Mysore J. Agric. Sci., 57 (2) : 246-256 (2023)

Molecular Detection and Virulence Profiling of Associated Bacterial Pathogens Causing Bacterial Blight in Rice

Namburi Karunakar Reddy, M. K. Prasanna Kumar and Swathi S. Patil
Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : karunakarpath18@gmail.com

Authors Contribution

Namburi Karunakar Reddy: Conceptualization, investigation, draft preparation and analysis; M. K. Prasanna Kumar : Conceptualization, draft correction and supervision; Swathi S. Patil : Draft correction and supervision

Corresponding Author :

Namburi Karunakar Reddy: Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065

Received : September 2022
Accepted : January 2023

Abstract

Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most destructive diseases. The disease severity of this differs from geographical regions, strain (race) and the host genotype. Hence, isolates were collected from different locations of Karnataka to know the virulence of *Xoo* and recorded disease severity. Cultured isolates were cultured in the laboratory and were confirmed by 16S rRNA sequencing as well isolated *Pantoea ananitis* associated with bacterial blight and producing similar kind of symptoms as that of *Xoo*. The ClustalW analysis of the 16S rRNA gene sequences revealed two groups in the case of both *Xoo* and *P. ananitis*. The second group containing only two isolates *Xoo* 4 and *Xoo* 9 are more similar to the MH444307 isolate from Maharashtra and is delineated from the rest of the *Xoo* isolates. The 35 isolates of *P. ananitis* also formed into two groups and had more similarity with Japan isolate AB304809 followed by South Korean isolates HE672167 and HE716948 respectively. Around 42 resistance genes of rice (*Xa* genes), have been identified to confer resistance against various strains of *Xoo*. An understanding of pathotype diversity within the target pathogen population is required to identify the *Xa* genes to be deployed for developing resistant rice cultivars. The isolates of *Xoo* and *P. ananitis* collected from different parts of Karnataka formed 2 major pathotypes of both *Xoo* and *P. ananitis* and were distinguished based on their reaction towards *Xa* genes in the monogenic lines. The resistance gene *Xa21* conferred resistance to more than 70 per cent of the *Xoo* and *P. ananitis* population. Further the amounts of exopolysaccharide (EPS) produced were quantified and varied depending on *Xoo* isolates. Isolate *Xoo* 3 produced a distinctly high amount of EPS (92.3 mg) followed by *Xoo* 6, *Xoo* 8 and *Xoo* 4. On the contrary, *Xoo* 2 produced distinctly less amount of EPS that has been co-related with the virulence nature of the pathogen on the rice cultivar, TN-1.

Keywords : *Xanthomonas oryzae* pv. *oryzae, Pantoea ananitis,* Pathotype, EPS

Rice, one of the most important food crop, is constantly challenged by bacterial pathogens, such as those causing bacterial leaf blight (BB), leaf streak, and bacterial panicle blight. Leaf blight is a serious problem in rice agroecosystems of many countries, causing significant economic losses worldwide (Chukwu *et al*., 2019). The impact of leaf blight on rice farming systems appears to be increasing, and causes yield loss up to 70 per cent in various countries**.** This disease is most prevalent and destructive in tropical Asia (Mew *et al.,* 1993). In India, the first report on BLB was made by Bhapkar *et al.* (1960) and it is one of the most devastating diseases during monsoon season and a major production constraint in rice cultivation particularly in irrigated and rainfed lowland ecosystems of rice growing states of India. For decades, the gram-negative bacterium *Xoo* has been widely regarded as the only causal agent of leaf blight in rice (Chien *et al*., 2019).

However, several studies in recent past have indicated that many species from the genus *Pantoea* are capable of causing the symptoms of leaf blight disease. *Pantoea* spp. is commonly associated with plants as epiphytes or pathogens (Deletoile *et al*., 2009). Recent evidence has provided additional support, where the *Pantoea* species have re-emerged as a threat to global rice production as they have been shown to cause various rice diseases in several rice growing areas of the world (Azizi *et al.,* 2020). The *Pantoea* spp. was described as a causal agent of leaf blight in India (Mondal *et al.,* 2011) and Korea (Lee *et al.,* 2010). Recent reports by Toh *et al*. (2019) and Azizi *et al*. (2020) indicated that *P. ananatis, P. dispersa* and *P. stewartii* were all recorded to be the causal agents of leaf blight outbreak in Malaysia.

Bacterial blight is characterized by a high degree of race cultivar specificity. *Xoo* races is differentially infecting rice cultivars and distributed in geographic regions over time periods. Hence, *Xoo* is a rapidly evolving pathogen (Salzberg *et al*., 2008). The selection of cultivated rice varieties may facilitate the race shift or result in the emergence of new races. Indeed, a prior study showed that the shifting of the major race over time in the Philippines might be caused by a dramatic change in the host genotypes (Quibod *et al*., 2016). The population dynamics of *Xoo* monitored in resistant and susceptible rice cultivars showed that bacterial populations in compatible and incompatible interactions increased almost equally in the initial stage of infection. There after, however, the virulent population multiplied more rapidly and extensively than the avirulent ones (Noda and Kaku*,* 1999).

Chemical control is not very successful in India and elsewhere for the management of the disease (Laha *et al.,* 2009). Therefore, deploying of resistance genes to increase host plant robustness is economically and environmentally advantageous and the most promising strategy to manage BLB. So far, more than 45 BLB resistance genes have been identified from diverse sources. Some of them have been introgressed to suitable agronomically important rice cultivars to develop resistance against BB in several countries (Yugander *et al*., 2018). The interlinked evolution of *Xoo* and rice harbours the potential for selecting emerging virulence factors (Mishra *et al*., 2013). Therefore, regular monitoring of BB pathogen is essential to identify the existing and evolving pathotypes of *Xoo* in a particular region as a prerequisite for developing of durable BB resistant rice cultivars.

Analysis of BLB pathogen population structure using both classical pathotyping on rice differentials, International Rice Bacterial Blight (IRBB near isogenic lines) and genetic diversity using molecular markers has been reported by several workers both in India and elsewhere (Yugander *et al*., 2017). The present study was undertaken to identify the pathotypes and virulence profile of *Xoo* and *P. ananitis* isolates collected from selected areas of Karnataka. The findings of this study will help for breeding BB resistant rice varieties suitable for the region.

