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Distribution of *Xanthomonas oryzae* pv. *oryzae* Pathotypes in Basmati-Rice-Growing Areas of Jammu and Kashmir, India

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Abstract: Rice is an important staple food for more than half of the world's population. Though the genetic potential of commonly cultivated varieties of rice is diminished due to various biotic and abiotic constraints, bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is considered one of its most destructive diseases in India. Based on morpho-cultural characteristics, bacterial pathogens isolated from the leaves of a rice plant showing typical BLB symptoms were identified as *Xanthomonas oryzae* pv. *oryzae*. Morphological studies revealed that the pathogen is Gram-negative, a short rod, with rounded ends, single or in pairs, light yellow, circular, whitish yellow to straw-colored, convex, yellow, slightly raised, motile with a single polar flagellum, capsulate and non-spore-forming. Biochemical tests, viz., the Gram reaction, KOH test and catalyst test, showed a positive reaction for all the isolates. Twenty isolates of *Xoo* were collected from the basmati-growing areas of the Jammu, Samba and Kathua districts in the Jammu sub-tropics during 2019, and their pathogenicity was confirmed on five susceptible rice cultivars, viz., Basmati-370, Pusa-1121, TN-1, SJR and Jaya, by the leaf-clipping method, and subsequently, Koch's postulate was established in each case. Seven *Xoo* pathotypes, viz., Pathotype 1, Pathotype 2, Pathotype 3, Pathotype 4, Pathotype 5, Pathotype 6 and Pathotype 7, were identified from the total sample of 20 isolates. Pathotype 2 was the most dominant (100%), followed by Pathotype 5 (44.44%), Pathotype 4 (40%), Pathotype 6 (40%), Pathotype 7 (33.33%), Pathotype 3 (22.22%) and Pathotype 1 (20%), in the Jammu sub-tropics. In Jammu district, Pathotype 5 was highly distributed (44.44%) followed by Pathotype 7 (33.33%) and Pathotype 3 (22.22%). Pathotype 4 and Pathotype 6 each showed a 40 percent distribution in Kathua district, followed by Pathotype 1 (20%). Only one pathotype, i.e., Pathotype 2, was recorded in Samba district with a 100 percent distribution. Five genes, viz., Xa13, Xa4, Xa13 and Xa5 + Xa13, showed complete resistance, whereas Xa4, Xa5, Xa7, Xa8, Xa21, Xa4 + Xa5 and Xa4 + Xa21 showed susceptible response against the test isolates. It was observed that most of the single BLB-resistant genes were moderately to highly susceptible to almost all the *Xoo* isolates, whereas combinations of BLB resistance genes possessed high resistance against all the *Xoo* isolates. The studies revealed that diverse pathogenic variations existed in the *Xoo* population in the basmati-growing region of Jammu and Kashmir. Based on the response exhibited by *Xoo* isolates on differential lines, seven pathotypes (Pathotype 1–7) were identified, and their virulence spectrum on rice differentials showed the occurrence of 5, 3, 10, 10, 20, 10 and 15 percent, respectively, in the Jammu sub-tropics. To develop

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durable and sustainable resistant cultivars, it is essential to identify predominate race(s) in a specific geographical area and continuously monitor the virulence pattern there.

Keywords: basmati rice; bacterial leaf blight; distribution; pathotypes; *Xanthomonas oryzae* pv. *oryzae*

1. Introduction

Basmati rice is described as 'scented pearls' because of its distinct and pleasant aroma after cooking, fluffy texture, sweetness, easy digestibility and longer shelf life [1,2]. Geographical specialty and escalating consumer demands in national and international markets bring enormous profitability to basmati rice growers [3]. In the Indian subcontinent, it has prominently been cultivated for the last 250 years [4–6]. During 2020–2021, India exported 4.63 million tonnes of basmati rice, thereby earning a foreign exchequer of USD 4019 million [7]. The unique quality characteristics of basmati rice are attained only when it is grown in the north-western foothills of the Himalayas [5]; therefore, its cultivation is mainly confined to Haryana, Punjab, Uttar Pradesh, Jammu and Kashmir, Uttarakhand and Himachal Pradesh [5], which contribute 41.88 and 42.56%; 36.13 and 39.09%; 16.58 and 14.53%; 4.10 and 2.69%; 0.99 and 0.74%; and 0.46 and 0.40% of area and production, respectively [8]. In the Union Territory of Jammu and Kashmir, it is grown over an area of 60.50 thousand hectares, out of which Jammu, Kathua and Samba districts have 40.50, 9.20 and 10.80 thousand hectares, respectively [9]. Cultivation of basmati rice is a major source of livelihood for the growers and also a source of the foreign exchequer for Jammu and Kashmir.

Among the different biotic and abiotic stress, bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a devastating disease because of its wide geographic distribution and destructiveness under favorable conditions [10,11]. It was first reported in Fukuoka Prefecture, Kyushu Island, Japan, in 1884–1885, and thereafter, the disease gradually spread throughout Japan in the 1950s, with high incidence in Kyushu Island [12]. It is a potentially devastating disease found worldwide in temperate and tropical regions, destroying up to 80 percent of crop, if the disease develops early in the season. Even if it develops late, it can severely diminish the quality and yield of the grain. In general, the disease is responsible for a yield loss of 20–50%, depending upon the stage of the crop, severity of infection, prevailing weather conditions and response of cultivars [13]. The disease has become a serious threat to rice production in South East Asia causing 25–30% yield losses in Japan [11]; 50% in Malaysia [14]; 20–80% in Indonesia [15]; 20–40% in Bangladesh [16]; 30–50% in the Philippines [17]; 50–80% in Mali [18]; 30–50% in China [19]; and 20–80% in India [20]. Infection at the tillering stage resulted in 100 percent crop failure [21]. In India, BLB was first reported in the Koloba district of Maharashtra in 1959; however, the disease gained importance with its epidemic outbreak in the Shahabad (Bhojpur) district of Bihar in 1963 [22]. *Xoo* enters the host through wounds, stomatal openings or hydathodes [23,24]. It proliferates in the epitheme, the tissue connecting the hydathodes to the xylem, and then the pathogen moves to the xylem vessels, multiplies and causes systemic infection [25,26]. To initiate the disease in rice, *Xoo* uses transcription activator-like effector (TALE) virulence proteins that are transported through the type III secretion system (TTSS) [27], which helps in overcoming PAMP (pathogen-associated molecular pattern)-triggered plant immunity [28–31].

BLB in basmati rice production can be managed by the application of synthetic chemicals [32–35], but due to their residual effects, their application is not advisable [36]. The use of biocontrol agents has gained importance [15,28,30,37], but their inconsistent field efficiency and meager commercialization have resulted in their slow adoption among farmers. Therefore, the deployment of resistant varieties emerges as an economically viable, environmentally safe, durable and promising strategy to manage BLB [38–40]. However, the durability of resistance in the field is short-lived, as it succumbs to the prevalence and

outbreak of various *Xoo* races. New pathotypes of the *Xoo* continue to evolve and overcome the resistance of the commonly grown cultivars. There is evidence of genome plasticity and rapid evolution within *Xoo*, resulting in its genomic variation and strain-specific adaptations, which explains the extraordinary diversity of *Xoo* genotypes and races [24,41,42]. The cultivated rice varieties facilitate the race shift and result in the emergence of new races. Out of 1024 strains of *Xoo*, eleven pathotypes have been identified in India [43], out of which seven distinct pathotypes (PbXo1 to PbXo-7) are from Punjab [44], seven from Andaman Islands [45] and ten from Andhra Pradesh [46]. For exploring new resistance genes and to develop efficient breeding programs, the identification of prevailing *Xoo* pathotypes in a specific geographical location and understanding their genetic makeup, pathogenicity, variability and host–pathogen interactions are pre-requisites [18]. The durability and efficiency of the deployed resistance genes in basmati rice cultivars for the management of BLB in Jammu and Kashmir lack proper characterization, and no specific data have been documented in this regard. Moreover, the epidemiology of BLB is still unexplored in the old traditional cultivars (Basmati 370 and Ranbir Basmati) possessing tall stature and thin stems. Considering the importance of basmati rice in Jammu and Kashmir and the severity and significance of damage caused by BLB, the present work was undertaken with the aim to develop the knowledge on the occurrence of *Xoo* in basmati-rice-growing areas and the response of basmati cultivars to BLB and to elucidate the diversity of *Xoo* strains, their structure and distribution. The study will be helpful in the identification of resistance genes, which can be incorporated in the development of a significant agro-morphologically superior source for their utilization in breeding programs in future and evolving disease management strategies against BLB.

2. Materials and Methods

The experiments under the present investigation on bacterial leaf blight (BLB) of rice were conducted at Advanced Centre for Horticulture Research, SKUAST-Jammu, Udheywala (32.7436° N, 74.8120° E), during 2020–2021.

2.1. Collection of Samples

Rice leaf samples showing typical BLB symptoms were collected from the farmers' fields in different basmati-producing zones of Union Territory of Jammu and Kashmir (Jammu, Samba and Kathua districts), during kharif season of 2019–2020, through simple random sampling (Figure 1). The typical symptoms included water-soaked yellow lesions with wavy margins on leaf blades, having characteristic bacterial ooze on the cut end of young lesions. Utmost care was taken while collecting the disease samples that showed young and progressive lesions. Each disease sample was tagged with information regarding collection date, site (location) and variety. The collected samples were carefully wrapped in blotting paper, put in a paper envelope, brought to the laboratory and kept at 4 °C until isolation of the pathogen was performed for further studies.

