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## RESEARCH ARTICLE

AN OVERVIEW OF BACTERIAL BLIGHT DISEASE OF RICE CAUSED BY  
*XANTHOMONAS ORYZAE* PV. *ORYZAE*.

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Rice is the most widely cultivated food crop of the world. Asia alone accounts for 90 per cent of the world's production coupled with consumption of rice also because of favorable hot and humid climate. Rice accounts for 35-75 per cent of the calories consumed by more than 3 billion Asian (Khush, 2004). It is also expected that by the year 2050, 90 per cent of world's projected 11 billion people will reside in the developing countries (Krattigar, 1996). The production is constrained mainly due to biotic and abiotic stress. Major advances have occurred in food production during the last seven decades due to adoption of improved techniques including high yielding varieties. This has also lead to emergence of new pests and diseases simultaneously. The loss of the yield in all crops due to biotic stress is approximately 500 billion. It has been estimated that in bacterial blight of rice the yield losses are as high as 6 to 60 per cent depending upon location, season, weather condition and cultivars (Srivastava, 1967). Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) reported as economically important disease in tropical Asia also (Mew, 1987 and 1989). The disease was known to occur in epidemic proportions in many parts of the world, incurring severe crop loss of up to 50 per cent. Crop loss assessment studies have revealed that this disease reduces grain yield to varying levels, depending on the stage of the crop, degree of cultivar susceptibility and to a great extent, the conduciveness of the environment in which it occurs.

Bacterial blight of rice is difficult to control through chemicals. Varietal resistance is considered the most practical and economic way of keeping the disease below the economic injury level. Variability in pathogenicity have now been fully recognized. Resistant varieties development has been targeted in almost all the breeding programmes of varietal improvement. In this context number of isogenic lines, differential having known genes and pyramids with more than two resistant genes have been also developed by different agencies. The literature pertaining to plant resistance and its nature of response in rice to *Xanthomonas oryzae* pv. *oryzae* has been reviewed under following heads:

### **Causal organisms:-**

The bacterial origin of the disease was first reported by Ishiyama in 1922. The disease was caused by the bacterium *Xanthomonas oryzae* pv. *oryzae*. The isolates and pathogenicity test of *Xoo* was first reported in India by Srinivasan et al. (1959).

### **Taxonomy:-**

- ❖ The nomenclature of the causal bacterium has changed over the years as under.
- ❖ *Bacillus oryzae* Hori and Bokura, 1911.
- ❖ *Pseudomonas oryzae* Uyeda and Ishiyama, 1922.
- ❖ *Bacterium oryzae* (Uyeda and Ishiyama) Nakata, 1927.
- ❖ *Phytomonas oryzae* (Uyeda and Ishiyama) Dowson, 1939.
- ❖ *Xanthomonas campestris* pv. *oryzae* (Uyeda & Ishiyama) Dye et al., 1980.
- ❖ *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings et al., 1990.

### **Symptoms:-**

Bacterial blight was a vascular disease. The infection was therefore, systemic. The pathogen normally enters the host through wounds and or natural openings such as the water pores. In either case, the bacterium ends up in the xylem tissues, multiplies and moves throughout the plant (Mew, 1987). Three types of symptoms were reported on rice: Kresek, leaf blight and yellow leaf. 'Kresek' was the more destructive manifestation of the disease, wherein the leaves of the entire plant turned pale yellow and wilted during the seedling to the early tillering stage, resulting in a partial or total crop failure (Gnanamanickam et al., 1999). 'Leaf blight' is the most common disease symptom that occurs widely. Lesions on the leaf blades may extend to the leaf sheath. The lesion enlarges in length and width, and may have wavy margins. It turns a whitish-straw colour from its initial water soaked grayish or yellowish hue in 1-2 weeks. Bacterial ooze may be observed in humid and warm condition on the lesion. Leaf blight may occur at all growth stages, but, it is common from maximum tillering until maturity. 'Yellow leaf' was also considered a syndrome of bacterial blight (Ou, 1985). However, Mew (1987) considered this a secondary effect of kresek or leaf blight, apparently due to the effect of toxin produced by the bacteria rather than to interruption of nutrients from roots to shoots.