Material and Methods

Collection of BB Diseased Leaf Samples

A field survey was undertaken in major rice growing regions of Karnataka India, during November 2020 and June 2021. A total of 45 samples were collected from different locations in Karnataka. Plants with typical bacterial leaf blight symptoms *viz.,* yellow water-soaked lesions at the margin of the leaf blade, the lesions run parallel along the leaf, presence of bacterial discharge on young lesion early in the morning resembling the milky dewdrop, drying up of leaf blade with white lesions as wavy margin were collected in blotting paper folds, labelled, and then in a paper envelope. The samples were brought to the laboratory and stored at 4 °C for isolation of the pathogen.

Isolation of the Pathogen

Isolation of pathogen was carried out from infected rice leaves collected during the survey. The leaves showing typical symptoms of leaf blighting, exuded bacterial ooze from the cut section were used for isolation. The diseased portion with healthy tissues was cut into 1.5 to 2 cm pieces. These diseased pieces

The Mysore Journal of Agricultural Sciences

were surface sterilized for 30 seconds in 0.1 per cent sodium hypochlorite solution, followed by three subsequent washing with sterilized distilled water in aseptic conditions to remove the traces of sodium hypochlorite (Bakade and Kumar, 2020). The bacterial suspension was prepared with the cut pieces of infected leaf samples. Following serial dilution, the suspension was streaked on Peptone Sucrose Agar (PSA) medium with the help of a sterilized wire loop. The inoculated plates were incubated at room temperature (27 ± 2 °C) for 48 hrs. Single colonies of cultures isolated from disease samples were picked up from PSA plates and the pure culture, thus obtained was preserved in slants for further investigations in the refrigerator at 4 °C for routine work and in 50 per cent glycerol at -80 °C for long term storage.

Pathogenicity Test

To confirm the pathogenicity of the bacteria isolated from the bacterial blight samples, the isolates were multiplied in a nutrient broth medium. The pathogenicity test was carried out on rice plants (cv. TN-1) in the glasshouse using leaf clip inoculation technique (Kaufmann, 1973). The pots were filled with sterilized soil and TN-1 seeds were sown in the prepared pots. Two seedlings per pot were raised for 50 days, covered with plastic to avoid air borne infection. The pots were labelled, watered gently and all the agronomical practices were adopted for growing the rice plants.

Molecular Confirmation of the Pathogen Using 16S rRNA and Phylogenetic Analysis

Genomic DNA of the bacterium was extracted as described by Zhang *et al*. (1998) with some modification. 0.3 g of washed bacterial cells pellet was suspended in 200 µl of Cetyltrimethylammonium bromide (CTAB) buffer (50 mMTris, pH 8.0; 0.7 mMNaCl; 10 mM EDTA; 2 Cetyltrimethylammonium hexade cyltrimethylammonium bromide) followed by 100 µl of 10 per cent sodium dodecyl sulfate and incubated at 65 °C for 10 min. DNA was purified with chloroform and precipitated with iso-propanol at -20 °C overnight. Purified DNA was washed with 70 per cent ethanol, then the pellet was air dried and resuspended in 30 µl of DNase free sterile distilled water. DNA concentration was measured using NanoDrop (De Novex) at 260 / 280 nm. DNA quality was checked on 0.8 per cent agarose gel in Tris-Acetate-EDTA (TAE) buffer (45 mM Tris-acetate, 1 mM EDTA, pH 8.0). After electrophoresis, the genomic DNA was further used for PCR amplification with universal 16S rRNA primers (F-5'-GAGTTTGATCCTGGCTCA-3'; R-5'-AGAAAGGAGGTGATCCAG-3'). PCR was performed in a thermal cycler (Eppendorf, Vapo protect), with 100 ng of genomic DNA, 1 µl of each primer, 10 µl of 2 x PCR master mix and sterile distilled water to make a final volume of 20 µl. The thermal cycler was programmed with an initial denaturation at 94 °C for four min followed by 35 cycles at 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min with the final extension at 72 °C for 10 min. The amplified products were purified and directly sequenced from both ends using a commercial facility (Eurofins Scientific India Pvt. Ltd.). Obtained DNA sequences were analysed and compared with the available NCBI database using BLAST analysis.

The phylogenetic analysis using 16S rRNA gene sequences from *Xoo* strains and *P. ananitis* was done using CLC genomic workbench (version 4.7.2) software programme using neighbor-joining method. The percent identity was determined using ClustalW multiple alignments of BioEdit software. The type strains of *P. ananatis* NR 026045, *Sphingomonas endophytica* MN932317, the common epiphytes in rice were kept as outgroups in determining the phylogenetic relationship among the *Xoo* strains. The GenBank accession numbers of 16S rRNA gene from *Xoo* strains are OP048090, OP048091, OP048092, OP048093, OP048094, OP048095, OP048096, OP048097, OP048098 and OP048099. The type strains of *Exiguobacterium* MH753639*, Xanthomonas oryzae* pv. *oryzae* AB68014, *Sphighomonas endophyticum* MN932317 the common epiphytes in rice were kept as outgroups in determining the phylogenetic relationship among the *P. ananitis* strains. The GenBank accessions of 16S rRNA genes from *P. ananitis* strains are

OK576910, OK576911, OK576912, OK576913, OK57614, OK576915, OK576916, OK576917, OK576918, OK576919, OK576920, OK576921, OK576922, OK576923, OK576924, OK576925, OK576926, OK576927, OK576928, OK576929, OK576930, OK576931, OK576932, OK576933, OK576934, OK576935, OK576936, OK576937, OK576938, OK576939, OK576940, OK576941, OK576941, OK576942, OK576943 and OK576944.