2.2. Isolation of Xanthomonas oryzae pv. oryzae

For the isolation of pathogens associated with BLB, the infected rice leaves were initially subjected to ooze test. A total of 20 samples were analyzed for the isolation of bacterium. Young disease lesions were cut into small bits (5 cm), and surface was disinfected with sodium hypochloride (0.1%) for 30 s and thrice rinsed thoroughly with sterile distilled water in order to neutralize the effect of chemical used for surface sterilization [47,48]. The bits were then blot-dried and transferred individually on sterilized watch glass containing 5 mL of sterilized distilled water under aseptic conditions. The infected bits were cut into 2 halves with sterilized scalpel and left for 10 min to permit the diffusion of bacterial cells from infected tissue into the water drops. A loopful of suspension was streaked quadrantally on sterilized Petri plates containing nutrient agar (NA) medium (Hi-media) with the help of sterilized inoculation needle. The inoculated plates were incubated in BOD at 27 ± 2 °C for 3–5 days. The bacterial colonies developed in the shape of straw

yellow droplets, from which single fresh and pure colony per sample was picked up with sterilized inoculation needle and transferred on slants having NA medium. Such culturally identical single colony was then transferred on NA medium slants, stored in a refrigerator at 4 °C for further experiments and maintained periodically, by the routine transfers after every fortnight.

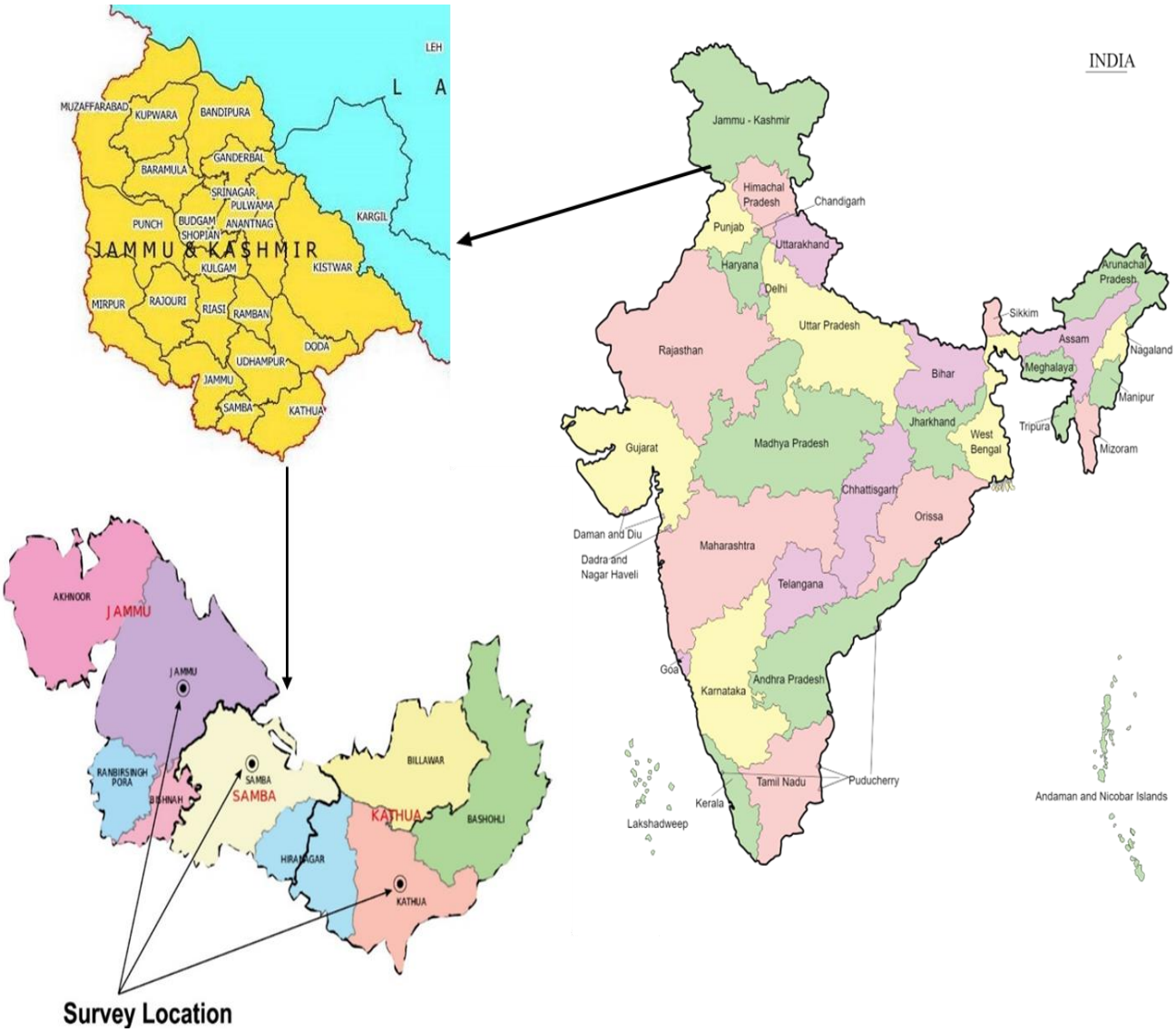

Figure 1. Collection of bacterial leaf blight samples from basmati-rice-growing area of Jammu.

2.3. Identification of Xanthomonas oryzae pv. oryzae

Pure cultures of all the 20 *Xoo* isolates were subjected to morphological, biochemical and molecular assays for identification.

2.3.1. Colony and Biochemical Characterization

All the *Xoo* isolates were examined for their colony characteristics on NA medium after an incubation period of 48 h at 28 ± 2 °C. Colony characteristics such as colony color, shape and nature were recorded. The isolates were further subjected to Gram reaction [48]. Bacterial smears were prepared on glass slides taken from 24 h old colonies, grown on NA medium. The smears were first heat-fixed; then, for 30 s, they were stained with crystal violet, followed by washing with distilled water. Prior to rinsing slightly with ethyl alcohol, Gram's iodine was applied on smears for one minute; later, safranin was applied on smears

for 30 s, followed by washing of smears with sterilized distilled water. The slides were air-dried, and a drop of immersion oil was placed on each smear and observed under 100× magnification of binocular microscope for Gram reactions. Further, potassium hydroxide (KOH) assay for the validation of Gram staining was conducted in which culture of each *Xoo* isolate (48 h old) was selected with sterilized toothpick and blended with a drop of KOH (3%) solution on sterilized glass slide under aseptic conditions [49]. For catalase assay, nutrient agar slants were inoculated aseptically with the 24 h old culture of the *Xoo* isolates and incubated at 28 + 20 °C for 24 h. After incubation, the slants were mixed with 5 drops of 3% hydrogen peroxide and observed for the formation of gas bubbles [50]. For esterase activity, sterilized Tween 80 @ 10 mL/liter was added aseptically just before pouring Sierra's medium into the Petri plates. *Xoo* isolates were spot-inoculated on the Petri plates and incubated for 7 days at 28 ± 2 °C [51]. Production of esterase was confirmed by the formation of opaque zones around the colonies.

2.3.2. Molecular Identification

Molecular identification was performed by sequencing the 16S rRNA region using universal primers [52]. *Xoo* isolates were grown overnight on Luria Bertani broth medium (Hi-media) at 37 °C and pelleted at 12,000 rpm. Total genomic DNA of each isolate was extracted from the resulting pellet using kit (QIAGEN) by following manufacturers' protocol. The purified genomic DNA was used as template to amplify 16S rRNA region using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTTA-3′) by polymerase chain reaction (PCR) using a thermocycler. The reaction mixture contained 100 ng of DNA, 2.0 µL 10X Buffer (with $MgCl_2$), 2.0 µL deoxy-nucleoside tri-phosphate (dNTPs) 2 mM, 2.0 µL each primer (5 µM) and 2 U of Taq polymerase (R001C TaKaRaTaq™) for a total volume of 20 µL. The reaction mixture was subjected to following temperature cycling profile: initial denaturation for 3 min at 95 °C, 32 cycles (denaturation, 30 s at 95 °C; annealing, 30 s at 55 °C; extension, 1 min at 72 °C) and 1 final extension cycle at 72 °C for 10 min. Amplified PCR product was analyzed on 1.5% agarose (Tris-Cl-sodium acetate-EDTA) stained with ethidium bromide and visualized under UV illumination. Bands were excised and eluted using Gel extraction kit (MinElute, QIAGEN) and sequenced using ABI 3130xl Genetic Analyzer (ABI, USA) as per manufacturers' instructions. The resulting nucleotide sequence was compared with those in the public database NCBI GenBank using Basic Local alignment Tool (BLASTn) to identify closely related sequences. Further, preparation of phylogenetic tree was performed using Molecular Evolutionary Genetics Analysis (MEGA 11) software [53].

2.3.3. Pathogenicity Assay

The pathogenicity test for confirming Koch's postulates and to determine the virulence of all the *Xoo* isolates, collected from different basmati-growing locations, were conducted on five susceptible basmati and non-basmati rice cultivars, viz., Basmati-370, Pusa-1121, TN-1, SJR and Jaya. Two-week-old seedlings of each cultivar were transplanted into plastic pots (25 × 14 cm) containing garden soil with decomposed farmyard manure (3:1). The potted plants were kept in the shade net house, and the experiment was conducted with completely randomized design with four replications. Inoculum of each *Xoo* isolate (10^7–10^8 cfu/mL) was prepared by suspending 50 mL distilled water in 48-day culture, grown on NA medium at 28 ± 2 °C. Three leaves of six-week-old rice seedlings of each cultivar were clipped off by sterilized scissor pre-dipped in the suspension of inoculum, and then the cut ends of the leaves were also submerged in the *Xoo* inoculum for 30 s. [54]. After inoculation, the pots were filled with water to maximize the relative humidity. The plants were watered at regular intervals and observed for disease development. Observations regarding the development of lesion length (cm) after 15 days of inoculation were recorded. After the development of typical symptoms on the inoculated potted plants, infected young leaves were again selected for the re-isolation of the pathogen under aseptic conditions on NA medium and then incubated in BOD at 28 ± 2 °C for the development of bacterium.

