min	
3	Annealing	60.8^0C	1 min	
4	Extension	72^0C	1 min	
5	Final extension	72^0C	7 min	
6	Hold	4^0C		

Table 10. Thermal profiling conditions for *xa5* (RM 122)

No.	Step	Temperature	Duration	
1	Initialization	94^0C	5 min	35 cycles
2	Denaturation	94^0C	1 min	
3	Annealing	59^0C	1 min	
4	Extension	72^0C	2 min	
5	Final extension	72^0C	7 min	
6	Hold	4^0C		

Table 11. Thermal profiling conditions for *xa13* (RM 230)

No.	Step	Temperature	Duration	
1	Initialization	94^0C	5 min	35 cycles
2	Denaturation	94^0C	30 sec	
3	Annealing	55^0C	30 sec	
4	Extension	72^0C	1 min	
5	Final extension	72^0C	10 min	
6	Hold	4^0C		

Table 12. Thermal profiling conditions for *xa13* (*xa13* prom)

No.	Step	Temperature	Duration	
1	Initialization	94^0C	5 min	35 cycles
2	Denaturation	94^0C	1 min	
3	Annealing	59^0C	1 min	
4	Extension	72^0C	2 min	
5	Final extension	72^0C	7 min	
6	Hold	4^0C		

Table 13. Thermal profiling conditions for *Xa21* (pTA 248)

No.	Step	Temperature	Duration	
1	Initialization	94^0C	4 min	35 cycles
2	Denaturation	94^0C	1 min	
3	Annealing	58^0C	1 min	
4	Extension	72^0C	1 min	
5	Final extension	72^0C	7 min	
6	Hold	4^0C		

Results

4. RESULTS

The present study on "Analysis of pathotypic variability of *Xanthomonas oryzae* pv. *oryzae,* the bacterial blight pathogen of rice and identification of new sources of resistance" was conducted at the Department of Plant Pathology, Regional Agricultural Research Station, Pattambi, Palakkad during the period 2017 to 2021. A survey was conducted on the occurrence and severity of bacterial blight of rice and diseased samples were collected from major rice growing areas of Palakkad, Malappuram, Thrissur, Alappuzha and Kottayam districts of Kerala. Pathotyping of isolates of *Xanthomonas oryzae* pv. *oryzae* was done using differentials/ Near Isogenic Lines (NILs) carrying bacterial blight resistant genes individually or in combinations. The genotypic screening was carried out to locate bacterial blight resistance genes *viz., Xa4, xa5, xa13* and *Xa21* in fifty germplasm accessions of rice maintained at RARS, Pattambi. Phenotypic screening of these 50 germplasm accessions for bacterial blight resistance was also carried out. The results obtained from all these experiments are given here.

4.1 Survey and occurrence of bacterial blight of rice

A purposive sampling survey was conducted to study the occurrence and intensity of bacterial blight disease of rice in major rice growing tracts of Kerala and to isolate pathogen from these places. Survey was conducted in five districts *viz,* Palakkad, Malappuram, Thrissur, Kottayam and Alappuzha covering 52 panchayats. The locations surveyed were coming under latitude 9.3107^0 to 11.0118^0 North and longitude 76.4305^0 to 76.3000^0 East (Plate 5-9). The details of survey locations, the varieties grown in different locations, stage of the crop, symptoms noticed and disease severity are given in tables 13 to 17. The plants showing typical bacterial blight symptoms *viz*., leaf blight or *kresek* were collected from different surveyed locations (Plate 10-11).

4.1.1 Bacterial blight severity in Palakkad district

In Palakkad districts, Kuthannur, Kuzhalmannam, Perigottukurissy, Kottayi, Kollengode, Pirayiri, Chittur, Pattanchery, Vallappuzha, Alathur, Thenkurissi, Kannambra, Kappur, Pattithara, Chalissery, Koppam, Vilayur, Pattambi and Ongallur panchayats was

surveyed during *kharif* season while Nagalassery, Anakkara, Perumatty, Muthalamada and Kumaramputhur was surveyed during *rabi* season. In Palakkad district among the 24 locations surveyed, the leaf blight severity ranging from 5.30 to 95.10 per cent was recorded (Table 14). The *kresek* symptom was noticed in six locations and the severity ranged from 5.28 to 60.18 per cent. The highest severity was recorded in eastern parts of the district *viz.,* Pattanchery (95.10%) and Chittur (90.28%) in variety Jyothi. During the period of survey, in most of the fields the crop was in reproductive phase which is highly susceptible to the disease. Among the 82 fields surveyed, in 24 fields the variety cultivated was Jyothi and in 35 fields the variety cultivated was Uma. The other varieties grown in the district affected with disease were ASD (10.08 - 40.88%), Kanchana (30.88%), Ponmani (15.33 to 50.85%), Mattatriveni (20.95%) and Annapoorna (10.85%). The upland varieties Kattamodan (10.20%), Karuthamodan (10%), Swarnaprabha (15.25%) and Vaisakh (35.78%) showed relatively less severity in upland condition. *Kresek* symptom was observed in varieties Jyothi, Uma and Ponmani in early transplanted stage in six fields surveyed (Plate 12).

4.1.2 Bacterial blight severity in Malappuram district

In Malappuram district, five Panchayats covering 19 fields were surveyed (Table 15). Survey was carried out in three panchayats *viz.,* Angadippuram, Perumpadappu during *rabi* season. Two panchayats, Vettathur and Thazhekode survey was conducted during *kharif* season. In five locations of Angadippuram panchayats, the variety Ponmani and in one location Jyothi was grown. The crop was in early transplanted stage and was affected with *kresek* phase of bacterial blight. In other fields surveyed covering three panchayats, Uma variety was in flowering stage. The leaf blight symptom ranging from 5.28 to 75.75 per cent severity was recorded. In one panchayat, Thazhekode, in two fields, the varieties Uma and Jyothi were in early transplanted condition where *kresek* symptom was recorded (5.33 - 15.05%) (Plate 13).

4.1.3 Bacterial blight severity inThrissur district

In Thrissur district 13 panchayats covering 29 locations in which 44 different fields were surveyed (Table 16). Survey was carried out in *rabi* season in Nadathara, Thrissur, Tholur, Kodakara, Varandarapalli, Vengittangu, Chelakkara, Thiruvillamala, Wadakkanchery and Thekkumkara panchayats, where as the survey was conducted in Elavally, Mundathikkode, Wadakkanchary and Pazhayannur during *kharif* season. In 32 fields the variety cultivated was Uma and the disease severity ranged from 5.25 per cent (Nadathara and Venkitangu) to 85.83 per cent (Kodakara). In two fields *kresek* symptom was noticed in 10.35-25.25 per cent severity. In eight fields surveyed variety Jyothi was cultivated. Disease severity ranged from 25-80.18 per cent. In two fields where direct sowing was practiced, *kresek* symptom was recorded at 35-50.15 per cent severity level in variety Jyothi and 10 to 25 per cent in variety Uma. The other varieties affected with leaf blight were Matta triveni (5.40-10.43%) in three fields of other panchayats and Shreyas in one location (50.25%) (Plate 14).

4.1.4 Bacterial blight severity in Alappuzha district

In Alappuzha district, survey was carried out in five panchayats, eight locations covering 11 fields (Table 17) during *rabi* season. Variety Uma was cultivated in seven fields and Jyothi was cultivated in four fields. The crop was in flowering to maturity stage. Bacterial leaf blight was noticed 10.63-70.68 per cent severity in variety Uma. In Jyothi 5.38- 50.70 per cent severity was noticed (Plate 15).

4.1.5 Bacterial blight severity in Kottayam district

In Kottayam district survey was carried out during *rabi* season. In four panchayats surveyed the variety Uma was grown in 10 fields and in one field IR5 was grown (Table 18). The crop was in maturity stage. In all the surveyed locations leaf blight symptom was recorded. The disease severity ranged from 5.45 to 50.63 per cent (Plate 16).

4.2 Isolation and purification of the pathogen

The pathogen was isolated from freshly collected bacterial blight infected plants samples collected from different locations. The isolation was done on Peptone Sucrose Agar media (PSA) and four to five days after incubation single colonies typical to that of *Xanthomonas oryzae* pv. *oryzae* were subcultured to PSA plates. These pure cultures were maintained in PSA slants. For long term storage these *Xoo* isolates were inoculated to 15 per cent glycerol in vials and stored in $^{-}20^{0}$C (Plate 17). A total of 168 isolates were purified and maintained (Plate 18).

4.3 Pathogenicity and virulence studies of *Xoo* isolates

Pathogenicity of the all the 168 isolates were proved by inoculation to susceptible variety Jyothi. The bacterial isolates produced symptoms on the test variety upon inoculation. The pathogen was reisolated from the symptomatic plants and upon reisolation the same bacterial isolate were obtained. The severity of symptoms obtained on the susceptible variety Jyothi varied with the isolate, showing the variability in virulence among the *Xanthomonas oryzae* pv. *oryzae* isolates. The data is given in Table 19. The disease severity varied from 14.37 per cent (Score 5) to 60.93 per cent (Score 9). Among the 168 isolates, 14 isolates (8.33%) produced highly susceptible reaction with score 9, 128 isolates (76.19 %) produced susceptible reaction with score 7 and 26 isolates (15.48%) produced moderately susceptible reaction with score 5 upon artificial inoculation on susceptible variety Jyothi (Plate 19).

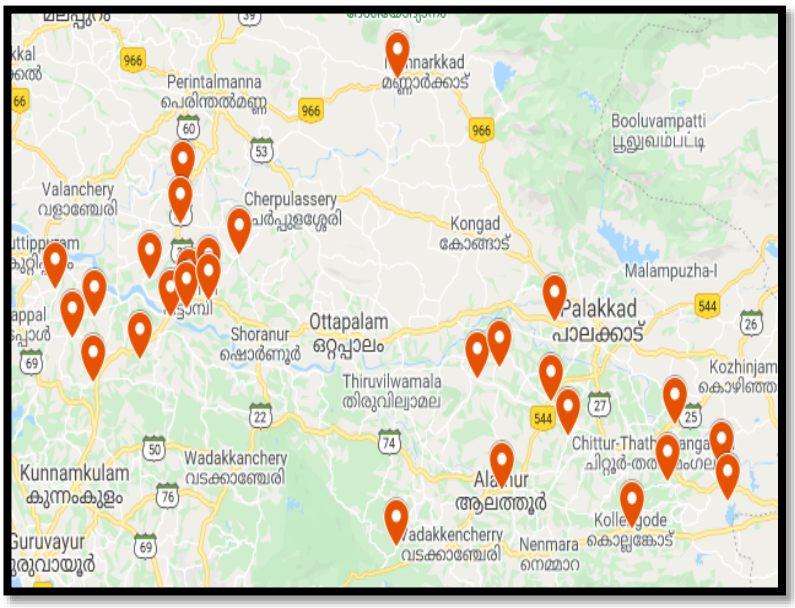

Plate 5. Details of survey locations of Palakkad district

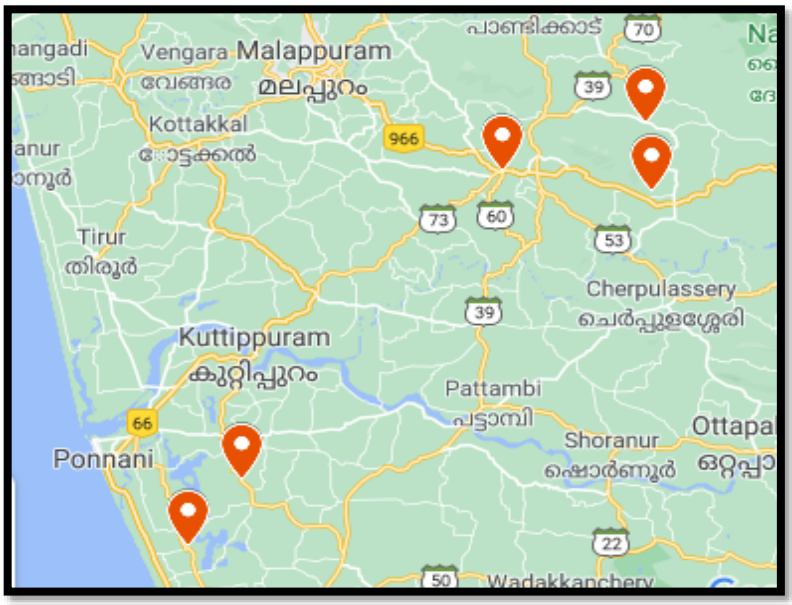

Plate 6. Details of survey locations of Malappuram district

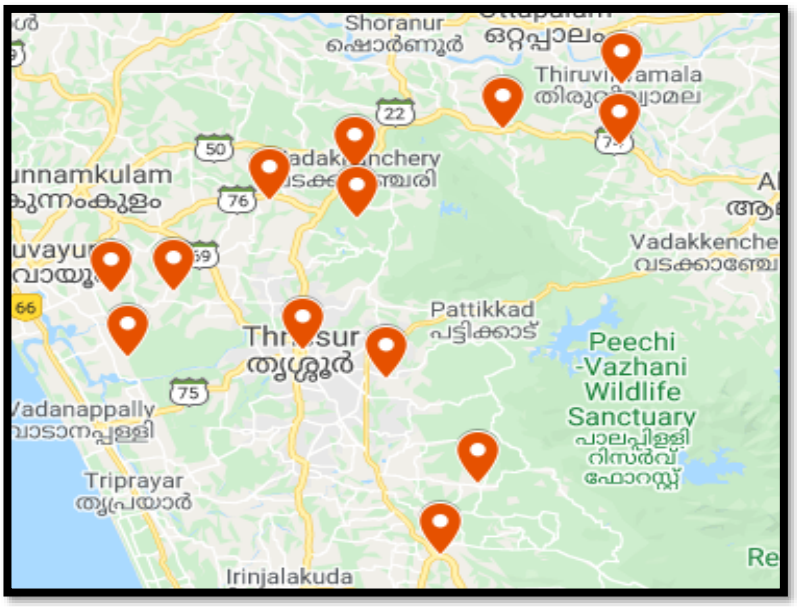

Plate 7. Details of survey locations of Thrissur district

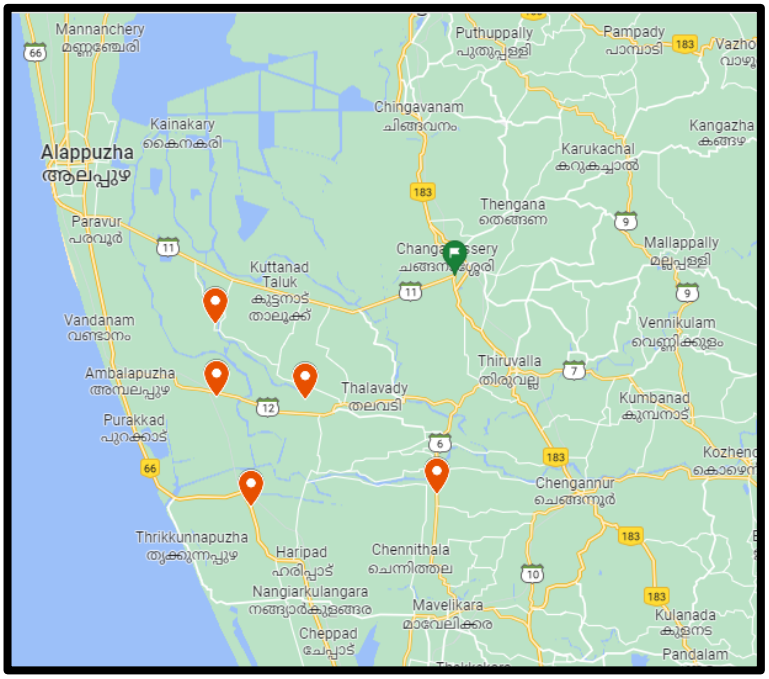

Plate 8. Details of survey locations of Alappuzha district

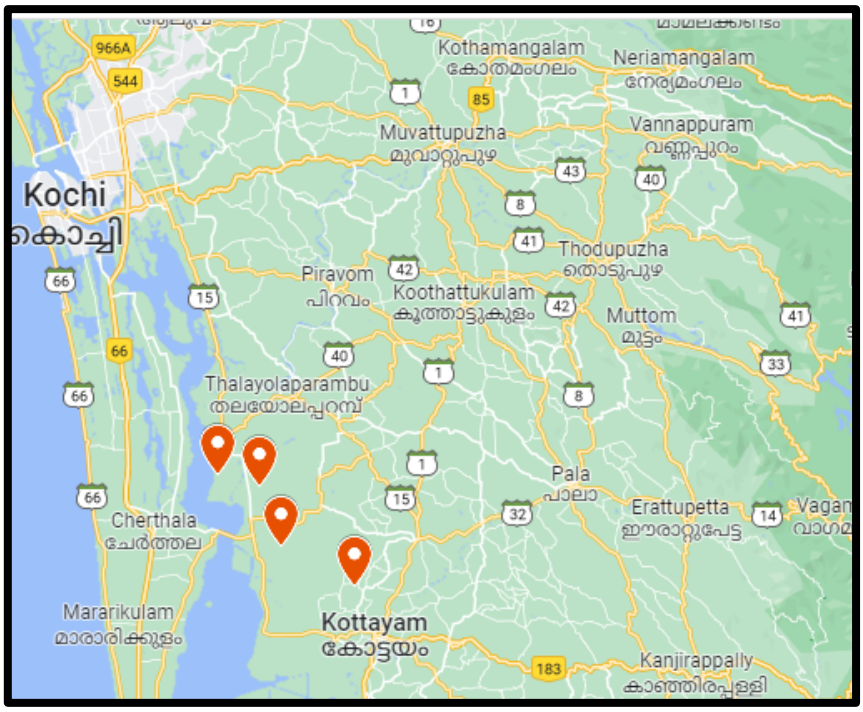

Plate 9. Details of survey locations of Kottayam district

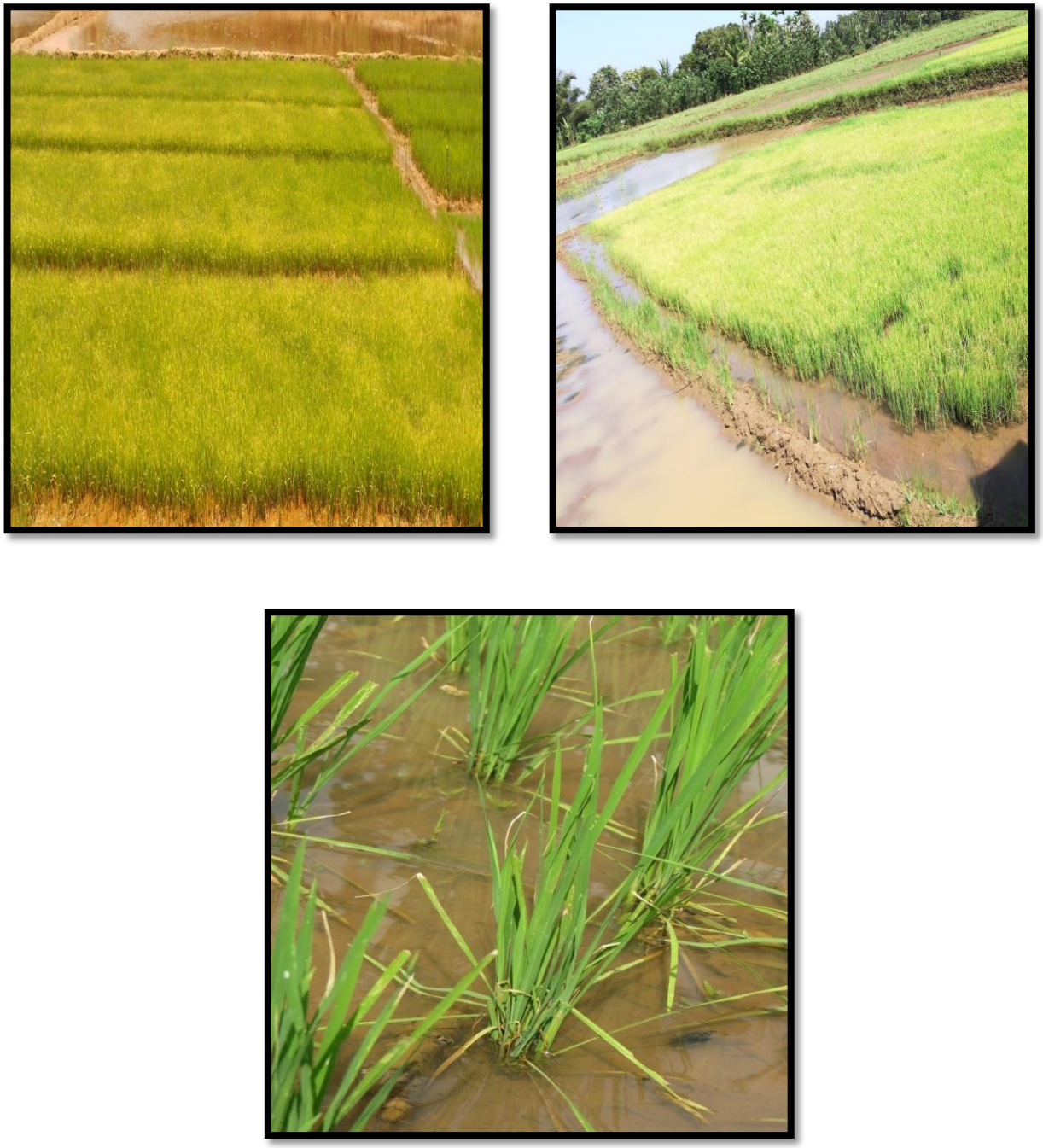

Plate 10. Bacterial blight of rice *Kresek* symptom

Plate 11. Bacterial blight of rice - Leaf blight symptom

Table 14. Crop details and severity of bacterial blight in different locations of Palakkad district

Panchayat	Location/ field	Variety	Stage of crop (days old)	Symptom	Disease severity %	*Xoo* isolate code
Kuthannur	L1 F1	ASD	100	Leaf blight	40.88	Xoo 1
	L1 F2	Uma	100	Leaf blight	40.95	Xoo 2
	L1F3	Jyothi	100	Leaf blight	40.10	Xoo 3
Kuzhalmannam	L1 F1	Jyothi	100	Leaf blight	40.15	Xoo 4
	L2F1	Jyothi	90	Leaf blight	75.48	Xoo 5
	L3F1	Kanchana	100	Leaf blight	30.18	Xoo 6
Peringottukurissy	L1F1	ASD	100	Leaf blight	10.08	Xoo 7
Kottayi	L1F1	Jyothi	110	Leaf blight	10.83	Xoo 8
	L2F1	Uma	90	Leaf blight	10.85	Xoo 73
Kollengode	L1F1	Jyothi	110	Leaf blight	5.30	Xoo 9
Pirayiri	L2F1	Uma	100	Leaf blight	10.70	Xoo 10
	L2F2	Uma	100	Leaf blight	15.33	Xoo 11
	L2F3	Uma	100	Leaf blight	10.08	Xoo 12
	L2F4	Uma	100	Leaf blight	90.50	Xoo13
Chittur	L1F1	Jyothi	100	Leaf blight	50.90	Xoo 39
	L2F1	Jyothi	100	Leaf blight	90.28	Xoo 74
Pattanchery	L1F1	Jyothi	90	Leaf blight	95.10	Xoo 76
	L1F2	Uma	100	Leaf blight	80.38	Xoo 77
	L1F3	Jyothi	100	Leaf blight	10.20	Xoo 88
	L1F4	Jyothi	90	Leaf blight	40.10	Xoo 89
	L1F5	Uma	90	Leaf blight	25.03	Xoo 164
Perumatty	L1F1	Uma	100	Leaf blight	20.08	Xoo 163
Muthalamada	L1F1	Jyothi	95	Leaf blight	40.18	Xoo 165
Vallappuzha	L1F1	Uma	75	Leaf blight	50.10	Xoo 168
Alathur	L1F1	Uma	95	Leaf blight	30.03	Xoo 58
	L1F2	Jyothi	100	Leaf blight	10.55	Xoo 59
	L2F1	Uma	100	Leaf blight	10.25	Xoo 60
Thenkurissi	L1F1	Jyothi	90	Leaf blight	30.80	Xoo 75
Kannambra	L1F2	Jyothi	30	*Kresek*	25.03	Xoo 103
	L1F3	Uma	30	*Kresek*	50.80	Xoo 104
Anakkara	L1F1	Ponmani	90	Leaf blight	30.80	Xoo 38
	L1F2	Uma	90	Leaf blight	50.15	Xoo 102
Kappur	L1F1	Uma	20	*Kresek*	40.75	Xoo 80
Pattithara	L1F2	Uma	90	Leaf blight	10.20	Xoo 44
	L1F3	Jyothi	90	Leaf blight	30.88	Xoo 45
Chalissery	L1F1	Ponmani	60	Leaf blight	15.33	Xoo 37
Nagalassery	L1F1	Jyothi	80	Leaf blight	10.13	Xoo 147
	L1F2	Jyothi	90	Leaf blight	25.85	Xoo 161
Koppam	L1F1	Uma	90	Leaf blight	10.00	Xoo 25
Vilayur	L1F1	Supriya	100	Leaf blight	30.98	Xoo 24
	L1F2	Jyothi	90	Leaf blight	25.85	Xoo 99

Table 14. (Cont.) Crop details and severity of bacterial blight in different locations of Palakkad district