Pathotype Analysis

The seeds of bacterial blight differential rice lines (IRBB-1, 3, 4, 5, 7, 8, 10,11, 13,14, 21,), the susceptible check line (IR24) and the resistant check line (RP Bio-226) were provided by the Zonal Agricultural Research Station (ZARS), Mandya. The plants were grown in pots in a greenhouse. Sowing of the differentials was done in pots and 50 days old plants were preferably used for inoculating the pathogen (Yugander *et al*., 2022). The trays were irrigated every day. Adequate plant protection measures were taken to ensure the healthy and vigorous growth of the plants. Plants were clip inoculated with bacterial suspensions of 10^9 cfu/ml. Four leaves per plant were inoculated for each isolate-cultivar combination for 50 days old plants. Disease observations were taken 14 days after inoculation by measuring lesion length. Lesion lengths ≤5 cm was considered as resistant; 5-10 cm was considered as moderately resistant and >10 cm was considered susceptible. Pathotype grouping was done based on the reaction pattern onto the differentials. Cluster analysis was carried out using the unweighted neighbor joining method and the robustness of the tree/cluster was assessed with 1000 bootstraps using the DARW in software.

Measurement of Exopolysaccharide (EPS)

The measurement of exopolysaccharide (EPS) was conducted as described by Jeong *et al*. (2008). A single colony of each *Xoo* isolate was inoculated in 40 ml of nutrient broth medium and incubated for 72 h at 28 °C with agitation. The optical density of the bacterial cultures was adjusted to 1.0 at 600 nm with NB. The culture supernatants were transferred into new 50-ml tubes and supplemented with 1.0 per cent potassium chloride (w/v; final concentration). Two volumes of absolute ethanol were added to each solution and the tubes were placed at - 20 °C overnight. The precipitated crude EPS was collected by centrifugation for 30 min at 83,000×g. The EPS pellets were dried at 55 °C for 12 h and the dry weight of each was measured.

Results and Discussion

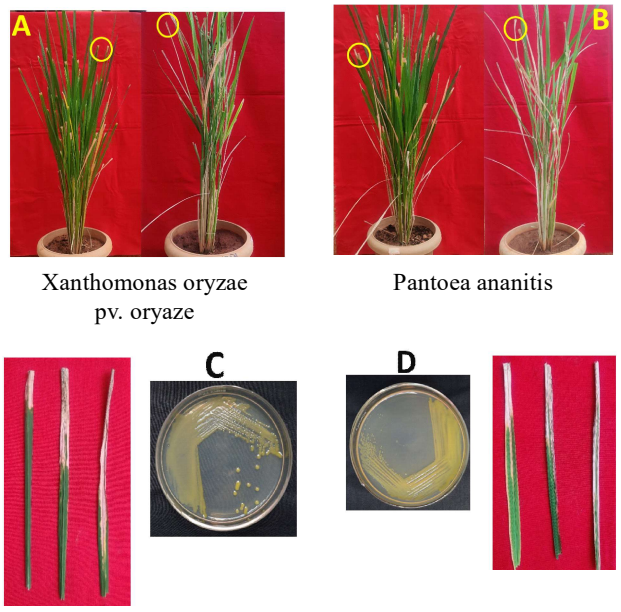
Survey and Collection of Infected Leaf Samples

The bacterial blight infected leaf samples of rice collected from different regions of Karnataka exhibited the characteristic symptom of yellow water-soaked lesions at the margin of the leaf blade and the lesions run parallel along the leaf with the bacterial discharge appears on young lesion early in the morning resembling the milky dew drop, as the disease progress the leaf dries up with white lesions and the leaf blade as wavy margin (Plate 1). Similarly, symptoms were reported by (Bakade and Kumar, 2020) collected infected rice leaf samples from different rice growing regions of Karnataka, Andhrapradesh and Tamil Nadu.

Plate 1: Symptoms of bacterial blight on rice leaves as observed under field conditions

Isolation and Pathogenicity Assay

The BLB infected rice samples collected during survey were homogenized in sterile distilled water for isolation of pathogen/s on PSA medium. Individual bacterial colonies on PSA medium were yellow, mucoid and convex as reported by Chauhan (1973) and Ou (1985). Pathogenicity of the 45 isolates was proved by adopting the leaf clip inoculation technique (Kaufmann, 1973). The bacterial suspension of all the isolates were inoculated on TN-1 (45 days old plants) in glass house. Symptoms of typical BLB were observed from the 3rd day of inoculation. The pathogen was re-isolated from the BB infected plants and thus confirmed its identity and pathogenicity (Plate 2). Based on the pathogenicity tests, the bacterium was confirmed as *Xanthomonas oryzae* pv. *oryzae.* This pattern of morphological characters of *Xoo* observed in the present study was in accordance with the description given by Chauhan (1973) and Ou (1985). Symptoms on the grown-up plant were manifested as rolling of leaves, greyish lesions with wavy margins, wilting of the plant (Laha *et al*., 2009). The 45 isolates were subjected to 16Sr RNA sequencing for molecular confirmation. Of the 45 strains, 35 were identified as



Xanthomonas oryzae pv. oryaze

Pantoea ananitis

Plate 2: A, B: Symptoms of bacterial blight on rice leaves as observed under glasshouse conditions upon inoculation with Xoo and *P. ananitis,* respectively; C, D: Colony morphology of Xoo and *P. ananitis,* respectively

P. ananitis and 10 as *Xoo*. Reported symptoms observed from *P. ananitis* infection are almost consistent with *Xoo* caused leaf blight in our study. Further, the plants infected with *P. ananitis* exhibited the tale like lesions along the leaf margin as described by Doni *et al*. (2019).