Single, yellow, separate, circular and semi-transparent colonies developed on NA medium in 48 h of incubation were picked and purified by re-streaking a pure bacterial colony on the NA medium in the Petri plates. The re-isolated pathogen from inoculated plants was then compared with the original culture to confirm Koch's postulates by various biochemical tests.

2.4. Virulence Profiling of Xanthomonas oryzae pv. oryzae Isolates

The virulence analysis of *Xoo* isolates was performed on a set of rice differentials (IRBB lines), which are near-isogenic lines (NILs) carrying different BLB resistance genes either singly or in different combinations, along with highly susceptible check TN1 (Table 1). Seeds of rice differentials were obtained from the All India Coordinated Rice Improvement Programme (AICRIP) Centre, Division of Genetics and Plant Breeding, Faculty of Agriculture, SKUAST-Jammu, Chatha. Under greenhouse conditions, all the differentials including susceptible check were sown directly in plastic pots (17 × 15 cm). Each pot was filled with three parts of soil and one-part farm yard manure (Figure 2). The soil in pots was thoroughly mixed and leveled properly, and all the differentials along with TN 1 were directly sown in the pots. For each differential, 3–4 plants were raised in each pot, and sufficient care was taken to raise healthy and vigorous plants. Fertilizers were also added to the pots as per the recommendations of SKUAST-Jammu, Packages of Practices, and irrigated every day [55]. Three-day-old cultures of each *Xoo* isolate, multiplied on nutrient broth medium (Hi-media), were taken to prepare suspension containing 10^7–10^8 cfu/mL. Inoculation was mainly performed at tillering stage (60 days after sowing) by clip inoculation with modification [54]. For each differential and susceptible check, 4–6 healthy, fully expanded leaves were inoculated for each strain–cultivar combination. The observations were recorded under pot and field experiments after 15 days of inoculation by measuring the lesion length (cm) using a graduation ruler by adopting SES scale [56] and using the standard area diagram [57]. Three observations were taken for each isolate on differentials along with susceptible check (16 nos.), and then average affected lesion area was calculated. Affected lesion area of up to 5% was taken as resistant (R), 6–12% as moderately resistant (MR), 13–25% as moderately susceptible (MS), 26–50% as susceptible (S) and 51–100% as highly susceptible [56]. For virulence characterization of *Xoo* isolates, lesion length up to 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). The isolates were then characterized into different groups based on their reactions (R, MR, MS, S and HS) on different differentials for pathotype designation [58].

Table 1. List of differentials used for studying virulence dynamics of *Xanthomonas oryzae* pv. *oryzae*.

S. No.	Differential Host	Gene	S. No.	Differential	Gene(s)
1	IRBB1	*Xa*1	9	IRBB13	*Xa*13
2	IRBB 3	*Xa*3	10	IRBB14	*Xa*14
3	IRBB4	*Xa*4	11	IRBB21	*Xa*21
4	IRBB5	*Xa*5	12	IRBB50	*Xa*4 + *Xa*5
5	IRBB7	*Xa* 7	13	IRBB51	*Xa*4 + *Xa*13
6	IRBB8	*Xa*8	14	IRBB 52	*Xa*4 + *Xa*21
7	IRBB10	*Xa*10	15	IRBB 53	*Xa*5 + *Xa*13
8	IRBB11	*Xa*11	16	TN1	Susceptible check

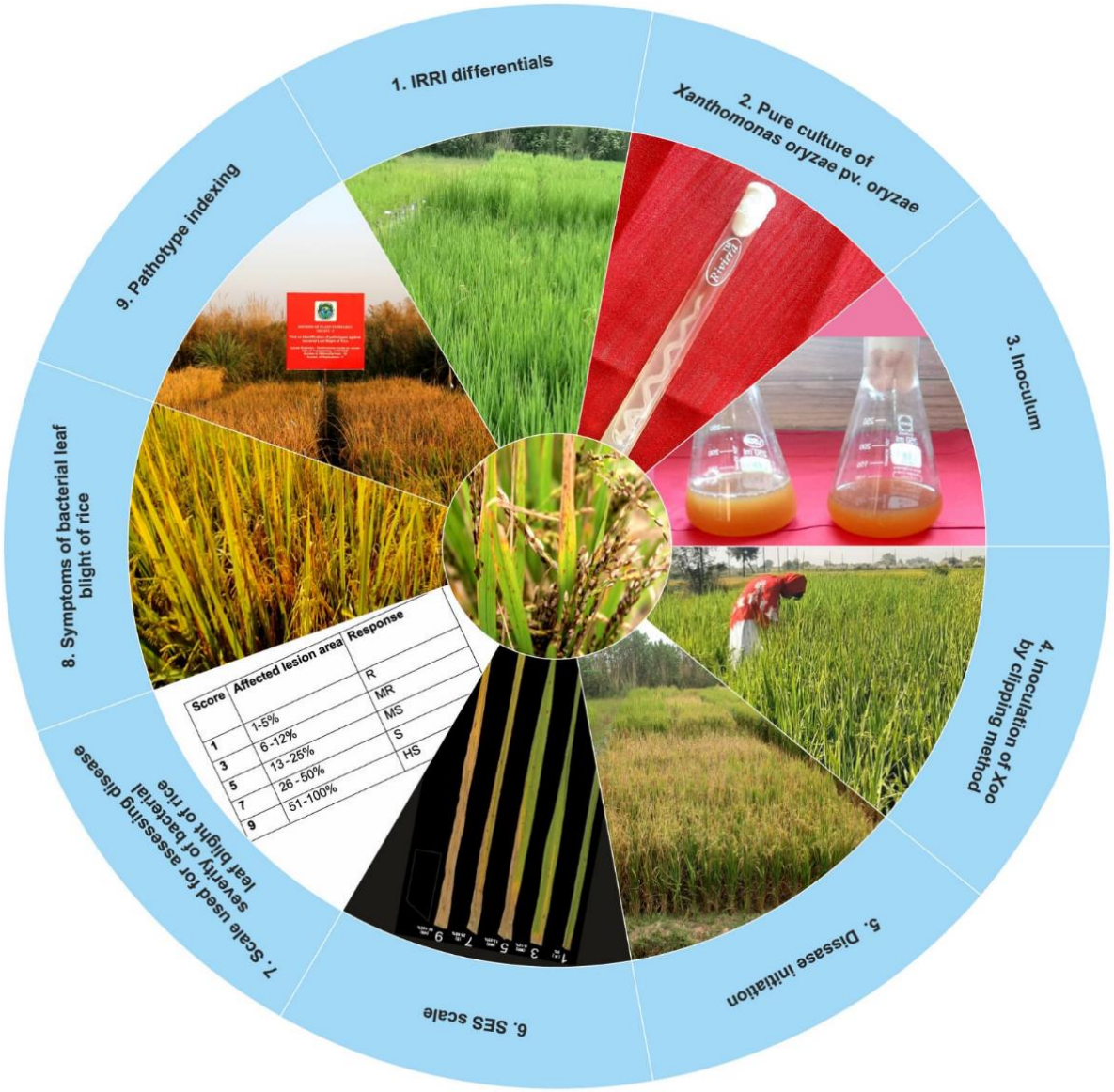

Figure 2. Detailed methodology adopted for pathotype indexing of *Xanthomonas oryzae* pv. *oryzae isolates* collected from basmati-rice-growing area of Jammu.

2.5. Virulence Frequencies of Xanthomonas oryzae pv. oryzae

Virulence frequency (%) of *Xoo* isolates was analyzed from the ratio of the number of *Xoo* isolates of each pathotype (representative strains) encountered and the total number of *Xoo* isolates analyzed [59].

2.6. Statistical Analysis

The data regarding lesion length (cm) of different *Xoo* isolates and their response to 16 tested lines under greenhouse and field conditions were represented in heat map generated by ggplot2 package [60].

3. Results

3.1. Isolation of Xanthomonas oryzae pv. oryzae Collected from Basmati-Rice-Growing Areas

Xanthomonas oryzae pv. *oryzae* (*Xoo*) was isolated on nutrient agar (NA) from the infected basmati rice leaves showing characteristic symptoms of bacterial leaf blight (BLB). One leaf per sample was selected for bacterial isolation, from different basmati cultivars, collected from various basmati-growing locations in Jammu Division (Table 2). The *Xoo*

isolates comprised *Xoo*-1 and *Xoo*-2 (Pusa 1121 and Pusa 1637) from Chatha, *Xoo*-3 and *Xoo*-4 (Basmati 370 and Ranbir Basmati) from R.S. Pura, *Xoo*-5 and *Xoo*-6 (Pusa 1728 and Basmati 564) from Marh and *Xoo*-7, *Xoo*-8 and *Xoo*-9 (Pusa 1637, Basmati 564 and Basmati 370) from Bishnah of Jammu district. Isolates *Xoo*-10, *Xoo*-11 and *Xoo*-12 (Pusa 1728, Pusa 1121 and Pusa1637) were from Vijaypur, *Xoo*-13 and *Xoo*-14 (Basmati 370 and Basmati 564) from Samba and *Xoo*-15 (Pusa 1121) from Ghagwal of Samba district. *Xoo*-16 and *Xoo*-17 (Jammu Basmati 138 and Jammu Basmati 123) were from Hiranagar, *Xoo*-18 (Basmati 370) from Nagri and *Xoo*-19 and *Xoo*-20 (Pusa 1121 and Basmati 370) were from Kathua representing Kathua district, Jammu Division. Colonies were purified by streaking the respective isolates on NA, and pure cultures were maintained on NA slants for further studies.