Panchayat	Location/ field	Variety	Stage of crop (days old)	Symptom	Disease severity %	*Xoo* isolate code
Pattambi	L1F1	Uma	90	Leaf blight	5.78	Xoo 20
	L1F2	Jyothi	90	Leaf blight	50.25	Xoo 21
	L1F1	Jyothi	20	*Kresek*	60.18	Xoo 50
	L1F2	Uma	80	Leaf blight	15.58	Xoo 160
	L1F3	Ponmani	75	Leaf blight	20.28	X00 162
	L2F1	Ponmani	75	Leaf blight	50.85	Xoo 127
	L2F2	Uma	75	Leaf blight	20.08	Xoo 128
	L2F3	Uma	80	Leaf blight	10.10	Xoo 129
	L2F4	Uma	80	Leaf blight	30.00	Xoo 130
	L2F5	Uma	80	Leaf blight	50.30	Xoo 131
	L2F6	Uma	80	Leaf blight	15.33	Xoo 132
	L2F7	Jyothi	80	Leaf blight	10.58	Xoo 133
	L2F8	Uma	80	Leaf blight	10.10	Xoo 134
	L3F1	Uma	75	Leaf blight	40.18	Xoo 116
	L3F2	Jyothi	75	Leaf blight	50.23	Xoo 117
	L3F3	Uma	75	Leaf blight	50.03	Xoo 118
	L3F4	Uma	75	Leaf blight	50.63	Xoo 119
	L3F5	Kanchana	75	Leaf blight	41.25	Xoo 144
	L3F6	Jyothi	90	Leaf blight	20.15	Xoo 145
	L3F7	Uma	80	Leaf blight	15.00	Xoo 146
	L4F1	Jyothi	100	Leaf blight	15.45	Xoo 86
	L4F2	Uma	100	Leaf blight	10.83	Xoo 87
	L4F3	Uma	75	Leaf blight	50.10	Xoo 120
	L5F1	Ponmani	40	*Kresek*	15.80	Xoo 78
	L5F2	Uma	40	*Kresek*	5.28	Xoo 79
	L5F3	Kattamodan	90	Leaf blight	10.20	Xoo 81
	L5F4	Karuthamodan	90	Leaf blight	10.00	Xoo 82
	L5F5	Swarnaprabha	90	Leaf blight	15.25	Xoo 83
	L5F6	Vaisakh	90	Leaf blight	35.78	Xoo 84
	L5F7	Mattatriveni	90	Leaf blight	20.95	Xoo 85
	L5F8	Jyothi	20	*Kresek*	30.83	Xoo 100
	L5F9	Jyothi	100	Leaf blight	40.18	Xoo 101
	L5F10	Annapoorna	90	Leaf blight	10.85	Xoo 148
Ongallur	L1F1	Uma	100	Leaf blight	40.10	Xoo 166
	L1F2	Jyothi	75	Leaf blight	40.18	Xoo 167
Kuamaramputhur	L1F1	Uma	100	Leaf blight	20.30	Xoo 135
	L1F2	Uma	100	Leaf blight	20.38	Xoo 136
	L1F3	Uma	100	Leaf blight	5.30	Xoo 137
	L1F1	Uma	60	Leaf blight	5.45	Xoo 138
	L1F2	Uma	75	Leaf blight	20.08	Xoo 139
	L1F3	Jyothi	75	Leaf blight	35.70	Xoo 140
	L1F1	Jyothi	75	Leaf blight	40.45	Xoo 141

Table 15. Crop details and severity of bacterial blight in different locations of Malappuram district

Panchayat	Location / field	Variety	Stage of crop (days old)	Symptom	Disease severity %	*Xoo* isolate code
Angadippuram	L1F1	Ponmani	50	*Kresek*	5.30	Xoo 14
	L2F1	Ponmani	60	*Kresek*	10.20	Xoo 15
	L2F2	Ponmani	60	*Kresek*	10.00	Xoo 16
	L3F3	Ponmani	20	*Kresek*	15.05	Xoo 17
	L3F2	Jyothii	90	Leaf blight	10.13	Xoo 18
	L4F1	Ponmani	45	*Kresek*	10.33	Xoo 19
Perumpadappa	L1F1	Uma	65	Leaf blight	10.38	Xoo 51
	L1F2	Uma	65	Leaf blight	20.08	Xoo 52
	L1F3	Uma	65	Leaf blight	10.00	Xoo 53
	L1F4	Uma	65	Leaf blight	10.70	Xoo 54
	L2F1	Uma	65	Leaf blight	5.28	Xoo 55
Alamkode	L1F1	Uma	60	Leaf blight	5.45	Xoo 56
	L1F2	Uma	60	Leaf blight	5.78	Xoo 57
Vettathur	L1F1	Uma	90	Leaf blight	70.20	Xoo 121
	L1F2	Uma	90	Leaf blight	70.60	Xoo 122
	L2F1	Uma	75	Leaf blight	50.80	Xoo 123
	L3F1	Uma	75	Leaf blight	75.75	Xoo 124
Thazhekode	L1F1	Uma	40	Leaf blight	50.15	Xoo 125
	L1F2	Jyothi	40	*Kresek*	50.10	Xoo 126

Table 16. Crop details and severity of bacterial blight in different locations of Thrissur district

Panchayat	Location / field	Variety	Stage of crop (days old)	Symptom	Diseases severity %	*Xoo* isolate code
Nadathara	L1F1	Uma	60	Leaf blight	20.23	Xoo 42
	L1F2	Uma	60	Leaf blight	5.20	Xoo 43
Thrissur	L2F1	Jyothi	75	Leaf blight	25.00	Xoo 142
Tholur	L1F1	Uma	49	Leaf blight	10.13	Xoo 32
	L2F1	Uma	50	Leaf blight	20.40	Xoo 33
	L3F1	Uma	65	Leaf blight	5.10	Xoo 34
	L4F1	Uma	65	Leaf blight	10.78	Xoo 35
	L5F1	Jyothi	90	Leaf blight	80.18	Xoo 36
Kodakara	L1F1	Uma	75	Leaf blight	85.83	Xoo 22
	L1F2	Jyothi	75	Leaf blight	30.75	Xoo 23
	L1F3	Jyothi	80	Leaf blight	20.53	Xoo 97
	L1F4	Jyothi	80	Leaf blight	50.28	Xoo 98
Varandarappally	L1F1	Uma	75	Leaf blight	25.35	Xoo 40
	L2F1	Uma	75	Leaf blight	20.03	Xoo 41
Vengittangu	L1F1	Mattatriveni	90	Leaf blight	10.43	Xoo 90
	L1F2	Mattatriveni	90	Leaf blight	5.65	Xoo 91
	L1F3	Mattatriveni	90	Leaf blight	5.40	Xoo 92
	L2F1	Uma	90	Leaf blight	10.08	Xoo 93
	L2F2	Uma	90	Leaf blight	10.23	Xoo 94
	L2F3	Uma	90	Leaf blight	5.25	Xoo 95
	L2F4	Uma	90	Leaf blight	5.05	Xoo 96
Elavally	L1F1	Jyothi	15	*Kresek*	35.05	Xoo 46
	L1F2	Jyothi	20	*Kresek*	50.15	Xoo 47
	L2F1	Uma	40	*Kresek*	25.20	Xoo 48
	L2F2	Uma	40	*Kresek*	10.35	Xoo 49
Mundathikkode	L1F1	Uma	50	Leaf blight	50.43	Xoo 26
	L1F2	Uma	50	Leaf blight	30.08	Xoo 27
	L2F1	Uma	60	Leaf blight	50.15	Xoo 28
	L2F2	Shreyas	50	Leaf blight	50.25	Xoo 29
	L3F1	Uma	60	Leaf blight	35.05	Xoo 30
Thekkumkara	L1F1	Uma	75	Leaf blight	50.43	Xoo 149
	L1F2	Uma	75	Leaf blight	45.53	Xoo 150
	L1F1	Uma	75	Leaf blight	30.03	Xoo 151
	L1F2	Uma	75	Leaf blight	30.83	Xoo 152
	L1F3	Uma	75	Leaf blight	20.23	Xoo 153
Wadakkanchery	L1F1	Uma	70	Leaf blight	30.55	Xoo 154
	L1F2	Uma	80	Leaf blight	60.90	Xoo 155
	L2F1	Uma	57	Leaf blight	60.18	Xoo 31
Pazhayannur	L1F1	Jyothi	90	Leaf blight	80.18	Xoo 72
Thiruvillamala	L1F1	Uma	90	Leaf blight	40.98	Xoo 143
Chelakkara	L1F1	Uma	110	Leaf blight	50.38	Xoo 156
	L1F2	Uma	110	Leaf blight	40.08	Xoo 157
	L1F3	Uma	110	Leaf blight	40.80	Xoo 158
	L1F3	Uma	110	Leaf blight	30.78	Xoo 159

Table 17. Crop details and severity of bacterial blight in different locations of Alappuzha district

Panchayat	Location / field	Variety	Stage of crop (days old)	Symptom	Disaese severity %	*Xoo* isolate code
Thakazhy	L1F1	Jyothi	90	Leaf blight	10.78	Xoo 61
Karuvatta	L1F1	Uma	90	Leaf blight	20.03	Xoo 62
	L2F1	Uma	60	Leaf blight	50.05	Xoo 63
	L3F1	Uma	90	Leaf blight	30.55	Xoo 64
Chambakkulam	L1F1	Jyothi	100	Leaf blight	50.70	Xoo 65
Edathua	L1F1	Uma	90	Leaf blight	10.63	Xoo 66
	L1F2	Uma	90	Leaf blight	50.70	Xoo 67
Mannar	L1F1	Uma	60	Leaf blight	70.68	Xoo 68
	L1F2	Uma	60	Leaf blight	30.58	Xoo 69
	L2F1	Jyothi	85	Leaf blight	5.38	Xoo 70
	L2F2	Jyothi	85	Leaf blight	5.30	Xoo 71

Table 18. Crop details and severity of bacterial blight in different locations of Kottayam district

Panchayat	Location /Field	Variety	Stage of crop (days old)	Symptom	Disease severity %	*Xoo* isolate code
Vaikom	L1F1	Uma	100	Leaf blight	30.18	Xoo 105
	L1F2	Uma	120	Leaf blight	25.85	Xoo 106
Vechoor	L1F1	Uma	135	Leaf blight	50.15	Xoo 107
	L1F2	Uma	135	Leaf blight	50.63	Xoo 108
Thalayazham	L1F1	Uma	125	Leaf blight	25.03	Xoo 109
	L1F2	Uma	125	Leaf blight	50.30	Xoo 110
	L1F3	Uma	125	Leaf blight	10.33	Xoo 111
	L1F1	IR 5	100	Leaf blight	5.80	Xoo 112
Arpookkara	L1F1	Uma	120	Leaf blight	10.55	Xoo 113
	L1F2	Uma	130	Leaf blight	10.23	Xoo 114
	L1F3	Uma	130	Leaf blight	5.45	Xoo 115

Table 19. Disease severity on rice variety Jyothi under artificial inoculation of *Xoo* isolates

Xoo code	Disease severity (%)	Score	Reaction
Xoo 1	41.18	7	S
Xoo 2	41.32	7	S
Xoo 3	24.13	5	MS
Xoo 4	30.64	7	S
Xoo 5	31.87	7	S
Xoo 6	47.68	7	S
Xoo 7	42.69	7	S
Xoo 8	31.91	5	MS
Xoo 9	54.31	9	HS
Xoo 10	59.70	9	HS
Xoo 11	30.98	7	S
Xoo 12	38.70	7	S
Xoo 13	51.17	9	HS
Xoo 14	44.48	7	S
Xoo 15	49.64	7	S
Xoo 16	44.97	7	S
Xoo 17	43.59	7	S
Xoo 18	49.82	7	S
Xoo 19	51.63	9	HS
Xoo 20	41.86	7	S
Xoo 21	43.13	7	S
Xoo 22	37.15	7	S
Xoo 23	40.02	7	S
Xoo 24	48.86	7	S
Xoo 25	30.49	7	S
Xoo 26	34.74	7	S
Xoo 27	39.61	7	S
Xoo 28	43.41	7	S
Xoo 29	43.47	7	S
Xoo 30	28.41	7	S
Xoo 31	39.12	7	S
Xoo 32	35.18	7	S
Xoo 33	42.12	7	S
Xoo 34	40.02	7	S
Xoo 35	24.18	5	MS
Xoo 36	43.96	7	S
Xoo 37	31.24	7	S
Xoo 38	25.52	7	S
Xoo 39	24.63	5	MS
Xoo 40	30.13	7	S
Xoo 41	37.29	7	S
Xoo 42	34.64	7	S
Xoo 43	40.49	7	S
Xoo 44	35.69	7	S

Xoo code	Disease severity(%)	Score	Reaction
Xoo 45	43.91	7	S
Xoo 46	15.15	5	MS
Xoo 47	44.46	7	S
Xoo 48	50.06	7	S
Xoo 49	60.93	9	HS
Xoo 50	40.54	7	S
Xoo 51	38.39	7	S
Xoo 52	60.07	9	HS
Xoo 53	47.66	7	S
Xoo 54	39.21	7	S
Xoo 55	55.09	9	HS
Xoo 56	41.59	7	S
Xoo 57	51.27	9	HS
Xoo 58	41.36	7	S
Xoo 59	28.66	7	S
Xoo 60	36.95	7	S
Xoo 61	42.97	7	S
Xoo 62	47.15	7	S
Xoo 63	57.81	9	HS
Xoo 64	32.82	7	S
Xoo 65	49.16	7	S
Xoo 66	34.10	7	S
Xoo 67	36.94	7	S
Xoo 68	57.55	9	HS
Xoo 69	39.38	7	S
Xoo 70	25.00	5	MS
Xoo 71	37.08	7	S
Xoo 72	21.05	5	MS
Xoo 73	45.84	7	S
Xoo 74	35.04	7	S
Xoo 75	45.80	7	S
Xoo 76	51.56	9	HS
Xoo 77	40.69	7	S
Xoo 78	43.11	7	S
Xoo 79	36.55	7	S
Xoo 80	46.87	7	S
Xoo 81	48.25	7	S
Xoo 82	43.67	7	S
Xoo 83	35.51	7	S
Xoo 84	30.94	7	S
Xoo 85	25.71	7	S
Xoo 86	23.44	5	MS
Xoo 87	30.94	7	S
Xoo 88	37.58	7	S

Plate 12.1. Njagattiri

Plate 12.2. Kodumunda

Plate 12.3. Kuthannur

Plate 12.4. Alathur

Plate 12. Bacterial blight infected fields in Palakkad district

Plate 13.1. Vettathur

Plate 13.2. Perumpadappa

Plate 13.3. Thazhekkode

Plate 13.4. Angadippuram

Plate 13. Bacterial blight infected fields in Malappuram district

Plate 14.1 Nadathara

Plate 14.2. Mundathikkode

Plate 14.3. Venkittangu

Plate 14.4. Kodakara

Plate 14. Bacterial blight infected field in Thrissur district

Plate 15.1. Mannar

Plate 15.2. Chambakkulam

Plate 15.3. Karuvatta

Plate 15.4. Thakazhi

Plate 15. Bacterial blight infected fields in Alappuzha district

Plate 16.1. Arpookkara

Plate 16.2. Thalayazham

Plate 16.3. Vechoor

Plate 16.4. Vaikam

Plate 16. Bacterial blight infected fields in Kottayam district

Plate 17. Yellow coloured colonies of *Xoo*

Xoo 8 Xoo 9 Xoo 12

Xoo 13 Xoo 20 Xoo 26

Xoo 28 Xoo 35 Xoo 50

Xoo 55 Xoo 56 Xoo 63

Plate 18. 1. Representative isolates of *Xoo* from different locations

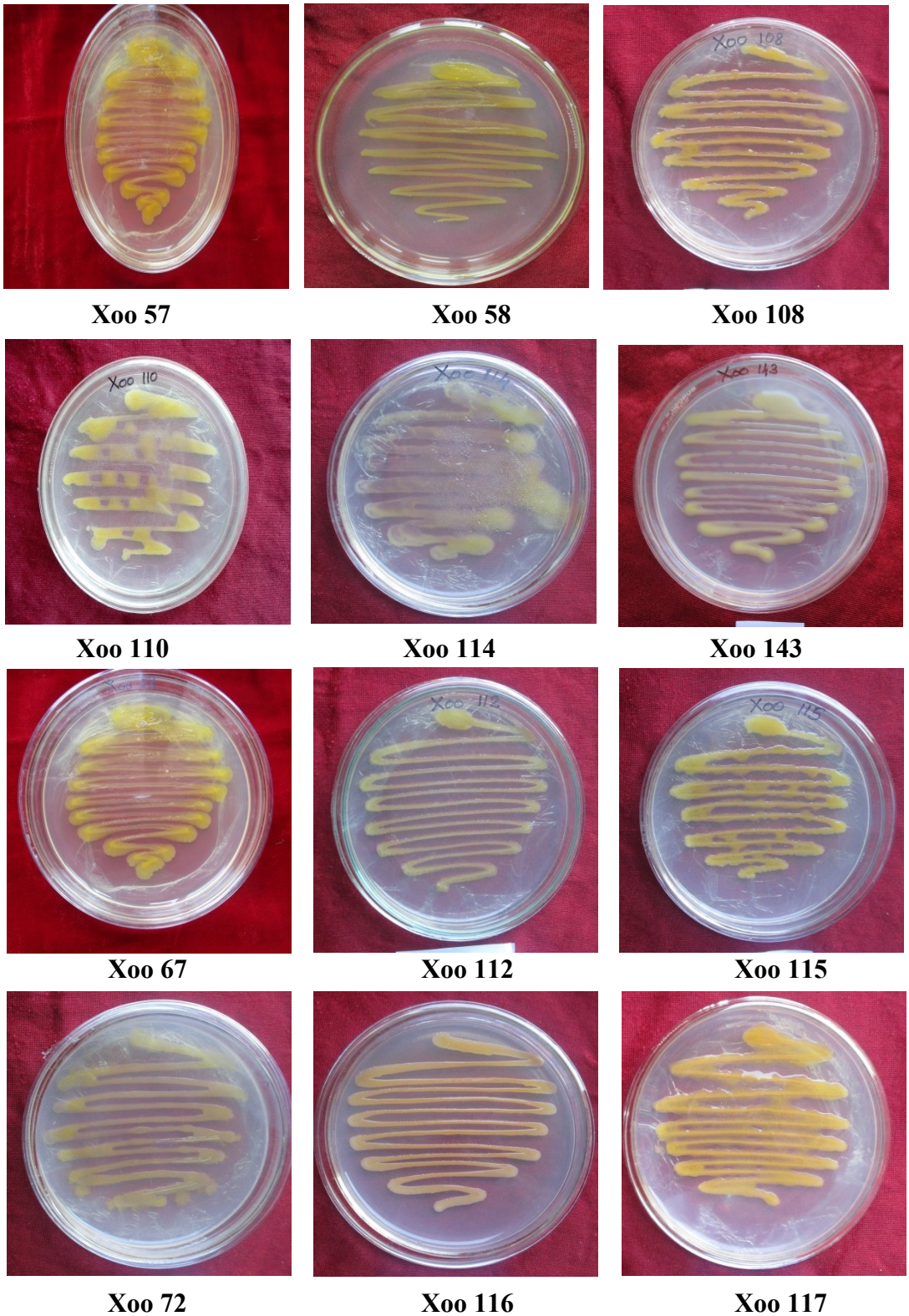

Plate 18. 2. Representative isolates of *Xoo* from different locations

Table 19. (Cont.) Disease severity on rice variety Jyothi under artificial inoculation of *Xoo* isolates

Xoo code	Disease severity(%)	Score	Reaction
Xoo 89	33.24	7	S
Xoo 90	32.41	7	S
Xoo 91	44.09	7	S
Xoo 92	32.57	7	S
Xoo 93	38.60	7	S
Xoo 94	55.47	9	HS
Xoo 95	31.21	7	S
Xoo 96	34.68	7	S
Xoo 97	36.07	7	S
Xoo 98	43.52	7	S
Xoo 99	27.44	7	S
Xoo 100	52.98	9	HS
Xoo 101	36.57	7	S
Xoo 102	24.32	5	S
Xoo 103	23.19	5	S
Xoo 104	24.10	5	S
Xoo 105	40.03	7	S
Xoo 106	27.03	7	S
Xoo 107	31.79	7	S
Xoo 108	44.93	7	S
Xoo 109	28.02	7	S
Xoo 110	32.56	7	S
Xoo 111	34.02	7	S
Xoo 112	37.14	7	S
Xoo 113	46.89	7	S
Xoo 114	33.70	7	S
Xoo 115	26.54	7	S
Xoo 116	26.37	7	S
Xoo 117	21.51	5	MS
Xoo 118	27.26	7	S
Xoo 119	41.06	7	S
Xoo 120	30.22	7	S
Xoo 121	17.96	5	MS
Xoo 122	27.17	7	S
Xoo 123	27.86	7	S
Xoo 124	27.48	7	S
Xoo 125	27.79	7	S
Xoo 126	23.60	5	MS
Xoo 127	33.01	7	S
Xoo 128	35.31	7	S

Xoo code	Disease severity (%)	Score	Reaction
Xoo 129	35.71	7	S
Xoo 130	42.53	7	S
Xoo 131	33.36	7	S
Xoo 132	45.95	7	S
Xoo 133	24.45	5	MS
Xoo 134	22.26	5	MS
Xoo 135	18.39	5	MS
Xoo 136	29.47	7	S
Xoo 137	26.27	7	S
Xoo 138	29.01	7	S
Xoo 139	44.74	7	S
Xoo 140	31.92	7	S
Xoo 141	20.61	7	S
Xoo 142	22.64	7	S
Xoo 143	32.79	7	S
Xoo 144	37.90	7	S
Xoo145	14.37	5	MS
Xoo 146	25.52	7	S
Xoo 147	22.05	5	MS
Xoo 148	29.05	7	S
Xoo 149	26.44	7	S
Xoo 150	21.76	5	MS
Xoo 151	21.84	5	MS
Xoo 152	33.21	7	S
Xoo 153	48.11	7	S
Xoo 154	26.64	7	S
Xoo 155	51.59	9	HS
Xoo 156	47.01	7	S
Xoo 157	31.14	7	S
Xoo 158	31.47	7	S
Xoo 159	29.13	7	S
Xoo 160	33.46	7	S
Xoo 161	31.51	7	S
Xoo 162	31.95	7	S
Xoo 163	33.11	7	S
Xoo 164	19.69	5	MS
Xoo 165	22.76	5	MS
Xoo 166	17.51	5	MS
Xoo 167	36.27	7	S
Xoo 168	17.95	5	MS

4.4 Morphological and biochemical characterization of *Xoo* isolates

4.4.1 Colony characters of *Xoo* isolates

The colony colours of *Xoo* isolates were light yellow, yellow, dark yellow and creamy yellow. *Xoo* colonies were raised and slimy or flattened slimy and were circular in shape (Table 20). All the isolates were purified and maintained in PSA slants and stored in refrigerator. For long term storage cultures were preserved in 15 per cent glycerol in sealed vials and stored at $^{-}20^{0}$C.

4.4.2 Biochemical characterization

The 100 *Xoo* isolates were subjected to 11 biochemical tests and the results are given here.

It was observed that all 100 *Xoo* isolates showed negative result for the indole production test. All the *Xoo* isolates gave negative result for VP test. MR test were positive for most of the isolates except Xoo 15, Xoo 24, Xoo 30, Xoo, 31, Xoo 42, Xoo 54, Xoo 77, Xoo 93 and Xoo 94. In MR test colour changed to red for positive result. VP test no colour change was observed. Most of the *Xoo* isolates showed positive result to starch hydrolysis but few isolates *viz.,* Xoo 7, Xoo 22, Xoo 28, Xoo 29, Xoo 53, Xoo 70 and Xoo 83 were negative. All the isolates were positive for tween80 hydrolysis by the formation of opaque zones around the bacterial colony. All the *Xoo* isolates were found negative for urease, oxidase and nitrate tests. *Xoo* isolates produced slimy thread when treated with 3 per cent KOH which indicating that positive result. The catalase test showed positive reaction by formation of bubbles when cultures were mixed with hydrogen peroxide. All the *Xoo* isolates produced acid from the glucose by the colour change to yellow (Table 21).