Molecular Confirmation Using 16SrRNA and Phylogenetic Analysis

The genomic DNA of 45 bacterial isolates were amplified using 16s rRNA universal primers and observed the amplicon of expected 1500bp size in all the isolates (Bakade and Kumar, 2020). The 16s rRNA gene of all the *Xoo* isolates were sequenced and compared with the previously published 16s rRNA gene sequences of *Xoo* using the NCBI-BLAST program. The sequence identities among these 10 isolates are 99-100 per cent. Phylogeny based on 16S rRNA gene revealed that all 10 strains of *Xoo* from Karnataka formed two clusters sharing 100 per cent identity among them, suggesting that geographical distance does not necessarily contribute to the variation in *Xoo* strains with respect to the gene loci, 16S rRNA (Fig. 1) (Kalyan, 2013). The ClustalW analysis of the 16S rRNA gene sequences revealed two groups. The first group includes almost all the isolates *viz., Xoo* 1, *Xoo* 2, *Xoo* 3, *Xoo* 5, *Xoo* 6, *Xoo* 7, *Xoo* 8 and *Xoo* 10. The second group contains only two isolates *Xoo*4 and *Xoo*9 which are from the Koppal district of Karnataka and are more similar to the MH444307 isolate from Maharashtra. The first cluster was divided into two sub clusters where the isolates *Xoo* 1, *Xoo* 2, *Xoo* 6 and *Xoo* 10 from Gangavathi showed high similarity with ON908986 *Xoo* from Shivamogga. The isolate *Xoo* 5 showed high similarity with JQ269244 *Xoo* from Tamilnadu, where, JQ269244 *Xoo* was found to be sub- ancestor for *Xoo* 3, *Xoo* 7 and *Xoo* 8. The 35 isolates of *P. ananitis* also formed into two groups and are more similar with Japan isolate AB304809 followed by South Korean isolates HE716948, HE672167 and all the *P. ananitis* isolate have clustered with in the Karnataka isolates (Fig. 2).

The use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy has been by far

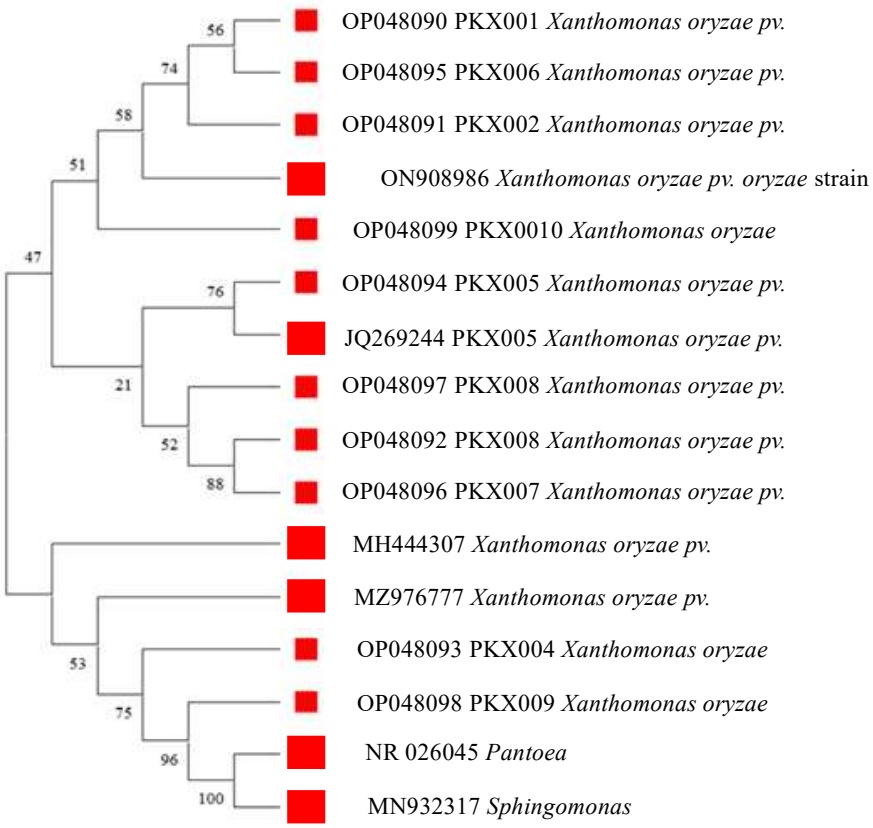


Fig 1: Phylogenetic tree construction using ClustalW analysis of 16S rRNA sequence of Indian Xoo isolates

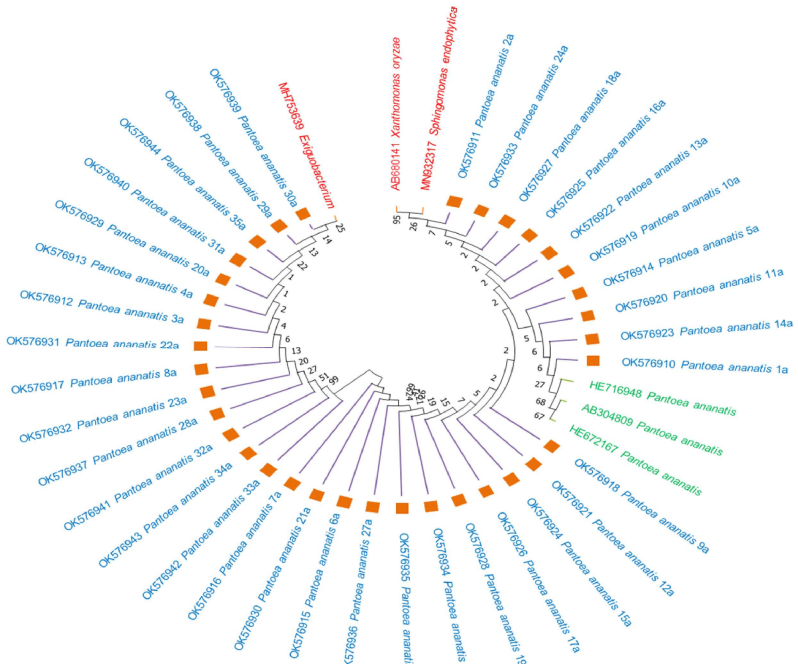


Fig 2: Phylogenetic tree construction using ClustalW analysis of 16S rRNA sequence of Indian *P. ananitis* isolates

the most common housekeeping genetic marker (Ashmawy *et al.,* 2015). 16S rRNA gene was used to identify the tested isolates and study the genetic variability among 35 isolates of *P. ananitis* and 10 isolates of *Xoo*. Results obtained from all tested isolates gave a band in the right expected molecular length. DNA sequences of tested isolates revealed that the sequences belong to *P. ananatis* and *Xoo*. The findings confirm with data from Krawczyk *et al.* (2010).