Table 2. *Xanthomonas oryzae* pv. *oryzae* isolates collected from Jammu sub-tropics.

District	Location	No. of Isolates Collected	Variety(ies)	Isolate(s)
Jammu	Chatha	2	Pusa Basmati 1121 and Pusa Basmati 1637	*Xoo*-1, *Xoo*-2
	R S Pura	2	Basmati 370 and Ranbir Basmati	*Xoo*-3, *Xoo*-4
	Marh	2	Pusa Basmati 1728 and Basmati 564	*Xoo*-5, *Xoo*-6
	Bishnah	3	Pusa Basmati 1637, Basmati 564 and Basmati 370	*Xoo*-7, *Xoo*-8, *Xoo*-9
Samba	Vijaypur	3	Pusa Basmati 1728, Pusa Basmati 1121 and Pusa Basmati 1637	*Xoo*-10, *Xoo*-11, *Xoo*-12
	Samba	2	Basmati 370 and Basmati 564	*Xoo*-13, *Xoo*-14
	Ghagwal	1	Pusa Basmati 1121	*Xoo*-15
Kathua	Hiranagar	2	Jammu Basmati 138 and Jammu Basmati 123	*Xoo*-16, *Xoo*-17
	Nagri	1	Basmati 370	*Xoo*-18
	Kathua	2	Pusa Basmati 1121 and Basmati 370	*Xoo*-19, *Xoo*-20

3.2. Identification of Xanthomonas oryzae pv. oryzae Collected from Basmati-Rice-Growing Areas

In Gram staining, all the isolated *Xoo* bacterial cells retained the pink or red color stain of safranin, indicating them as Gram-negative. The bacterium was aerobic, produced yellow water-insoluble pigment, was non-diffusible, had oxidative metabolism and was non-spore-forming. Bacterial colonies were circular, convex with entire margins, whitish yellow to straw yellow in color. Further, all the *Xoo* isolates were positive for the KOH test, exhibiting typical thread-like slime confirming Gram-negative reaction. The formation of opaque zones around the colonies and bubbles indicated the presence of esterase and catalase, respectively, confirming that all the isolates were of *Xoo*. In the present study, *Xoo* strain V1 was isolated from the leaf, and 16S rRNA genotyping was performed using universal primers. A 1506 bp DNA sequence was obtained and deposited to the NCBI Genbank database, with Accession number MN718665. A phylogenetic tree was constructed, and as expected, the amplified 16S rRNA gene sequence of the *Xoo* strain V1 showed clustering with other *Xoo* strains (Table 3 and Figure 3).

Table 3. Cultural and morphological characteristics of *Xanthomonas oryzae* pv. *oryzae* isolates.

Isolate	Cultural Characteristics	Morphological Characteristics	Biochemical Tests			
			Gram Reaction	KOH Test	Catalyst Test	Esterase Test
*Xoo*1	Light yellow, circular	Short rods with rounded ends single or in pairs	+ve	+ve	+ve	+ve
*Xoo*2	Light yellow, circular					
*Xoo*3	Yellow, circular					
*Xoo*4	Yellow, circular					
*Xoo*5	Whitish yellow to straw-colored, convex					
*Xoo*6	Whitish yellow to straw-colored, convex					
*Xoo*7	Whitish yellow to straw-colored, convex					
*Xoo*8	Yellow, slightly raised					
*Xoo*9	Yellow, slightly raised					
*Xoo*10	Yellow, slightly raised					
*Xoo*11	Yellow, slightly raised					
*Xoo*12	Yellow, slightly raised					
*Xoo*13	Yellow, slightly raised					
*Xoo*14	Light yellow, convex					
*Xoo*15	Light yellow, convex					
*Xoo*16	Light yellow, circular					
*Xoo*17	Yellow, circular					
*Xoo*18	Yellow, circular					
*Xoo*19	Whitish yellow					
*Xoo*20	Yellow					

3.3. Pathogenicity Assay Xanthomonas oryzae pv. oryzae Isolates on Rice Cultivars

The pathogenicity test of twenty *Xoo* isolates was conducted on five susceptible rice cultivars, viz., Basmati 370, Ranbir Basmati, TN 1, SJR and Jaya. The isolates produced initial symptoms of the BLB within 15–18 days of inoculation. The average lesion length caused by the isolates ranged between 15.90 ± 0.17 and 18.56 ± 0.69 cm in Basmati 370, 15.56 ± 0.17 and 17.03 ± 0.86 cm in Ranbir Basmati, 17.33 ± 0.27 and 19.46 ± 0.20 cm in TN 1, 11.36 ± 0.60 and 14.30 ± 0.23 cm in SJR and 13.10 ± 0.50 and 14.36 ± 0.06 cm in Jaya (Table 4). In Basmati 370, *Xoo* 10 exhibited a maximum lesion length of 18.56 ± 0.69 cm followed by *Xoo* 7, *Xoo* 9, *Xoo* 4 and *Xoo* 2 with lesion lengths of 18.33 ± 0.60,18.10 ± 0.79, 17.73 ± 0.93 and 17.60 ± 0.43 cm, respectively, whereas *Xoo*-1 exhibited a minimum lesion length of 15.90 ± 0.17 cm. In Ranbir Basmati, *Xoo* 6 exhibited a maximum lesion length of 17.03 ± 0.86 cm, followed by *Xoo* 11, *Xoo* 4, *Xoo* 9 and *Xoo* 5 with lesion lengths of 16.93 ± 0.29, 16.83 ± 0.52, 16.66 ± 0.18 and 16.63 ± 0.31 cm, respectively, whereas *Xoo* 20 exhibited a minimum lesion length of 15.56 ± 0.17 cm. In TN 1, *Xoo* 17 exhibited a maximum lesion length of 19.46 ± 0.20 cm followed by *Xoo* 18, *Xoo* 6, *Xoo* 13 and *Xoo* 7 with lesion lengths of 19.00 ± 0.20, 18.96 ± 0.68, 18.93 ± 0.36 and 18.83 ± 0.73 cm, respectively, whereas *Xoo* 16 exhibited a minimum lesion length of 17.33 ± 0.27 cm. In SJR, *Xoo*-5 exhibited a maximum lesion length of 14.30 ± 0.23 cm followed by *Xoo* 2, *Xoo*-14, *Xoo*-8 and *Xoo*-17 with lesion lengths of 13.70 ± 0.26, 13.40 ± 0.75, 13.40 ± 0.66 and 13.36 ± 0.75 cm, respectively, whereas *Xoo*-11 exhibited a minimum lesion length of 11.36 ± 0.60 cm. In Jaya, *Xoo*-8 exhibited a maximum lesion length of 14.36 ± 0.06 cm followed by *Xoo*-13,

Xoo-10 and *Xoo*-11, with lesion lengths of 14.33 ± 1.00, 14.30 ± 0.23 and 14.13 ± 0.31 cm, respectively, whereas *Xoo*-9 exhibited a minimum lesion length of 13.10 ± 0.50 cm.

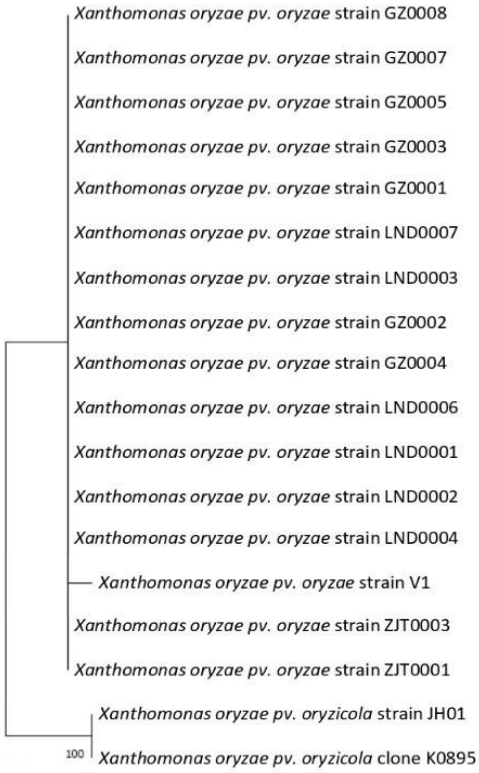

Figure 3. A neighbor-joining tree based on the nucleotide sequence of 16S rRNA. The 16S rRNA sequences of 50 *Xoo* were gathered from the NCBI (National Center for Biotechnology Information) database by a BLASTN search using the 1506 bp PCR products obtained from *Xoo* isolates.

Table 4. Pathogenicity assay of *Xanthomonas oryzae* pv. *oryzae* isolates on rice cultivars.