Plate 19.1.a. Plants before inoculation

Plate 19.1.b. Plants after inoculation

Plate 19.1. Pathogenicity studies of *Xoo* isolates on susceptible variety Jyothi

Xoo 129

Xoo 140

Xoo 138

Xoo 132

Plate 19.2. Pathogenicity studies of *Xoo* isolates on susceptible variety Jyothi

Xoo 9

Xoo 18

Xoo 79

Xoo 124

Plate 19.3 Pathogenicity studies of *Xoo* isolates on susceptible variety Jyothi

Table 20. Colony characters of *Xoo* isolates

Isolate	Colony colour	Shape	Appearance	Isolate	Colony colour	Shape	Appearance
Xoo 1	Light yellow	circular	Raised and slimy	Xoo 25	Light yellow	circular	Flattened and Slimy
Xoo 2	Yellow	circular	Raised and slimy	Xoo 26	Creamy yellow	circular	Raised and Slimy
Xoo 3	Yellow	circular	Raised and slimy	Xoo 27	Creamy yellow	circular	Raised and Slimy
Xoo 4	Light yellow	circular	Raised and slimy	Xoo 28	Light yellow	circular	Flattened and Slimy
Xoo 5	Yellow	circular	Raised and slimy	Xoo 29	Light yellow	circular	Flattened and Slimy
Xoo 6	Yellow	circular	Raised and slimy	Xoo 30	Yellow	circular	Raised and Slimy
Xoo 7	Yellow	circular	Raised and slimy	Xoo 31	Light yellow	circular	Raised and Slimy
Xoo 8	Yellow	circular	Raised and slimy	Xoo 32	Light yellow	circular	Flattened and Slimy
Xoo 9	Yellow	circular	Raised and slimy	Xoo 33	Creamy yellow	circular	Raised and Slimy
Xoo 10	Yellow	circular	Raised and slimy	Xoo 34	Yellow	circular	Raised and Slimy
Xoo 11	Yellow	circular	Raised and slimy	Xoo 35	Yellow	circular	Raised and Slimy
Xoo 12	Yellow	circular	Raised	Xoo 36	Dark yellow	circular	Raised and Slimy
Xoo 13	Dark yellow	circular	Raised and slimy	Xoo 37	Yellow	circular	Raised
Xoo 14	Dark yellow	circular	Raised and slimy	Xoo 38	Yellow	circular	Raised and Slimy
Xoo 15	Creamy yellow	circular	Raised andslimy	Xoo 39	Yellow	circular	Raised andslimy
Xoo 16	Yellow	circular	Raised and slimy	Xoo 40	Yellow	circular	Raised and Slimy
Xoo 17	Dark yellow	circular	Raised and slimy	Xoo 41	Yellow	circular	Raised and Slimy
Xoo 18	Yellow	circular	Raised and slimy	Xoo 42	Light yellow	circular	Raised and Slimy
Xoo 19	Yellow	circular	Raised and slimy	Xoo 43	Yellow	circular	Raised and Slimy
Xoo 20	Yellow	circular	Raised andslimy	Xoo 44	Yellow	circular	Raised andslimy
Xoo 21	Yellow	circular	Raised and slimy	Xoo 45	Yellow	circular	Raised and Slimy
Xoo 22	Dark yellow	circular	Raised and slimy	Xoo 46	Yellow	circular	Raised and Slimy
Xoo 23	Yellow	circular	Raised and slimy	Xoo 47	Yellow	circular	Raised and Slimy
Xoo 24	Light yellow	circular	Raised and slimy	Xoo 48	Yellow	circular	Raised and Slimy

Table 20. (Cont.) Colony characters of *Xoo* isolates

Isolate	Colony colour	Shape	Appearance	Isolate	Colony colour	Shape	Appearance
Xoo 49	Yellow	circular	Raised and slimy	Xoo 72	Light yellow	circular	Flattened and Slimy
Xoo 50	Creamy yellow	circular	Raised and slimy	Xoo 73	Yellow	circular	Raised and Slimy
Xoo 51	Creamy yellow	circular	Raised and slimy	Xoo 74	Yellow	circular	Raised and Slimy
Xoo 52	Yellow	circular	Raised and slimy	Xoo 75	Light yellow	circular	Raised and Slimy
Xoo 53	Yellow	circular	Raised and slimy	Xoo 76	Yellow	circular	Raised and Slimy
Xoo 54	Dark yellow	circular	Raised and slimy	Xoo 77	Yellow	circular	Raised and Slimy
Xoo 55	Yellow	circular	Flattened and slimy	Xoo 78	Yellow	circular	Raised and Slimy
Xoo 56	Creamy yellow	circular	Raised and slimy	Xoo 79	Creamy yellow	circular	Raised and Slimy
Xoo 57	Dark yellow	circular	Raised and slimy	Xoo 80	Yellow	circular	Raised and Slimy
Xoo 58	Dark yellow	circular	Raised andslimy	Xoo 81	Yellow	circular	Raised and slimy
Xoo 59	Yellow	circular	Raised and slimy	Xoo 82	Yellow	circular	Raised and Slimy
Xoo 60	Yellow	circular	Raised and slimy	Xoo 83	Yellow	circular	Raised and Slimy
Xoo 61	Yellow	circular	Raised and slimy	Xoo 84	Yellow	circular	Raised and Slimy
Xoo 62	Dark yellow	circular	Raised and slimy	Xoo 85	Yellow	circular	Raised and Slimy
Xoo 63	Yellow	circular	Raised and slimy	Xoo 86	Yellow	circular	Raised and Slimy
Xoo 64	Yellow	circular	Raised andslimy	Xoo 87	Light yellow	circular	Raised and slimy
Xoo 65	Dark yellow	circular	Flattened and slimy	Xoo 88	Dark yellow	circular	Raised and Slimy
Xoo 66	Yellow	circular	Raised and slimy	Xoo 89	Yellow	Circular	Raised and Slimy
Xoo 67	Yellow	circular	Raised and slimy	Xoo 90	Light yellow	Circular	Raised and Slimy
Xoo 68	Yellow	circular	Raised and slimy	Xoo 91	Yellow	Circular	Raised and Slimy
Xoo 69	Yellow	circular	Raised and slimy	Xoo 92	Yellow	Circular	Raised and Slimy
Xoo 70	Yellow	circular	Raised and slimy	Xoo 93	Creamy yellow	Circular	Raised and Slimy
Xoo 71	Light yellow	circular	Flattened and slimy	Xoo 94	Dark yellow	Circular	Raised and Slimy

Table 20. (Cont.) Colony characters of *Xoo* isolates

Isolate	Colony colour	Shape	Appearance	Isolate	Colony colour	Shape	Appearance
Xoo 95	Yellow	Circular	Raised and slimy	Xoo 118	Yellow	Circular	Raised and Slimy
Xoo 96	Yellow	Circular	Raised and slimy	Xoo 119	Yellow	Circular	Raised and Slimy
Xoo 97	Creamy yellow	Circular	Raised and slimy	Xoo 120	Yellow	Circular	Raised and Slimy
Xoo 98	Yellow	Circular	Raised and slimy	Xoo 121	Light yellow	Circular	Raised and Slimy
Xoo 99	Yellow	Circular	Raised and slimy	Xoo 122	Yellow	Circular	Raised and Slimy
Xoo 100	Light yellow	Circular	Flattened and slimy	Xoo 123	Yellow	Circular	Raised and Slimy
Xoo 101	Yellow	Circular	Raised and slimy	Xoo 124	Yellow	Circular	Raised and Slimy
Xoo 102	Yellow	Circular	Raised and slimy	Xoo 125	Yellow	Circular	Raised and Slimy
Xoo 103	Yellow	Circular	Raised and slimy	Xoo 126	Yellow	Circular	Raised and Slimy
Xoo 104	Dark yellow	Circular	Raised and slimy	Xoo 127	Yellow	Circular	Raised and slimy
Xoo 105	Light yellow	Circular	Raised and slimy	Xoo 128	Yellow	Circular	Raised and Slimy
Xoo 106	Yellow	Circular	Raised and slimy	Xoo 129	Light yellow	Circular	Raised and Slimy
Xoo 107	Dark yellow	Circular	Raised and slimy	Xoo 130	Light yellow	Circular	Raised and Slimy
Xoo 108	Yellow	Circular	Raised and slimy	Xoo 131	Yellow	Circular	Flattened and Slimy
Xoo 109	Light yellow	Circular	Flattened and slimy	Xoo 132	Yellow	Circular	Raised and Slimy
Xoo 110	Dark yellow	Circular	Flattened and slimy	Xoo 133	Yellow	Circular	Raised and slimy
Xoo 111	Yellow	Circular	Flattened and slimy	Xoo 134	Yellow	Circular	Raised and Slimy
Xoo 112	Light yellow	Circular	Flattened and slimy	Xoo 135	Dark yellow	Circular	Raised and Slimy
Xoo 113	Yellow	Circular	Raised and slimy	Xoo 136	Dark yellow	Circular	Raised and Slimy
Xoo 114	Light yellow	Circular	Raised and slimy	Xoo 137	Yellow	Circular	Raised and Slimy
Xoo 115	Yellow	Circular	Raised and slimy	Xoo 138	Yellow	Circular	Raised and Slimy
Xoo 116	Dark yellow	Circular	Raised and slimy	Xoo 139	Yellow	Circular	Raised and Slimy
Xoo 117	Yellow	Circular	Raised and slimy	Xoo 140	Yellow	Circular	Raised and Slimy

Table 20. (Cont.) Colony characters of *Xoo* isolates

Isolate	Colony colour	Shape	Appearance
Xoo 141	Light yellow	Circular	Raised and slimy
Xoo 142	Yellow	Circular	Raised and slimy
Xoo 143	Light yellow	Circular	Raised and slimy
Xoo 144	Dark yellow	Circular	Raised and slimy
Xoo 145	Yellow	Circular	Raised and slimy
Xoo 146	Yellow	Circular	Raised and slimy
Xoo 147	Dark yellow	Circular	Raised and slimy
Xoo 148	Yellow	Circular	Raised and slimy
Xoo 149	Yellow	Circular	Raised and slimy
Xoo 151	Yellow	Circular	Raised and slimy
Xoo 152	Yellow	Circular	Raised and slimy
Xoo 153	Yellow	Circular	Flattened and slimy
Xoo 154	Yellow	Circular	Raised and slimy
Xoo 155	Creamy yellow	Circular	Raised and slimy
Xoo 156	Yellow	Circular	Raised and slimy
Xoo 157	Yellow	Circular	Raised and slimy
Xoo 158	Yellow	Circular	Raised and slimy
Xoo 159	Creamy yellow	Circular	Raised and slimy
Xoo 160	Yellow	Circular	Raised and slimy
Xoo 161	Yellow	Circular	Raised and slimy
Xoo 162	Yellow	Circular	Raised and slimy
Xoo 163	Yellow	Circular	Raised and slimy
Xoo 164	Yellow	Circular	Raised and slimy
Xoo 165	Yellow	Circular	Raised and slimy
Xoo 166	Yellow	Circular	Raised and slimy
Xoo 167	Creamy yellow	Circular	Flattened and slimy
Xoo 168	Creamy yellow	Circular	Raised and slimy

Table 21. Biochemical characterization of *Xoo* isolates

Isolates	Indole production test	MR test	VP test	Starch hydrolysis test	Tween 80 hydrolysis test	Urease productiontest	KOH test	Catalasetest	Oxidase test	Nitrate reduction test	Carbohydrate utilization test
Xoo 1	-	+	-	+	+	-	+	+	-	-	+
Xoo 2	-	+	-	+	+	-	+	+	-	-	+
Xoo 3	-	+	-	+	+	-	+	+	-	-	+
Xoo 4	-	+	-	+	+	-	+	+	-	-	+
Xoo 5	-	+	-	+	+	-	+	+	-	-	+
Xoo 6	-	+	-	+	+	-	+	+	-	-	+
Xoo 7	-	+	-	-	+	-	+	+	-	-	+
Xoo 8	-	+	-	+	+	-	+	+	-	-	+
Xoo 9	-	+	-	+	+	-	+	+	-	-	+
Xoo 10	-	+	-	+	+	-	+	+	-	-	+
Xoo 11	-	+	-	+	+	-	+	+	-	-	+
Xoo 12	-	+	-	+	+	-	+	+	-	-	+
Xoo 13	-	+	-	+	+	-	+	+	-	-	+
Xoo 14	-	+	-	+	+	-	+	+	-	-	+
Xoo15	-	-	-	+	+	-	+	+	-	-	+
Xoo 16	-	+	-	+	+	-	+	+	-	-	+
Xoo 17	-	+	-	+	+	-	+	+	-	-	+
Xoo 18	-	+	-	+	+	-	+	+	-	-	+
Xoo 19	-	+	-	+	+	-	+	+	-	-	+
Xoo 20	-	+	-	+	+	-	+	+	-	-	+
Xoo 21	-	+	-	+	+	-	+	+	-	-	+
Xoo 22	-	+	-	-	+	-	+	+	-	-	+
Xoo 23	-	+	-	+	+	-	+	+	-	-	+
Xoo 24	-	-	-	+	+	-	+	+	-	-	+
Xoo 25	-	+	-	+	+	-	+	+	-	-	+
Xoo 26	-	+	-	+	+	-	+	+	-	-	+

Table 21. (Cont.) Biochemical characterization of *Xoo* isolates

Isolates	Indole production test	MR test	VP test	Starch hydrolysis test	Tween 80 hydrolysis test	Urease production test	KOH test	Catalase test	Oxidase test	Nitrate reduction test	Carbohydrate utilization test
Xoo 27	-	+	-	+	+	-	+	+	-	-	+
Xoo 28	-	+	-	-	+	-	+	+	-	-	+
Xoo 29	-	+	-	-	+	-	+	+	-	-	+
Xoo 30	-	-	-	+	+	-	+	+	-	-	+
Xoo 31	-	-	-	+	+	-	+	+	-	-	+
Xoo 32	-	+	-	+	+	-	+	+	-	-	+
Xoo 33	-	+	-	+	+	-	+	+	-	-	+
Xoo 34	-	+	-	+	+	-	+	+	-	-	+
Xoo 35	-	+	-	+	+	-	+	+	-	-	+
Xoo 36	-	+	-	+	+	-	+	+	-	-	+
Xoo 37	-	+	-	+	+	-	+	+	-	-	+
Xoo 38	-	+	-	+	+	-	+	+	-	-	+
Xoo 39	-	+	-	+	+	-	+	+	-	-	+
Xoo 40	-	+	-	+	+	-	+	+	-	-	+
Xoo 41	-	+	-	+	+	-	+	+	-	-	+
Xoo 42	-	-	-	+	+	-	+	+	-	-	+
Xoo 43	-	+	-	+	+	-	+	+	-	-	+
Xoo 44	-	+	-	+	+	-	+	+	-	-	+
Xoo 45	-	+	-	+	+	-	+	+	-	-	+
Xoo 46	-	+	-	+	+	-	+	+	-	-	+
Xoo 47	-	+	-	+	+	-	+	+	-	-	+
Xoo 48	-	+	-	+	+	-	+	+	-	-	+
Xoo 49	-	+	-	+	+	-	+	+	-	-	+
Xoo 50	-	+	-	+	+	-	+	+	-	-	+
Xoo 51	-	+	-	+	+	-	+	+	-	-	+
Xoo 52	-	+	-	+	+	-	+	+	-	-	+

Table 21. (Cont.) Biochemical characterization of *Xoo* isolates

Isolates	Indole production test	MR test	VP test	Starch hydrolysis test	Tween 80 hydrolysis test	Urease production test	KOH test	Catalase test	Oxidase test	Nitrate reduction test	Carbohydrate utilization test
Xoo 53	-	+	-	-	+	-	+	+	-	-	+
Xoo 54	-	-	-	+	+	-	+	+	-	-	+
Xoo 55	-	+	-	+	+	-	+	+	-	-	+
Xoo 56	-	+	-	+	+	-	+	+	-	-	+
Xoo 57	-	+	-	+	+	-	+	+	-	-	+
Xoo 58	-	+	-	+	+	-	+	+	-	-	+
Xoo 59	-	+	-	+	+	-	+	+	-	-	+
Xoo 60	-	+	-	+	+	-	+	+	-	-	+
Xoo 61	-	+	-	+	+	-	+	+	-	-	+
Xoo 62	-	+	-	+	+	-	+	+	-	-	+
Xoo 63	-	+	-	+	+	-	+	+	-	-	+
Xoo 64	-	+	-	+	+	-	+	+	-	-	+
Xoo 65	-	+	-	+	+	-	+	+	-	-	+
Xoo 66	-	+	-	+	+	-	+	+	-	-	+
Xoo 67	-	+	-	+	+	-	+	+	-	-	+
Xoo 68	-	+	-	+	+	-	+	+	-	-	+
Xoo 69	-	+	-	+	+	-	+	+	-	-	+
Xoo 70	-	+	-	-	+	-	+	+	-	-	+
Xoo 71	-	+	-	+	+	-	+	+	-	-	+
Xoo 72	-	+	-	+	+	-	+	+	-	-	+
Xoo 73	-	+	-	+	+	-	+	+	-	-	+
Xoo 74	-	+	-	+	+	-	+	+	-	-	+
Xoo 75	-	+	-	+	+	-	+	+	-	-	+
Xoo 76	-	+	-	+	+	-	+	+	-	-	+
Xoo 77	-	-	-	+	+	-	+	+	-	-	+
Xoo 78	-	+	-	+	+	-	+	+	-	-	+

Table 21. (Cont.) Biochemical characterization of *Xoo* isolates

Isolates	Indole production test	MR test	VP test	Starch hydrolysis test	Tween 80 hydrolysis test	Urease production test	KOH test	Catalase test	Oxidase test	Nitrate reduction test	Carbohydrate utilization test
Xoo 79	-	+	-	+	+	-	+	+	-	-	+
Xoo 80	-	+	-	+	+	-	+	+	-	-	+
Xoo 81	-	+	-	+	+	-	+	+	-	-	+
Xoo 82	-	+	-	+	+	-	+	+	-	-	+
Xoo 83	-	+	-	-	+	-	+	+	-	-	+
Xoo 84	-	+	-	+	+	-	+	+	-	-	+
Xoo 85	-	+	-	+	+	-	+	+	-	-	+
Xoo 86	-	+	-	+	+	-	+	+	-	-	+
Xoo 87	-	+	-	+	+	-	+	+	-	-	+
Xoo 88	-	+	-	+	+	-	+	+	-	-	+
Xoo 89	-	+	-	+	+	-	+	+	-	-	+
Xoo 90	-	+	-	+	+	-	+	+	-	-	+
Xoo 91	-	+	-	+	+	-	+	+	-	-	+
Xoo 92	-	+	-	+	+	-	+	+	-	-	+
Xoo 93	-	-	-	+	+	-	+	+	-	-	+
Xoo 94	-	-	-	+	+	-	+	+	-	-	+
Xoo 95	-	+	-	+	+	-	+	+	-	-	+
Xoo 96	-	+	-	+	+	-	+	+	-	-	+
Xoo 97	-	+	-	+	+	-	+	+	-	-	+
Xoo 98	-	+	-	+	+	-	+	+	-	-	+
Xoo 99	-	+	-	+	+	-	+	+	-	-	+
Xoo 100	-	+	-	+	+	-	+	+	-	-	+
Xoo 101	-	+	-	+	+	-	+	+	-	-	+
Xoo 102	-	+	-	+	+	-	+	+	-	-	+
Xoo 103	-	+	-	+	+	-	+	+	-	-	+
Xoo 104	-	+	-	+	+	-	+	+	-	-	+

Table 21. (Cont.) Biochemical characterization of *Xoo* isolates

Isolates	Indole production test	MR test	VP test	Starch hydrolysis test	Tween 80 hydrolysis test	Urease production test	KOH test	Catalase test	Oxidase test	Nitrate reduction test	Carbohydrate utilization test
Xoo 105	-	+	-	+	+	-	+	+	-	-	+
Xoo 106	-	+	-	+	+	-	+	+	-	-	+
Xoo 107	-	+	-	+	+	-	+	+	-	-	+
Xoo 108	-	+	-	+	+	-	+	+	-	-	+
Xoo 109	-	+	-	+	+	-	+	+	-	-	+
Xoo 110	-	+	-	+	+	-	+	+	-	-	+
Xoo 111	-	+	-	+	+	-	+	+	-	-	+
Xoo 112	-	+	-	+	+	-	+	+	-	-	+
Xoo 113	-	+	-	+	+	-	+	+	-	-	+
Xoo 114	-	+	-	+	+	-	+	+	-	-	+
Xoo 115	-	+	-	+	+	-	+	+	-	-	+
Xoo 116	-	+	-	+	+	-	+	+	-	-	+
Xoo 117	-	+	-	+	+	-	+	+	-	-	+
Xoo 118	-	+	-	+	+	-	+	+	-	-	+
Xoo 119	-	+	-	+	+	-	+	+	-	-	+
Xoo 120	-	+	-	+	+	-	+	+	-	-	+
Xoo 121	-	+	-	+	+	-	+	+	-	-	+
Xoo 122	-	+	-	+	+	-	+	+	-	-	+
Xoo 123	-	+	-	+	+	-	+	+	-	-	+
Xoo 124	-	+	-	+	+	-	+	+	-	-	+
Xoo 125	-	+	-	+	+	-	+	+	-	-	+
Xoo 126	-	+	-	+	+	-	+	+	-	-	+
Xoo 127	-	+	-	+	+	-	+	+	-	-	+
Xoo 128	-	+	-	+	+	-	+	+	-	-	+
Xoo 129	-	+	-	+	+	-	+	+	-	-	+
Xoo 130	-	+	-	+	+	-	+	+	-	-	+

Table 21. (Cont.) Biochemical characterization of *Xoo* isolates

Isolates	Indole production test	MR test	VP test	Starch hydrolysis test	Tween 80 hydrolysis test	Urease production test	KOH test	Catalase test	Oxidase test	Nitrate reduction test	Carbohydrate utilization test
Xoo 131	-	+	-	+	+	-	+	+	-	-	+
Xoo 132	-	+	-	+	+	-	+	+	-	-	+
Xoo 133	-	+	-	+	+	-	+	+	-	-	+
Xoo 134	-	+	-	+	+	-	+	+	-	-	+
Xoo 135	-	+	-	+	+	-	+	+	-	-	+
Xoo 136	-	+	-	+	+	-	+	+	-	-	+
Xoo 137	-	+	-	+	+	-	+	+	-	-	+
Xoo 138	-	+	-	+	+	-	+	+	-	-	+
Xoo 139	-	+	-	+	+	-	+	+	-	-	+
Xoo 140	-	+	-	+	+	-	+	+	-	-	+
Xoo 141	-	+	-	+	+	-	+	+	-	-	+
Xoo 142	-	+	-	+	+	-	+	+	-	-	+
Xoo 143	-	+	-	+	+	-	+	+	-	-	+
Xoo 144	-	+	-	+	+	-	+	+	-	-	+
Xoo 145	-	+	-	+	+	-	+	+	-	-	+
Xoo 146	-	+	-	+	+	-	+	+	-	-	+
Xoo 147	-	+	-	+	+	-	+	+	-	-	+
Xoo 148	-	+	-	+	+	-	+	+	-	-	+
Xoo 149	-	+	-	+	+	-	+	+	-	-	+
Xoo 150	-	+	-	+	+	-	+	+	-	-	+
Xoo 151	-	+	-	+	+	-	+	+	-	-	+
Xoo 152	-	+	-	+	+	-	+	+	-	-	+
Xoo 153	-	+	-	+	+	-	+	+	-	-	+
Xoo 154	-	+	-	+	+	-	+	+	-	-	+
Xoo 155	-	+	-	+	+	-	+	+	-	-	+
Xoo 156	-	+	-	+	+	-	+	+	-	-	+

Table 21. (Cont.) Biochemical characterization of *Xoo* isolates

Isolates	Indole production test	MR test	VP test	Starch hydrolysis test	Tween 80 hydrolysis test	Urease production test	KOH test	Catalase test	Oxidase test	Nitrate reduction test	Carbohydrate utilization test
Xoo 157	-	+	-	+	+	-	+	+	-	-	+
Xoo 158	-	+	-	+	+	-	+	+	-	-	+
Xoo 159	-	+	-	+	+	-	+	+	-	-	+
Xoo 160	-	+	-	+	+	-	+	+	-	-	+
Xoo 161	-	+	-	+	+	-	+	+	-	-	+
Xoo 162	-	+	-	+	+	-	+	+	-	-	+
Xoo 163	-	+	-	+	+	-	+	+	-	-	+
Xoo 164	-	+	-	+	+	-	+	+	-	-	+
Xoo 165	-	+	-	+	+	-	+	+	-	-	+
Xoo 166	-	+	-	+	+	-	+	+	-	-	+
Xoo 167	-	+	-	+	+	-	+	+	-	-	+
Xoo 168	-	+	-	+	+	-	+	+	-	-	+

4.4 Pathotyping of *Xoo* isolates on differentials/ NILs

4.4.1 Virulence analysis of *Xoo* isolates

The reaction of 100 *Xoo* isolates on differentials are given in table 22. Virulence spectrum of 100 isolates of *Xoo* was characterized based on these reactions. The reaction of *Xoo* isolates on NILs varied widely which showed that the effectiveness of the resistance genes varied with *Xoo* isolates. The effectiveness of resistance genes against the *Xoo* isolates was analysed by grouping moderately resistant and resistant reactions into one category as "effective" and moderately susceptible and susceptible reactions into another category as " non-effective". The *Xoo* isolates which showed moderately resistant reaction on susceptible variety Jyothi were not considered while analyzing the effectiveness of bacterial blight resistance genes. The effectiveness of different single bacterial blight resistance genes as well as their combinations on 94 *Xoo* isolates is presented in (Table 23). None of the single genes tested *viz., Xa1, Xa3, Xa4*, *xa5, Xa7, xa8, Xa10, Xa11, xa13, Xa14* and *Xa21* showed broad spectrum resistance widely effective against *Xoo* isolates tested. NILs carrying single genes were not effective against 38.30 to 92.55 per cent of the isolates. Differentials *viz.,* IRBB 1 (*Xa1*), IRBB 3 (*Xa3*), IRBB 10 (*Xa10*), IRBB 11 (*Xa11*) and IRBB 14 (*Xa14*) showed susceptibility/ moderate susceptibility to 92.55 per cent of the *Xoo* isolates. IRBB 7 (*Xa7*) showed susceptibility to 81.91 per cent of the *Xoo* isolates. IRBB 8 (*xa8*) showed susceptible / moderately susceptible reaction to 74.47 per cent of the *Xoo* isolates tested. IRBB 4 (*Xa4*) showed moderately susceptible or susceptible reaction to 57.45 per cent of the *Xoo* isolates tested. IRBB 5 carrying recessive gene *xa5* showed susceptibility or moderate susceptibility to 53.19 per cent of the *Xoo* isolates. IRBB 13 (*xa13*) showed susceptibility/ moderate susceptibility to 46.81 per cent of *Xoo* isolates. IRBB21 carrying *Xa21* gene was susceptible / moderately susceptible to 38.30 per cent of the *Xoo* isolates tested. Among the single genes tested *Xa21* was comparatively effective, followed by *xa13*.

Among the two gene combinations tested IRBB 50 carrying *Xa4* and *xa5* genes showed susceptibility to 47.87 per cent of the *Xoo* isolates. Only 13.83 per cent of *Xoo*

isolates were virulent on IRBB 51 carrying *Xa4+xa13* showing that it is moderately effective. IRBB 52 carrying *Xa4+Xa21* also moderately effective which showed susceptibility only to 14.89 per cent of *Xoo* isolates. IRBB 53 containing *xa5* and *xa13* genes was widely effective among the two gene combinations on which only 12.77 per cent of *Xoo* isolates were virulent. IRBB 54 with *xa5* and *Xa21* showed susceptible, moderately susceptible reaction to 20.21 per cent of *Xoo* isolates. IRBB 55 having two gene combinations of *xa13+Xa21* was susceptible to 14.89 per cent of *Xoo* isolates. Widely used *Xa21* gene was effective against 61.70 per cent of the *Xoo* isolates tested. *xa13* was effective against 53.19 per cent of the isolates. BB resistance genes *xa5* and *Xa4* were effective against 46.81 to 42.55 per cent of isolates tested respectively. BB resistance genes *Xa1*, *Xa3*, *Xa10*, *Xa11* and *Xa14* were non effective, *Xa7* and *xa8* were effective against 18.09 and 25.53 per cent of *Xoo* isolates respectively (Plate 20).

4.4.2 Grouping of *Xoo* isolates into different pathotypes

Based on the reaction on 31 rice differentials, the *Xoo* isolates were grouped into 15 pathotypes designated XoPt 1 to XoPt 15. According to the virulence, pathotypes were arranged starting from least virulent to highly virulent types (Table 24). The pathotypes XoPt 1 was least virulent which produced moderate resistance reaction on susceptible variety TN-1 as well as on Jyothi. This pathotype showed resistant reaction on all the NILs carrying single genes or combinations of resistance genes. XoPt 2 showed susceptible reaction on TN-1 as well as on Jyothi. In all other NILs it showed resistance reaction. All other pathotypes were virulent on single genes *Xa1*, *Xa3*, *Xa10*, *Xa11* and *Xa14.* Pathotype XoPt 3 was also less virulent. It exhibited moderately resistant reaction on IRBB 4 (*Xa4*) and IRBB 5 (*xa5*) and was virulent on *xa8,* showing susceptible reaction on IRBB 8. Pathotype 4 showed virulence reaction on *Xa4* but was avirulent on *xa8*. Pathotype 5 was virulent on *xa5* and moderately resistant reaction on *xa8* and *Xa4* which is the difference from XoPt 4. XoPt 6 and XoPt 7 were virulent on *Xa4*, *xa5* and *Xa7.* XoPt 7 was additionally virulent on *xa8* and showed slightly less resistance on *xa13* and *Xa21* (moderately resistant reaction as against resistant reaction in XoPt 6). Pathotype XoPt 8 showed virulence on *xa5* and *xa13* and showed moderate resistance reaction on *Xa4*.