Pathotype Analysis

The phylogenetic neighbour - joining tree constructed using phenotypic data of differential reaction on differential lines divided 35 isolates of *P. ananitis* into two major clusters and a sub-cluster under each (Fig. 3). The clusters were designated as pathotypes and were classified based upon the similarity coefficient given based on differential reaction produced by the differential lines upon on inoculation with the pathogen. Pathotype I, the largest cluster, contained 19 *P. ananitis* isolates while pathotype II contained 16 *P. ananitis* isolates. Pathotype I and II were further divided into two pathotypes IA, IIA, IB and IIB (Table 1). The frequency of the isolates under pathotypes IA, IB, IIA, and IIB is 42, 11, 25 and 20 per cent respectively. The results indicate that majority of the isolates fall under pathotype IA since most of the collections were obtained from Gangavathi under the Tungabhadra command area where BB was drastic during the respective year 2020. The results indicate a strong correlation between pathotype diversity since the isolates were collected only from two command areas of Karnataka and within the cluster the isolates exhibited pathogenic diversity based on the differential reaction produced by the monogenic differential lines were in pace with results as obtained by (Mishra *et al*., 2013). Considering the individual R-genes, *Xa21* appeared as the most broadly effective, conferring resistance against 85 per cent of the isolates, followed in decreasing order by *Xa11* (54%), *Xa13* (45%), *Xa14* (45 %), *Xa8* (28 %), *Xa5* (28 %), *Xa3* (22 %), *Xa4* (20 %) (20 %), *Xa7* (14 %), *Xa10* (14 %), *Xa1* (5 %) (Fig. 4). The R-genes *Xa1, Xa3, Xa4, Xa7* and *Xa10* were the least effective against any pathotypes.

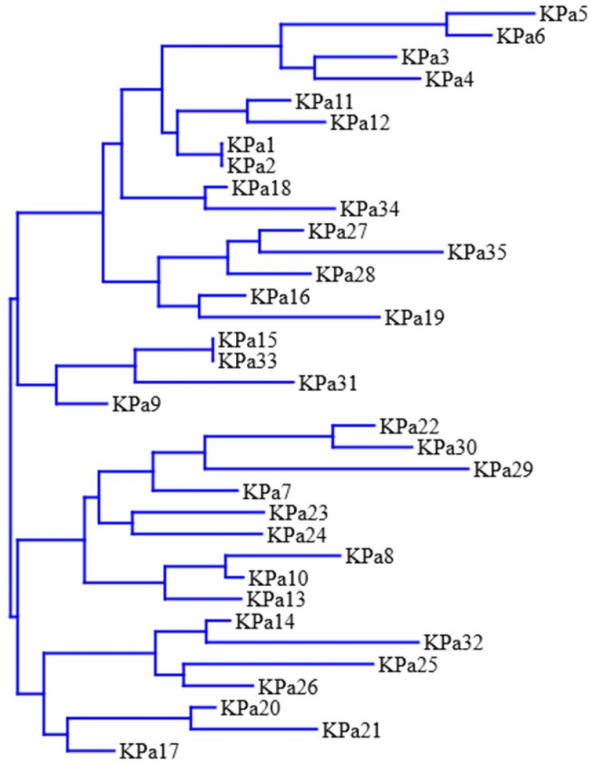


Fig. 3: Dendrogram showing the pathogenic relatedness of 35 *P. ananitis* isolates collected from Karnataka

TABLE 1

Details of the number of pathotypes and isolates belonging to each pathotype

Patho-types	No. of isolates	*Pantoea* isolates
I A	15	KPa #- 1-6, 11-12, 16, 18-19, 27-28, 34-35
I B	4	KPa #- 9, 15, 31, 33
II A	9	KPa #- 7-8, 10, 13, 22-24, 29-30
II B	7	KPa #- 14, 17, 20-21, 25-26, 32

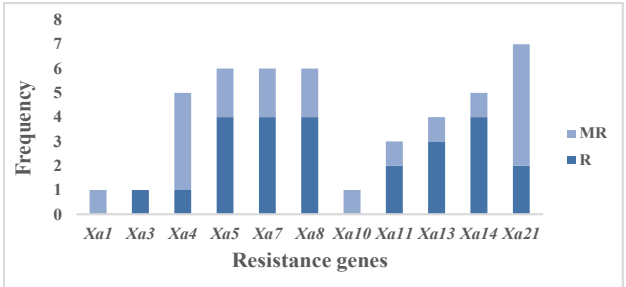


Fig. 4: Effectiveness of Xa genes against Indian *P. ananitis* isolates. The x-axis indicates resistance gene and the y-axis indicates the frequency of isolates against which this gene confers resistance

Similarly, pathotype analysis was performed for the 10 *Xoo* isolates, pathotype I is the largest cluster, containing 8 *Xoo* isolates and pathotype II contained 2 *Xoo* isolates (Fig. 5). The results did not indicate a strong correlation between pathotype diversity since the isolates were collected only from one command area of Karnataka *i.e.,* Tungabhadra command area. Within the cluster, the isolates exhibited the pathogenic diversity based on the differential reaction produced by the monogenic differential lines. Considering the individual R-genes, *Xa21* appeared as the most broadly effective, conferring resistance against 70 per cent of the isolates, followed in decreasing order by *Xa5* (60 %), *Xa7* (60 %), *Xa8* (60 %), *Xa14* (50 %), *Xa4* (50 %) *Xa13* (40 %) (Fig. 6). The R-genes *Xa1, Xa3, Xa10* and *Xa11* were least effective against any of the pathotypes. With all the pathotypes of *Xoo* and *P. ananitis, Xa21* exhibited more than 70 percent of resistance. These *Xa21*-mediated basal pathways included mainly those related to the basic material and energy metabolisms and many related to phytohormones such as cytokinin, suggesting that *Xa21* triggered redistribution of energy, phytohormones and resources among essential cellular activities before invasion thus providing broader resistance (Peng *et al*., 2015).