Isolate	Lesion Length (cm) of Bacterial Leaf Blight on Selected Rice Varieties				
	Basmati 370	**Pusa 1121**	**TN 1**	**SJR 5**	**Jaya**
*Xoo*1	15.90 ± 0.17	15.90 ± 0.34	18.60 ± 0.43	12.20 ± 1.11	13.73 ± 0.46
*Xoo*2	17.60 ± 0.43	16.53 ± 0.52	18.43 ± 0.55	13.70 ± 0.26	13.96 ± 0.56
*Xoo*3	16.66 ± 0.68	15.96 ± 0.76	17.80 ± 0.36	12.96 ± 0.61	13.73 ± 0.55
*Xoo*4	17.73 ± 0.93	16.83 ± 0.52	18.40 ± 0.45	11.53 ± 1.08	13.86 ± 0.38
*Xoo*5	17.16 ± 0.20	16.63 ± 0.31	18.16 ± 0.37	14.30 ± 0.23	13.16 ± 0.56
*Xoo*6	16.50 ± 0.72	17.03 ± 0.86	18.96 ± 0.68	12.40 ± 1.24	13.63 ± 0.61
*Xoo*7	18.33 ± 0.60	16.06 ± 0.39	18.83 ± 0.73	11.70 ± 1.05	14.10 ± 0.17
*Xoo*8	16.03 ± 0.68	16.53 ± 0.54	18.83 ± 0.34	13.23 ± 1.21	14.36 ± 0.06
*Xoo*9	18.10 ± 0.79	16.66 ± 0.18	17.73 ± 0.33	11.76 ± 0.24	13.10 ± 0.50
*Xoo*10	18.56 ± 0.69	15.66 ± 0.12	17.93 ± 0.38	13.26 ± 0.51	14.30 ± 0.23
*Xoo*11	17.00 ± 0.86	16.93 ± 0.29	18.13 ± 0.67	11.36 ± 0.60	14.13 ± 0.31
*Xoo*12	16.56 ± 0.54	16.36 ± 0.60	18.63 ± 0.70	11.73 ± 0.47	13.83 ± 0.20
*Xoo*13	16.30 ± 0.60	16.56 ± 0.61	18.93 ± 0.36	12.00 ± 0.15	14.33 ± 1.00
*Xoo*14	16.53 ± 0.63	15.96 ± 0.43	18.73 ± 0.56	13.40 ± 0.75	13.60 ± 0.58
*Xoo*15	16.36 ± 0.67	16.13 ± 0.33	17.96 ± 0.23	12.13 ± 0.69	13.46 ± 0.52

Table 4. *Cont.*

Isolate	Lesion Length (cm) of Bacterial Leaf Blight on Selected Rice Varieties				
	Basmati 370	**Pusa 1121**	**TN 1**	**SJR 5**	**Jaya**
*Xoo*16	16.30 ± 0.30	16.13 ± 0.26	17.33 ± 0.27	12.03 ± 1.19	13.56 ± 0.57
*Xoo*17	16.53 ± 0.69	16.56 ± 0.67	19.46 ± 0.20	13.36 ± 0.75	13.46 ± 0.29
*Xoo*18	17.46 ± 0.98	16.60 ± 0.46	19.00 ± 0.20	13.40 ± 0.66	14.06 ± 0.13
*Xoo*19	16.13 ± 0.43	16.60 ± 0.60	18.20 ± 0.85	12.83 ± 0.73	14.13 ± 0.27
*Xoo*20	16.36 ± 1.02	15.56 ± 0.17	18.43 ± 0.60	12.93 ± 1.12	13.33 ± 0.37
p-value	≤0.195	≤0.795	≤0.405	≤0.466	≤0.695

3.4. Virulence Profiling of Xanthomonas oryzae pv. oryzae Isolates

The characterization of the virulence profile of the *Xoo* isolates was performed on a set of rice differentials which consisted of fifteen near-isogenic lines (IRBB lines), having different BLB resistance genes either singly or in combination, along with susceptible check TN 1, under greenhouse conditions during the kharif season of 2020–2021 (Table 5). Isolates *Xoo*-1, *Xoo*-2, *Xoo*-3 and *Xoo*-4 exhibited susceptible reaction (>26–50% affected lesion area or score 7) on IRBB1 (Xa1), IRBB4 (Xa4), IRBB5 (Xa5), IRBB7 (Xa7), IRBB8 (Xa8), IRBB10 (Xa10), IRBB11 (Xa11), IRBB14 (Xa14) and TN1. Moderately susceptible response (>13–25% affected lesion area or score 5) was seen on IRBB3 (Xa3) and IRBB50 (Xa4 + Xa5), whereas moderately resistant reaction (>6–12% affected lesion area or score 3) was observed on IRBB21 (Xa21) and IRBB52 (Xa4 + Xa21) and resistant reaction (>1–5% affected lesion area or score 1) on IRBB13 (Xa13), IRBB51 (Xa4 + Xa13) and IRBB53 (Xa5 + Xa13). Isolates *Xoo*-5 and *Xoo*-6 exhibited susceptible response on IRBB1 (Xa1), IRBB3 (Xa3), IRBB10 (Xa10), IRBB11 (Xa11), IRBB14 (Xa14) and TN1, moderately susceptible reaction on IRBB21 (Xa21), moderately resistant reaction on IRBB4 (Xa4), IRBB5 (Xa5) and IRBB7 (Xa7) and resistant reaction on IRBB8 (Xa8), IRBB13 (Xa13), IRBB50 (Xa4 + Xa5), IRBB51 (Xa4 + Xa13), IRBB52 (Xa4 + Xa21) and IRBB53 (Xa5 + Xa13). Isolates *Xoo*-7, *Xoo*-8 and *Xoo*-9 exhibited susceptible reaction on IRBB1 (Xa1), IRBB3 (Xa3), IRBB5 (Xa5), IRBB7 (Xa7), IRBB8 (Xa8), IRBB10 (Xa10), IRBB11 (Xa11), IRBB14 (Xa14) and TN 1, moderately susceptible reaction on IRBB4 (Xa4), moderately resistant reaction on IRBB21 (Xa21), IRBB50 (Xa4 + Xa5) and IRBB52 (Xa4 + Xa21) and resistant reaction on IRBB51 (Xa4 + Xa13), IRBB53 (Xa5 + Xa13) and IRBB13 (Xa13). Isolates *Xoo*-10, *Xoo*-11, *Xoo*-12, *Xoo*-13, *Xoo*-14 and *Xoo*-15 showed susceptible reaction on IRBB1 (Xa1), IRBB10 (Xa10), IRBB11 (Xa11) and TN 1, moderately susceptible reaction on IRBB3 (Xa3) and IRBB14 (Xa14), resistant response on IRBB4 (Xa4), IRBB5 (Xa5), IRBB7 (Xa7), IRBB8 (Xa8), IRBB13 (Xa13), IRBB21 (Xa21), IRBB50 (Xa4 + Xa5), IRBB51 (Xa4 + Xa13), IRBB52 (Xa4 + Xa21) and IRBB53 (Xa5 + Xa13). Isolates *Xoo*-16 and *Xoo*-17 showed susceptible reaction on IRBB1 (Xa1), IRBB3 (Xa3), IRBB4 (Xa4), IRBB5 (Xa5), IRBB7 (Xa7), IRBB10 (Xa10), IRBB11 (Xa11) and TN 1, moderately resistant lesions on IRBB21 (Xa21) and resistant reaction on IRBB8 (Xa8), IRBB13 (Xa13), IRBB50 (Xa4 + Xa5), IRBB51 (Xa4 + Xa13), IRBB52 (Xa4 + Xa21) and IRBB53 (Xa5 + Xa13). Isolate *Xoo*-18 expressed susceptible reaction on IRBB1 (Xa1), IRBB3 (Xa3), IRBB10 (Xa10), IRBB11 (Xa11), IRBB14 (Xa14), IRBB7 (Xa7) and TN 1, moderately susceptible reaction on IRBB4 (Xa4) and IRBB5 (Xa5), moderately resistant reaction on IRBB21 (Xa21) and resistant reaction on IRBB8 (Xa8), IRBB13 (Xa13), IRBB50 (Xa4 + Xa5), IRBB51 (Xa4 + Xa13), IRBB52 (Xa4 + Xa21) and IRBB53 (Xa5 + Xa13). Isolates *Xoo*-19 and *Xoo*-20 expressed susceptible lesions on IRBB1 (Xa1), IRBB3 (Xa3), IRBB10 (Xa10), IRBB11 (Xa11), IRBB14 (Xa14), IRBB21 (Xa21) and TN 1, moderately susceptible reaction on IRBB4 (Xa4), IRBB5 (Xa5), IRBB7 (Xa7) and IRBB52 (Xa4 + Xa21) and resistant reaction on IRBB8 (Xa8), IRBB13 (Xa13), IRBB50 (Xa4 + Xa5), IRBB51 (Xa4 + Xa13) and IRBB53 (Xa5 + Xa13).

Table 5. Virulence profiling of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates on rice differentials under greenhouse conditions during *kharif* 2020–2021.