Pathotype 9 showed moderately resistant reaction on *xa5* and moderately susceptible reaction on *xa13*. It differs from pathotype 8 on the reaction on *Xa4* which was susceptible to this pathotype and resistant on *xa8*. Pathotype 10 showed moderately susceptible / susceptible reaction on all single genes except *xa5* and *Xa21.* It showed moderate resistant reaction on *xa5* and resistant reaction on *Xa21*. Pathotype 9 showed resistance on *xa8*, however XoPt 10 was virulent on *xa8*. Pathotype 11 showed moderate resistance reaction on *xa5* and *xa13* and virulent on other single genes including *Xa21*. Pathotype 12 showed virulence on most of the single genes except *xa8* which showed moderate virulence. Pathotype 13 to 15 showed moderately susceptible to susceptible reaction to most of the single genes. In Pathotype 13, *xa5* showed moderate resistance and in XoPt 14, *xa8* showed moderate resistance. In both these *Xa21* was susceptible. In Pathotype 15 all single genes were susceptible.

Pathotype 12 showed susceptibility to all single genes except *Xa4* additionally it showed moderate susceptibility to *Xa4+xa5* combination (IRBB 50) also. This pathotype showed moderately resistant to moderately susceptible reaction to IRBB 54 containing *xa5+Xa21* and IRBB 55 (*xa13+Xa21*). IRBB 61 carrying three gene combination (*Xa4+xa5+Xa7*) showed moderately susceptible reaction. Improved Samba Mashuri carrying (*xa5+xa13+Xa21*) was moderately susceptible to this pathotype. Pathotype XoPt13 showed susceptibility to two gene combination *Xa4+xa5, Xa14+xa13, xa5+Xa21* and moderate resistance to moderate susceptibility to *xa13+Xa21.* This pathotype showed moderate resistance to *Xa4+Xa21* and *xa5+xa13*. Three gene combinations *Xa4+xa5+xa13*, *Xa4+xa13+Xa21*, *Xa4+Xa7+Xa21* were moderately susceptible. The four gene and five gene combinations were resistant to this pathotype. Improved Samba mashuri was also susceptible to Pathotype 13.

Pathotype 14 was virulent on all the single genes except *xa8* which showed moderately resistant reaction. It showed susceptibility to two gene combinations *Xa4+xa5* and moderately susceptible to *Xa4+Xa21*. This pathotype showed moderately resistant to moderately susceptible reaction to *xa5+xa13*, *xa5+Xa21* and *xa13+Xa21*. Among the three gene combinations, *Xa4+xa5+xa13*, *Xa4+xa13+Xa21* and*xa5+xa13+Xa21* showed

moderately resistant to moderately susceptible reaction. The four gene combination *Xa4+xa5+xa13+Xa21* was moderately resistant to this pathotype. Five gene combination *Xa4+xa5+Xa7+xa13+Xa21* also showed resistance reaction to this pathotype. Pathotype 15 showed susceptible reaction on all single genes and moderately susceptible reaction to all two gene combinations tested. It showed moderately susceptible reaction on all three gene combinations except *xa5+xa13+Xa21*. Four gene combination *Xa4+xa5+xa13+Xa21* and five gene combination *Xa4+xa5+Xa7+xa13+Xa21* showed resistance reaction. The *Xoo* isolates coming under each pathotype is given in table 25 and the pathotype 8 contain highest number of isolates(13). The isolates from different districts were grouped into same pathotype. Likewise isolates from same region grouped into different pathotypes. Indicating the wide spread distribution.

4.4.3 Frequency distribution of *Xoo* pathotypes in Kerala

Out of 100 *Xoo* isolates collected from five districts of Kerala *viz*., Palakkad, Malappuram, Thrissur, Alappuzha and Kottayam, 49 were from Palakkad, 25 from Thrissur, 12 from Malappuram and seven each from Kottayam and Alappuzha (Table 26). All the 15 pathotypes were found in Palakkad district. The results showed that predominant pathotype was XoPt 8 comprising of 13 isolates, followed by XoPt 4 and XoPt 11 having 10 isolates each. Pathotype XoPt 5 and XoPt 13 each had 4 isolates. Most virulent pathotype XoPt 14 and XoPt 15 comprised of seven isolates each and least frequent pathotype XoPt 10 consists of two isolates. Pathotype XoPt 1 comprising six per cent of isolates and were distributed over Palakkad and Thrissur, in which 66.66 per cent isolates were from Thrissur district. Pathotype XoPt 2 consists of seven per cent of isolates and was from Palakkad , Malappuram, Thrissur district. Eight isolates were included in XoPt 3 and from Palakkad, Thrissur and Alappuzha district. Second predominant pathotype XoPt 4 was found in Palakkad, Thrissur, Alappuzha districts which comprises 10 per cent of isolates. XoPt 5 which consists of four per cent isolates were present in Palakkad, Malappuram and Thrissur districts. Pathotype XoPt 6 and XoPt 10 was only present in Palakkad district which comprises eight per cent and two per cent of isolates respectively. XoPt 7 were present in Palakkad and Malappuram district in total

isolates belongs to this isolates were three. Most predominant pathotype XoPt 8, isolates were from Palakkad, Malappuram and Thrissur districts and 53.84 per cent of isolates from Palakkad district. XoPt 9 consists of three per cent of isolates which were collected from Palakkad and Thrissur districts. Pathotype XoPt 11 were present in all the distrcts except Kottayam comprises 10 per cent of isolates. Eight per cent of isolates comes under pathotype XoPt 12 and were from Palakkad, Thrissur, Malappuram and Kottayam districts. XoPt 14 comprises seven per cent of isolates and were present in Palakkad, Thrissur and Kottayam districts. Most virulent pathotype XoPt 15 were present in Palakkad, Malappuram and Thrissur districts which comprises seven per cent of isolates.

Table 22. Reaction of different *Xoo* isolates on differentials

Sl. No.	IRBB Line	Xoo 1	Xoo 2	Xoo 5	Xoo 6	Xoo 7	Xoo 8	Xoo 9	Xoo 10	Xoo 12	Xoo 13	Xoo 14	Xoo 15	Xoo 16	Xoo 17	Xoo 18	Xoo 20	Xoo 21	Xoo 22
1	IRBB 1(*Xa1*)	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R
2	IRBB 3(*Xa3*)	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R
3	IRBB 4(*Xa4*)	MR	S	MR	S	S	S	MS	S	S	S	S	S	MS	R	R	R	R	R
4	IRBB 5(*xa5*)	S	S	S	S	S	S	MR	MR	S	S	S	S	MR	R	R	R	R	R
5	IRBB 7(*Xa7*)	MS	S	MS	S	S	S	MS	S	S	S	S	S	MS	R	R	R	R	R
6	IRBB 8(*xa8*)	MR	MS	MR	S	MS	S	S	MS	S	MR	S	S	S	R	R	R	R	R
7	IRBB 10(*Xa10*)	S	MS	S	S	MS	S	S	S	S	S	S	S	S	R	R	R	R	R
8	IRBB 11(*Xa11*)	S	MS	S	S	MS	S	S	S	S	S	S	S	S	R	R	R	R	R
9	IRBB 13*(xa13*)	R	R	R	MR	R	S	S	MR	S	MS	MR	S	S	R	R	R	R	R
10	IRBB 14(*Xa14*)	MS	S	MS	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R
11	IRBB 21(*Xa21*)	R	R	R	MR	R	S	S	S	S	S	MR	S	S	R	R	R	R	R
12	IRBB 50(*Xa4+xa5*)	MR	MR	MR	MR	MR	S	S	MR	S	S	MR	S	S	R	R	R	R	R
13	IRBB 51(*Xa4+xa13*)	R	R	R	R	R	S	S	MR	S	R	MR	S	S	R	R	R	R	R
14	IRBB 52(*Xa4+Xa21*)	R	R	R	R	R	MS	MR	MR	MS	MS	R	MS	MR	R	R	R	R	R
15	IRBB 53(*xa5+xa13*)	R	R	R	R	R	MS	MR	R	MS	MS	MR	MS	MR	R	R	R	R	R
16	IRBB 54(*xa5+Xa21*)	R	MR	R	R	MS	MS	S	R	MS	MS	MR	MS	S	R	R	R	R	R
17	IRBB 55(*xa13+Xa21*)	R	R	R	R	R	MS	MS	R	MS	MS	R	MS	MR	R	R	R	R	R
18	IRBB 56(*Xa4+xa5+xa13*)	R	R	R	R	R	MS	MS	R	MS	MR	R	MS	MS	R	R	R	R	R
19	IRBB 57(*Xa4+xa5+Xa21*)	R	R	R	R	R	MS	MS	R	MS	MS	R	MS	MS	R	R	R	R	R
20	IRBB 58(*Xa4+xa13+Xa21*)	R	R	R	R	R	MS	MS	R	MS	MR	R	MS	MS	R	R	R	R	R
21	IRBB 59(*xa5+xa13+Xa21*)	R	R	R	R	R	MS	R	R	MS	MR	R	MS	R	R	R	R	R	R
22	IRBB 60(*Xa4+xa5+xa13+Xa21*)	R	R	R	R	R	R	MR	R	R	MR	R	R	MR	R	R	R	R	R
23	IRBB 61(*Xa4+xa5+Xa7*)	R	R	R	R	R	MS	MS	R	MS	MS	R	MS	MS	R	R	R	R	R
24	IRBB 62(*Xa4+Xa7+Xa21*)	R	R	R	R	R	MS	MS	R	MS	MS	R	MS	MS	R	R	R	R	R
25	IRBB 63(*xa5+Xa7+xa13*)	R	R	R	R	R	MS	MR	R	MS	MR	R	MS	MS	R	R	R	R	R
26	IRBB 64(*Xa4+xa5+Xa7+Xa21*)	R	R	R	R	R	MS	MR	R	MS	MR	R	MS	MR	R	R	R	R	R
27	IRBB 65(*Xa4+Xa7+xa13+Xa21*)	R	R	R	R	R	MR	R	R	MR	MS	R	MR	R	R	R	R	R	R
28	IRBB 66(*Xa4+xa5+Xa7+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
29	Improved Samba Mahsuri	R	R	R	R	R	S	S	R	S	S	R	S	S	R	R	R	R	R
30	TN-1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	MR	MR
31	Jyothi	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	MR	MR

Table 22. (Cont.) Reaction of different *Xoo* isolates on differentials

Sl. No.	IRBB Line	Xoo 23	Xoo 24	Xoo 25	Xoo 27	Xoo 28	Xoo 29	Xoo 31	Xoo 32	Xoo 33	Xoo 34	Xoo 36	Xoo 37	Xoo 38	Xoo 41	Xoo 43	Xoo 44	Xoo 45	Xoo 47
1	IRBB 1(*Xa1*)	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S
2	IRBB 3(*Xa3*)	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S
3	IRBB 4(*Xa4*)	R	R	R	R	R	R	S	S	MR	S	S	MR	S	S	MR	MR	S	S
4	IRBB 5(*xa5*)	R	R	R	R	R	R	R	MR	S	S	MR	S	MR	MR	S	S	MR	S
5	IRBB 7(*Xa7*)	R	R	R	R	R	R	MR	MR	MS	S	MR	MS	S	MS	MS	MS	MS	S
6	IRBB 8(*xa8*)	R	R	R	R	R	R	R	R	S	S	R	S	MS	R	S	S	R	MR
7	IRBB 10(*Xa10*)	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S
8	IRBB 11(*Xa11*)	R	R	R	R	R	R	MS	S	S	S	S	S	S	S	S	S	S	S
9	IRBB 13*(xa13*)	R	R	R	R	R	R	MR	MR	MS	S	MR	MS	MR	MS	MS	MS	MS	MS
10	IRBB 14(*Xa14*)	R	R	R	R	R	R	MS	S	MS	S	S	MS	S	S	MS	MS	S	S
11	IRBB 21(*Xa21*)	R	R	R	R	R	R	R	R	MR	S	R	MR	S	MR	MR	MR	MR	S
12	IRBB 50(*Xa4+xa5*)	R	R	R	R	R	R	MR	MR	MS	S	R	MS	MR	MR	MS	MS	MR	S
13	IRBB 51(*Xa4+xa13*)	R	R	R	R	R	R	R	R	R	S	R	MR	MR	MR	MR	MR	MR	R
14	IRBB 52(*Xa4+Xa21*)	R	R	R	R	R	R	R	R	R	MS	R	R	MR	MR	MR	MR	R	MS
15	IRBB 53(*xa5+xa13*)	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	R	MR
16	IRBB 54(*xa5+Xa21*)	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	R	MR
17	IRBB 55(*xa13+Xa21*)	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	R	MR
18	IRBB56(*Xa4+xa5+xa13*)	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	R	MR
19	IRBB 57(*Xa4+xa5+Xa21*)	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	R	MS
20	IRBB 58(*Xa4+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	R	MR
21	IRBB 59(*xa5+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	MR
22	IRBB 60(*Xa4+xa5+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MR
23	IRBB 61(*Xa4+xa5+Xa7*)	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	R	MS
24	IRBB 62(*Xa4+Xa7+Xa21*)	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	R	MS
25	IRBB 63(*xa5+Xa7+xa13*)	R	R	R	R	R	R	R	R	R	MS	R	R	R	R	R	R	R	MS
26	IRBB 64(*Xa4+xa5+Xa7+Xa21*)	R	R	R	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	MR
27	IRBB 65(*Xa4+Xa7+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	MS
28	IRBB 66(*Xa4+xa5+Xa7+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
29	Improved Samba Mahsuri	R	R	R	R	R	R	R	R	MR	S	R	MR	R	R	MS	MS	R	S
30	TN-1	S	MR	S	MR	MR	MR	S	S	S	S	S	S	S	S	S	S	S	S
31	Jyothi	S	MR	S	MR	MR	MR	S	S	S	S	S	S	S	S	S	S	S	S

Table 22. (Cont.) Reaction of different *Xoo* isolates on differentials

Sl. . No.	IRBB Line	Xoo 48	Xoo 49	Xoo 50	Xoo 53	Xoo 55	Xoo 56	Xoo 57	Xoo 58	Xoo 60	Xoo 61	Xoo 62	Xoo 63	Xoo 65	Xoo 67	Xoo 68	Xoo 69	Xoo 71	Xoo 72
1	IRBB 1(*Xa1*)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	IRBB 3(*Xa3*)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	IRBB 4(*Xa4*)	S	MR	MR	S	MR	MR	S	S	S	S	S	MS	S	S	S	MR	MR	MR
4	IRBB 5(*xa5*)	S	S	S	MR	S	S	S	MR	MR	MR	MR	MR	MR	MR	MR	MR	S	S
5	IRBB 7(*Xa7*)	S	MS	S	S	S	MS	S	S	S	S	S	MS	S	S	MR	S	MS	MS
6	IRBB 8(*xa8*)	MR	S	MS	MS	MS	S	S	MS	MS	MS	MS	S	MS	MS	R	S	S	S
7	IRBB 10(*Xa10*)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	MS	S	S
8	IRBB 11(*Xa11*)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	MS	S	S
9	IRBB 13*(xa13*)	MS	MS	MS	MR	MS	MS	S	MR	MR	MR	MR	S	MR	MR	R	MR	S	MS
10	IRBB 14(*Xa14*)	S	MS	S	S	S	MS	S	S	S	S	S	S	S	S	MS	MS	MS	MS
11	IRBB 21(*Xa21*)	S	MR	S	S	S	MR	S	S	S	S	S	S	S	S	R	R	R	R
12	IRBB 50(*Xa4+xa5*)	S	MS	MS	MR	MS	MS	S	MR	MR	MR	MR	S	MR	MR	R	R	MS	MS
13	IRBB 51(*Xa4+xa13*)	R	MR	R	MR	R	MR	S	MR	MR	MR	MR	S	MR	MR	R	R	MR	R
14	IRBB 52(*Xa4+Xa21*)	MS	MR	MR	MR	MR	R	MS	MR	MR	MR	MR	MR	MR	MR	R	R	MR	MR
15	IRBB 53(*xa5+xa13*)	MR	R	MS	R	MS	R	MS	R	R	R	R	MR	R	R	R	R	R	R
16	IRBB 54(*xa5+Xa21*)	MR	R	MR	R	MS	R	MS	R	R	R	R	S	R	R	R	R	R	R
17	IRBB 55(*xa13+Xa21*)	MR	R	MR	R	MR	R	MS	R	R	R	R	MR	R	R	R	R	R	R
18	IRBB 56(*Xa4+xa5+xa13*)	MR	R	MR	R	MR	R	MS	R	R	R	R	MS	R	R	R	R	R	R
19	IRBB 57(*Xa4+xa5+Xa21*)	MS	R	MR	R	MR	R	MS	R	R	R	R	MR	R	R	R	R	R	R
20	IRBB 58(*Xa4+xa13+Xa21*)	MR	R	MR	R	R	R	MS	R	R	R	R	MS	R	R	R	R	R	R
21	IRBB 59(*xa5+xa13+Xa21*)	MS	R	R	R	R	R	MS	R	R	R	R	R	R	R	R	R	R	R
22	IRBB 60(*Xa4+xa5+xa13+Xa21*)	MR	R	R	R	R	R	R	R	R	R	R	MR	R	R	R	R	R	R
23	IRBB 61(*Xa4+xa5+Xa7*)	MS	R	MS	R	MS	R	MS	R	R	R	R	MS	R	R	R	R	R	R
24	IRBB 62(*Xa4+Xa7+Xa21*)	MS	R	MR	R	R	R	MS	R	R	R	R	MS	R	R	R	R	R	R
25	IRBB 63(*xa5+Xa7+xa13*)	MS	R	R	R	R	R	MS	R	R	R	R	MS	R	R	R	R	R	R
26	IRBB 64(*Xa4+xa5+Xa7+Xa21*)	MR	R	R	R	R	R	MS	R	R	R	R	MR	R	R	R	R	R	R
27	IRBB 65(*Xa4+Xa7+xa13+Xa21*)	MS	R	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	R
28	IRBB 66(*Xa4+xa5+Xa7+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
29	Improved Samba Mahsuri	S	MS	MS	R	MS	MS	S	R	R	R	R	S	R	R	R	R	MS	MR
30	TN-1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
31	Jyothi	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 22. (Cont.) Reaction of different *Xoo* isolates on differentials

Sl. No.	IRBB Line	Xoo 73	Xoo 74	Xoo 75	Xoo 76	Xoo 77	Xoo 78	Xoo 79	Xoo 80	Xoo 81	Xoo 82	Xoo 83	Xoo 87	Xoo 88	Xoo 91	Xoo 93	Xoo 94	Xoo 98	Xoo 100
1	IRBB 1(*Xa1*)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	IRBB 3(*Xa3*)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	IRBB 4(*Xa4*)	S	S	S	S	MR	S	MR	MR	S	MR	MR	S	MR	S	MR	MR	MR	S
4	IRBB 5(*xa5*)	S	S	S	S	MR	MR	S	S	MR	S	S	S	S	MR	S	S	S	S
5	IRBB 7(*Xa7*)	S	S	S	S	S	MR	S	S	MR	MS	S	S	MS	S	MS	S	S	S
6	IRBB 8(*xa8*)	S	MR	MS	S	S	R	MS	S	R	S	S	MS	S	MS	MR	MS	S	MR
7	IRBB 10(*Xa10*)	S	S	MS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
8	IRBB 11(*Xa11*)	S	S	MS	S	S	MS	S	S	S	S	S	S	S	S	S	S	S	S
9	IRBB 13*(xa13*)	S	MS	R	MR	MR	MR	MS	MS	MR	MS	S	R	S	MR	R	MS	MS	MS
10	IRBB 14(*Xa14*)	S	S	S	S	S	MS	S	S	MS	MS	S	S	MS	S	MS	S	S	S
11	IRBB 21(*Xa21*)	S	S	R	MR	R	R	S	MR	R	R	MR	R	R	S	R	S	R	S
12	IRBB 50(*Xa4+xa5*)	S	S	MS	MR	R	R	MS	MS	R	MS	MS	MS	MS	MR	MR	MS	MS	S
13	IRBB 51(*Xa4+xa13*)	S	R	R	MR	R	R	R	R	R	R	R	R	R	MR	R	R	MR	R
14	IRBB 52(*Xa4+Xa21*)	MS	MS	R	MR	R	R	MR	MR	R	R	R	R	R	MR	R	MR	MR	MS
15	IRBB 53(*xa5+xa13*)	MS	MS	R	MR	R	R	MS	R	R	R	R	R	R	R	R	MS	R	MS
16	IRBB 54(*xa5+Xa21*)	MS	MR	MR	MR	R	R	MR	R	R	R	R	MR	R	R	R	MR	R	MR
17	IRBB 55(*xa13+Xa21*)	MS	MR	R	R	R	R	MR	R	R	R	R	R	R	R	R	MS	R	MR
18	IRBB 56(*Xa4+xa5+xa13*)	MS	MR	R	R	R	R	MR	R	R	R	R	R	R	R	R	MR	R	MR
19	IRBB 57(*Xa4+xa5+Xa21*)	MS	MS	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MS
20	IRBB 58(*Xa4+xa13+Xa21*)	MS	MR	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	MS
21	IRBB 59(*xa5+xa13+Xa21*)	MS	MS	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MR
22	IRBB 60(*Xa4+xa5+xa13+Xa21*)	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MR
23	IRBB 61(*Xa4+xa5+Xa7*)	MS	MS	R	R	R	R	MS	R	R	R	R	R	R	R	R	MS	R	MS
24	IRBB 62(*Xa4+Xa7+Xa21*)	MS	MS	R	R	R	R	R	R	R	R	R	R	R	R	R	MR	R	MS
25	IRBB 63(*xa5+Xa7+xa13*)	MS	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MR
26	IRBB 64(*Xa4+xa5+Xa7+Xa21*)	MS	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MR
27	IRBB 65(*Xa4+Xa7+xa13+Xa21*)	MR	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MR
28	IRBB 66(*Xa4+xa5+Xa7+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
29	Improved Samba Mahsuri	S	S	R	R	R	R	MS	MR	R	MR	MS	R	MR	R	R	MS	MS	S
30	TN-1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
31	Jyothi	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 22. (Cont.) Reaction of different *Xoo* isolates on differentials

Sl. No.	IRBB Line	Xoo 105	Xoo 107	Xoo 108	Xoo 111	Xoo 112	Xoo 113	Xoo 114	Xoo 119	Xoo 120	Xoo 124	Xoo 125	Xoo 127	Xoo 129	Xoo 130	Xoo 132	Xoo 139	Xoo 140	Xoo 143
1	IRBB 1(*Xa1*)	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
2	IRBB 3(*Xa3*)	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
3	IRBB 4(*Xa4*)	S	S	S	S	S	S	S	S	MS	R	MR	MS	S	MS	MS	S	S	S
4	IRBB 5(*xa5*)	S	S	S	MR	S	S	MR	R	S	R	S	S	S	S	S	MS	MS	MS
5	IRBB 7(*Xa7*)	S	S	S	MR	S	S	MR	MR	MS	R	MS	MS	S	MS	MS	S	S	S
6	IRBB 8(*xa8)*	MS	S	S	R	MS	MS	R	R	S	R	MR	S	MS	S	S	S	S	R
7	IRBB 10(*Xa10*)	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S
8	IRBB 11(*Xa11*)	S	S	S	MS	S	S	S	S	S	R	S	S	S	S	S	S	S	S
9	IRBB 13*(xa13*)	MS	MS	MS	R	MS	MS	R	MR	S	R	R	S	MS	S	S	S	S	MS
10	IRBB 14(*Xa14*)	S	S	S	MS	S	S	MS	MS	S	R	MS	S	S	S	S	S	S	S
11	IRBB 21(*Xa21*)	S	S	S	R	S	S	R	R	S	R	R	S	S	S	S	R	R	MR
12	IRBB 50(*Xa4+xa5*)	S	S	S	MR	MS	S	R	MR	S	R	MR	S	S	S	S	MR	MS	MS
13	IRBB 51(*Xa4+xa13*)	R	MR	MR	R	R	R	R	R	S	R	R	S	R	S	S	MR	MS	MS
14	IRBB 52(*Xa4+Xa21*)	MR	MS	MS	R	MR	MR	R	R	MR	R	R	MR	MR	MR	MR	R	R	R
15	IRBB 53(*xa5+xa13*)	MR	R	R	R	MR	MR	R	R	MR	R	R	MR	MR	MR	MR	R	R	R
16	IRBB 54(*xa5+Xa21*)	MS	MS	MS	R	MR	MS	R	R	S	R	R	S	MR	S	S	R	R	R
17	IRBB 55 (*xa13+Xa21*)	MS	MS	MR	R	MR	MS	R	R	MR	R	R	MR	MR	MS	MR	R	R	R
18	IRBB 56(*Xa4+xa5+xa13*)	MR	MS	MR	R	MR	MR	R	R	MS	R	R	MS	MR	MS	MS	R	R	R
19	IRBB 57(*Xa4+xa5+Xa21*)	MR	MS	MS	R	MR	MR	R	R	MR	R	R	MR	MR	MR	MR	R	R	R
20	IRBB 58 (*Xa4+xa13+Xa21*)	MR	MR	MR	R	MR	MR	R	R	MR	R	R	MR	MR	MR	MR	R	R	R
21	IRBB 59(*xa5+xa13+Xa21*)	R	MS	MS	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
22	IRBB 60(*Xa4+xa5+xa13+Xa21*)	R	MR	MR	R	R	R	R	R	MR	R	R	MR	R	MR	MR	R	R	R
23	IRBB 61(*Xa4+xa5+Xa7*)	MS	MS	MS	R	MS	MS	R	R	MS	R	R	MS	MS	MS	MS	R	R	R
24	IRBB 62 (*Xa4+Xa7+Xa21*)	MR	MS	MS	R	MR	MR	R	R	MS	R	R	MS	MR	MS	MS	R	R	R
25	IRBB 63(*xa5+Xa7+xa13*)	R	MS	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
26	IRBB 64(*Xa4+xa5+Xa7+Xa21*)	R	MR	MR	R	R	R	R	R	MR	R	R	MR	R	MR	MR	R	R	R
27	IRBB 65(*Xa4+Xa7+xa13+Xa21*)	R	MR	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
28	IRBB 66(*Xa4+xa5+Xa7+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
29	Improved Samba Mahsuri	MS	S	S	R	MS	MS	R	R	S	R	R	S	MS	S	S	R	R	R
30	TN-1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
31	Jyothi	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 22. (Cont.) Reaction of different *Xoo* isolates on differentials