### **Host range:-**

The weed hosts of *Xanthomonas oryzae* Japan were first reported under artificial inoculation (Goto et al., 1953), the infected weeds were also later found in nature. Togami and Mizukami (1962) reported that *Leersia sayanuka* Ohwi, was the most important as it served as a common over-wintering host. *L. oryzoides* (L) Sw., *L. japonica* and *Zizania itifolia* were also naturally infected. In the tropics, *Leptochloa chinensis* (L.), Ness *L. filiformis* and *L. panacea* had been found weed host in the Philippines *Cyprus rotundus* L. and *C. defformis* were also reported from India as being alternative host found under natural conditions (Chattopadhyay and Mukherjee, 1968).

### **Variability:-**

For many years, it was unclear whether there was differential interaction between rice and *Xoo*. Many scientist opinions differed on the existence 'races' of the bacterial blight of rice pathogen. The confusion was centered on the question of a system that could clearly differentiate pathogenicity of bacterial pathogen. With two different cultivars and two different isolates, scientists often looked for the host pathogen association, when the disease was either present or absent in the cultivars isolates combinations. Resistance in rice to *X. oryzae* pv. *oryzae* was therefore often viewed in the absolute rather than in the relative terms, neither pathogenic specialization nor specific resistance occurs alone.

### **Variation in pathogenicity:-**

The degree of specialization reported to differs in various host-pathogen associations (Browder and Eversmeyer, 1986). Variation in the virulence of *Xoo* from one location to another location and from one country to another (particularly in Asia) has long been recognized (Buddenhagen et al., 1969; Buddenhagen and Reddy, 1972; Kauffman and Rao, 1972; Reddy and Ou, 1976; Mew and Vera Cruz, 1979, 1988; Srinivasan and Singh, 1982; Durgapal, 1986; Gupta et al., 1986; Noda et al., 1990; Gopinath et al., 1991; Nayak and Reddy, 1993). Isolates from India have been reported more virulent than the isolates from Japan (Wakimoto, 1967; AICRIP, 1971; Reddy, 1980; Nayak and Reddy, 1993). Since the breakdown of resistance, scientist from Japan made attempts to classify rice varieties and bacterial isolates in Japan (Kuhara et al., 1965; Washio et al., 1966 and Ezuka and Horino, 1974).

