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EVALUATION OF RESISTANCE OF RICE VARIETIES AGAINST BACTERIAL BLIGHT CAUSED BY *XANTHOMONAS ORYZAE* PV. *ORYZAE*.

AMNA NOOR, ZUBEDA CHAUDHRY, HAMID RASHID AND BUSHRA MIRZA*

Agricultural Biotechnology Programme (ABP),
Institute of Agricultural Biotechnology & Genetic Resources (IABGR),
National Agricultural Research Center (NARC), Park Road, Islamabad, Pakistan
**Department of Biological Sciences, Quaid-i- Azam University, Islamabad, Pakistan.*

Abstract

Eight different isolates of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) procured from International Rice Research Institute (IRRI) Manila, Philippine were subjected to pathogencity test to check virulence on three basmati rice varieties in order to find out the source of resistance against Bacterial blight and to pick the most resistant and susceptible varieties.

Rice nursery was grown in the glass house and pathogenicity of each strain was tested on 4 week, 8 week and 12-week-old rice plants by using clipping method of artificial inoculation, in which five plants successively inoculated, after dipping the scissors in the bacterial suspension. A control of each variety was also maintained, for this, scissors dipped in sterile water was used for clipping off leaves. The symptom development was rated by counted lesion number, size and progress of blightening, which indicated the resistance of particular variety against particular strain.

It was evident from the results that all the three rice varieties were susceptible to *Xoo* but Super basmati was highly susceptible to all the exotic strains of *Xoo* with maximum percentage disease incidence (89.5%) for PXO 340 at the seedling stage (4 week), with 84.54% at maximum tillering stage and with 56.21% for PXO 61 at leaf flag stage. On the other hand Basmati 2000 was most resistant variety at all the two growth stages and susceptible at maximum tillering stage for PXO 280, with maximum percentage disease incidence (75.96%) and PXO 340 with 71.53%. The reaction of eight different strains of *Xoo* was variable against the Basmati 385. At seedling stage, it showed susceptibility against PXO 61 with maximum percentage disease incidence 65.33%, for PXO 339 with 58.18% at maximum tillering stage and with highest rate of maximum percentage disease incidence i.e. 75.68% for PXO 341 at leaf flag stage. The reaction of a bacterial strain was variable to different rice varieties, the reaction of different bacterial strains was also found variable against the same rice varieties.

Introduction

Rice, *Oryza sativa* occupies the enviable prime place among the food crops cultivated around the world. (Mahadevappa, 2004). It a staple food for 2.7 billion people, almost half of the world population and 90% of the total rice is grown in Asia. (Salim *et al*., 2003). In Pakistan, rice is cultivated on an area of 2225.2 million ha with a production of 4478.5 million tons giving an average yield of2013 kgs per hectare (Anon., 2002-2003).

Pakistani rice especially basmati rice is a major commercial variety grown in rice growing areas of Pakistan and is famous in the world for its particular aroma. Total area under basmati production is 1377.3 hectare in which 1316.8 hectare is for Punjab. Total production of basmati rice is 2175.5, Punjab contributes 2304.2 million tons with a 1673 kgs per hectare yield of basmati rice Basmati rice, the best quality scented rice produced

in Pakistan commands the international market have four times greater price than in the domestic market. Pakistan exports 7% of the total world market (Rashid *et al*., 2001) Despite all these, yield per unit area of rice in Pakistan is far below from the world average and low from many neighboring countries. The production of basmati rice is severely affected by various stresses, including diseases. (Viral, bacterial and fungal origin) which are one of the reasons for the low yields of rice.

Bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama; Swings *et al.,* 1990) is one of the most destructive diseases of rice throughout the world (Mew, 1987). This disease is also serious rice problem in other parts of Asia (Alim, 1967, Ou, 1985) during the heavy rains of the monsoon season. In many Asian countries, bacterial blight has become endemic on rice following repeated cultivation (Mew *et al.,* 1993). This disease has become serious because many improved, high yielding cultivars, when managed with high nitrogen levels and close spacing, have inadequate resistance to the pathogen. (Eamchit & Mew, 1982). It was first recorded in Japan in 1884. In the 1960's, bacterial blight became prevalent in other rice-growing regions of Asia with the introduction of 'improved' cultivars such as TNI and IR8, which were susceptible to the disease. In addition to Asia, bacterial blight occurs in Australia, Africa, Latin America, the Caribbean and the United States. Economically, it has had the greatest impact in Asia, where several epidemics have occurred in the past two decades, and in West Africa, particularly in Niger, where irrigated rice was extensively damaged in 1982. Yield losses of 10-50% from leaf blight have been reported. (Webster & Gunnell, 1992).

In Pakistan the disease was recorded for the first time by Mew & Majid (1977), later Ahmad & Majid (1980) observed it on rice varieties IR 6, Palman, Basmati-198 at Rice Research Institute, Kala Shah Kaku and farmer's field. During rice travelling seminar in 1985 its incidence on farmers field was recorded as 10-15, 15-20, 20-25% in Sindh, Punjab and NWFP respectively (Akhtar & Sarwar, 1986). Nineteen rice cultivars under NURYT trial in 1985 were tested at 10 locations and its occurrence was noted in almost all provinces of Pakistan (Akhtar & Akram, 1987). Khan *et al.,* (2000) narrated that BB incidence is increasing in Pakistan in recent years especially in Kallar belt that is famous for producing high quality rice. In 1997, in Muridke, Narang and adjoining areas BLB was observed in patches showing 5-10% disease BLB was found only the prevalent disease in Dhinga and Farkhandabad was recorded 40-50% in some fields of this area while in some fields of nearby villages 70-80% or even 90-95% infection was observed showing sever epidemic. Akhtar *et al.,* (2003) conducted a survey during the crop year 2002 for monitoring bacterial blight incidence and severity in Punjab, Sindh, Baluchistan, NWFP and Azad Jammu & Kashmir rice growing areas and to study the latest situation of this menace. They reported that Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* created a serious problem in rice during the crop year 2002. They concluded that bacterial blight incidence and severity in Punjab remained high during Sep-Oct., due to conducive environment (sudden strong wind and rain) at the time of panicle initiation and flowering of rice cultivars.

This study was conducted to evaluate responses of three different Basmati rice cultivars against 8 different exotic strains of *Xanthomonas oryzae pv*. *oryzae* collected from International Rice Research Institute (IRRI) Manila. In the present study, the clipping method, a faster method for inoculation was used to inoculate the rice plants. In this study, 28-30days, 48-60 days and 80-90 days old rice plants were inoculated with 8 different strains of *Xoo*, the causing organism of bacterial blight.

Materials and Methods

The experiment was conducted under glasshouse at the ABP, NARC during March-November 2003. Three different varieties of the Basmati rice viz., Super Basmati, Basmati 2000 and Basmati 385 were selected to study the Bacterial bright caused by *Xanthomonas oryzae*. The seeds of these varieties were procured from IABGR, NARC, Islamabad.

Thirty to thirty-five seeds of each variety were sown in a separate pot and three such pots were maintained for each variety. At the three-leaf stage, these seedlings were shifted to separate pots so that each pot consisted 2-3 plants.

The seeds of each variety were dehusked. Then the healthy seeds of each variety were chosen and separately soaked by placing them on the wet filter paper in the separate Petri plate to break the dormancy and obtain the synchronous growth. After germination 15-20 seeds were sown in a separate pot and five such pots were maintained for each of three varieties. At three-leaf stage, these seeding were shifted in separate pots so that one pot contained 2-3 plants. Plant aged 30 days, 60 days and 90 days were used to study bacterial blight of rice.