Sl. No.	IRBB Line	Xoo 152	Xoo 153	Xoo 155	Xoo 156	Xoo 158	Xoo 160	Xoo 161	Xoo 162	Xoo 163	Xoo 167
1	IRBB 1(*Xa1*)	S	S	S	S	S	S	S	S	R	S
2	IRBB 3(*Xa3*)	S	S	S	S	S	S	S	S	R	S
3	IRBB 4(*Xa4*)	S	MR	MR	S	MR	MS	MR	MR	R	MR
4	IRBB 5(*xa5*)	MR	MR	MR	S	MR	MR	MR	MR	R	MR
5	IRBB 7(*Xa7*)	MR	MS	MS	S	MS	MS	MS	MS	R	MS
6	IRBB 8(*xa8*)	R	S	S	S	S	S	S	S	R	S
7	IRBB 10(*Xa10*)	S	MS	MS	S	MS	S	S	S	R	MS
8	IRBB 11(*Xa11*)	MS	MS	MS	S	MS	S	S	S	R	MS
9	IRBB 13*(xa13*)	MR	R	MR	S	MR	S	MR	R	R	R
10	IRBB 14(*Xa14*)	MS	MS	S	S	MS	S	MS	MS	R	MS
11	IRBB 21(*Xa21*)	R	R	R	S	R	S	R	R	R	R
12	IRBB 50(*Xa4+xa5*)	MR	R	R	S	R	S	R	R	R	R
13	IRBB 51(*Xa4+xa13*)	R	R	R	S	R	S	R	R	R	R
14	IRBB 52(*Xa4+Xa21*)	R	R	R	MS	R	MR	R	R	R	R
15	IRBB 53(*xa5+xa13*)	R	R	R	MS	R	MR	R	R	R	R
16	IRBB 54(*xa5+Xa21*)	R	R	R	MS	R	S	R	R	R	R
17	IRBB 55(*xa13+Xa21*)	R	R	R	MS	R	MS	R	R	R	R
18	IRBB 56 (*Xa4+xa5+xa13*)	R	R	R	MS	R	MS	R	R	R	R
19	IRBB 57(*Xa4+xa5+Xa21*)	R	R	R	MS	R	MS	R	R	R	R
20	IRBB 58(*Xa4+xa13+Xa21*)	R	R	R	MS	R	MS	R	R	R	R
21	IRBB 59(*xa5+xa13+Xa21*)	R	R	R	MR	R	R	R	R	R	R
22	IRBB 60(*Xa4+xa5+xa13+Xa21*)	R	R	R	R	R	MR	R	R	R	R
23	IRBB 61(*Xa4+xa5+Xa7*)	R	R	R	MS	R	MS	R	R	R	R
24	IRBB 62(*Xa4+Xa7+Xa21*)	R	R	R	MS	R	MS	R	R	R	R
25	IRBB 63(*xa5+Xa7+xa13*)	R	R	R	MS	R	MR	R	R	R	R
26	IRBB 64(*Xa4+xa5+Xa7+Xa21*)	R	R	R	MR	R	MR	R	R	R	R
27	IRBB 65(*Xa4+Xa7+xa13+Xa21*)	R	R	R	MR	R	R	R	R	R	R
28	IRBB 66(*Xa4+xa5+Xa7+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R
29	Improved Samba Mahsuri	R	R	R	S	R	S	R	R	R	R
30	TN-1	S	S	S	S	S	S	S	S	S	S
31	Jyothi	S	S	S	S	S	S	S	S	S	S

Table 23. Virulence of *Xoo* isolates on *Xa* gene/gene combinations

Sl. No.	IRBB Line	BB resistant gene or gene combinations	No of virulent isolates	Per cent
1	IRBB 1	*Xa1*	87	92.55
2	IRBB 3	*Xa3*	87	92.55
3	IRBB 4	*Xa4*	54	57.45
4	IRBB 5	*xa5*	50	53.19
5	IRBB 7	*Xa7*	77	81.91
6	IRBB 8	*xa8*	70	74.47
7	IRBB 10	*Xa10*	87	92.55
8	IRBB 11	*Xa11*	87	92.55
9	IRBB 13	*xa13*	44	46.81
10	IRBB 14	*Xa14*	87	92.55
11	IRBB 21	*Xa21*	36	38.30
12	IRBB 50	*Xa4+xa5*	45	47.87
13	IRBB 51	*Xa4+xa13*	13	13.83
14	IRBB 52	*Xa4+Xa21*	14	14.89
15	IRBB 53	*xa5+xa13*	12	12.77
16	IRBB 54	*xa5+Xa21*	19	20.21
17	IRBB 55	*xa13+Xa21*	14	14.89
18	IRBB 56	*Xa4+xa5+xa13*	12	12.77
19	IRBB 57	*Xa4+xa5+Xa21*	17	18.09
20	IRBB 58	*Xa4+xa13+Xa21*	12	12.77
21	IRBB 59	*xa5+xa13+Xa21*	10	10.64
22	IRBB 60	*Xa4+xa5+xa13+Xa21*	0	0.00
23	IRBB 61	*Xa4+xa5+Xa7*	26	27.66
24	IRBB 62	*Xa4+Xa7+Xa21*	18	19.15
25	IRBB 63	*xa5+Xa7+xa13*	12	12.77
26	IRBB 64	*Xa4+xa5+Xa7+Xa21*	7	7.45
27	IRBB 65	*Xa4+Xa7+xa13+Xa21*	3	3.19
28	IRBB 66	*Xa4+xa5+Xa7+xa13+Xa21*	0	0.00

Table 24. Grouping of *Xoo* isolates into different pathotypes based on their reaction on differentials

Sl. No.	IRBB Line	Pathotypes														
		Xopt 1	**Xopt 2**	**Xopt 3**	**Xopt 4**	**Xopt 5**	**Xopt 6**	**Xopt 7**	**Xopt 8**	**Xopt 9**	**Xopt 10**	**Xopt 11**	**Xopt 12**	**Xopt 13**	**Xopt 14**	**Xopt 15**
1	IRBB 1 (*Xa1*)	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S
2	IRBB 3(*Xa3*)	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S
3	IRBB 4(*Xa4*)	R	R	MR	S	MR	S	S	MR	S	S	S	MR	MS	S	S
4	IRBB5(*xa5*)	R	R	MR	R/MR	S	S	S	S	MR	MR	MR	S	MR	S	S
5	IRBB 7(*Xa7*)	R	R	MS/S	MR	MS	S	S	S	MS/S	S	S	S	MS	S	S
6	IRBB 8(*xa8*)	R	R	S	R	MR	MS	S	S	R	S	MS	MS	S	MR	S
7	IRBB 10(*Xa10*)	R	R	S/MS	S	S	S/MS	S	S	S	S	S	S	S	S	S
8	IRBB 11(*Xa11*)	R	R	S/MS	S/MS	S	S/MS	S	S	S	S	S	S	S	S	S
9	IRBB 13*(xa13*)	R	R	R/MR	R/MR	R	R	MR	MS/S	MS	S	MR	MS	S	MS	S
10	IRBB 14(*Xa14*)	R	R	S/MS	S/MS	MS	S	S	S	S	S	S	S	S	S	S
11	IRBB 21(*Xa21*)	R	R	R	R	R	R	MR	R/MR	MR	R	S	S	S	S	S
12	IRBB 50(*Xa4+xa5*)	R	R	R	R/MR	MR	MR/MS	MR	MS	MR/MS	MR/MS	MR	S/MS	S	S	S
13	IRBB 51(*Xa4+xa13*)	R	R	R	R	R	R	R/MR	R/MR	MR/MS	MR/MS	MR	R	S	R/MR	S
14	IRBB 52(*Xa4+Xa21*)	R	R	R	R	R	R	R/MR	R/MR	R/MR	R	MR	MR	MR	MS	MS
15	IRBB 53(*xa5+xa13*)	R	R	R	R	R	R	R/MR	R	R	R	R	MR	MR	MR/MS	MS
16	IRBB54 (*xa5+Xa21*)	R	R	R	R	R	MR/MS	R/MR	R	R	R	R	MR/MS	S	MR/MS	MS
17	IRBB 55 (*xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	MR/MS	MR/MS	MR/MS	MS
18	IRBB56 (*Xa4+xa5+xa13*)	R	R	R	R	R	R	R	R	R	R	R	MR	MS	MR/MS	MS
19	IRBB 57 (*Xa4+xa5+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R/MR	MR/MS	MS	MS
20	IRBB58 (*Xa4+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R/MR	MS	MR/MS	MS
21	IRBB 59 (*xa5+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	R	MR/MS	MR/MS
22	IRBB 60 (*Xa4+xa5+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	MR	MR	R
23	IRBB 61 (*Xa4+xa5+Xa7*)	R	R	R	R	R	R	R	R	R	R	R	MS	MS	MS	MS
24	IRBB 62 (*Xa4+Xa7+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R/MR	MS	MS	MS
25	IRBB 63 (*xa5+Xa7+xa13*)	R	R	R	R	R	R	R	R	R	R	R	R	MR/MS	MR/MS	MS
26	IRBB 64 (*Xa4+xa5+Xa7+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	MR	MR	MR/MS
27	IRBB 65 (*Xa4+Xa7+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	R	MR/MS	MR
28	IRBB 66 (*Xa4+xa5+Xa7+xa13+Xa21*)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
29	Improved Samba Mahsuri	R	R	R	R	R	R	R	MR/MS	R	R	R	MS	S	S	S
30	TN-1	MR	S	S	S	S	S	S	S	S	S	S	S	S	S	S
31	Jyothi	MR	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	No. of isolates	**6**	**7**	**8**	**10**	**4**	**8**	**3**	**13**	**3**	**2**	**10**	**8**	**4**	**7**	**7**

Table 25. Details of *Xoo* isolates in different pathotypes

Pathotype	*Xoo* isolates
XoPt 1	Xoo 21, Xoo 22, Xoo 24, Xoo 27, Xoo 28, Xoo 29
XoPt 2	Xoo 17, Xoo 18, Xoo 20, Xoo 23, Xoo 25, Xoo 124, Xoo 163
XoPt 3	Xoo 153, Xoo 69, Xoo 77, Xoo 155, Xoo 158, Xoo 161, Xoo 162, Xoo 167
XoPt 4	Xoo 31, Xoo 32, Xoo 36, Xoo 68, Xoo 78, Xoo 81, Xoo 111, Xoo 114, Xoo 119, Xoo 152
XoPt 5	Xoo 1, Xoo 5, Xoo 93, Xoo 125
XoPt 6	Xoo 2, Xoo 7, Xoo 75, Xoo 87, Xoo 120, Xoo 127, Xoo 130, Xoo 132
XoPt 7	Xoo 6, Xoo 14, Xoo 76
XoPt 8	Xoo 33, Xoo 37, Xoo 43, Xoo 44, Xoo 49, Xoo 56, Xoo 71, Xoo 72, Xoo 82, Xoo 88, Xoo 80, Xoo 83, Xoo 98
XoPt 9	Xoo 41, Xoo 45, Xoo 143
XoPt 10	Xoo 139, Xoo 140
XoPt 11	Xoo 10, Xoo 38, Xoo 53, Xoo 61, Xoo 91, Xoo 58, Xoo 60, Xoo 62, Xoo 65, Xoo 67
XoPt 12	Xoo 55, Xoo 79, Xoo 94, Xoo 129, Xoo 50, Xoo 112, Xoo 113, Xoo 105
XoPt 13	Xoo 9, Xoo 16, Xoo 63, Xoo 160
XoPt 14	Xoo 13, Xoo 74, Xoo 100, Xoo 47, Xoo 48, Xoo 107, Xoo 108
XoPt 15	Xoo 8, Xoo 12, Xoo 15, Xoo 34, Xoo 73, Xoo 57, Xoo 156

Table 26. District wise *Xoo* isolates in different pathotypes

Pathotype	No. of isolates					
	Palakkad	**Malappuram**	**Thrissur**	**Alappuzha**	**Kottayam**	**Total**
XoPt 1	2	0	4	0	0	6
XoPt 2	3	3	1	0	0	7
XoPt 3	4	0	3	1	0	8
XoPt 4	3	0	4	1	2	10
XoPt 5	2	1	1	0	0	4
XoPt 6	8	0	0	0	0	8
XoPt 7	2	1	0	0	0	3
XoPt 8	7	1	5	0	0	13
XoPt 9	1	0	2	0	0	3
XoPt 10	2	0	0	0	0	2
XoPt 11	4	1	1	4	0	10
XoPt 12	3	1	1	0	3	8
XoPt 13	2	1	0	1	0	4
XoPt 14	3	0	2	0	2	7
XoPt 15	3	2	2	0	0	7

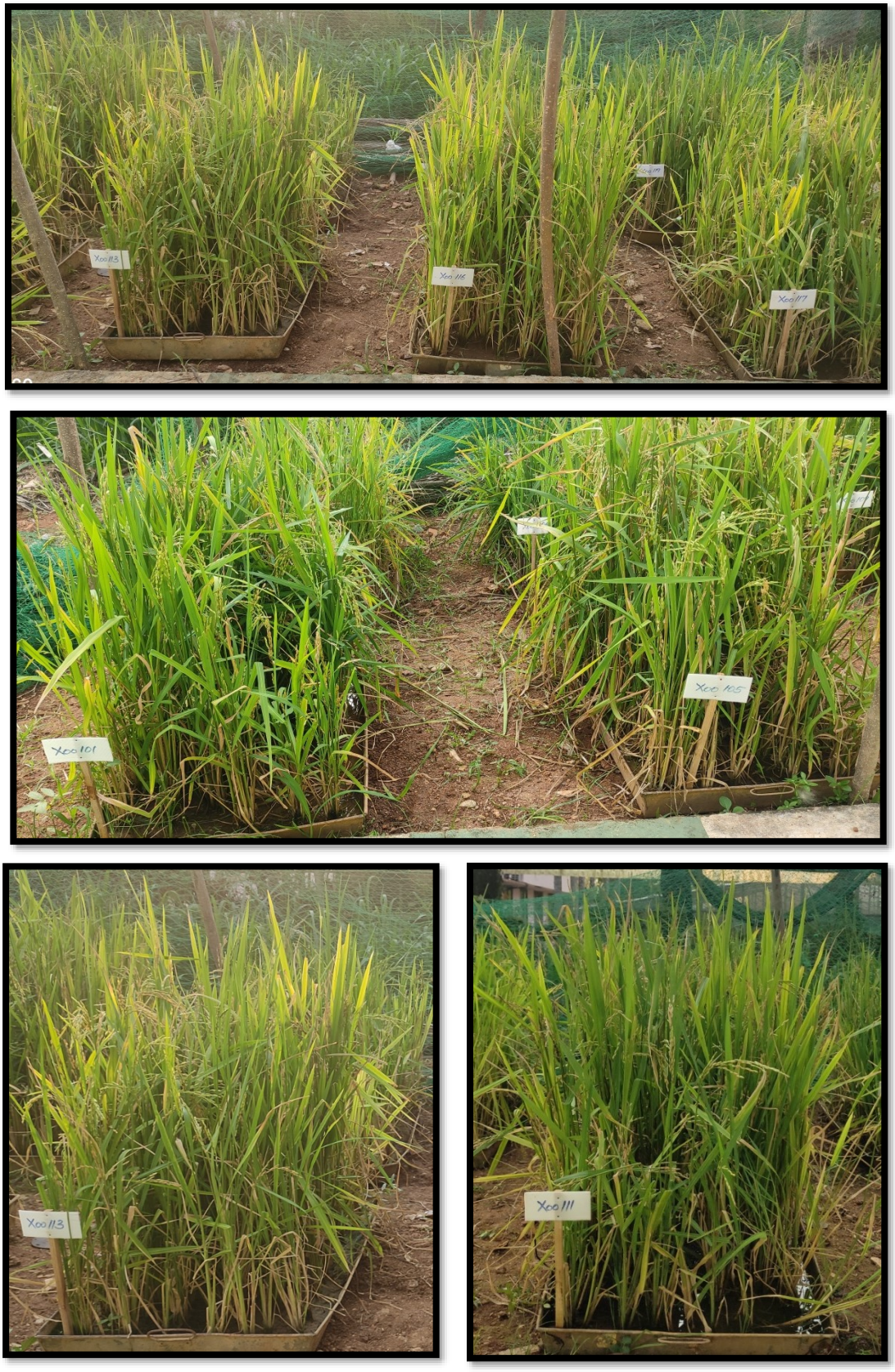

Plate 20. Reaction of *Xoo* isolates on differentials

4.6 Phenotypic screening of germplasm accessions for bacterial blight resistance

Fifty germplasm accessions along with susceptible check IR24 and local susceptible variety Jyothi were screened against three virulent isolates of bacterial blight pathogen *viz*., Xoo 13, Xoo 57 and Xoo 63 following artificial inoculation. The observations were taken 14 days after inoculation. The disease severity were recorded by measuring the lesion length and leaf length. The results are given in table 27. Allgenotypes showed the symptoms of bacterial blight upon artificial inoculation. The symptom initiated as water soaked lesion from the cut ends of the leaf blade extending downwards. Out of 50 accessions screened against Xoo13 isolates two were moderately resistant (score 3), 21 were moderately susceptible (score 5), 26 were susceptible (score 7) and one accession was highly susceptible (score 9). Among the 50 accessions screened against Xoo 57 none were resistant or moderately resistant. Four accessions were moderately susceptible 39 were susceptible and 7 were highly susceptible. Out of the 50 accessions six accessions showed moderate resistance, 12 were moderately susceptible and 27 accessions were susceptible and five were highly susceptible to the strain Xoo 63 (Plate 21-22).

Out of the 50 germplasm accessions, six accessions *viz*., Gandhasala (2), Kothambalarikayama (1), Mundon (Cheruli), Krishnakamod, Wayanad 2 and Ottadi were moderately resistant against the isolate Xoo63. Among these, two accessions Krishnakamod and Ottadi were moderately resistant to the isolate *Xoo*13 too. None of these accessions were resistant to Xoo 57. Ottadi showed moderately susceptible reaction and Krishnakamod was susceptible reaction to this isolate.

Table 27. Reaction of rice germplasm accessions to *Xanthomonas oryzae* pv. *oryzae* isolates

Sl. No.	Germplasm accessions	Disease score (0-9 scale)		
		Isolate		
		Xoo 13	Xoo 57	Xoo 63
1	Eruvakkali (2050)	7	7	5
2	Mandupakki (2053)	7	7	5
3	Mangalapuram (2071)	7	7	7
4	Chenkayama (Ambalapara) (2085)	5	7	5
5	Ponmani (2095)	7	7	7
6	Chettivirippu (2097)	7	9	7
7	Vellapokkali (2098)	7	9	7
8	Virippu (2105)	5	7	9
9	Bolamgittikayama (2112)	5	7	7
10	Vellakkayama (2113)	7	7	5
11	Mundakan (2117)	7	7	5
12	Anakkodan (2118)	9	7	5
13	Cheriya orpandy (2122)	7	9	7
14	Gandhasala (1) (2151)	5	7	7
15	Parambuvattan (2153)	7	7	7
16	Champan (2157)	5	5	7
17	Gandhasala (2) (2159)	5	7	3
18	Jeerakasala (2161)	5	7	5
19	Kokkankoli (2162)	5	9	5
20	Pandi Champan (2164)	7	7	9
21	Kalladiaryan (Red rice) (2168)	7	7	9
22	Kothambalarikayama (1) (2171)	5	7	3
23	Njavara (Black) (2201)	7	9	7
24	Mundon (Cheruli) (2219)	5	7	3
25	Veliyan (1) (2221)	7	9	7
26	Basmati (2228)	5	5	7
27	Krishnakamod (2229)	3	7	3
28	Karutha njavara (2230)	5	7	9
29	Kalluruli upland (2231)	5	7	7

Table 27. (Cont.) Reaction of rice germplasm accessions to *Xanthomonas oryzae* pv. o*ryzae* isolates

Sl. No.	Germplasm accessions	Disease score (0-9 scale)		
		Isolate		
		Xoo 13	Xoo 57	Xoo 63
30	Chitteni (Alathur) (3040)	7	7	7
31	Wayanad 2 (3049)	5	7	3
32	Black Chitteni (Thavanur) (3075)	7	7	7
33	Chembavu	7	7	7
34	Cheruvellari	5	9	7
35	Ithikandan	7	7	7
36	Kariyadukkan	7	7	7
37	Kokkan	5	7	7
38	Kothambalarikayama (2)	7	7	5
39	Kunnamkulamban	5	7	7
40	Kuruva	5	7	7
41	Mallimatta	5	7	7
42	Mannuveliyan	7	7	5
43	Marathondi	5	7	5
44	Mullankayama	7	7	7
45	Mundon	7	7	7
46	Odiyan	7	7	7
47	Ottadi	3	5	3
48	Thondi	7	7	7
49	Veliyan (2)	7	5	5
50	Vellari	5	7	7
51	Jyothi (Susceptible check)	9	9	9
52	IR24 (Susceptible check)	9	9	9

4.7 Genotypic screening of germplasm accessions for bacterial blight resistance

The genomic DNA of 50 germplasm were isolated by adapting CTAB method. The quality and quantity of genomic DNA were quantified using spectrophotometer by measuring A260/A280 (Table 28).

50 germplasm accessions were screened for the presence/absence of four bacterial blight resistance genes *Xa4, xa5, xa13* and *Xa21*. PCR amplification was carried out using PCR based SSR/STS markers synthesised by IDT USA *viz*., MP for *Xa4,* RM 390 and RM 122 for *xa5*, RM 230 and *xa13* prom for *xa13* and pTA 248 for *Xa21.* The resistant checks IRBB 4, IRBB 5, IRBB 13 and IRBB 21 and susceptible check IR24 were also included. PCR results were analysed by visualization of amplicons corresponding to each gene. The marker RM 390 for *xa5* gene did not give any polymorphism between resistant check IRBB 5 and susceptible check IR24. Similiarlythe marker RM 230 for *xa13* gene also did not give polymorphism between resistant and susceptible checks. Hence the results obtained using RM 122 for *xa5* gene and *xa13* promfor *xa13* gene were considered. The amplicon size corresponding to positive and negativecontrols were 150 bp and 120 bp for *Xa4*, 240 bp and 230 bp for *xa5*, 450 bp and 220 bp for *xa13* and 950 bp and 725 bpfor *Xa21* respectively. The results of genotypic screening of 50 germplasm accessions are presented in table 29 and electrophoretic patterns of molecular markers linked to bacterial blight resistance genes are shown in Figure 1-6.

The amplicons specific to *Xa21* and *xa13* alleles were not detected in these germplasm accessions indicating the absence of these genes in any of the germplasm accessions except in positive controls IRBB 21 and IRBB 13. Amplicons of size 150 bp corresponding to the marker MP linked to *Xa4* gene were detected in 25 accessions indicating the presence of this gene. In five accessions showed the resistant alleles in heterozygous pattern. In 17 accessions amplicon corresponding to the linked marker was absent indicating the absence of *Xa4* gene.

Plate 21.1 Germplasm accessions affected with bacterial blight

Ottadi

Krishnakamod

Plate 21. Germplasm accessions showing bacterial blight symptom

Karutha njavara

Eruvakkali

Bolamgittikayama

Pandi champan

Plate 22. Germplasm accessions showing moderately susceptible to susceptible reaction

The SSR marker RM 122 amplified 240 bp sized fragment in positive check and 230 bp sized fragment in negative control (IR24). Out of the 50 germplasm accessions 21 accessions were positive for the presence of 240 bp amplicon indicating the presence of *xa5* gene. In two accessions both resistant and susceptible alleles were present and 22 accessions showed the presence of susceptible allele.

10 accessions showed the presence of resistant alleles corresponding to *Xa4* and *xa5* genes. These include Mandupakki, Ponmani, Gandhasala (2), Kothambalarikayama 1 and Kothambalarikayama 2, Njavara (Black), Chembavu, Kunnamkulamban, Veliyan (2) and Vellari. Five accessions *viz*., Vellapokkali, Virippu, Bolamgittikayama, Kothambalarikayama (1), Mundon (Cheruli) carry either *Xa4* or *xa5* genes or both together in heterozygous condition. These germplasm accessions carrying single or combination of *Xa4* and *xa5* genes could be used as donors for these genes in breeding programme.