Kozaka (1969) presented a system of varietal classification. On the basis of differential reaction, rice varieties were derived into four groups viz., Kinmaze, Kogyoku, Rantjemas and Wase Aikoku. The bacterial isolates were put into three groups I, II and III accordingly. Devdath and Padmanabhan (1969) tested nine isolates on 20 cultivars and found differences in virulence. They noticed that cultivars resistant in Japan or in Philippines became susceptible in India and found differential interaction between isolates and cultivars. Xia and Fu (1983) studied variability and strain separation of *Xoo* in 1979-83. Nearly 200 isolates of the bacterium from rice in each major epidemic region of 46 countries and cities in Fujian province were tested on set of differentials in China and some differential cultivars from Japan and IRRI with known resistance genes. Results showed that the Fujian isolates differed in virulence from strains in Japan and Philippines. Some cultivars including Jiggang 30, Zaiyeqing 8, Honken 57, Zhike, Nan 15, IR-26 and 76 C-Fan 7 could be used as a set of differentials in Fujian to classify 5 strain groups (0, I, II, III and IV) and 9 sub groups. Noda et al. (1990) distinguished seven races in Japan using five Japanese differential, but when they used 18 differential varieties, from several Asian countries including Japan, race I, II and III could be subdivided further into 10, 17 and 13 subgroups respectively. Five cultivars (Chugoku 45, IR 20, Tongil, Milyang 42 and DV 85) were resistant to all the isolates tested. Studied at All India Co-ordinated Rice Improvement Projects (AICRP) had showed the existence of only two pathotypes. Pathotype I was prevalent in all the bacterial blight epidemic regions of the country whereas, distribution of pathotype II was restricted to areas in Orissa and West Bengal (Reddy, 1980). Durgapal (1986) obtained the isolates from rice in the epiphytic year. On the basis of reaction on BJ-1, TKM-6, T-65, and T(N)-1, the isolates were made differential into five pathogenic groups designated pathotypes I, II, III, IV and V. Reddy and Reddy (1990) collected, 130 isolates of *Xoo* from 25 locations from different regions of India, where bacterial blight of rice was endemic and grouped into pathotypes Ia, Ib and II based on the reactions on six differential cultivars. Gopinath et al. (1991) tested the pathogenicity of 12 of 37 isolates of *Xanthomonas oryzae* on 17 rice cultivars. All the 12 isolates were virulent on all cultivars tested. Pathotypes studies conducted under the All India Co-ordinated Rice Improvement Project (AICRIP) had identified two major pathotypes within the *Xoo* populations in India (Reddy and Reddy, 1992). Das et al. (1994) reported isolate HC 44, which was most virulent out of four different isolates of the bacterial blight pathogen tested. This isolate produced early infection and large lesions on the leaves during their testing. Nayak and Reddy (1993) constituted a set of differentials comprising in rice varieties IR-8, IET-8585, PN-13, TKM-6 and DV-85 and/ or Malagkit Sungsong to classify the isolates of bacterial blight pathogen occurring in India into virulence groups. Based upon the reaction, 36 bacterial isolates (CR x CO1 – CR x CO36) were distinguished into 6 groups or pathogens. The DV-85 showed resistance against Indian group I, III, IV and IR-8 showed susceptibility against all isolate groups (I-IV). Thrimurthy *et al.* (1993) isolated 6 isolates (6, 6R, 6-4R, 6-5R, 6-6R and 6-7R) of *Xanthomonas oryzae* from rice in different parts of Chhattisgarh region. These isolates showed variations in their reaction on isogenic lines, differential varieties and MP rice germplasm genotypes. They found that isolate 6-5R and 6-4R were most virulent. Among the isogenic lines, IRBB-1(t) was resistant against isolate 6R, 6-5R and 6-7R. Among the differential varieties, BJ-1, IR-20, IR-13427 and IR-1543-338 were resistant against three isolates. Isolate 6-5R showed virulent reaction on maximum number of differentials followed by 6-4R. All the isolates showed variable disease reaction on the Madhya Pradesh germplasm rice genotypes. BJ-400-1 and RP-2151-33-2 were found resistant against maximum number of isolates (4). The overall reaction of the isolates on the 48 varieties (10 isogenic + 24 differential + 14 MP germplasm) showed that isolate 6-5R was avirulent on only two varieties [IRBB-1(t) and S-971] followed by 6-4R on 4 varieties (BJ-400-1, RP-2151-33-2, BJ-1 and IR-24). IRBB-1 was resistant against isolate 6R.

#### **Isolation, purification and testing the pathogenicity:-**

The isolates of *Xanthomonas oryzae* were recovered from infected rice samples. The causal bacterium was isolated from green leaves with yellow BB young lesions. The bacterial exudates from fresh lesion were better isolation material as compared to infected tissue because of less contamination. This was also observed by Isaka (1970). Ishiyama (1922) reported *Xanthomonas oryzae* pv. *oryzae* to be associated with bacterial blight of rice. It was first time isolated in 1884 from Japan. The media used for isolation were yeast extract dextrose calcium carbonate agar medium (YDCA), Wakimoto semi synthetic agar (WSSA) and Nutrient agar (NA). The Xanthomonad produced yellow, mucoid, dome shaped, shiny colonies on YDCA medium. These cultural characteristics corresponds to the results of Lelliot and Stead (1987). The bacterium when grown on WSSA medium produced viscous, smooth, circular colonies. Similar results had been reported by Wakimoto (1955, 1967). On NA medium the bacterium produced light yellow, circular dome shaped colonies. The yellow colour and mucoid colonies were cultural characteristics of Xanthomonads due to the production of extra cellular polysaccharides (EPS in media containing sugar). Ou (1977) observed lesions on upland rice as well as on weeds both near to and far from rice fields in several Latin American countries. He suspected that the causative agent was similar to *Xoo*, but this was not confirmed by isolation, pathogenicity or other bacteriological tests. Lazano (1977) isolated a bacterial pathogen from infected rice