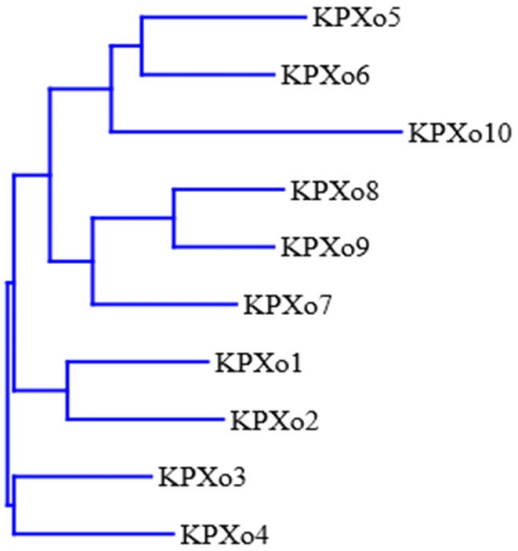


Fig. 5: Dendrogram showing the pathogenic relatedness of Xoo isolates collected from Karnataka

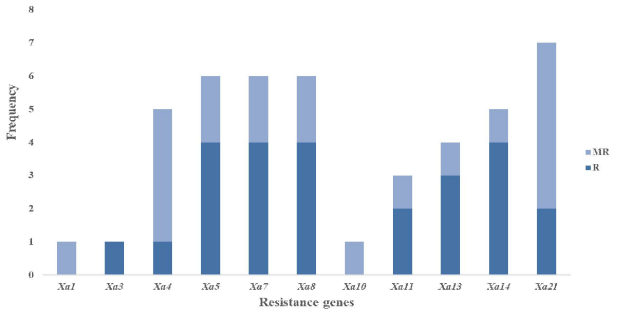


Fig. 6 : Effectiveness of Xa genes against Indian Xoo isolates. The x-axis indicates resistance gene and the y-axis indicates the frequency of isolates against which this gene confers resistance

Variation in the Production of Exopolysaccharide (EPS) by *Xoo* Isolates

The production of exopolysaccharide (EPS) is a characteristic feature of *Xanthomonads*. A biochemical assay was carried out to assess the EPS accumulation in different isolates. Isolate *Xoo* 3 produced a distinctly high amount of EPS in this study (92.3 mg) followed by *Xoo* 6, *Xoo* 8 and *Xoo* 4. On the contrary isolates *Xoo* 2 produced distinctly less amount of EPS. The isolates *Xoo* 1, *Xoo* 5 and *Xoo* 7 gave 35-55 mg dry weight of EPS (Fig. 7). To assess the severity of disease infection by different isolates, the susceptible cultivar TN1 was challenged with *Xoo*. All tested isolates caused leaf blight on leaf surface on the 6th day after inoculation. Symptoms of BB appeared on leaves as pale green to grey-green water-soaked streaks near the leaf tip and margin. These lesions coalesced and became yellowish-white with wavy edges. On the leaf sheath of susceptible cultivars, the affected leaves will turn yellow, roll up and wilt rapidly. Under greenhouse inoculation, the

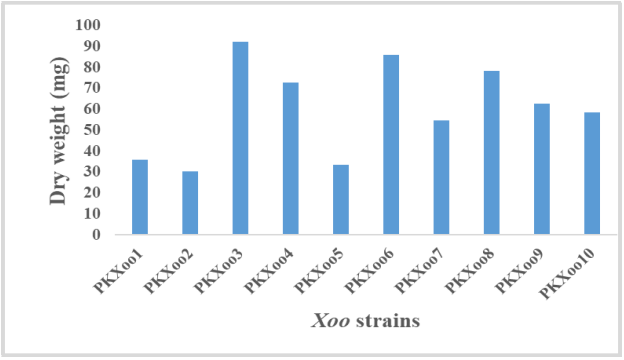
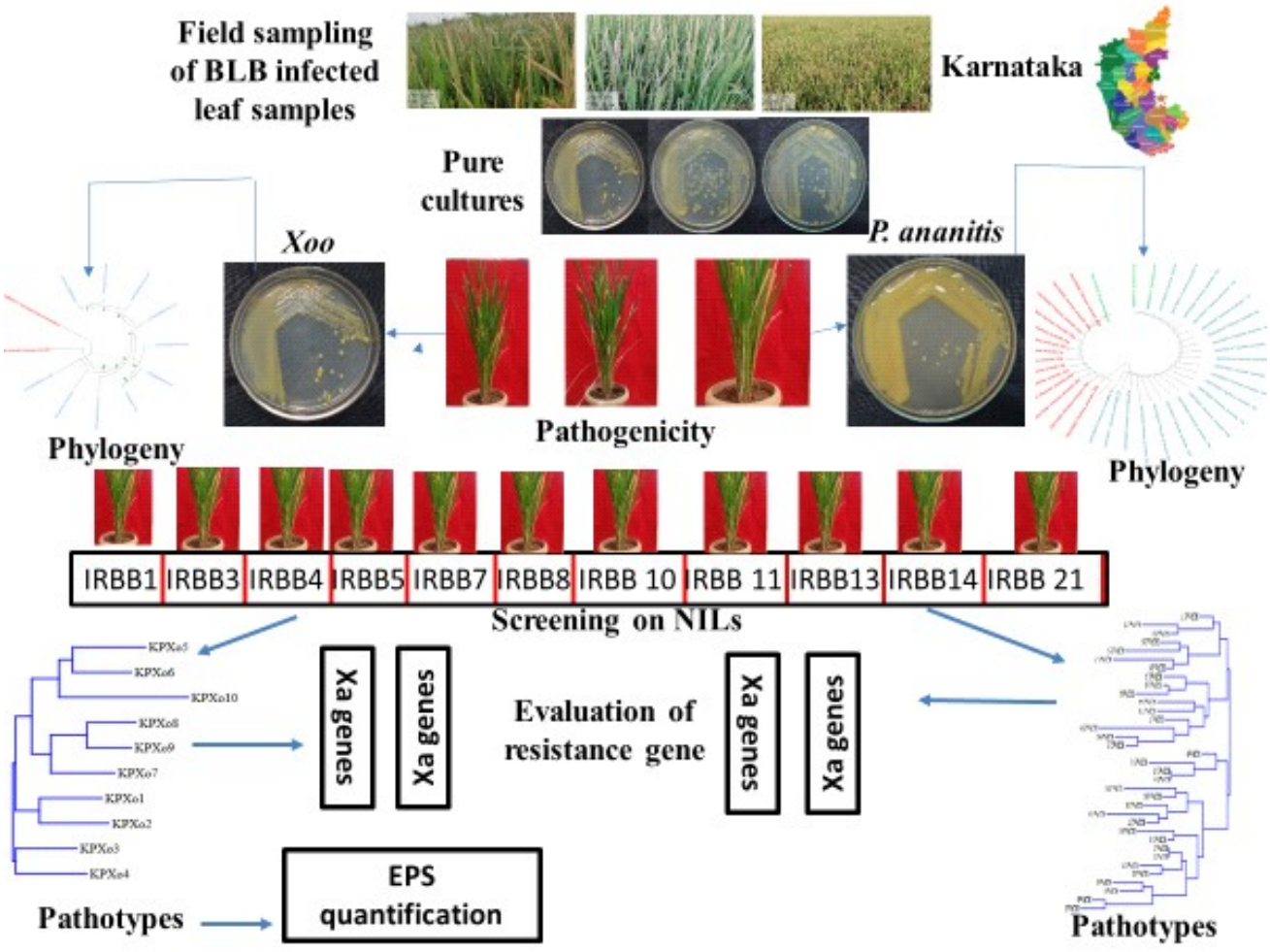