Table 28. Quality and quantity of DNA of germplasm accessions

Sl.No.	Germplasm accessions	DNA ng/µl	260/280
1	Eruvakkali (2050)	4825	1.98
2	Mandupakki (2053)	630	1.79
3	Mangalapuram (2071)	3330	2.02
4	Chenkayama (Ambalapara) (2085)	780	2.21
5	Ponmani (2095)	2650	1.98
6	Chettivirippu (2097)	880	1.91
7	Vellapokkali (2098)	1755	2.07
8	Virippu (2105)	4480	2.02
9	Bolamgittikayama (2112)	1280	2.07
10	Vellakkayama (2113)	2170	2.05
11	Mundakan (2117)	2855	1.94
12	Anakkodan (2118)	2160	2.01
13	Cheriya orpandy (2122)	1820	1.92
14	Gandhasala (1) (2151)	3985	2.02
15	Parambuvattan (2153)	1240	1.89
16	Champan (2157)	3830	2.09
17	Gandhasala (2) (2159)	1430	2.06
18	Jeerakasala (2161)	4905	2.02
19	Kokkankoli (2162)	1770	1.84
20	Pandi Champan (2164)	1840	2.01
21	Kalladiaryan (Red rice) (2168)	1900	2.11
22	Kothambalarikayama (1) (2171)	4720	2.01
23	Njavara (Black) (2201)	1140	1.83
24	Mundon (Cheruli) (2219)	1700	1.98
25	Veliyan (1) (2221)	3950	1.98
26	Basmati (2228)	330	1.85
27	Krishnakamod (2229)	6515	1.88
28	Karutha njavara (2230)	5235	1.90
29	Kalluruli upland (2231)	3395	1.97
30	Chitteni (Alathur) (3040)	600	1.84

Table 28. (Cont.) Quality and quantity of DNA of germplasm accessions

Sl. No.	Germplasm accessions	DNA ng/µl	260/280
31	Wayanad 2 (3049)	6590	1.90
32	Black Chitteni (Thavanur) (3075)	1155	1.87
33	Chembavu	705	1.74
34	Cheruvellari	1065	1.81
35	Ithikandan	935	1.63
36	Kariyadukkan	890	1.65
37	Kokkan	640	1.60
38	Kothambalarikayama (2)	1945	1.92
39	Kunnamkulamban	915	1.79
40	Kuruva	985	1.72
41	Mallimatta	870	1.63
42	Mannuveliyan	1145	1.91
43	Marathondi	720	1.62
44	Mullankayma	885	1.67
45	Mundon	815	1.65
46	Odiyan	160	1.78
47	Ottadi	1860	1.80
48	Thondi	800	1.89
49	Veliyan (2)	1770	1.81
50	Vellari	1475	1.78
51	IR24	1245	1.85
52	Jyothi	1540	1.92

Table 29. Status of bacterial blight resistance genes in germplasm accessions

Sl. No.	Germplasm accessions	*Xa4*	*xa5*	*xa13*	*Xa21*
1	Eruvakkali	-	-	-	-
2	Mandupakki	+	+	-	-
3	Mangalapuram	+	-	-	-
4	Chenkayama (Ambalapara)	+	-	-	-
5	Ponmani	+	+	-	-
6	Chettivirippu	-	+	-	-
7	Vellapokkali	+	+ -	-	-
8	Virippu	+ -	+ -	-	-
9	Bolamgittikayama	+ -	-	-	-
10	Vellakkayama	+	-	-	-
11	Mundakan	-	+	-	-
12	Anakkodan	-	+	-	-
13	Cheriya orpandy	-	+	-	-
14	Gandhasala (1)	-	+	-	-
15	Parambuvattan	+	-	-	-
16	Champan	-	+	-	-
17	Gandhasala (2)	+	+	-	-
18	Jeerakasala	-	+	-	-
19	Kokkankoli	+	-	-	-
20	Pandi Champan	-	-	-	-
21	Kalladiaryan (Red rice)	+	-	-	-
22	Kothambalarikayama (1)	+ -	+	-	-
23	Njavara (Black)	+	+	-	-
24	Mundon (Cheruli)	+ -	+	-	-
25	Veliyan (1)	-	-	-	-
26	Basmati	+ -	-	-	-
27	Krishnakamod	-	+	-	-
28	Karutha njavara	-	-	-	-
29	Kalluruli upland	+	-	-	-

Table 29. (Cont.) Status of bacterial blight resistance genes in germplasm accessions

Sl. No.	Germplasm accessions	*Xa4*	*xa5*	*xa13*	*Xa21*
30	Chitteni (Alathur)	-	-	-	-
31	Wayanad 2	-	-	-	-
32	Black Chitteni (Thavanur)	-	+	-	-
33	Chembavu	+	+	-	-
34	Cheruvellari	+	-	-	-
35	Ithikandan	+	-	-	-
36	Kariyadukkan	+	-	-	-
37	Kokkan	+	-	-	-
38	Kothambalarikayama (2)	+	+	-	-
39	Kunnamkulamban	+	+	-	-
40	Kuruva	+	-	-	-
41	Mallimatta	+	-	-	-
42	Mannuveliyan	+	-	-	-
43	Marathondi	+	-	-	-
44	Mullankayama	+	-	-	-
45	Mundon	+	-	-	-
46	Odiyan	-	-	-	-
47	Ottadi	-	-	-	-
48	Thondi	+	-	-	-
49	Veliyan (2)	+	+	-	-
50	Vellari	+	+	-	-
51	IR24	-	-	-	-

Figure 1. Agarose gel electrophoretic patterns of germplasm accessions generated by using SSR marker MP

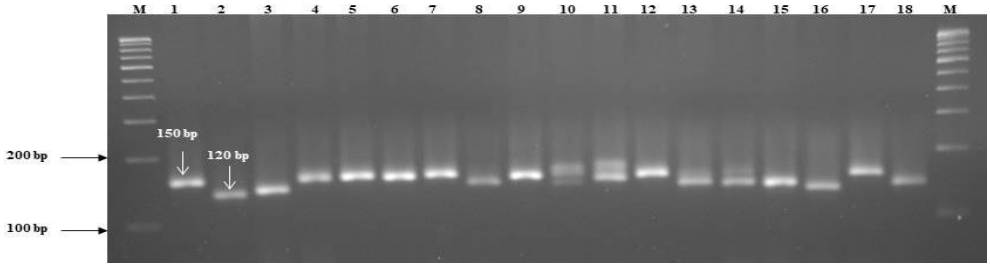

Fig. 1.1 Banding pattern generated using the marker MP for *Xa4* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 4, lane 2. IR24, lane 3 = Eruvakkali, lane 4 = Mandupakki, lane 5 = Mangalpuram, lane 6 = Chenkayama (Ambalapara), lane 7 = Ponmani, lane 8 = Chettivirippu, lane 9 = Vellapokkali, lane 10 = Virippu, lane 11 = Bolamgittikayama, lane 12 = Vellakkayama, lane 13 = Mundakan, lane 14 = Anakkodan, lane 15 = Cheriya orpandy, lane 16 = Gandhasala (1), lane 17 = Parambuvattan , lane 18 = Champan,, lane M = 100 bp DNA ladder

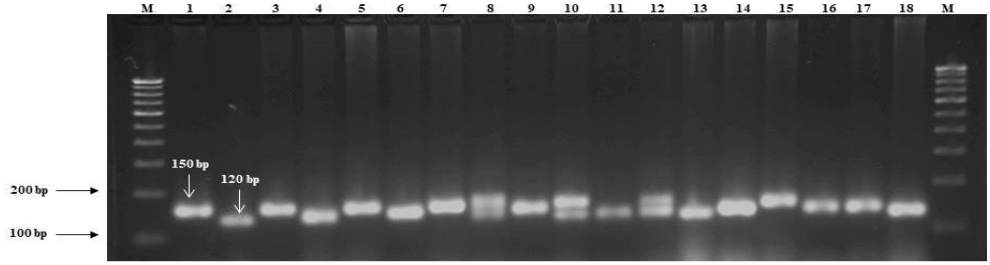

Fig. 1.2 Banding pattern generated using the marker MP for *Xa4* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 4, lane 2 = IR24, lane 3 = Gandhasala (2), lane 4 = Jeerakasala, lane 5 = Kokkankoli, lane 6 = Pandi Champan, lane 7 = Kalladiaryan (Red rice), lane 8 = Kothambalarikayama (1), lane 9 = Njavara (Black), lane 10 = Mundon (Cheruli), lane 11 = Veliyan (1), lane 12 = Basmati, lane 13 = Krishnakamod, lane 14 = Karutha njavara, lane 15 = Kalluruli upland, lane 16 = Chitteni (Alathur), lane 17 = Wayanad 2, lane 18 = Black Chitteni (Thavanur), lane M = 100 bp DNA ladder

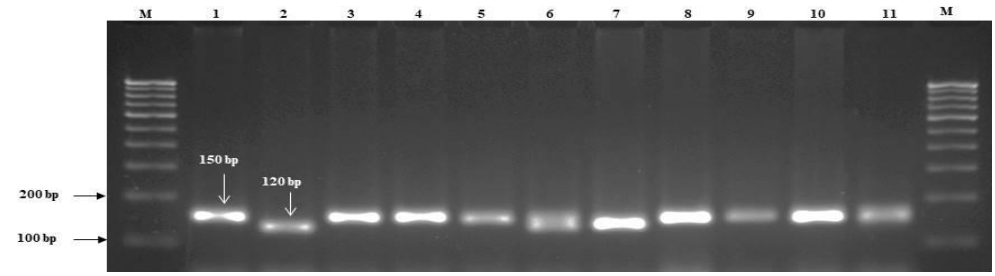

Fig. 1.3 Banding pattern generated using the marker MP for *Xa4* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 4, lane 2 = IR24, lane 3 = Chembavu, lane 4 = Cheruvellari, lane 5 = Ithikandan, lane 6 = Kariyadukkan, lane 7 = Kokkan, lane 8 = Kothambalarikayama (2), lane 9 = Kunnamkulamban, lane 10 = Kuruva, lane 11 = Mallimatta, lane M = 100 bp DNA ladder

Figure 1. Agarose gel electrophoretic patterns of germplasm accessions generated by using SSR marker MP

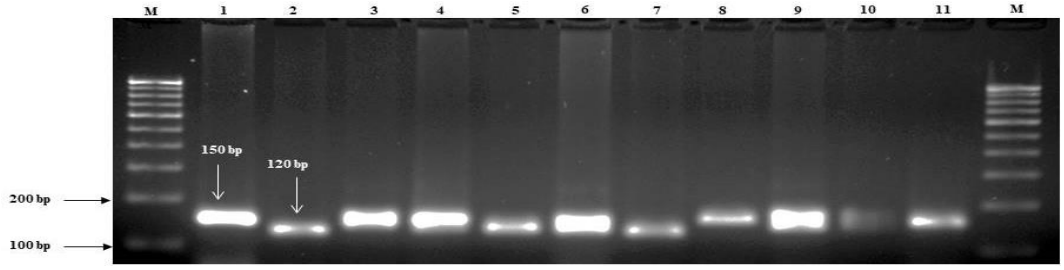

Fig. 1.4 Banding pattern generated using the marker MP for *Xa4* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 4, lane 2 = IR24, lane 3 = Mannuveliyan, lane 4 = Marathondi, lane 5 = Odiyan, lane 6 = Mullankayama, lane 7 = Ottadi lane 8 = Mundon, lane 9 = Thondi, lane 10 = Veliyan (2), lane 11 = Vellari, lane M = 100 bp DNA ladder

Figure 2. Agarose gel electrophoretic patterns of germplasm accessions generated by using SSR marker RM 390

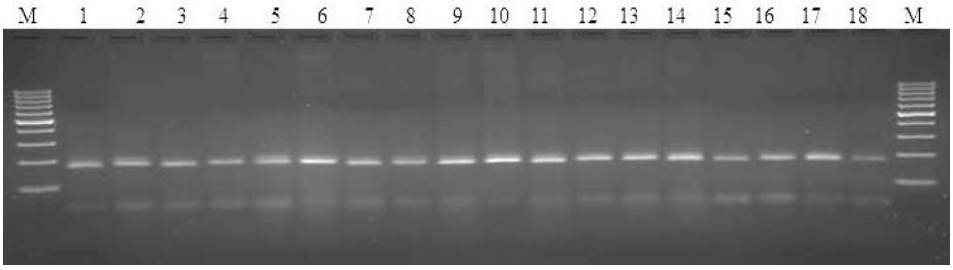

Fig. 2. 1 Banding pattern generated using the marker RM 390 for *xa5* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 5, lane 2. IR24, lane 3 = Eruvakkali, lane 4 = Mandupakki, lane 5 = Mangalpuram, lane 6 = Chenkayama (Ambalapara), lane 7 = Ponmani, lane 8 = Chettivirippu, lane 9 = Vellapokkali, lane 10 = Virippu, lane 11 = Bolamgittikayama, lane 12 = Vellakkayama, lane 13 = Mundakan, lane 14 = Anakkodan, lane 15 = Cheriya orpandy, lane 16 = Gandhasala (1), lane 17 = Parambuvattan , lane 18 = Champan, lane M = 100 bp DNA ladder

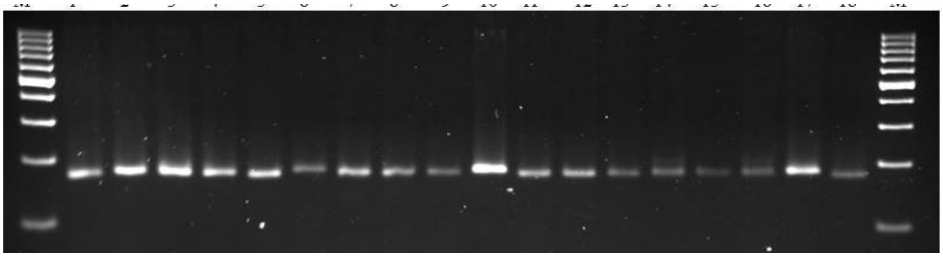

Fig. 2. 2 Banding pattern generated using the marker RM 390 for *xa5* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 5, lane 2 = IR24, lane 3 = Gandhasala (2), lane 4 = Jeerakasala, lane 5 = Kokkankoli, lane 6 = Pandi Champan, lane 7 = Kalladiaryan (Red rice), lane 8 = Kothambalarikayama (1), lane 9 = Njavara (Black), lane 10 = Mundon (Cheruli), lane 11 = Veliyan (1), lane 12 = Basmati, lane 13 = Krishnakamod, lane 14 = Karutha njavara, lane 15 = Kalluruli upland, lane 16 = Chitteni (Alathur), lane 17 = Wayanad 2, lane 18 = Black Chitteni (Thavanur), lane

Figure 2. Agarose gel electrophoretic patterns of germplasm accessions generated by using SSR marker RM 390

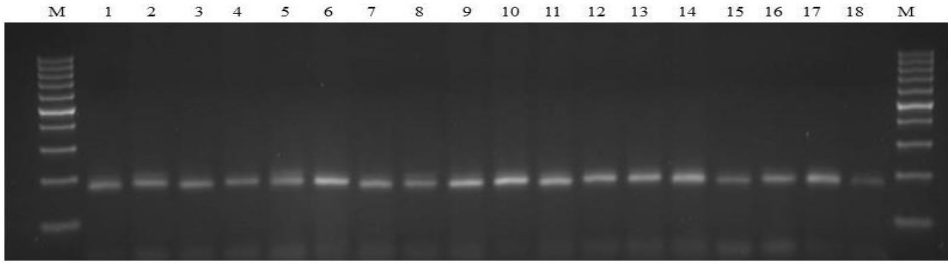

Fig. 2. 3 Banding pattern generated using the marker RM 390 for *xa5* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 5, lane 2 = IR24, lane 3 = Chembavu, lane 4 = Cheruvellari, lane 5 = Ithikandan, lane 6 = Kariyadukkan, lane 7 = Kokkan, lane 8 = Kothambalarikayama (2), lane 9 = Kunnamkulamban, lane 10 = Kuruva, lane 11 = Mallimatta, , lane 12 = Mannuveliyan, lane 13 = Marathondi, lane 14 = Odiyan, lane 15 = Mullankayama, lane 16 = Ottadi, lane 17 = Mundon, lane 18 = Thondi, lane M = 100 bp DNA ladder

Figure 3. Agarose electrophoretic patterns of germplasm accessions generated by using SSR marker RM 122

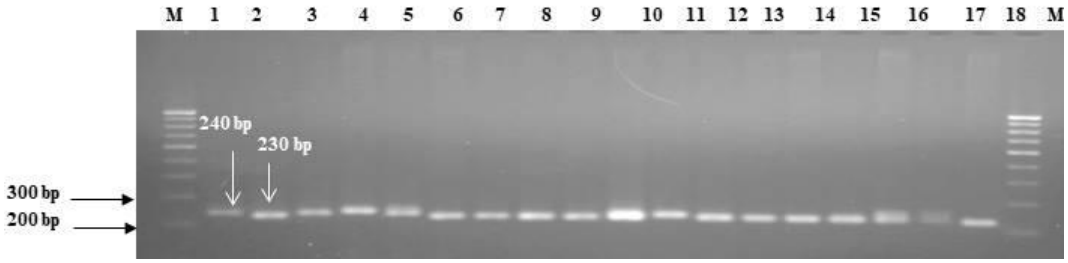

Fig. 3. 1 Banding pattern generated using the marker RM 122 for *xa5* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 5, lane 2 = IR24, lane 3 = Ponmani, lane 4 = Black Chitteni (Thavanur), lane 5 = Krishnakamod, lane 6 = Kokkankoli, lane 7 = Kalladiaryan (Red rice), lane 8 = Eruvakkali, lane 9 = Kalluruli upland, lane 10 = Mandupakki, lane 11 = Gandhasala (1), lane 12 = Parambuvattan, lane 13 = Vellakayama, lane 14 = Bolamgittikayama, lane 15 = Basmati, lane 16 = Mundon (Cheruli), lane 17 = Kothambalarikayama (1), lane 18 = Chitteni (Alathur), lane M = 100 bp DNA ladder

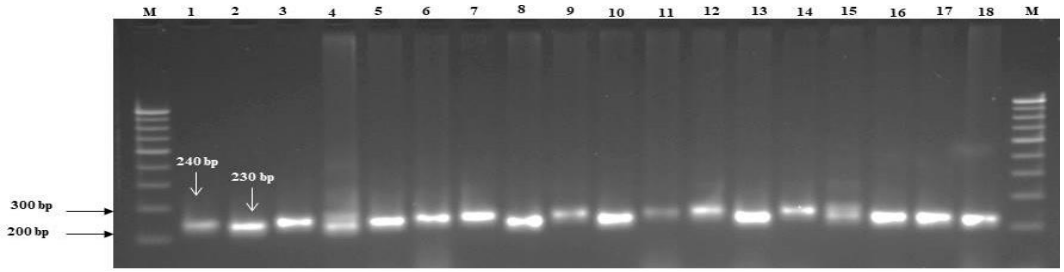

Fig. 3. 2 Banding pattern generated using the marker RM 122 for *xa5* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 5, lane 2 = IR24, lane 3 = Njavara (black), lane 4 = Virippu, lane 5 = Cheriya orpandy, lane 6 = Champan, lane 7 = Chettivirippu, lane 8 = Mangalapuram, lane 9 = Gandhasala (2), lane 10 = Pandi champan, lane 11 = Jeerakasala, lane 12 = Anakkodan, lane 13 = Wayanad 2, lane 14 = Mundakan, lane 15 = Vellapokkali, lane 16 = Veliyan (1), lane 17 = Chenkayama (Ambalapara), lane 18 = Karutha njavara, lane M = 100 bp DNA ladder

Figure 3. Agarose gel electrophoretic patterns of germplasm accessions generated by using SSR marker RM 122

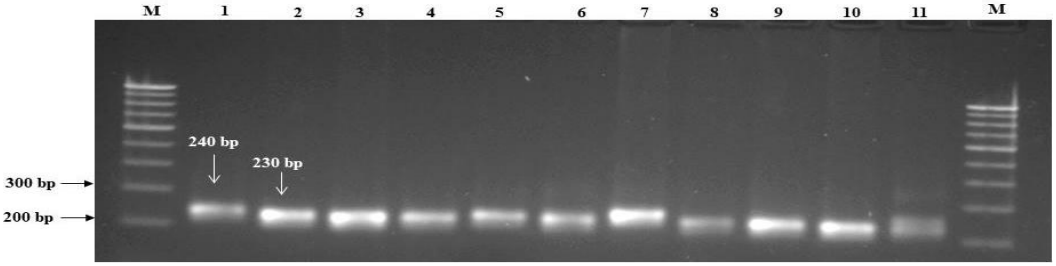

Fig. 3. 3 Banding pattern generated using the marker RM 122 for *xa5* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 5, lane 2 = IR24, lane 3 = Kariyadukkan, lane 4 = Ithikandan, lane 5 = Mullankayama, lane 6 = Marathondi, lane 7 = Kothambalarikayama, lane 8 = Kuruva, lane 9 = Cheruvellari, lane 10 = Mallimatta, lane 11 = Kokkan lane, M = 100 bp DNA ladder

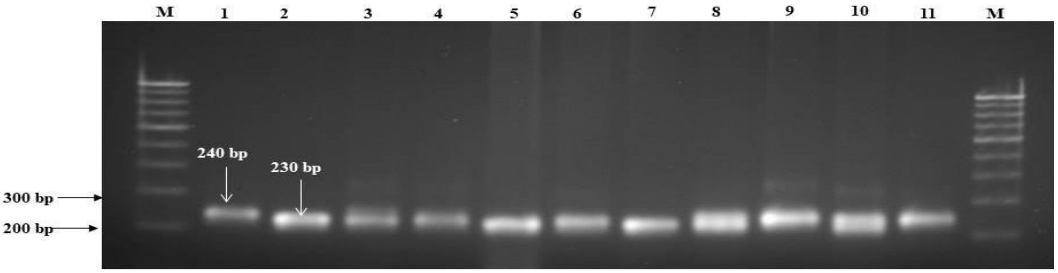

Fig. 3. 4 Banding pattern generated using the marker RM 122 for *xa5* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 5, lane 2 = IR24, lane 3 = Mundon, lane 4 = Thondi, lane 5 = Odiyan, lane 6 = Mannuveliyan, lane 7 = Ottadi, lane 8 = Veliyan (2), lane 9 = Vellari, lane 10 = Chembavu, lane 11 = Kunnamkulamban, lane M = 100 bp DNA ladder

Figure 4. Agarose gel electrophoretic patterns of germplasm accessions generated by using SSR marker RM 230

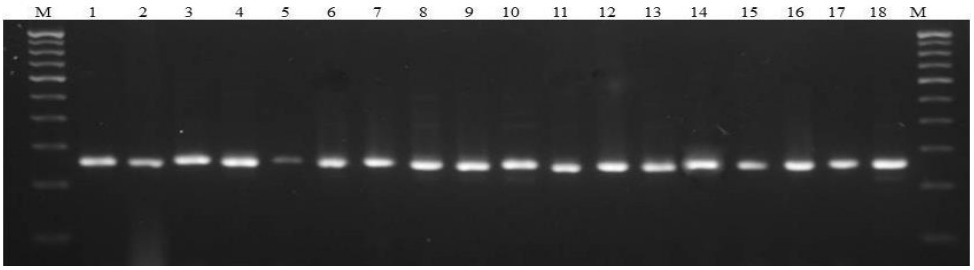

Fig. 4.1 Banding pattern generated using the marker RM 230 for *xa13* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB13, lane 2. IR24, lane 3 = Eruvakkali, lane 4 = Mandupakki, lane 5 = Mangalpuram, lane 6 = Chenkayama (Ambalapara), lane 7 = Ponmani, lane 8 = Chettivirippu, lane 9 = Vellapokkali, lane 10 = Virippu, lane 11 = Bolamgittikayama, lane 12 = Vellakkayama, lane 13 = Mundakan, lane 14 = Anakkodan, lane 15 = Cheriya orpandy, lane 16 = Gandhasala (1), lane 17 = Parambuvattan , lane 18 = Champan, lane M = 100 bp DNA ladder

Figure 4. Agarose gel electrophoretic patterns of germplasm accessions generated by using SSR marker RM 230

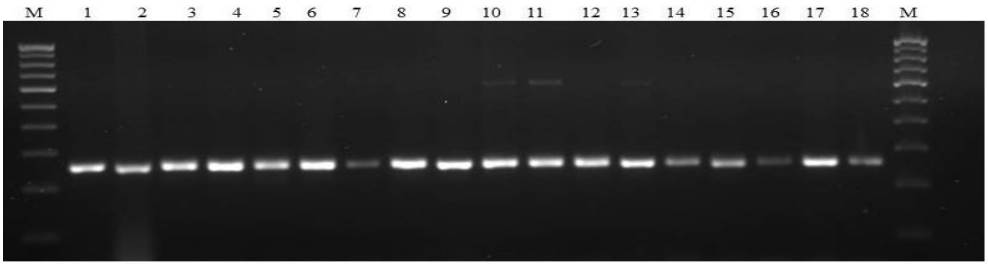

Fig. 4.2 Banding pattern generated using the marker RM 230 for *xa13* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 13, lane 2 = IR24, lane 3 = Gandhasala (2), lane 4 = Jeerakasala, lane 5 = Kokkankoli, lane 6 = Pandi Champan, lane 7 = Kalladiaryan (Red rice), lane 8 = Kothambalarikayama (1), lane 9 = Njavara (Black), lane 10 = Mundon (Cheruli), lane 11 = Veliyan (1), lane 12 = Basmati, lane 13 = Krishnakamod, lane 14 = Karutha njavara, lane 15 = Kalluruli upland, lane 16 = Chitteni (Alathur), lane 17 = Wayanad 2, lane 18 = Black Chitteni (Thavanur), lane M = 100 bp DNA ladder

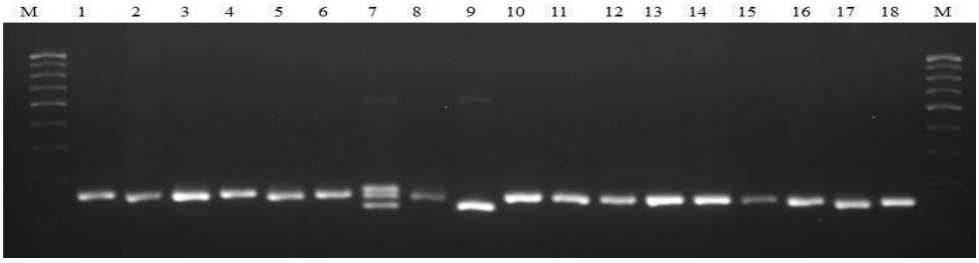

Fig. 4.3 Banding pattern generated using the marker RM 230 for *xa13* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 13, lane 2 = IR24, lane 3 = Chembavu, lane 4 = Cheruvellari, lane 5 = Ithikandan, lane 6 = Kariyadukkan, lane 7 = Kokkan, lane 8 = Kothambalarikayama (2), lane 9 = Kunnamkulamban, lane 10 = Kuruva, lane 11 = Mallimatta, lane 12 = Mannuveliyan, lane 13 = Marathondi, lane 14 = Odiyan, lane 15 = Mullankayama, lane 16 = Ottadi, lane 17 = Mundon, lane 18 = Thondi, lane M = 100 bp DNA ladder

Figure 5. Agarose gel electrophoretic patterns of germplasm accessions generated by using SSR marker *xa13* prom

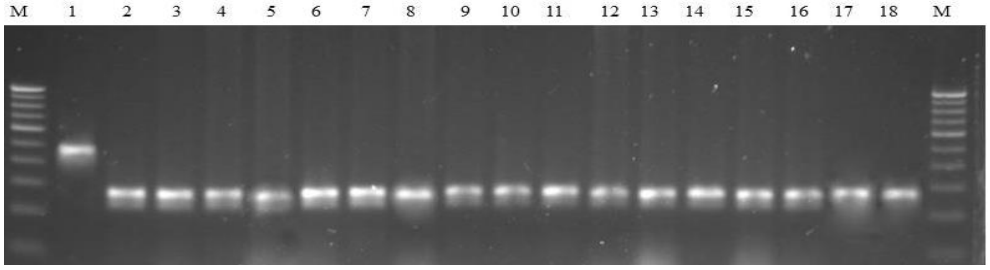

Fig. 5.1 Banding pattern generated using the marker *xa13* prom for *xa13* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 13, lane 2 = IR24, lane 3 = Eruvakkali, lane 4 = Mandupakki, lane 5 = Mangalpuram, lane 6 = Chenkayama (Ambalapara), lane 7 = Ponmani, lane 8 = Chettivirippu, lane 9 = Vellapokkali, lane 10 = Virippu, lane 11 = Bolamgittikayama, lane 12 = Vellakkayama, lane 13 = Mundakan, lane 14 = Anakkodan, lane 15 = Cheriya orpandy, lane 16 = Gandhasala (1), lane 17 = Parambuvattan , lane 18 = Champan, lane M = 100 bp DNA ladder

Figure 5. Agarose gel electrophoretic patterns of germplasm accessions generated by using SSR marker *xa13* prom

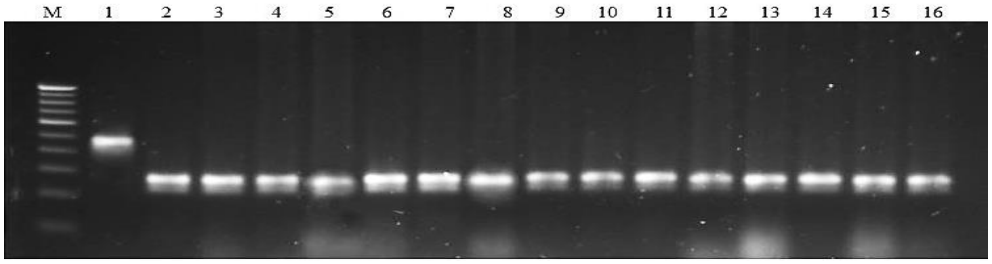

Fig. 5.2 Banding pattern generated using the marker *xa13* prom for *xa13* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB13, lane 2 = IR24, lane 3 = Gandhasala (2), lane 4 = Jeerakasala, lane 5 = Kokkankoli, lane 6 = Pandi Champan, lane 7 = Kalladiaryan (Red rice), lane 8 = Kothambalarikayama (1), lane 9 = Njavara (Black), lane 10 = Mundon (Cheruli), lane 11 = Veliyan (1), lane 12 = Basmati, lane 13 = Krishnakamod, lane 14 = Karutha njavara, lane 15 = Kalluruli upland, lane 16 = Chitteni (Alathur), lane M = 100 bp DNA ladder