in the Caribbean region and identified it as *Xoo*. No further reports of BB in the American continent emerged until July 1987, when a rice disease exhibiting symptoms similar to those of bacterial blight was observed in Texas and Louisiana in the United States. Bacterial isolates from infected tissues showed the cultural and bacteriological characteristics and pathogenicity of *Xoo*. This was perhaps the first time the disease was reported in the US. In India, bacterial blight was first isolated from diseased leaves by Srinivasan *et al.* (1959) at Bombay region in 1951. Dinh *et al.* (2008) collected diseased rice leaf samples from 13 provinces, used for isolation and evaluated for the variation in pathogenicity. Forty one isolates were used for inoculation on 10 differential rice varieties containing different single resistant genes, the results showed that the infection response was clearly compatible and incompatible reactions on differential rice varieties. Gene xa 5 was highest effective against the disease, the next were Xa 7, Xa 21 and xa 13. Six pathogenic races were identified and their distribution was varied across the provinces in Mekong Delta. Race A, E and F were predominant, they occurred and infected on rice in 6 to 8 provinces, while race B, C and D in 3 to 5 provinces in Mekong Delta. Khan and Hingorani (1970) studied the pathogenicity of *Xanthomonas oryzae* pv. *oryzae* by three methods of inoculation viz., clipping, pin-prick and paint brush were tested both on detached leaves and on attached leaves in-vitro and in-vivo. They observed all the three methods were effective for artificial inoculation, but pin-prick method was found to be more efficient in detached leaf assay (in-vitro) based on lesion size. The paint brush method of inoculation was found to be less effective as compared to other two methods but it was more similar to natural infection. Mew *et al.* (1981) compared the needle prick, clip and spray technique and found that neither lesion length nor disease score were affected by inoculation method, although the incubation period was longer in spray inoculated plants than in other plants. The pin-prick method of inoculation developed by Muko and Yoshida (1951) and Yoshida and Muko (1961) proved most suitable for quantitative determination of causal bacterium. The cell sap coming from injured tissue due to pin-pricking usually serves as nutrient for the bacteria. This causes bacterium to multiply and increase in hypertrophic region. After that the bacterium moves towards non-hypertrophic intercellular spaces. Kauffman *et al.* (1973) proved that the clipping method of inoculation was quick and efficient in glass house and field assay. The leaves of each plant are grasped and the top of the leaves were clipping by a pair of scissor dipped in bacterial suspension. The cut ends provide entry for pathogen and might be one of the main causes for spreading BB disease in rice plants. Ogawa *et al.* (1990) used the clipping inoculation method for their genetic studies and found this method most efficient for genetic analysis of resistance cultivars.

#### **Age factor influence on resistance:-**

A varied response of rice plant to *Xanthomonas oryzae* pv. *oryzae* at different growth stages has been reported. Crop growth stages with reference to resistance were studied by few research workers. Mew *et al.* (1981) reported that leaf and plant age influenced the infection which varied from young to old plants. Noda (1982) observed that the reaction of cultivars at different growth stages found to be helpful in classifying the pathogen isolates into different groups. The disease pressures at adult stages were lower than that recorded at the seedling stage. Kinmaza, Sigadagabo, Tetep and Nagomasari were selected to classify bacterial isolates into four groups by artificial inoculation at the seedling stage. Hossain *et al.* (1982) developed 39 mutant strains through gamma rays irradiation and EMS treatment of the rice cv. Nizersail and evaluated for resistance to *Xanthomonas campestris* pv. *oryzae* at seedling, maximum tillering and flowering stages. Disease severity increased with age in both the mother cultivar and the mutant strain. Sharma *et al.* (1982) studied both seedling resistance and adult plant resistance among the 428 cultivars. Resistance at both the stages was associated with the genes Xa 1, Xa 2, Xa3 and Xa 5, whereas, cultivar IR 20 carrying Xa 4 gene was susceptible at both the stages. Few of the cultivars tested were resistance either at seedling stage or during the adult plant. In contradiction to the previous finding Zhang *et al.* (1984) observed in a study of 11 varieties with adult plant resistance to *Xanthomonas oryzae*, one with overall resistance and one with susceptibility, the responses of those with adult plant resistance changed from susceptible to resistant in the 55-68 the days of the growth period. The varieties differed in their response to the three isolates used, some showing adult plant resistance to same isolates and overall resistance to others. Qi and Mew (1985) reported that selected rice cultivars expressed more resistance to *Xanthomonas campestris* pv. *oryzae* as plants advanced in age, this being comparable with adult plant resistance to diseases in other crops. Some cultivars were resistant to four races and other to only one or two and the disease severity on some cultivars gradually decreased from seedling to the flag leaf, whereas, in others it showed a distinct change to a resistant reaction on a certain leaf. The leaf showing resistance varied with cultivars. Wase Aikoku 3 had adult plant resistance to three races, but seedling resistance to another. The adult plant resistance of rice to bacterial blight appears to be race specific. Similar findings were also reported by Yoshimura *et al.* (1985). Guo *et al.* (1987) tested 36 varieties by inoculating with *Xanthomonas campestris* pv. *oryzae* strain KS-6-6. Among these, 6 indica varieties showed resistance throughout the growth period; 5 indica and 5 japonica varieties were susceptible throughout the whole season and 4 indica and 16 japonica