***Xoo* Isolate**	**Differential (IRBB) Lines**																**Pathotype**
	IRBB 1	**IRBB 3**	**IRBB 4**	**IRBB 5**	**IRBB 7**	**IRBB 8**	**IRBB 10**	**IRBB 11**	**IRBB 13**	**IRBB 14**	**IRBB 21**	**IRBB 50**	**IRBB 51**	**IRBB 52**	**IRBB 53**	**TN1**	
*Xoo*1	7 (S)	5 (MS)	7 (S)	7 (S)	7 (S)	7 (S)	7 (S)	7(S)	1 (R)	7 (S)	3 (MR)	5 (MS)	1 (R)	3 (MR)	1 (R)	7 (S)	
*Xoo*2	7 (S)	5 (MS)	7 (S)	7 (S)	7 (S)	7 (S)	7 (S)	7(S)	1 (R)	7 (S)	3 (MR)	5 (MS)	1 (R)	3 (MR)	1 (R)	7 (S)	Pathotype 5
*Xoo*3	7 (S)	5 (MS)	7 (S)	7 (S)	7 (S)	7 (S)	7 (S)	7(S)	1 (R)	7 (S)	3 (MR)	5 (MS)	1 (R)	3 (MR)	1 (R)	7 (S)	
*Xoo*4	7 (S)	5 (MS)	7 (S)	7 (S)	7 (S)	7 (S)	7 (S)	7(S)	1 (R)	7 (S)	3 (MR)	5 (MS)	1 (R)	3 (MR)	1 (R)	7 (S)	
*Xoo*5	7 (S)	7 (S)	3 (MR)	3 (MR)	3 (MR)	1 (R)	7 (S)	7 (S)	1(R)	7 (S)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	Pathotype 3
*Xoo*6	7 (S)	7 (S)	3 (MR)	3 (MR)	3 (MR)	1 (R)	7 (S)	7 (S)	1(R)	7 (S)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	
*Xoo*7	7 (S)	7 (S)	5 (MS)	7 (S)	7 (S)	7 (S)	7 (S)	7 (S)	1(R)	7 (S)	3 (MR)	3 (MR)	1 (R)	3 (MR)	1 (R)	7 (S)	Pathotype 7
*Xoo*8	7 (S)	7 (S)	5 (MS)	7 (S)	7 (S)	7 (S)	7 (S)	7 (S)	1(R)	7 (S)	3 (MR)	3 (MR)	1 (R)	3 (MR)	1 (R)	7 (S)	
*Xoo*9	7 (S)	7 (S)	5 (MS)	7 (S)	7 (S)	7 (S)	7 (S)	7 (S)	1(R)	7 (S)	3 (MR)	3 (MR)	1 (R)	3 (MR)	1 (R)	7 (S)	
*Xoo*10	7 (S)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	7 (S)	1 (R)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	
*Xoo*11	7 (S)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	7 (S)	1 (R)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	
*Xoo*12	7 (S)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	7 (S)	1 (R)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	Pathotype 2
*Xoo*13	7 (S)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	7 (S)	1 (R)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	
*Xoo*14	7 (S)	5 (MS)	1(R)	1 (R)	1 (R)	1 (R)	7 (S)	7 (S)	1 (R)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	
*Xoo*15	7 (S)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	7 (S)	1 (R)	5 (MS)	1 (R)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	
*Xoo*16	7 (S)	7 (S)	7 (S)	7 (S)	7 (S)	1 (R)	7 (S)	7 (S)	1 (R)	7 (S)	3 (MR)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	Pathotype 6
*Xoo*17	7 (S)	7 (S)	7 (S)	7 (S)	7 (S)	1 (R)	7 (S)	7 (S)	1 (R)	7 (S)	3 (MR)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	
*Xoo*18	7 (S)	7 (S)	5 (MS)	5 (MS)	7 (S)	1 (R)	7 (S)	7 (S)	1 (R)	7 (S)	3 (MR)	1 (R)	1 (R)	1 (R)	1 (R)	7 (S)	Pathotype 6
*Xoo*19	7 (S)	7 (S)	5 (MS)	5 (MS)	5 (MS)	1 (R)	7 (S)	7 (S)	1 (R)	7 (S)	7 (S)	1 (R)	1 (R)	5 (MS)	1 (R)	7 (S)	Pathotype 6
*Xoo*20	7 (S)	7 (S)	5 (MS)	5 (MS)	5 (MS)	1 (R)	7 (S)	7 (S)	1 (R)	7 (S)	7 (S)	1 (R)	1 (R)	5 (MS)	1 (R)	7 (S)	

S: Susceptible (>26–50% lesion area or score 7). MS: Moderately Susceptible (>13–25% lesion area or score 5). MR: Moderately Resistant (>6–12% lesion area or score 3). R: Resistant (>1–5% lesion area or score 1).

3.5. Pathotyping of Xanthomonas oryzae pv. oryzae Isolates

During the course of the study, from the basmati rice fields of the Jammu, Samba and Kathua districts of Jammu Division, on the basis of the virulence or avirulence response of twenty *Xoo* isolates on resistance genes present in the fifteen differential lines, seven diverse pathotypes, viz., Pathotype 1, 2, 3, 4, 5, 6 and 7, were identified (Table 6).

Pathotype 1. Isolate *Xoo*-18 collected from Kathua district during 2020 was identified as Pathotype 1 (Table 6). It exhibited complete virulence against Xa1 (IRBB1), Xa3 (IRBB3), Xa7 (IRBB7), Xa10 (IRBB10), Xa11 (IRBB11), Xa14 (IRBB14) and 0 (TN-1), with the lesion lengths of 16.2, 15.8, 15.9, 16.4, 18.6, 15.6 and 18.7 cm, respectively. It was moderately susceptible against Xa4 (IRBB4) and Xa5 (IRBB5) with lesion lengths of 14.3 and 11.9 cm, respectively, and moderately resistant against Xa21 (IRBB21), with a lesion length of 7.8 cm, and avirulence was observed against Xa8 (IRBB8), Xa13 (IRBB13), Xa4 + Xa5 (IRBB50), Xa4 + Xa13 (IRBB51), Xa4 + Xa21 (IRBB52) and Xa5 + Xa13 (IRBB53) with lesion lengths of 2.3, 4.3, 3.7, 1.9, 2.9 and 3.8 cm, respectively.

Pathotype 2. Data (Table 6) further exhibited that *Xoo* isolates *Xoo*-10, 11, 13, 14 and 15, collected from Samba district, were characterized as Pathotype 2, and they exhibited virulence to Xa1 (IRBB1), Xa10 (IRBB10), Xa11 (IRBB11) and 0 (TN-1), with lesion lengths of 15.6, 16.7, 17.2 and 19.4 cm, respectively. They were moderately susceptible against Xa3 (IRBB3) and Xa14 (IRBB14) with lesion lengths of 12.3 and 12.8 cm, respectively, and showed avirulence to Xa4 (IRBB4), Xa5 (IRBB5), Xa7 (IRBB7), Xa8 (IRBB8), Xa13 (IRBB13), Xa21 (IRBB21), Xa4 + Xa5 (IRBB50), Xa4 + Xa13 (IRBB51), Xa4 + Xa21 (IRBB52) and Xa5 + Xa13 (IRBB53) with lesion lengths of 2.9, 1.8, 2.7, 4.4, 4.3, 3.3, 4.4, 2.9, 3.4 and 3.7 cm, respectively.

Pathotype 3. *Xoo* isolates *Xoo*-5 and 6 collected from Jammu district were identified as Pathotype 3 (Table 6), showing virulence against Xa1 (IRBB1), Xa3 (IRBB3), Xa10 (IRBB10), Xa11 (IRBB11), Xa14 (IRBB14) and 0 (TN-1), with lesion lengths of 15.7, 16.4, 17.1, 15.6, 15.3 and 17.8 cm, respectively. Both isolates were moderately susceptible against Xa21 (IRBB21) with a lesion length of 11.8 cm and moderately resistant against Xa4 (IRBB4), Xa5 (IRBB5) and Xa7 (IRBB7) with lesion lengths of 9.3, 6.5 and 6.3 cm, respectively, and they exhibited avirulence against Xa8 (IRBB8), Xa13 (IRBB13), Xa4 + Xa5 (IRBB50), Xa4 + Xa13 (IRBB51), Xa4 + Xa21 (IRBB52) and Xa5 + Xa13 (IRBB53) with lesion lengths of 1.8, 4.6, 4.2, 3.9, 3.6 and 1.9 cm, respectively.

Pathotype 4. The *Xoo* isolates *Xoo*-19 and 20 collected from Kathua district were characterized as Pathotype 4 (Table 6), which showed virulence to Xa1 (IRBB1), Xa3 (IRBB3), Xa10 (IRBB10), Xa11 (IRBB11), Xa14 (IRBB14), Xa21 (IRBB21) and 0 (TN-1), with lesion lengths of 16.7, 15.6, 17.3, 17.5, 16.7, 16.9 and 17.8 cm, respectively, were moderately susceptible against Xa4 (IRBB4), Xa5 (IRBB5), Xa7 (IRBB7) and Xa4 + Xa21 (IRBB52), with lesion lengths of 13.8, 14.8, 11.6 and 11.8 cm, respectively, and exhibited avirulence to Xa8 (IRBB8), Xa13 (IRBB13), Xa4 + Xa5 (IRBB50), Xa4 + Xa13 (IRBB51) and Xa5 + Xa13 (IRBB53) with lesion lengths of 2.6, 4.3, 3.4, 2.9 and 4.6 cm, respectively.

Pathotype 5. The *Xoo* isolates 1, 2, 3 and 4, collected from Jammu district, were categorized as Pathotype 5 (Table 6). They showed virulence against Xa1 (IRBB1), Xa4 (IRBB4), Xa5 (IRBB5), Xa7 (IRBB7), Xa8 (IRBB8), Xa10 (IRBB10), Xa11 (IRBB11), Xa14 (IRBB14) and 0 (TN-1), with lesion lengths of 15.4, 15.6,16.4, 16.6, 17.6, 17.8, 15.4, 16.7 and 18.7 cm, respectively, moderate susceptibility to Xa3 (IRBB3) and Xa4 + Xa5 (IRBB50), with lesion lengths of 12.5 and 11.9 cm, respectively, moderate resistance against Xa21 (IRBB21) and Xa4 + Xa21 (IRBB52), with lesion lengths of 7.3 and 7.1 cm, respectively, and avirulence against Xa13 (IRBB13), Xa4 + Xa13 (IRBB51) and Xa5 + Xa13 (IRBB53) with lesion lengths of 3.8, 4.1 and 4.9 cm, respectively.