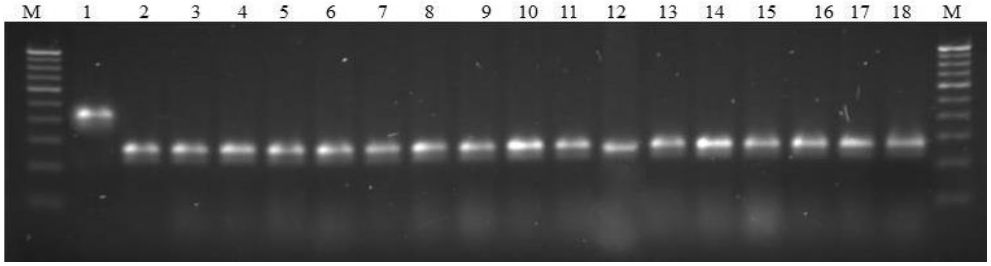

Fig. 5. 3 Banding pattern generated using the marker *xa13* prom for *xa13* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 13, lane 2 = IR24, lane 3 = Chembavu, lane 4 = Cheruvellari, lane 5 = Ithikandan, lane 6 = Kariyadukkan, lane 7 = Kokkan, lane 8 = Kothambalarikayama (2), lane 9 = Kunnamkulamban, lane 10 = Kuruva, lane 11 = Mallimatta, lane 12 = Mannuveliyan, lane 13 = Marathondi, lane 14 = Odiyan, lane 15 = Mullankayama, lane 16 = Ottadi, lane M = 100 bp DNA ladder

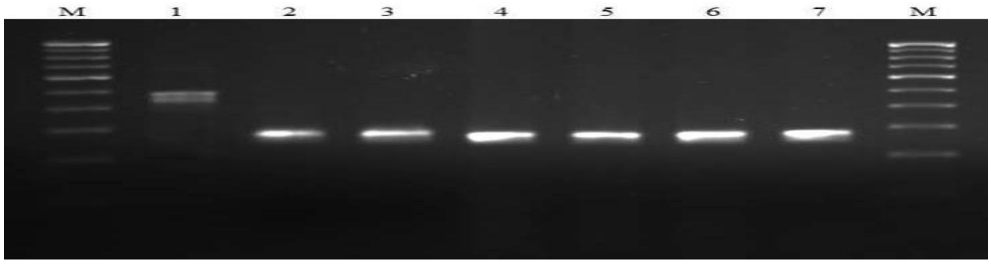

Fig. 5.4 Banding pattern generated using the marker *xa13* prom for *xa13* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB13, lane 2 = IR24, lane 3 = Wayanad 2, lane 4 = Black Chitteni (Thavanur), lane 5 = Thondi, lane 6 = Veliyan (2), lane 7 = Vellari, lane M = 100 bp DNA ladder

Figure 6. Agarose gel electrophoretic patterns of germplasm accessions generated by using STS marker pTA 248

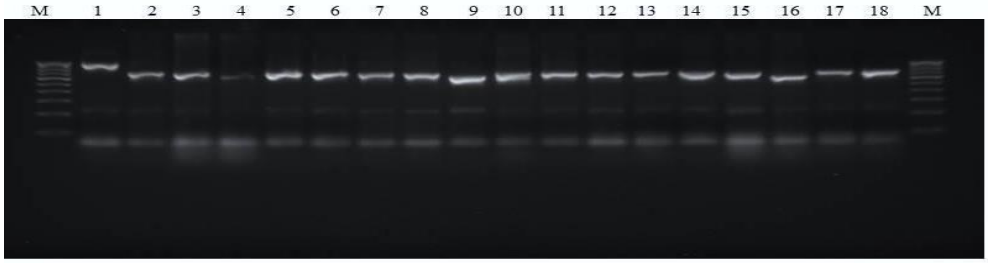

Fig. 6.1 Banding pattern generated using the marker pTA248 for *Xa21* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB21, lane 2. IR24, lane 3 = Eruvakkali, lane 4 = Mandupakki, lane 5 = Mangalpuram, lane 6 = Chenkayama (Ambalapara), lane 7 = Ponmani, lane 8 = Chettivirippu, lane 9 = Vellapokkali, lane 10 = Virippu, lane 11 = Bolamgittikayama, lane 12 = Vellakkayama, lane 13 = Mundakan, lane 14 = Anakkodan, lane 15 = Cheriya orpandy, lane 16 = Gandhasala (1), lane 17 = Parambuvattan , lane 18 = Champan,, lane M = 100 bp DNA ladder

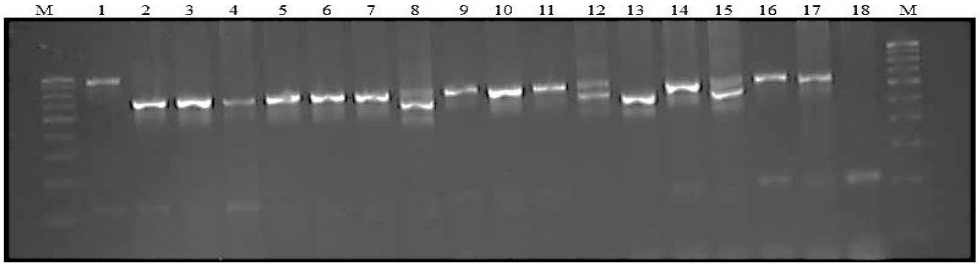

Fig. 6.2 Banding pattern generated using the marker pTA248 for *Xa21* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB21, lane 2 = IR24, lane 3 = Gandhasala (2), lane 4 = Jeerakasala, lane 5 = Kokkankoli, lane 6 = Pandi Champan, lane 7 = Kalladiaryan (Red rice), lane 8 = Kothambalarikayama (1), lane 9 = Njavara (Black), lane 10 = Mundon (Cheruli), lane 11 = Veliyan (1), lane 12 = Basmati, lane 13 = Krishnakamod, lane 14 = Karutha njavara, lane 15 = Kalluruli upland, lane 16 = Chitteni (Alathur), lane 17 = Wayanad 2, lane 18 = Black Chitteni (Thavanur), lane M = 100 bp DNA ladder

Figure 6. Agarose gel electrophoretic patterns of germplasm accessions generated by using STS marker pTA 248

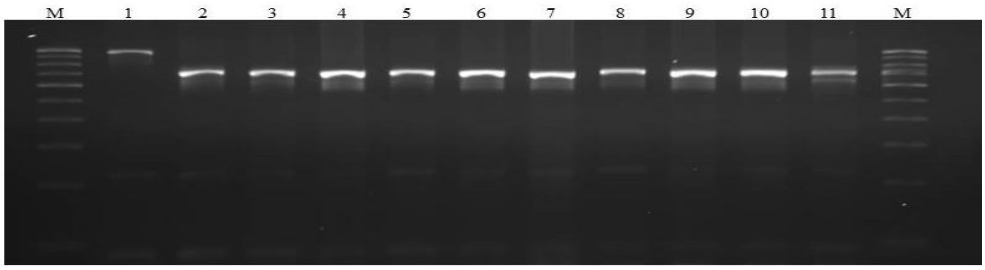

Fig. 6.3 Banding pattern generated using the marker pTA248 for *Xa21* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 21, lane 2 = IR24, lane 3 = Chembavu, lane 4 = Cheruvellari, lane 5 = Ithikandan, lane 6 = Kariyadukkan, lane 7 = Kokkan, lane 8 = Kothambalarikayama (2), lane 9 = Kunnamkulamban, lane 10 = Kuruva, lane 11 = Mallimatta, lane M = 100 bp DNA ladder

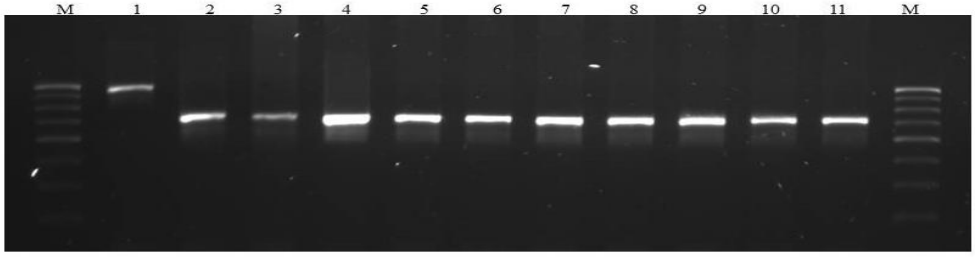

Fig. 6.4 Banding pattern generated using the marker pTA248 for *Xa21* gene
Lane M = 100 bp DNA ladder, lane 1 = IRBB 21, lane 2 = IR24, lane 3 = Mannuveliyan, lane 4 = Marathondi, lane 5 = Odiyan, lane 6 = Mullankayama, lane 7 = Ottadi lane 8 = Mundon, lane 9 = Thondi, lane 10 = Veliyan (2), lane 11 = Vellari, lane M = 100 bp DNA ladder

Discussion

5. DISCUSSION

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is a devastating disease of rice causing considerable yield loss world wide. The losses due to this disease depends on the crop stage, variety of the crop and climatic conditions. The yield loss ranging from 20 to 80 per cent has been reported by various researchers. (Raina *et al*., 1981; Mew, 1987; Mew *et al.,* 1992).

Understanding the pathogen population prevailing in a geographical area is important for the development of management strategies. Periodic survey to assess the disease occurrence and study of pathogen population is essential for understanding evolution new pathotypes of the pathogen. Many of the BB resistance genes have been used and transferred to several cultivars (Srinivasan and Gnanamanickam, 2005; Kumar*et al*., 2012). However large scale and long-term cultivation of the varieties would result in its breakdown of resistance.

Survey and study of the pathogen population have helped to report resistance status of predominant varieties as well as the structure of pathogen population (Laha *et al.*, 2009; Patil *et al.,* 2017). In Kerala, bacterial blight of rice is causing yield loss recently in every year particularly during *kharif* season. After the floods experienced during the years 2018 and 2019, the incidence of bacterial blight has increased in all the major rice growing areas of the state. Understanding the extent of severity of the disease in predominantly cultivated varieties of the state and the structure of the pathogen population prevailing in the state is essential for the development of control measures of bacterial blight of rice in Kerala. The present study was taken up to assess the severity of bacterial blight in major rice growing districts of the Kerala *viz*., Palakkad, Thrissur, Malappuram, Alappuzha and Kottayam and to collect pathogen for studying the pathotypic variability of bacterial blight pathogen of rice, *Xanthomonas oryzae* pv. *oryzae* in Kerala. An attempt was made in this study to identify sources of bacterial blightresistance from the germplasm comprising local landraces and other cultivars of rice maintained at the Regional Agricultural Research Station, Pattambi, Kerala, India.

A purposive sampling survey was carried out in major growing districts of Kerala *viz.,* Palakkad, Thrissur, Malappuram, Alappuzha and Kottayam. During the period of survey variety, stage of the crop, symptom and disease severity were noted. The survey conducted in major rice growing tracts of Kerala revealed the wide spread occurrence of bacterial blight of rice.

The predominantly cultivated varieties in the surveyed areas were Uma and Jyothi which were affected by bacterial blight in moderate to severe intensities. Among the districts surveyed, the disease severity was high in Palakkad district.

In variety Jyothi the highest severity recorded was 90.50 per cent and in Uma it was 95.10 per cent. This was followed by Thrissur district, where the highest disease severity of 85.35 per cent and 80.18 per cent was recorded in varieties Uma and Jyothi respectively. The highest disease severity recorded in variety Uma was 75.75 per cent. Jyothi was cultivated in only one field surveyed where the disease severity was low (10.13%). In Alappuzha district, the highest disease severity recorded in variety Uma was 70.68 per cent and in variety Jyothi was 50.70 per cent. In Kottayam district, in all the locations surveyed except one, the variety cultivated was Uma and the highest severity recorded was 50.63 per cent.

The incidence of bacterial blight as major disease of rice has been reported from the states of Punjab, Haryana, Uttaranchal, Bihar, West Bengal, Tripura, Assam, Tamil Nadu, Uttar Pradesh, Andhra Pradesh, Andaman and Nicobar Islands, Maharashtra, Chhattisgarh, Gujarat, Himachal Pradesh, Karnataka and Kerala (Laha *et al.,* 2009). The bacterial blight epidemics causing yield loss due to bacterial blight ranging from 30 to 90 per cent have been reported by several researchers depending on the variety cultivated, stage of the crop and climatic conditions. (Raina *et al.,* 1981; Mew, 1987; Mew *et al.,* 1992; Srinivasan and Gnanamanickam, 2005). The yield loss 10-20 per cent is common. In the state of Kerala, after the floods experienced during the year 2018 and 2019, the incidence and spread of bacterial blight has increased considerably. The systematic collection of diseased samples, isolation of pathogen and study of its virulence is

important to develop effective management strategies including development of resistant varieties. Similar studies were conducted in the states of India such as Punjab and Andhra Pradesh. Rajarajeswari and Muralidharan (2008) conducted a study to assess the occurrence of bacterial blight of rice in four rice growing districts of Nellore (Andhra Pradesh), Rangareddy (Andhra Pradesh), West Godavari (Andhra Pradesh), Karnal (Hariyana). Singh *et al.* (2003) reported the prevalence and intensity of bacterial blight in 10 districts of Punjab. Rafi *et al.* (2013) conducted a survey and reported the disease incidence and severity of bacterial blight in all rice growing zones of Pakistan.

Bacterial blight symptoms both *kresek* and leaf blight were observed in the field. The *kresek* symptom were observed in crops of 15-40 days old while bacterial blight symptom were seen in 60-135 days old plants. In Palakkad district from *kresek* symptom was observed in three locations *viz.,* Kannambra, Kappur and Pattambi. In Malappuram and Thrissur *kresek* symptom were noted in two (Angadippuram and Thazhekkode) and one (Elavally) locations respectively. Leaf blight symptom was observed in later stages of crop development. It was most common in the maximum tillering stage to the panicle initiation stage. It was observed that disease severity was highest during the time of panicle initiation.

Kresek causes either the death of the whole plant or wilting of few leaves. It is the more destructive phase of the disease, wherein the leaves of the entire plant turn pale yellow and wilt during the seedling to the early tillering stage, which causes partial or total crop failure. The *kresek* symptom appears during the early stage of the crop, one to two weeks after transplanting. Leaf blight symptoms are observed at the tillering stage, disease incidence increases with plant growth and maximum at the panicle initiation time. It starts from the lower portion of the plant and proceeds gradually towards the upper leaves. The upper half of the leaf or the whole leaf turns pale before drying up (Gnanamanickam *et al.,* 1999).

Reddy and Ou (1976) reported leaf blight symptom starts with small water-soaked lesions that will appear and later turn to yellowish-white color expanding from the equal

sides in a square form to produce elongated circular to quite uneven lesions. The occurrence of bacterial ooze from infected leaves has been observed in warm and humid climates and this also helps in the spread of the disease. Damage due BB is maximum when *kresek* phase. Post flowering infections have very little effect on grain yield. However, when infection occurs during panicle initiation or subsequently during stages that precede flowering, severe impairment of grain development and a consequent increase in sterility was observed.

The pathogen was isolated from diseased samples collected and isolation was done on Peptone Sucrose Agar media (PSA). A total of 168 isolates were isolated based on cultural and morphological characters. Out of 168 isolates 83 were from Palakkad, 44 from Thrissur, 19 from Malappuram, eleven each from Alappuzha and Kottayam districts.

The inoculation of the isolates in susceptible variety Jyothi revealed that the isolates from different locations are highly virulent capable of producing moderately susceptible to highly susceptible disease response. This indicate the chances of epidemics in predominant varieties like Jyothi is very high under favourable climatic conditions due to the prevailing pathogen population in different rice growing tracts of the state. Among the isolates 168 isolates collected, all the isolates are virulent on susceptible variety Jyothi causing a reaction of moderately susceptible (score 5) and above upto maximum score of 9 (highly susceptible). The disease response varies with the isolates indicating the variability among the isolates. The disease severity varied from 14.37 per cent (Xoo 145) to 60.93 per cent (Xoo 49). Among the 168 isolates, 14 isolates (8.33%) produced highly susceptible reaction with score 9, 128 isolates (76.19%) produced susceptible reaction with score 7 and 26 isolates (15.48%) produced moderately susceptible reaction with score 5 upon artificial inoculation on susceptible variety Jyothi (Fig.7).

Gonzalez *et al.* (2007) tested virulence of twenty six *X. oryzae* pv. *oryzae* by leaf clip inoculation method on the rice variety Nipponbare. Akthar *et al.* (2008) inoculated one month old rice plants with *Xoo* isolate suspension on rice plants by clip inoculation and reported symptoms of bacterial blight appeared after two weeks. Yellow

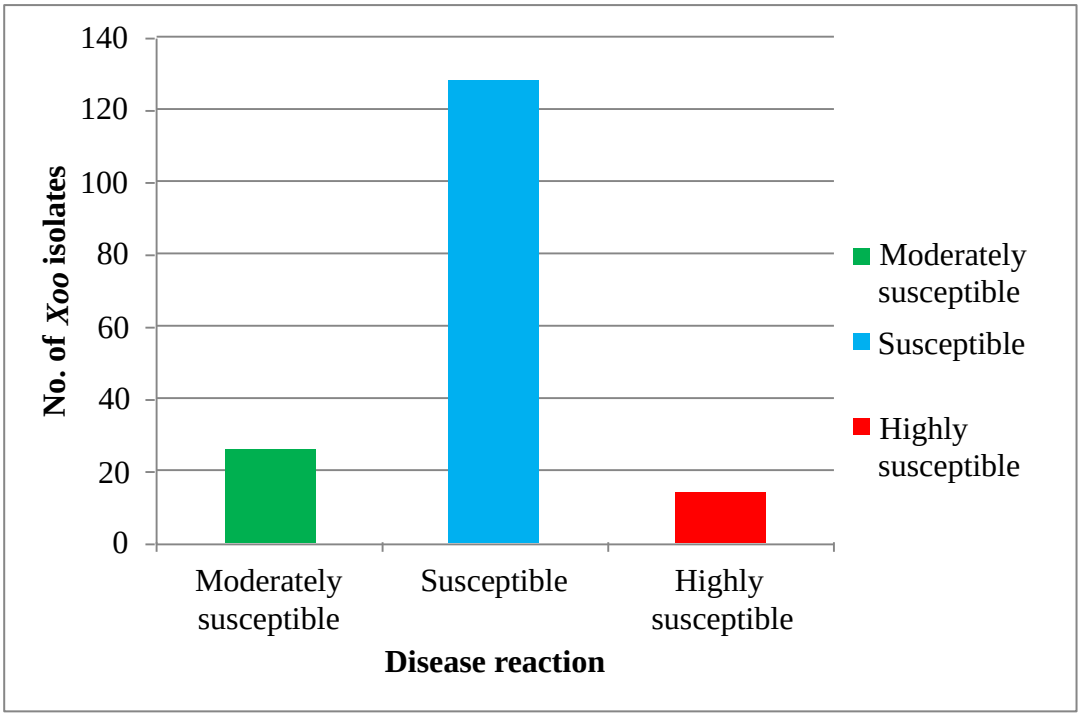

Figure 7. Disease reaction of *Xoo* isolates on susceptible variety Jyothi

lesion with a wavy margin appeared on the leaf margin and the leave became yellowish and dry. Muneer *et al*. (2007) proved the pathogenicity of forty nine isolateson six rice cultivars including 1RR1 6, KS-282, JP 5, Basmati 385, Dilrosh 97 and Kashmir Basmati 385 by clip inoculation method.

Bakade and Kumar (2020) studied the virulence of *Xoo* isolates collected from different geographical regions of South India by inoculation of one susceptible variety TN-1. They reported high variability among the isolates in their virulence.

Morphological and biochemical characterization revealed the identity of pathogen as *Xanthomonas oryzae* pv. *oryzae.* Isolates were negative for indole production, oxidase and urease production. Such results were reported by Anonymous (2001) and Padmaja *et al.* (2017). *Xoo* isolates were reported to be positive for starch hydrolysis (Swings *et al.,* 1990). In this study few isolates showed negative reaction . such results were obtained for Bradbury (1986). Other tests MR, VP test, tween80 hydrolysis, urease production, nitrate reduction and carbohydrate utilization also confirmed the pathogen as *X. oryzae* pv. *oryzae.*

Pathotyping of 100 isolates of *Xanthomonas oryzae* pv. *oryzae* was carried out using 28 NIL's (IRBB lines) and one bacterial blight resistant three gene pyramided variety, Improved Samba Mashuri and susceptible checks TN-1 and Jyothi. Understanding pathotypic diversity among the pathogen population is essential to develop rice varieties with bacterial blight resistance. The present study analyzed the virulence of 100 isolates of *Xanthomonas oryzae* pv. *oryzae* the bacterial blight pathogen of rice in Kerala. The *Xoo* isolates collected from 52 panchayats covering major rice growing areas of Palakkad, Thrissur, Malappuram, Alappuzha and Kottayam districts.

The disease reaction of various isolates of *Xoo* collected from different locations on differentials varied widely. None of single genes tested *viz*., *Xa1, xa3, Xa4, xa5, Xa7, xa8, Xa11, xa13, Xa14* and *Xa21* were widely effective against the *Xoo* isolates tested. Most of the NILs with single genes were non effective to about 53.19 to 92.55 per cent except *Xa21* which showed susceptibility to 38.30 per cent of the isolates and *xa13* whichshowed

susceptibility to 46.81 per cent of the *Xoo* population tested and *xa5* which showed susceptibility to 53.12 per cent. The bacterial blight pathogen of rice is highly dynamic in nature which evolve very fast. To over come new pathotype resistance is always a potential threat in rice production (Leonard and Czochor, 1980; Eamchit and Mew, 1982).

Xa21 showed less susceptibility to (38.30%) of *Xoo* isolates compared to other single genes tested. Followed by *Xa21*, *xa13* gene showed next lowest susceptibility to *Xoo* isolates (46.81%). *xa13* is also widely used bacterial blight resistance gene. Yugander *et al*. (2017) reported that *xa13* gene showed moderate susceptibility to about 35 per cent of Indian isolates of *Xoo.*

The BB resistance genes *xa5* showed susceptibility to 53.19 per cent of the *Xoo* population. Followed by *xa5, Xa4* recorded susceptibility to 57.46 per cent of *Xoo* population tested. These two genes which were utilized widely in breeding programme particularly in conventional breeding during 1980s. But later these were found susceptible. Yugander *et al.* (2017) reported the susceptibility of these two single genes for Indian isolates of *Xoo.*

Continuous and widespread deployment of *Xa4* gene in cultivars during 1970 and 1980 period has resulted in breakdown of resistance (Mew *et al.,* 1992). The pathotype analysis of *Xoo* isolates was carried out in India and other countries revealed the susceptibility of single resistance genes (Singh *et al.,* 2003; Lore *et al*., 2011; Yugander *et al*., 2014; Yugander *et al*., 2017). Among the single genes *Xa21* showed susceptibility to *Xoo* population (58.30%). This is the commonly used bacterial blight resistance gene. Several other workers also reported the susceptibility of *Xa21* (Priyadarsini and Gnanamanickam 1999; Mishra *et al*., 2013; Yugander *et al*., 2017).

xa8 was susceptible to 74.47 per cent of the isolates and *Xa7* was susceptible to 81.91 per cent of the isolates tested. Susceptibility of *Xa7* and *xa8* BB resistance gene was reported to Indian isolate including *Xoo* isolates from Kerala was reported earlier by Mishra *et al.* (2013) and Yugander *et al*. (2014). The other single genes tested *Xa1, Xa3, Xa10, Xa11* and *Xa14* were found susceptible to 92.55 per cent *Xoo* isolates tested. High

virulence of *Xoo* isolates on these genes were reported by other workers from India (Lore *et al*., 2011; Yugander *et al*., 2014).

Six differentials carrying two gene combination were also evaluated against the *Xoo* isolates. Among the differentials tested IRBB 53 (*xa5*+*xa13*) showed lowest susceptibility of 12.77 per cent to *Xoo* isolates tested. Yugander *et al*. (2017) and Hunjan *et al.* (2014), also obtained similar results. High efficiency of IRBB 53 against Indian isolates of BB pathogens compared to other two gene combinations. IRRBB 51 (*Xa4*+*xa13*), IRBB 52 (*Xa4*+*Xa21*), IRBB 55 (*xa13*+*Xa21*) were having almost similar range of effectiveness. These showed susceptibility ranging from 13.83 to 14.89 per cent. The two gene combination *xa5*+*Xa21* (IRBB 54) showed 20.21 per cent susceptibility to *Xoo* isolates tested.

Even though the BB resistance gene, *Xa4* alone was not widely effective against the *Xoo* isolates, in combination with *xa13* and *Xa21* it showed better resistance indicating the complimentary effects. Li *et al.* (2001) and Shanti *et al*. (2010) reported similar complimentary effect of *Xa4* and *Xa21*. BB resistance gene *xa5* when alone was not found widely effective but in combination with *xa13* and *Xa21* it showed broad spectrum resistance. Similarly, *xa13* in combination with *Xa21* and *Xa4* gave broad spectrum resistance. Such additive effect of the single gene was reported by Yugander *et al.* (2014).

Among the three gene pyramid *xa5*+*xa13*+*Xa21* followed by *Xa4*+*xa13*+*Xa21* were effective to which showed susceptibility to 10.64 per cent and 12.77 per cent of *Xoo* isolates. IRBB 59 carrying *xa5*+*xa13*+ *Xa21* showed susceptibility only to 10.64 per cent *Xoo* isolates tested. Even though the BB resistance genes *Xa4* and *Xa7* were having low resistance to *Xoo* isolates. These two genes in combination with *xa13* (IRBB 13) and *Xa21* (IRBB 62) showed increased resistance. Among the four gene pyramids evaluated IRBB 60 carrying *Xa4*+*xa5*+*xa13*+*Xa21* was highly effective. None of the *Xoo* isolates tested showed virulence on this. IRBB 65 (*Xa4*+*Xa7*+*xa13*+*Xa21*) showed susceptibility to only 3.19 per cent of the tested followed by IRBB 64 (7.45 per cent susceptibility) four gene

pyramid IRBB 66 carrying *Xa4+xa5+Xa7+xa13+Xa21* did not show susceptible reaction to any of the isolates tested. Yugander *et al.* (2022) also reported the effectivenessof IRBB 60 and IRBB 66 to the isolates of *Xoo* from Andhra Pradesh.