varieties showed adult plant resistance. These findings revealed in the resistance expressed in different ways. This was clearly depicted in Yangeng 2 and Dazhi 1 which were resistant from the 7 to 8 leaf stage, Nangeng 11 was only fully resistant after the 11 to 12 leaf stage. Liu (1990) studied 24 varieties throughout their development, some had a clear stage of adult plant resistance to *Xanthomonas campestris* pv. *oryzae*, while others developed the infection gradually. In some varieties, adult plant resistance was shown only to certain strains of the pathogen. Sahu (1987) screened about 200 rice cultivars and some hybrid progenies against *Xanthomonas campestris* pv. *oryzae* for resistance at the seedling, maximum tillering and boot leaf stages. Plants that were resistant to the disease at the seedling stage remained resistant at later growth stages, but the reverse was not true. Identification of resistant plants at the seedling stage was possible if the genotypes involved possessed the Xa 5 and or Xa 10 resistance genes. Plants with Xa 4 conferred resistance at the maximum tillering stage, while cultivars possessing Xa3 genes could only be identified at the boot leaf stage. Therefore, it was recommended that cultivars be screened at the all stages. Sahu (1988) screened 200 rice cultivars from the IRRI gene bank at seedling, maximum tillering and boot stage. Resistances at tillering and panicle initiation stages were closely linked in some of the cultivars. Noda and Ohuchi (1989) extensively studied, involving a wide range of varieties, and reported information on differences in varietal resistance to the pathogen during the seedling stage, genetic analysis of resistance to the pathogen in varieties during both the seedling and adult plant stages, classification of isolates of the pathogen on the basis of virulence to varieties at the seedling stage and interaction between the kresek and leaf blight phases of bacterial blight of rice. Koch and Mew (1991a) observed that lesions of all cultivars decreased about equally in absolute length with plant age, but the relative increase in lesion length was greater with moderately resistant cultivars than with highly susceptible. The change in quantitative resistant cultivars with increasing plant age was different. The resistance immature leaves that were still extending showed susceptibility than the adjacent mature, fully expanded leaves, but immature leaves of moderately resistant cultivars were less susceptible than those of highly susceptible cultivars. Koch and Mew (1991b) reported that younger plants and susceptible cultivar had higher rates of increase than older plants and moderately susceptible cultivars, respectively. The rate of expansion of *Xanthomonas campestris* pv. *oryzae* decreased with time, for strains PXO 71 and PXO 99 and increased or remained the same for strains PXO 86. There were no major differences among cultivars for the change in the rate of lesion expansion over time. The susceptible cultivar IR-24, which was used as susceptible control in screening trial displayed resistance at adult plant growth stage. Further, resistance conveyed by Xa 21 was evident in 21 days old plants, and the level of resistance increased with plant maturity (Mazzola et al., 1994). Goel and Singh (1995) examined 27 genotypes at three growth stages (seedling, tillering and flowering), and among them the BR-51, 50-3-4, IETS 8326 and IETS 12174 showed overall resistance to all five pathotypes of *Xanthomonas oryzae* pv. *oryzae* and others showed adult plant resistance to one or more pathotypes. The expression of adult plant resistance was both cultivar and pathotype specific. Resistance at the seedling stage did not change to susceptibility at later growth stages in any of the cultivar / pathotype combinations studied. Malagkit Sungsong, Zenith, IR-944 and IR-1695, possessing Xa 6 gene conferring resistance to *Xanthomonas oryzae* were susceptible at the seedling stage but resistant at the leaf stages of 11 and 12. Malagkit Sungsong showed resistance on earlier leaves than did the other cultivars. On staggering the planting to synchronize inoculation, resistance increased with ascending leaf position. Lesions on susceptible T(N)1 developed faster at 33/25 °C than at 25/20 °C. The overall resistance of IRI545-339 was not altered by high temperature. The adult plant resistance of the cultivars tested was not affected by changes in temperature. The bacterial population in leaves 6, 9 and 10 of the variety Zenith was at a maximum three days after inoculation; on leaf 12 the population was lower (Qi and Mew, 1984). The degree of infection was studied at early tillering, maximum tillering and booting in 12 rice varieties carrying Xa 4 resistance gene, after inoculation in dry season with races 1 and 2 of *Xanthomonas campestris* pv. *oryzae*. Based on hill infection, the relative resistance index (calculated by comparison with susceptible cv. IR-24) of the varieties infected with race 1 (avirulent) increased as the plant matured. The same occurred with race 2 (virulent). It was suggested that these varieties contain background resistance in addition to Xa4 and increased as the plant matures (Mew and Vera Cruz, 1988). The early japonica lines G 38, carrying the gene Xa 5 for resistance to *Xanthomonas campestris* pv. *oryzae*, was used as resistance donor in crosses with the recurrent parent Xiushui 11, which had adult plant resistance. As a result of successive backcrosses, three late japonica lines, D601, D602 and D603, were bred with resistance at all stages. Genetic analysis showed that the lines combined with gene Xa 5 and Xa 3 were more resistant than G 38 and Xiushui 11 (Xu et al., 1996). Khare and Thrimurthy (2006) evaluated 86 cultivars in 1999 and 2000 for resistance to *Xanthomonas oryzae* pv. *oryzae* at the seedling, maximum tillering and flowering stages. At the seedling stage, disease incidence ranged from 6.2% (IR-54/BK-1170-33-13-1-1-32) to 1.6% (R-714-1-19-1-7). At the maximum tillering stage, disease incidence ranged from 1.9% (R 296-260) to 0.3% (IRBB-207) and at 50% flowering stage, the disease incidence ranged from 0.6% (R 435-1585) to 10.8% (RP-2526-14767-1143) among the cultivars studied. Thrimurthy et al. (2000) studied Ajaya (IET-8585) bacterial blight resistance variety possessing donor level resistance. They observed out of the inoculated

leaves, 48 per cent showed resistant, 42 per cent moderate and 10 per cent susceptible reaction. Only 7 per cent leaves showed water soaked symptom irrespective of lesion length. Narrow broken lesions were also observed with intermediate healthy area in 6 per cent leaves. The AUDPC values of the inoculated leaves showed that 2<sup>nd</sup> leaf was more prone to blighting than younger first leaf. The susceptible check TN-1 showed 100 per cent susceptibility with water soaked and no broken lesions during the same period. Thrimurthy *et al.* (2005) studied 65 rice genotypes developed from MAS. The composition of the genotypes were xa 5 (6 entries), xa 13 (6 entries), Xa 21 (6 entries), xa 5 + xa 13 (5 entries), xa 5 + Xa 21 (5 entries), xa 5 + xa 13 + Xa 21 (17 entries). Variation in disease reaction among the MAS selected plants of a genotype were also recorded ranges from susceptible to resistant reaction. They further ascribed that the virulence of the pathogen and congenial condition, does play more importance for such variations. Similarly, in other crops also the host pathogen interaction was studied.

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