Table 6. Pathotypes of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) prevalent in Jammu, based on the disease reaction of *Xoo* isolates collected from various districts, on fifteen differential lines.

Pathotype	Collected from District	*Xoo* Isolate	Virulent against (Differential Lines)	Lesion Length (cm)	Moderately Susceptible against (Differential Lines)	Lesion Length (cm)	Moderately Resistant against (Differential Lines)	Lesion Length (cm)	Avirulent against (Differential Lines)	Lesion Length (cm)
Pathotype 1	Kathua	*Xoo*-18	Xa1 (IRBB1) Xa3 (IRBB3) Xa7 (IRBB7) Xa10 (IRBB10) Xa11 (IRBB11) Xa14 (IRBB14) TN-1	16.2 15.8 15.9 16.4 18.6 15.6 18.7	Xa4 (IRBB4) Xa5 (IRBB5)	14.3 11.9	Xa21 (IRBB21)	7.8	Xa8 (IRBB8) Xa13 (IRBB13) Xa4 + Xa5 (IRBB50) Xa4 + Xa13 (IRBB51) Xa4 + Xa21 (IRBB52) Xa5 + Xa13 (IRBB53)	 2.3 4.3 3.7 1.9 2.9 3.8
Pathotype 2	Samba	*Xoo*-10 *Xoo*-11 *Xoo*-13 *Xoo*-14 *Xoo*-15	Xa1 (IRBB1) Xa10 (IRBB10) Xa11 (IRBB11) TN-1	15.6 16.7 17.2 19.4	Xa3 (IRBB3) Xa14 (IRBB14)	12.3 12.8	-	-	Xa4 (IRBB4) Xa5 (IRBB5) Xa7 (IRBB7) Xa8 (IRBB8) Xa13 (IRBB13) Xa21 (IRBB21) Xa4 + Xa5 (IRBB50) Xa4 + Xa13 (IRBB51) Xa4 + Xa21 (IRBB52) Xa5 + Xa13 (IRBB53)	2.9 1.8 2.7 4.4 4.3 3.3 4.4 2.9 3.4 3.7
Pathotype 3	Jammu	*Xoo*-5 *Xoo*-6	Xa1 (IRBB1) Xa3 (IRBB3) Xa10 (IRBB10) Xa11 (IRBB11) Xa14 (IRBB14) TN-1	15.7 16.4 17.1 15.6 15.3 17.8	Xa21 (IRBB21)	11.8	Xa4 (IRBB4) Xa5 (IRBB5) Xa7 (IRBB7)	9.3 6.5 6.3	Xa8 (IRBB8) Xa13 (IRBB13) Xa4 + Xa5 (IRBB50) Xa4 + Xa13 (IRBB51) Xa4 + Xa21 (IRBB52) Xa5 + Xa13 (IRBB53)	 1.8 4.6 4.2 3.9 3.6 1.9

Table 6. *Cont.*

Pathotype	Collected from District	*Xoo* Isolate	Virulent against (Differential Lines)	Lesion Length (cm)	Moderately Susceptible against (Differential Lines)	Lesion Length (cm)	Moderately Resistant against (Differential Lines)	Lesion Length (cm)	Avirulent against (Differential Lines)	Lesion Length (cm)
Pathotype 4	Kathua	*Xoo*-19 *Xoo*-20	Xa1 (IRBB1) Xa3 (IRBB3) Xa10 (IRBB10) Xa11 (IRBB11) Xa14 (IRBB14) Xa21 (IRBB21) TN-1	16.7 15.6 17.3 17.5 16.7 16.9 17.8	Xa4 (IRBB4) Xa5 (IRBB5) Xa7 (IRBB7) Xa4 + Xa21 (IRBB52)	13.8 14.8 11.6 11.8			Xa8 (IRBB8) Xa13 (IRBB13) Xa4 + Xa5 (IRBB50) Xa4 + Xa13 (IRBB51) Xa5 + Xa13 (IRBB53)	2.6 4.3 3.4 2.9 4.6
Pathotype 5	Jammu	*Xoo*-1 *Xoo*-2 *Xoo*-3 *Xoo*-4	Xa1 (IRBB1) Xa4 (IRBB4) Xa5 (IRBB5) Xa7 (IRBB7) Xa8 (IRBB8) Xa10 (IRBB10) Xa11 (IRBB11) Xa14 (IRBB14) TN-1	15.4 15.6 16.4 16.6 17.6 17.8 15.4 16.7 18.7	Xa3 (IRBB3) Xa4 + Xa5 (IRBB50)	12.5 11.9	Xa21 (IRBB21) Xa4 + Xa21 (IRBB52)	7.3 7.1	Xa13 (IRBB13) Xa4 + Xa13 (IRBB51) Xa5 + Xa13 (IRBB53)	3.8 4.1 4.9
Pathotype 6	Kathua	*Xoo*-16 *Xoo*-17	Xa1(IRBB1) Xa3 (IRBB3) Xa4 (IRBB4) Xa5 (IRBB5) Xa7 (IRBB7) Xa10 (IRBB10) Xa11 (IRBB11) Xa14 (IRBB14) TN-1	17.8 16.8 17.5 16.3 15.3 15.6 16.5 16.7 19.2	-	-	Xa21 (IRBB21)	7.8	Xa8 (IRBB8) Xa13 (IRBB13) Xa4 + Xa5 (IRBB50) Xa4 + Xa13 (IRBB51) Xa4 + Xa21 (IRBB52) Xa5 + Xa13 (IRBB53)	3.6 4.6 3.2 2.9 4.8 3.8

Table 6. *Cont.*

Pathotype	Collected from District	*Xoo* Isolate	Virulent against (Differential Lines)	Lesion Length (cm)	Moderately Susceptible against (Differential Lines)	Lesion Length (cm)	Moderately Resistant against (Differential Lines)	Lesion Length (cm)	Avirulent against (Differential Lines)	Lesion Length (cm)
Pathotype 7	Jammu	*Xoo*-7 *Xoo*-8 *Xoo*-9	Xa1 (IRBB1) Xa3 (IRBB3) Xa5 (IRBB5) Xa7 (IRBB7) Xa8 (IRBB8) Xa10 (IRBB10) Xa11 (IRBB11) Xa14 (IRBB14) TN-1	16.4 17.3 16.4 17.9 18.3 16.7 16.4 17.2 18.7	Xa4 (IRBB4)	11.2	Xa21 (IRBB21) Xa4 + Xa5 (IRBB50) Xa4 + Xa21 (IRBB52)	5.8 7.4 7.3	Xa13 (IRBB13) Xa4 + Xa13 (IRBB51) Xa5 + Xa13 (IRBB53)	4.2 1.9 4.3

Pathotype 6. The *Xoo* isolates 16 and 17, collected from Kathua district, were identified as Pathotype 6 (Table 6). They had virulence against Xa1 (IRBB1), Xa3 (IRBB3), Xa4 (IRBB4), Xa5 (IRBB5), Xa7 (IRBB7), Xa10 (IRBB10), Xa11 (IRBB11), Xa14 (IRBB14) and 0 (TN-1), with lesion lengths of 17.8, 16.8, 17.5, 16.3, 15.3, 15.6, 16.5, 16.7 and 19.2 cm, respectively, moderate resistance against Xa21 (IRBB21) with a lesion length of 7.8 cm and avirulence to Xa8 (IRBB8), Xa13 (IRBB13), Xa4 + Xa5 (IRBB50), Xa4 + Xa13 (IRBB51), Xa4 + Xa21 (IRBB52) and Xa5 + Xa13 (IRBB53) with lesion lengths of 3.6, 4.6, 3.2, 2.9, 4.8 and 3.8 cm, respectively.

Pathotype 7. The *Xoo* isolates, *Xoo*-7, 8 and 9, collected from Jammu district, were characterized as Pathotype 7 (Table 6). They showed virulence to Xa1 (IRBB1), Xa3 (IRBB3), Xa5 (IRBB5), Xa7 (IRBB7), Xa8 (IRBB8), Xa10 (IRBB10), Xa11 (IRBB11), Xa14 (IRBB14) and 0 (TN-1), with lesion lengths of 16.4, 17.3, 16.4, 17.9, 18.3, 16.7, 16.4, 17.2 and 18.7 cm, respectively, moderate susceptibility to Xa4 (IRBB4) with a lesion length of 11.2 cm, moderate resistance to Xa21 (IRBB21), Xa4 + Xa5 (IRBB50) and Xa4 + Xa21 (IRBB52) with lesion lengths of 5.8, 7.4 and 7.3 cm, respectively, and avirulence against Xa13 (IRBB13), Xa4 + Xa13 (IRBB51) and Xa5 + Xa13 (IRBB53) with lesion lengths of 4.2, 1.9 and 4.3 cm, respectively.