Fig. 7: EPS dry weights of ten Indian Xoo isolates

systemic infection produced tannish grey to white lesions along the vein. Differences in virulence among the *Xoo* isolates were quantified according to the lesion length of the necrotic area. *Xoo* 3, *Xoo* 6, *Xoo* 8, *Xoo* 4 and isolates were shown to cause symptoms on day 5 after inoculation while *Xoo* 7, *Xoo* 9 and *Xoo* 10 isolates were shown symptoms on 6 dpi followed by *Xoo* 1, *Xoo* 2, *Xoo* 5 on 7 dpi. It also showed the maximum disease development and increased virulence to susceptible rice cultivar TN-1 at 21 d post inoculation (Fig. 8). The average lesion length was 10-15 cm at 21 d post inoculation. Isolate *Xoo* 2 showed the lowest lesion length and *Xoo* 3 was the highest lesion length at day 21 post inoculation. The isolate *Xoo* 3 revealed a high yield of EPS which

Fig. 8: Leaf phenotype of TN-1 showing increased susceptibility to pathogen isolates, lesion length count in cm up to 21 dpi, leaves were photographed on 21 days after inoculation



Schematic representation of sampling of infected BLB leaf samples, screening on nearly isogenic lines, grouping of isolates in to pathotypes and EPS quantification

is further evident by the higher virulence of *Xoo* that shows the aggressiveness in the disease severity index. On contrary with *Xoo* 2 revealed a low yield of EPS with less aggressiveness in the disease index (Bakade and Kumar, 2020). The screening of isolates for virulence-related genes of *Xoo* needs further evaluation toward revealing the complex and overlapped bacterial pathogenesis mechanisms, especially in the early stage of infection.

In conclusion, while the most commonly reported causal agents for leaf blight disease in rice are still the various *Xoo* strains, the involvement of *Pantoea* spp. in also causing the disease cannot be ignored. The various strains of *Xoo* have been extensively studied and that will help to incorporate genes conferring resistance to *Xoo* associated leaf blight. The recent findings of the association of *Pantoea* spp. as a pathogen in causing leaf blight create new challenges in combating this disease (Doni *et al*., 2021). Although the full impacts of this new pathogenic species are yet to be determined, a thorough investigation of the role of *Pantoea* spp. such as the range of symptoms, mode of infection, infestation, pathogenicity, genetics and evolutionary shift from *Xoo,* will be crucial for ensuring a successful control and management of *Pantoea* spp. associated leaf blight.

Acknowledgement : The authors would like to express gratitude to the Dr. Pramesh Devanna, Scientist, Rice Pathology, Agriculture Research Station, Gangavathi, Karnataka for providing necessary help during collection of the samples.

References

Ashmawy, N. A., Jadalla, N. M., Shoeib, A. A. and El-Bebany, A. F., 2015, Identification and genetic characterization of *Pectobacterium* spp. and related Enterobacteriaceae causing potato soft rot diseases. *Egyp. J. Pure Appl. Microbiol*., **9** (3) : 1847 - 1858.

Azizi, M. M. F., Ismail, S. I., Ina-Salwany, M. Y., Hata, E. M. and Zulperi, D., 2020, The emergence of *Pantoea* species as a future threat to global rice production. *J. Plant Prot. Res.,* **60** : 327 - 335.

Bakade, R. and Prasanna Kumar, M. K., 2020, Variation in virulence in *Xanthomonas oryzae* pv. *oryzae* isolates collected from South India. *Int. J. Curr. Microbiol. App. Sci.,* **9** (12) : 425 - 436.

Bhapkar, D. G., Kulkarni, N. B. and Chavan, V. M., 1960, Bacterial blight of paddy. Pune Agricultural College Magazine, **51** : 36 - 46.

Chauhan, H. L., 1973, Studies on bacterial leaf blight of paddy (*Oryza sativa* L.) caused by *Xanthomonas oryzae* (Uyeda & Ishiyama) M.Sc. (Agri.) Thesis (pub.) Sardarkrushinagar Dantiwada Agric. Univ., Dantiwada.

Chien, C. C., Chou, M. Y., Chen, C. Y. and Shih, M. C., 2019, Analysis of genetic diversity of *Xanthomonas oryzae* pv. *oryzae* populations in Taiwan. *Sci. Rep.,* **9** : 316 - 329.

Chukwu, S. C., Rafi, M. Y., Ramlee, S. I., Ismail, S. I., Hasan, M. M., Oladosu, Y. A., Magaji, U. G., Akos, I. and Olalekan, K. K., 2019, Bacterial leaf blight resistance in rice: a review of conventional breeding to molecular approach. *Mol. Biol. Rep.* **46**: 1519 - 1532.

Deletoile, A., Decre, D., Courant, S., Passet, V., Audo, J., Grimont, P., Arlet, G. and Brisse, S., 2009, Phylogeny and identification of *Pantoea* species and typing of *Pantoea agglomerans* strains by multilocus gene sequencing. *J. Clin. Microbiol.,* **47** (2) : 300 - 310.