Based on the reaction on a set of 31 differentials *Xoo* isolates were classified into 15 pathotypes. The pathotypes were arranged chronologically based on their virulence from least virulent to most virulent. The pathotype 1 and pathotype 2 were avirulent on all differentials pathotype XoPt 14 and XoPt 15 were highly virulent. These two pathotypes were virulent on *xa13* and *Xa21.* All the pyramided lines carrying different gene combinations were found effective against this pathotype. Pathotype 11 was present in Palakkad (4), Malappuram (1), Thrissur (1) and Alappuzha (4) districts (Fig 8-9). XoPt 11 showed moderate resistance reaction on *xa21.* However all other gene combinations showed resistance to this pathotype. Pathotype XoPt 15 the most virulent one was virulent on all NILs carrying single genes as well as two gene pyramids with moderately susceptible to susceptible reaction. In three gene combination *xa5+xa13+Xa21* two isolates showed moderately resistance and four isolate showed moderate susceptibility. Other three gene pyramid *Xa4+xa5+xa13* (IRBB 56), *Xa4+xa5+Xa21* (IRBB 57), *Xa4+Xa13+Xa21* (IRBB 58), *Xa4+xa5+Xa7* (IRBB 61) and *xa5+Xa7+xa13* (IRBB 63) also showed moderately susceptible reaction to this pathotype.This indicates the evolution of more virulent strains of *Xoo* in the state. Yugander *et al*. (2017) reported the prevalence of *Xoo* strains having high virulence from Kerala, which showed virulence on *Xa4+xa5+xa13* and *Xa4+xa5+Xa21*. The differential IRBB 60 carrying four gene combination *Xa4+xa5+xa13+Xa21*, IRBB 64 (*Xa4+Xa7+xa13+Xa21*) and IRBB 66 (*Xa4+xa5+xa13+Xa21*) showed resistance tothese pathotype.

Pathotype XoPt 8 showed highest frequency distribution. Thirteen per cent of the isolates belongs to their pathotype. This was present in Palakkad (7), Malappuram (1) and Thrissur (5) districts. This pathotype was virulent on *xa5* these pathotype was virulent on *xa5* and *xa13*. But *Xa21* was effective against this showing MR/R reaction. All the two gene combinations except (*Xa4+xa5*) and other three, four and five gene combinations tested were found effective against this pathotype. The next pathotype were XoPt 4 and

XoPt 11 comprised of 10 isolates each. XoPt 4 was distributed over Palakkad (3), Thrissur (4), Alappuzha (1) and Kottayam (2) this pathotype was virulent on *Xa4* but was avirulent on *xa8.* This pathotype showed R/MR reaction on *xa5* and *xa13* genes and R reaction on *Xa21*.

The study revealed the existence of 15 pathotypes among the bacterial blight pathogen isolates tested indicating high variability. Similar diversity of *Xoo* population was reported from other states such as Punjab (Lore *et al.,* 2011). They reported existence of seven pathotypes in Punjab. Yugander *et al.* (2014) characterized the *Xoo* isolates from Andhra Pradesh into 10 pathotypes. Yugander *et al.* (2017) studied the virulence spectrum of 400 isolates of *Xoo* from different rice growing states of India including Kerala and grouped the pathogen into 22 different pathotypes.

From the study the bacterial blight pathogen of rice Kerala vary widely in their virulence. None of the single bacterial blight resistance gene can give wide spectrum resistance to the pathogen population prevailing in the state. Two gene combinations *xa5*+*xa13* is effective compared to other two gene combinations. However, three gene combination *xa5*+*xa13*+*xa21* is more effective than *xa5*+*xa13.* Other three gene combinations which can give broad resistance are *Xa4*+*xa5*+*xa13, Xa4*+*xa13*+*Xa21* and *xa5*+ *Xa7*+*xa13*. The four gene combination, *Xa4*+ *xa5*+*xa13*+*Xa21* can give broad spectrum resistance to bacterial blight in Kerala. The results of the study could be utilized for the deployment of effective genes for the development of bacterial blight resistant variety for the state of Kerala.

In view of the limited success of the other management practices the most effective, economical and environmentally friendly approach is the development of resistant varieties (Patil *et al*., 2017). Until now forty seven bacterial blight resistant genes conferring resistance to *Xoo* have been identified from various rice cultivars, wild relatives of rice, and mutation populations (Lin *et al*., 1996). Large scale and long term cultivation of rice varieties carrying a single resistance gene results in breakdown of resistance due to high pathogen variability (Mew *et al.,* 1992; Laha *et al*., 2009; Shanti *et al.,* 2010).

Incorporation of two or more genes of bacterial blight resistance can enhance the durability of resistance (Sundaram *et al*., 2008; Hajira *et al.,* 2014).

The effectiveness of resistance genes varies over locations due to the geographical structuring of the pathogen. There is a huge potential of utilizing the untapped sources of resistance from the germplasm for development of disease resistant rice varieties with other quality parameters. Utilizing different sources of resistance for introgression of the traits will be useful for broadening the genetic base rather than utilizing the same source. Several researchers have studied the local germplasm for the identification of multiple bacterial blight resistance genes (Amgai *et al.,* 2015; Yadav *et al.,* 2013; Banerjee *et al.,* 2018; Zhao *et al.,* 2022). An attempt was made in this study to identify sources of bacterial blight resistance from the germplasm comprising local landraces and othercultivars of rice maintained at the Regional Agricultural Research Station, Pattambi, Kerala, India.

Phenotypic screening of 50 germplasm accessions of rice obtained from germplasm collection maintained at the Regional Agricultural Research Station, Pattambi, Kerala were used for the study. Along with these, IR24 as well as local susceptible variety Jyothi were also included. As part of the study three most virulent *Xoo* isolates were used phenotypic screening out of 168 isolates isolated from major rice growing parts of Kerala. The three isolates used for checking the disease reaction on germplasms were Xoo 13*,* Xoo 57 and Xoo 63 collected from Palakkad, Malappuram and Alappuzha respectively. The observations were taken 21 days after inoculation. Bacterial blight symptom was observed on all the accessions tested for phenotypic screening. The symptom initiated as water soaked lesion on leaf blade then later it turn into straw colour.

Among the 50 accessions screened, two accessions *viz*., Krishnakamod and Ottadi were moderately resistant to the isolate Xoo 13 of *Xanthomonas oryzae* pv. *oryzae* with the score of 3. None of the accessions screened were resistant or moderately resistant to the most virulent isolate Xoo 57. The accession Ottadi showed moderate susceptibility with score 5 to this isolate. These two accessions Krishnakamod and Ottadi showed moderate resistance to the isolate Xoo 63 also. Four other accessions Gandhasala (1),

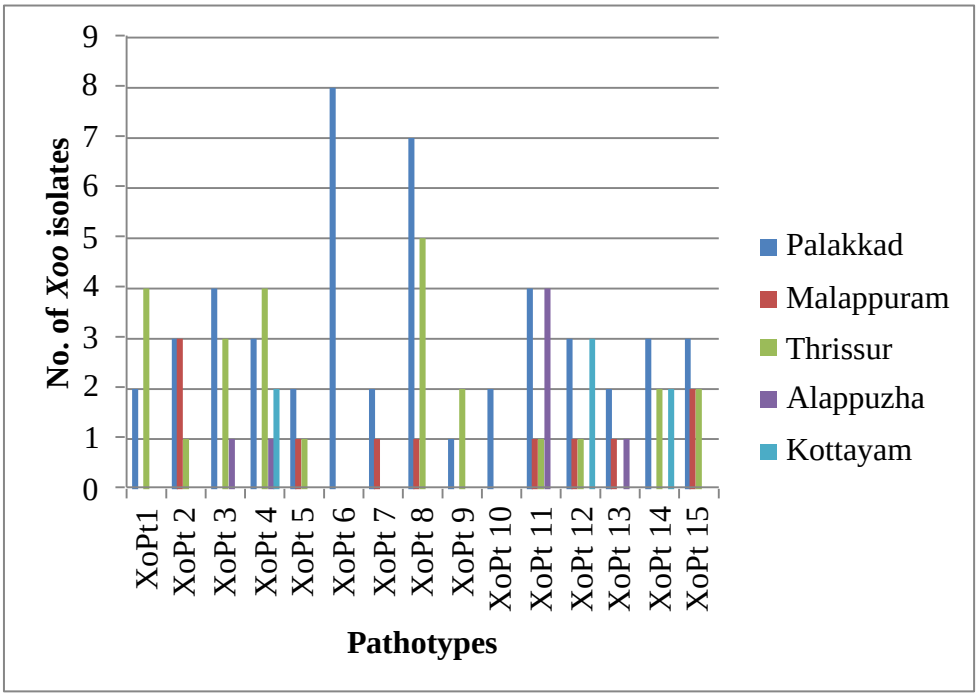

Figure 8. Geographical distribution of *Xoo* isolates

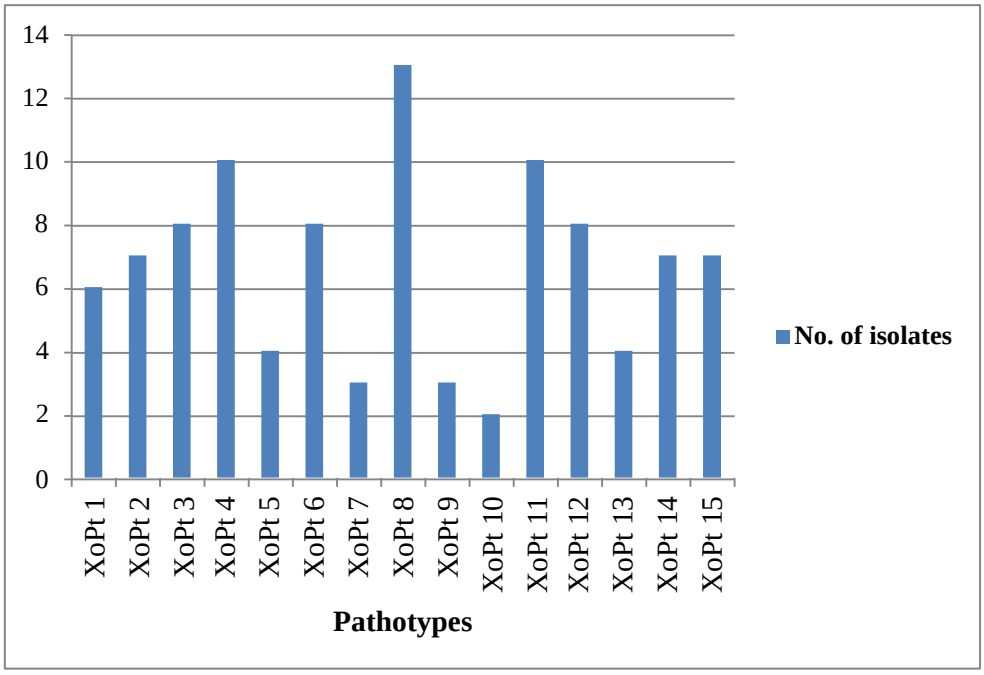

Figure 9. Number of *Xoo* isolates under each pathotype

Mundon (Cheruli), Kothambalarikayama (1) and Wayanad 2 were moderately resistant to the bacterial blight pathogen isolate Xoo 63. Many of cultivars/ landraces available in the germplasm are potential sources of resistance to diseases. Unless we know the trait qualities, we would not be able to utilize these directly for cultivation or to utilize for breeding purpose. In the present study none of the accessions were resistant to bacterial blight phenotypically under artificial inoculation. However the accessions with moderate resistance under artificial inoculation particularly the accessions which showed moderate resistance to more than one virulent isolates (Krishnakamod and Ottadi) could be utilized as a source for resistance to bacterial blight for the improvement of existing high yielding varieties for bacterial blight resistance or to develop new bacterial blight resistant varieties. Several researchers located landraces/ cultivars/ wild rice accessions with resistance/ tolerance to bacterial blight earlier (Thimmegowda *et al.,* 2011; Yadav *et al.,* 2013; Singh *et al.,* 2015; Banerjee *et al.,* 2018; Majumder *et al.,* 2020; Zhao *et al.,* 2022). The germplasm accessions identified in this study would be of specifically useful for the state of Kerala where bacterial blight is a severe problem. By understanding the phenotypic resistance, these germplasm accessions could be utilized for cultivation directly after their performance evaluation or for conventional breeding for disease resistance.

Conventional breeding is time consuming and laborious tool for gene pyramiding. To get broad spectrum durable resistance against bacterial blight, incorporation of more than one BB resistance genes is more desirable (Sundaram *et al.,* 2008; Rajpurohit *et al.,* 2011; Dasari *et al.,* 2022). To achieve this marker assisted breeding is an efficient tool. For this approach donors with known resistance genes are essential. Once the resistance genes in our landraces are identified, these can be further utilized for molecular breeding. In this study, we report the resistance spectrum of 50 germplasm accessions and the status of bacterial blight resistance genes *Xa4, xa5, xa13 and Xa21.*

The effectiveness of these genes in various combinations for enhancing bacterial blight resistance has already been reported by several workers. The high yielding rice variety Jalmagna was improved by incorporating three resistance genes, *xa5, xa13* and

Xa21 (Pradhan *et al.,* 2015). Four resistance genes *Xa4, xa5, xa13* and *Xa21* were pyramided to a popular variety Ranidhan and developed a variety with broad spectrum resistance to bacterial blight (Pradhan *et al.,* 2022). Improvement of rice variety Basmati 385 was done by pyramiding of *Xa4, xa5, xa13* and *Xa21* genes by Ullah *et al*. (2022).

Among the germplasm accessions of rice identified with moderate phenotypic resistance to bacterial blight pathogen isolates, the accession namely Krishnakamodcarry only *xa5* gene. In Ottadi none of these genes were present. This suggest thecontribution of some other genes also towards the phenotypic resistance shown by these varieties. To utilize these two accessions for molecular breeding further search for other genes is required. Among the other accessions exhibited moderate resistance to other isolate *Xoo63* possess two resistance genes surveyed *viz*., Gandhasala (1) (*Xa4* and *xa5*), Mundon (Cheruli) (*Xa4* (+ -) and *xa5*) and Kothambalarikayama (1) (*Xa4* (+-) and *xa5*). The accession Wayanad 2 which showed phenotypic moderate resistance to the isolate *Xoo63* does not carry any of these four genes. So as in Ottadi this also may harboursource other resistance genes. These accessions having moderate phenotypic resistance along with known resistance genes could be utilized as potential donors for bacterial blight resistance breeding. Some of the accessions which apparently did not show resistance reaction also carries *Xa4* and *xa5* genes such as Mandupakki, Ponmani, Gandhasala (2), Vellapokkali, Njavara (black), Chembavu, Kunnumkulamban, Veliyan (2) and Vellari. This may be due to the wide range of genetic background. Similar results of occurrence of one or more R genes in moderately susceptible germplasm accessions were earlier reported (Zhao *et al*., 2022). None of the genotypes screened had *Xa21* and *xa13* genes. Similar observation was reported by Singh *et al.* (2015). The presence of *Xa21* gene in germplasm is very rare as the gene was introgressed originally from wild rice, *Oryza longistaminata* (Khush and Angeles, 1999).

Summary

6. SUMMARY

The present study entitled “Analysis of pathotypic variability of *Xanthomonas oryzae* pv. *oryzae,* the bacterial blight pathogen of rice and identification of new sourcesof resistance” was conducted during the period of 2017 to 2021.

A purposive sampling survey was conducted to assess the occurrence and severity of bacterial blight of rice in major rice growing districts of Kerala state *viz*., Alappuzha, Kottayam, Thrissur, Palakkad and Malappuram during the years 2018 and 2019 and to isolate bacterial blight pathogen.

In Palakkad district 24 panchayats were surveyed and disease severity varied from 5.28 to 95.10 per cent. The highest disease severity was observed in Pattanchery (95.10%) innvariety Jyothi. Five panchayats were surveyed in Malappuram district. The disease severity ranged from 5.28 to 75.75 per cent and highest severity was noticed in Vettathur. Severity of *kresek* symptom varied from 5.30 to 50.10 per cent and leaf blight varied from 5.28 to 75.75 per cent. In Thrissur district 13 panchayats were surveyed and both the symptom of bacterial blight *kresek* and leaf blight were observed. The disease severity ranged from 5.05 to 85.83 per cent. In Alappuzha districts five panchayats were surveyed covering 11 fields. Varieties infected with bacterial blight were Uma and Jyothi and disease severity ranged from 5.30 to 70.68 per cent. Leaf blight symptom only found in the field. Four panchayats were surveyed in Kottayam districtand only leaf blight symptom were noticed. Disease severity varied from 5.45 to 50.63 per cent. Majorvarieties with bacterial blight infected were Uma and IR5. To all the districts surveyed predominant varieties were Uma and Jyothi and both were affected widely by bacterial blight.

The pathogen was isolated from diseased samples collected from different locations of five districts surveyed, in peptone sucrose agar medium. Yellow pigmented bacterial colonies typical to that of *Xanthomonas oryzae* pv. *oryzae* were sub cultured. A total of 168 isolates were obtained from different locations surveyed.

Pathogenicity of 168 isolates were proved by inoculation to susceptible variety Jyothi. All the bacterial isolates produced symptoms of bacterial blight on variety Jyothi upon inoculation. The severity ranged from 14.37 to 60.93 per cent. The pathogen was reisolated from the symptomatic plants and upon reisolation the bacterial isolate typical to that of original one were obtained.

The colony character of *Xoo* isolates were studied. The colonies were smooth raised/ flattened, circular with light yellow, yellow, dark yellow or creamy yellow in colour.

Biochemical characterization of *Xoo* isolates were carried out by 11 tests. Indole production test, MR-VP test, starch hydrolysis test, tween 80 hydrolysis test, urease production test, KOH test, catalase test, oxidase test, nitrate reduction and carbohydrate utilization test were conducted and the results confirmed that the pathogen isolates were *Xanthomonas oryzae* pv. *oryzae.*

Pathotyping and virulence analysis of 100 isolates of *Xoo* selected from the pathogenicity studies based on the virulence and representing all the locations surveyed was carried out using a set of 31 differentials. These include NILs carrying bacterial blight resistance genes and gene combinations in the background of IR24 (IRBB lines), Improved Samba Mashuri, a bacterial blight resistant variety released from IIRR, Hyderabad and susceptible varieties TN-1 and Jyothi. Based on the reaction of *Xoo* isolates on differentials, they were categorized into 15 pathotypes. The pathotype XoPt 1 was the least virulent and pathotype XoPt 15 was the most virulent. Pathotype XoPt 8 was the most predominant one (13% of the isolates) and distributed in Palakkad, Thrissur and Malappuram districts. This pathotype was virulent on *xa5* and *xa13* genes but wasavirulent on *Xa21*. This was followed by XoPt 4 (10%) and XoPt 11 (10%). The pathotype XoPt 15 was virulent on all the single genes. None of single genes tested *viz*., *Xa1, Xa3, Xa4, xa5, Xa7, Xa11, xa13, Xa14* and *Xa21* were widely effective against the *Xoo* isolates tested. Most of the NILs with single genes were non- effective to about 53.19 to 92.55 per cent of the isolates tested except *Xa21* which showed susceptibility to 38.30 per

cent of the isolates and *xa13* which showed susceptibility to 46.81 per cent. Thetwo gene combination *xa5*+*xa13* was effective compared to other two gene combinations. However, the three gene combination *xa5*+*xa13*+*xa21* was more effective than *xa5*+*xa13*. Other three gene combinations which showed broad resistance were *Xa4*+ *xa5*+*xa13*, *Xa4*+*xa13*+*Xa21* and *xa5*+*Xa7*+*xa13.* The four gene combination,*Xa4*+*xa5*+*xa13*+*Xa21* and the five gene combination *Xa4*+*xa5*+*Xa7*+ *xa13*+*Xa21* were highly effective against all the *Xoo* isolates.

Phenotypic screening of 50 germplasm accessions for bacterial blight resistance were carried out by inoculating with three *Xoo* isolates *viz*., Xoo 13, Xoo 57 and Xoo 63. None of the accessions tested were resistant or moderately resistant to the most virulent isolate Xoo 57. Out of the 50 accessions tested, six were moderately resistant to *Xoo* 63 and two accessions were moderately resistant to Xoo 13. Moderate resistance were observed in two accessions Krishnakamod and Mundon to two isolates of the bacterial blight pathogen *viz*., Xoo 13 and Xoo 63. Six accessions *viz*., Gandhasala (2), Kothambalarikayama (1) Mundon (Cheruli), Krishnakamod, Wayanad 2 and Ottadi were moderately resistant to Xoo 63. All other accessions were moderately susceptible to highly susceptible in nature.

The genotypic screening to locate bacterial blight resistance genes using SSR/STS molecular markers *viz*., MP (*Xa4*), RM 390 and RM 122 (*xa5*), RM 230 and *xa13* prom (*xa13*), and pTA 248 (*Xa21*) was carried out. No amplicons specific to *xa13* and *Xa21* were detected showing the absence of these two genes in the germplasm accessions screened. Twenty five accessions amplified 150 bp size fragment corresponding to *Xa4* gene. Twenty accessions amplified 240 bp fragment specific to *xa5* gene and 10 accessions amplified both indicating the presence of both *Xa4* and *xa5* genes. Out of the two accessions which showed moderate resistance to two isolates of the pathogen in phenotypic screening, one contains only *xa5* gene and other does not carry any of these four genes studied indicating the involvement of some other genes contributing to its resistance.

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Appendices

APPENDICES

1. **Peptone Sucrose Agar medium**

   Peptone – 10 g

   Sucrose – 10 g

   Glutamic acid – 1g

   Distilled water – 1000 ml

2. **Starch Agar medium**

   Soluble starch – 20 g

   Peptone – 5 g

   Beef extract – 3g

   Agar – 20 g

   Distilled water – 1000 ml

3. **Nitrate Broth**

   Peptone – 5g

   Meat extract- 3 g

   KNO_3 – 1 g

   NaCl – 30 g

4. **CTAB Extraction buffer**

   CTAB – 20 ml

   Tris HCl – 10 ml

   EDTA – 4 ml

   NaCl – 28 ml

   Distilled water – 37.8 ml

   β-mercaptoethanol - 200 µl

Abstract

ANALYSIS OF PATHOTYPIC VARIABILITY OF *Xanthomonas oryzae* pv. *oryzae*, THE BACTERIAL BLIGHT PATHOGEN OF RICE AND IDENTIFICATION OF NEW SOURCES OF RESISTANCE

By

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ABSTRACT OF THE THESIS

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Abstract

Bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is becoming a major production constraint worldwide. In India, the disease is causing considerable yield loss in all rice producing states. In Kerala, the disease is appearing in an epidemic form in recent years in Palakkad, Thrissur and Alappuzha districts. Host plant resistance offers a viable option for the management of the disease. Understanding the pathogen population prevailing in a geographical area is essential for the development of varieties having broad spectrum resistance. The present study was undertaken to analyse the pathotypic variability of *Xoo* in major rice growing areas of Kerala and to identify new sources of resistance.

A purposive sampling survey was conducted in Palakkad, Malappuram, Thrissur, Alappuzha and Kottayam districts to assess the intensity of the disease and to isolate pathogen from different locations. The predominantly cultivated rice varieties *viz*., Uma and Jyothi were affected in farmers field in all the districts surveyed. The survey revealed the occurrence of both *kresek* as well as leaf blight symptoms in the field. The pathogen was isolated from diseased plants showing typical symptoms. A total of 168 isolates typical to that of *Xoo* were obtained. The pathogenicity of the isolates was proved on susceptible variety Jyothi. The disease severity varied from 14.37 to 60.93 per cent indicating the variability in virulence of *Xoo* isolates.

Pathotyping and virulence analysis of 100 isolates of *Xoo* selected from the pathogenicity studies based on the virulence and representing all the locations surveyed was carried out using a set of 31 differentials. These include NILs carrying bacterial blight resistance genes and gene combinations in the background of IR24 (IRBB lines), Improved Samba Mahsuri, a bacterial blight resistant variety released from IIRR, Hyderabad and susceptible varieties TN-1 and Jyothi. Based on the reaction of *Xoo* isolates on differentials, they were categorized into 15 pathotypes. The pathotype XoPt1 was the least virulent and pathotype XoPt15 was the most virulent. Pathotype XoPt8 was the most predominant one (13% of the isolates) and distributed in Palakkad, Thrissur and Malappuram districts. This pathotype was virulent on *xa5* and *xa13* genes but was avirulent on *Xa21*. This was followed by XoPt4 (10%) and XoPt11 (10 %). The pathotype XoPt15 was virulent on all the single genes. None of single genes tested *viz*., *Xa1, Xa3, Xa4, xa5, Xa7, Xa11, xa13, Xa14* and *Xa21* were widely effective against the *Xoo* isolates tested. Most of the NILs with single genes were non- effective to about 53.19 to 92.55 per cent of the isolates tested except *Xa21* which

showed susceptibility to 38.30 per cent of the isolates and *xa13* which showed susceptibility to 46.81 per cent. The two gene combination *xa5+xa13* was effective compared to other two gene combinations. However, the three gene combination *xa5+xa13+xa21* was more effective than *xa5+xa13*. Other three gene combinations which showed broad resistance were *Xa4+xa5+xa13*, *Xa4+xa13+Xa21* and *xa5+Xa7+xa13.* The four gene combination, *Xa4+xa5+xa13+Xa21* and the five gene combination *Xa4+xa5+Xa7+xa13+Xa21* were highly effective against all the *Xoo* isolates.

Fifty germplasm accessions were screened using three virulent isolates of *Xoo*. Two accessions were moderately resistant against two *Xoo* isolates (Xoo13 and Xoo63). None of the accessions tested were resistant or moderately resistant to the most virulent isolate Xoo57. The genotypic screening to locate bacterial blight resistance genes using SSR/STS molecular markers *viz*., MP (*Xa4*), RM 390 and RM 122 (*xa5*), RM 230 and *xa13* prom (*xa13*), and pTA 248 (*Xa21*) was carried out. No amplicons specific to *xa13* and *Xa21* were detected showing the absence of these two genes in the germplasm accessions screened. Twenty five accessions amplified 150 bp size fragment corresponding to *Xa4* gene. Twenty accessions amplified 240 bp fragments specific to *xa5* gene and 10 accessions amplified both indicating the presence of both *Xa4* and *xa5* genes. Out of the two accessions which showed moderate resistance to two isolates of the pathogen in phenotypic screening, one contains only *xa5* gene and other does not carry any of these four genes studied indicating the involvement of some other genes contributing to its resistance.