3.6. Pathotype Abundance

Twenty *Xoo* isolates collected from the Jammu, Samba and Kathua districts of Jammu Division were assessed to ascertain the regional distribution of *Xoo* pathotypes (Table 7). In Jammu district, Pathotype 5 was highly distributed (44.44%), followed by Pathotype 7 (33.33%) and Pathotype 3 (22.22%), whereas, in Samba district, only Pathotype 2 was recorded among all the collected isolates. However, Pathotypes 4 and 6 (40% each) and Pathotype 3 (20%) were distributed in Kathua district. Among the seven *Xoo* pathotypes, Pathotype 2 was the most dominant one, having an occurrence of 30 percent, followed by Pathotype 5 (20%), Pathotype 7 (15%), Pathotypes 3, 4 and 6 (10% each) and Pathotype 1 (5%), in all the three basmati-growing districts of Jammu Division.

Table 7. Distribution of *Xanthomonas oryzae* pv. *oryzae* pathotypes in various districts of Jammu sub-tropics.

Pathotype	Jammu	Samba	Kathua	Total	Percent Distribution
Pathotype 1	-	-	1/5 (20)	1	5
Pathotype 2	-	6/6 (100)	-	6	30
Pathotype 3	2/9 (22.22)	-	-	2	10
Pathotype 4	-	-	2/5 (40)	2	10
Pathotype 5	4/9 (44.44)	-	-	4	20
Pathotype 6	-	-	2/5 (40)	2	10
Pathotype 7	3/9 (33.333)	-	-	3	15
(Isolates)	3/9 *	1/6 *	3/5 *	7/20 *	100

* The number of isolates. Values in parentheses denote isolate percentages.

3.7. Frequency Virulence of Xanthomonas oryzae pv. oryzae Isolates

Xoo isolates, collected from the basmati-rice-growing fields of Jammu Division during 2020, varied in their virulence frequency against rice differential hosts (Table 8). Differentials IRBB8 and IRBB21 possessing BLB-resistant genes Xa8 and Xa21 showed susceptibility against 35 and 20 percent of the isolates, respectively, and IRBB13 possessing Xa13 showed resistance against all the isolates. Different IRBB lines with a single BLB resistance gene were susceptible to 20 to 100 percent of the isolates. Differentials possessing only one BLB resistance gene were the least effective for all the *Xoo* isolates, showing a high level of susceptibility. Differentials IRBB51 and IRBB53, possessing two resistant gene combinations of Xa4 + Xa13 and Xa5 + Xa13, respectively, showed resistance against all the *Xoo* isolates. However, IRBB50 and IRBB52 possessing the BLB-resistant gene combination of Xa4 +

Xa5 and Xa4 + Xa21, respectively, showed resistance to only 20 and 10 percent of the isolates, respectively.

Table 8. Virulence frequency of *Xanthomonas oryzae* pv. *oryzae* isolates against *Xa* genes.

Differential Line	BB Resistance Gene (s)	Virulent Isolate	Virulence Frequency (%)
IRBB1	*Xa*1	20	100
IRBB3	*Xa*3	20	100
IRBB4	*Xa*4	12	60
IRBB5	*Xa*5	12	60
IRBB7	*Xa*7	12	60
IRBB8	*Xa*8	7	35
IRBB10	*Xa*10	20	100
IRBB11	*Xa*11	20	100
IRBB13	*Xa*13	0	0
IRBB14	*Xa*14	20	100
IRBB21	*Xa*21	4	20
IRBB50	*Xa*4 + *Xa*5	4	20
IRBB51	*Xa*4 + *Xa*13	0	0
IRBB52	*Xa*4 + *Xa*21	2	10
IRBB53	*Xa*5 + *Xa*13	0	0
TN1	0	20	100

4. Discussion

Traditional basmati varieties such as Ranbir Basmati and Basmati 370, though old, are still cultivated in Jammu and Kashmir, due to their excellent cooking quality, aroma and consumer preference. Both of these varieties have special consideration for the farmers, although the prevalence of biotic and abiotic stresses and undesired morphological characteristics (tall stature and thin stems) diminish their potential yield [61]. BLB has emerged as a major production limitation for rice production. The disease was introduced into India during the 1960s with the advent of semi-dwarf rice cultivation [62]. Losses due to BLB can be more than 50 percent depending on the type of cultivar, severity of infection, stage of the crop and season, amount of nitrogen fertilizer applied and environmental conditions [61].

Due to the nonavailability of chemicals or biocontrol agents for the management of BLB at farmers' fields, the cultivation of resistant varieties is the only durable and sustainable approach for the management of the disease [3]. However, the continuous evolution of new races/pathotypes of *Xoo* has always impeded the use of major resistance (R) genes(s) for the development of resistance varieties [63]. Globally, more than 38 R-genes (Xa/xa) have been explored [36,64]. Additionally, more than 42 R-genes have been identified from Japonica varieties, *Oryza sativa* sp. *indica* cultivars and their related wild species [38–40].

In the present study, 20 *Xoo* isolates were collected from BLB-infected basmati rice in Jammu Division. In Jammu district, isolates *Xoo*-1 and *Xoo*-2 were collected from Chatha, *Xoo*-3 and *Xoo*-4 from R.S. Pura, *Xoo*-5 and *Xoo*-6 from Akhnoor and *Xoo*-7, *Xoo*-8 and *Xoo*-9 from Bishnah. In Samba district, *Xoo*-10, *Xoo*-11 and *Xoo*-12 were collected from Vijaypur, *Xoo*-13 and *Xoo*-14 from Samba and *Xoo*-15 from Ghagwal. In Kathua district, *Xoo*-16 and *Xoo*-17 were collected from Hiranagar, *Xoo*-18 from Nagri and *Xoo*-19 and *Xoo*-20 from Kathua. All the *Xoo* isolates (*Xoo*-1 to *Xoo*-20) were Gram-negative and exhibited positive reactions for KOH, esterase and catalase activity tests. The potassium hydroxide (KOH)

test is an excellent validation assay for Gram staining [49]. Catalase enzyme decomposes hydrogen peroxide to water and oxygen [65] and esterase activity [66].

Phenotypic analysis of 20 *Xoo* isolates on the rice differentials having a BLB resistance gene either singly or in combinations along with TN 1 revealed that most of the single BLB-resistant genes were moderately to highly susceptible to almost all the *Xoo* isolates, except xa13, under greenhouse and field conditions. *Xoo* isolates from a neighboring state (Punjab) were grouped into eight pathotypes based on their response on near-isogenic lines (NILs) which exhibited that xa13 was more effective than Xa21. In the present study, combinations of BLB resistance genes (Xa4 + Xa13 and Xa5 + Xa13) possessed high resistance against all the *Xoo* isolates. Although individually BLB resistance gene Xa4 was ineffective against 60 percent of the isolates, its amalgamation with Xa13 (IRBB51) and Xa21 (IRBB52) exhibited a broader effect on all the isolates of *Xoo*, indicating quantitative complementation [67–69]. In Punjab, the highest virulence frequency of *Xoo* was recorded in IRBB14 carrying resistant gene Xa14 and differential line IRBB13 [59].

On the basis of the response exhibited by different *Xoo* isolates on differential lines, seven pathotypes (Pathotypes 1–7) were identified. The occurrence of a new pathotype may result from mutation, somatic hybridization, migration, recombination and host selection [15,70]. The introduction of new varieties with diverse resistance genes results in variation in the diversity of the *Xoo* population by overcoming the deployed resistance gene [71,72]. Twenty-six pathotypes of *Xoo* were identified from the analysis of 171 strains of *Xoo*, collected from eight rice-producing zones in Nepal, using 11 near-isogenic rice lines, each containing a single gene for resistance [73]. Meanwhile, in Pakistan, five pathotypes were recorded from 105 isolates, collected from different rice-producing areas on the basis of their virulence on 12 isogenic lines [21]. In India, 9 pathotypes have been categorized from *Xoo* populations from eastern India [70], 7 major pathotypes from Punjab [44], 11 pathotypes from throughout India except Jammu and Kashmir [43], 10 pathotypes from Andhra Pradesh [47] and 11 pathotypes from the Udham Singh Nagar district of Uttarakhand [74]. Further, from the virulence profiling of *Xoo* isolates (400) in India, on a set of differentials comprising 22 near-isogenic lines, 22 pathotypes were recorded [58]. The pathogenic diversity of 300 *Xoo* isolates, collected from 17 districts of Punjab province, Pakistan, exhibited 29 pathotypes (Pt1-29), based on their virulence spectrum on rice differentials [42]. Pathotypes 1 to 7 showed the occurrence of 5, 3, 10, 10, 20, 10 and 15 percent, respectively, in the Jammu sub-tropics; in Jammu district, Pathotype 5 was predominant (44.44%), followed by Pathotype 7 (33.33%) and Pathotype 3 (22.22%); in Samba district, only Pathotype 2 (100%) was prevalent; and Pathotypes 4 and 6 (40% each) were distributed in Kathua district. The studies revealed that diverse pathogenic variations existed in the *Xoo* population in the basmati-growing region of Jammu and Kashmir. As the new pathotypes continue to emerge, the majority of them varied in their virulence depending upon areas and fields within an area [75,76]. Thus, a substantial approach is needed to develop resistant cultivars with the help of the identification of predominate races in that specific geographical area, and the continuous monitoring of the virulence structure of the pathogen in a particular area is a pre-requisite to make resistance breeding more durable and sustainable.

The present study is a pioneering work in the basmati-growing area of Jammu and Kashmir, where work regarding the identification of the prevailing *Xoo* pathotypes had not been undertaken earlier. It is essential to identify and continuously monitor the virulence pattern of the predominate race(s) in a specific geographical area in order to develop durable and sustainable resistant cultivars against bacterial leaf blight of rice.

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