Doni, F., Suhaimi, N. S. M., Mohamed, Z., Ishak, N. and Mispan, M. S., 2019, *Pantoea*: A newly identified causative agent for leaf blight disease in rice. *J. Plant Dis. Prot.*, **126** : 491 - 494.

Doni, F., Suhaimi, N. S. M., Irawan, B., Mohamed, Z. and Mispan, M. S., 2021, Associations of Pantoea with rice plants: As friends or foes?. *Agriculture*, **11** (12) : 1278 - 1290.

Jeong, K. S., Lee, S. E., Han, J. W., Yang, S. U., Lee, B. M., Noh, T. H. and Cha, J. S., 2008, Virulence reduction and differing regulation of virulence genes in mutants of rpf *Xanthomonas oryzae* pv. *oryzae. Plant Pathol. J.,* **24** : 143 - 151.

Kalyan, K. M., 2013, Advancements in the diagnosis of bacterial plant pathogens: An overview. *Biotechnol. Mol. Biol. Rev*., **8** (1), Pp. : 1 - 11.

Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y. and Merca, S. D., 1973, An improved technique for evaluating

resistance of rice varieties to *Xanthomonas oryzae* pv. *oryzae. Plant Diseases. Rep*., **57** : 537 - 541.

Krawczyk, K., Kamasa, J., Zwolinska, A. and Pospieszny, H., 2010, First report of *Pantoea ananatis* associated with leaf spot disease of maize in Poland. *J. Plant Pathol.,* **12** (6) : 807 - 811.

Laha, G. S., Reddy, C. S., Krishnaveni, D., Sundaram, R. M., Srinivas, P. M. and Ram, T., 2009, Bacterial blight of rice and its management. DRR Technical Bulletin, No. 41, Directorate of Rice Research (ICAR), Hyderabad, (India).

Laha, G. S., Virakthamath, B. C. and Priyanka, 2009, Bacterial blight of rice and its management. Technical Bulletin No.4. Directorate of Rice Research (ICAR), Hyderabad (India).

Lee, H. B., Hong, J. P. and Kim, S. B., 2010, First report of leaf blight caused by *Pantoea agglomerans* on rice in Korea. *Plant Dis.,* **94** : 1372 - 1386.

Mew, T. W., Alvarez, A. M., Leach, J. E. and Swings, J., 1993. Focus on bacterial blight of rice. *Plant Dis*., **77** : 5 - 12.

Mishra, D., Vishnupriya, M. R., Anil, M. G., Kotilingam, K., Raj, Y. and Sonti, R. V., 2013, Pathotype and Genetic diversity amongst Indian isolates of *Xanthomonas oryzae pv. oryzae*. *PLoS One*, **8** : e81996.

Mondal, K. K., Mani, C., Singh, J., Kim, J. G. and Mudgett, M. B., 2011, A new leaf blight of rice caused by *Pantoea ananatis* in India. *Plant Dis*., **95** : 1582 - 1594.

Noda, T. and Kaku, H., 1999, Growth of *Xanthomonas oryzae* pv. *oryzae* in planta and in guttation fluid of rice. *Ann. Phytopathol. Soc. Jpn.,* **65** : 9 - 14.

Ou, S. H., 1985, Rice Diseases. 2nd Edition. Common wealth Mycological Institute, UK, Pp. : 61 - 96.

Peng, H., Chen, Z., Fang, Z., Zhou, J., Xia, Z., Gao, L., Chen, L., Li, L., Li, T., Zhai, W. and Zhang, W., 2015, Rice *Xa21* primed genes and pathways that are critical for combating bacterial blight infection. *Sci. Rep*., **5** (1) : 1 - 12.

Quibod, I. L., Quintero, A. P., Booher, N. J., Dossa, G. S., Grande, G., Szurek, B., Cruz, C. V., Bogdanove, A. J. and Oliva, R., 2016, Effector diversification contributes to *Xanthomonas oryzae pv. oryzae* phenotypic adaptation in a semi-isolated environment. *Sci. Rep.,* **6** : 341 - 362.

Salzberg, S. L., Sommer, D. D., Schatz, M. C., Phillippy, A. M., Rabinowicz, P. D., Tsuge, S., Furutani, A., Ochiai, H., Delcher, A. L., Kelley, D., Madupu, R., Puiu, D., Radune, D., Shumway, M., Trapnell, C., Aparna, G., Jha, G., Pandey, A., Patil, P. B., Ishihara, H., Meyer, D. F., Szurek, B., Verdier, V., Koebnik, R., Dow, J. M., Ryan, R. P., Hirata, H., Tsuyumu, S., Won Lee, S., Seo, Y. S., Sriariyanum, M., Ronald, P. C., Sonti, R. V., Van Sluys, M. A., Leach, J. E., White, F. F. and Bogdanove, A. J., 2008, Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99A. *BMC Genom*., **204** : 1 - 16.

Toh, W. K., Loh, P. C. and Wong, H. L., 2019, First report of leaf blight of rice caused by *Pantoea ananatis* and *Pantoea dispersa* in Malaysia. *Plant Dis*., **103** : 7 - 19.

Yugander, A., Sundaram, R. M., Ladhalakshmi, D., Hajira, S. K., Prakasam, V. and Prasad, M. S., 2017, Virulence profiling of *Xanthomonas oryzae* pv. *oryzae* isolates, causing bacterial blight of rice in India. *Eur. J. Plant Pathol*., **149** : 171 - 191.

Yugander, A., Sundaram, R. M., Singh, K., Ladhalakshmi, D., Subbarao, L. V. and Madhav, M. S., 2018, Incorporation of the novel bacterial blight resistance gene *Xa38* into the genetic background of elite rice variety Improved Samba Mahsuri. *PLOS One*, **13** : e0198260.

Yugander, A., Ershad, M., Muthuraman, P. P., Prakasam, V., Ladhalakshmi, D., Sheshu Madhav, M., Srinivas Prasad, M., Sundaram, R. M. and Laha, G. S., 2022, Understanding the variability of rice bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* in Andhra Pradesh, India. *J. Basic Microbiol*., **62** (2) : 185 - 196.

Zhang, Y. P., Uyemoto, J. K. and Kirkpatrick, B. C., 1998, A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. *J. Virol. Methods*, **71** (1) : 45 - 50.