Eight different strains of *Xanthomonas oryzae*. *pv*. *Oryzae* viz., PXO145, PXO 86, PXO336, PXO280, PXO112, PXO340, PXO61, PXO341 were used to study bacterial blight of rice. These strains were obtained during 1993-1997 from the International Rice Research Institute (IRRI) Manila and provided by IABGR, NARC, Islamabad. All these strains were in dry preserved form at 4^oC is small vials. Bacteria were grown on two agar media for revival: Modified Wakimoto's medium and Yeast Dextrose Calcium Carbonate Agar medium (YDC medium), but Modified Wakimoto's medium proved to be better to obtain pure and clear culture as compared to YDC medium. For inoculums preparation half ml or 500 ul of distiledl water was added in vials containing preserved bacterial culture mix will and them bacterial culture was streaked on the Petri plates with solid modified Wakimoto's medium. These plates were incubated at 28 C^0 for two to three days depend upon colony development. Approximately one third of the test tubes were filled with modified wakimoto medium after autoclaving these were placed up word in slanting position to make slant, after these slants were incubated at $30C^0$ for 24 hours to observe the presence of contamination. Slants free of contamination were used for the streaking of bacteria these slants were incubated at $28C^0$ for 48 hrs. For the preparation of fresh innoculum 10ml of sterile distilled water was added to the 48 hrs culture of *Xanthmonas oryzae* in the slants for the preparation of bacterial suspension.

For pathogenecity test clip method was used for the inoculation of the rice plants with *Xanthomonas oryzae* pv. *oryzae*. A control of each variety was also maintained, for this scissors dipped in sterile water was used for clipping off the leaves.the pathgenicity of each strain was tested on 4 week, 8 week and 12 week old rice plants of all three varieties, at 35 ± 2^oC in the glass house.

Following the inoculation, the plants were surveyed after every 24h interval to note the appearance of disease symptoms and final data was recorded after 14 days of inoculation. Percent disease incidence was calculated according to (Gnanamanickam *et al*., 1999) formula as follows:

$$\% \text{ Disease incidence} = \frac{\text{Total lesion length}}{\text{Total leaf length}} \times 100$$

Rating the disease reaction was based on a 0-9 scale of the standard evaluation system for rice (Anon., 1996). Scoring was at 12-14 days after inoculation or when the susceptible check showed maximum disease incidence.

The leaves of rice plants inoculated with *Xanthomonas oryzae*, showing the symptoms of bacterial blight i.e., yellow lesions were used for isolation of the bacteria in order to confirm the bacterial blight. The infected tissues were cut into 2x7mm section from advancing portion of the lesions. Leaf tissues were sterilized in 70% alcohol for I minute and were placed on solid modified Wakimoto's medium and incubated at 28^{o}C for 72-96 h. After72-96 h, plates were observed for the presence of *X. oryzae*

Results and Discussion

Selected 8 isolates of Xoo collected from 1994-1997 were tested for pathogenicity and to check their virulence to the three different basmati rice cultivars to find out the susceptible and resistant cultivars. On each cultivar, lesions developed uniformly downward from the point of inoculation. The length from the leaf tip varied, however, among cultivars. Initial symptoms in the present study were leaf curling which appeared after 3 days in case of basmati 370 while in all the other varieties it appeared after 4 to 5 days of inoculation at seedling stage. This was supported by the work of Kauffman (1973) as they reported that the disease symptoms first appeared 4 to 5 days of inoculation in the form of leaf curling near the cut off portion when they evaluated the resistance of IR 8 and IR 20 against PXO 25.

In this study the lesions caused by infection through leaves results in water soaked lesions at the margin of the cutoff portions of the leaves. Lesions started at the margins, a few cms from the tip, as water soaked stripes. The lesions enlarge both in length and in width, have a wavy margin, which turn yellow within a few days the region adjoining the healthy part shows water soaking. As the disease, advances the lesions cover the entire blade, turn white and later become grayish as reported by Salim *et al*., (2003).

The pathogenicity of eight different strains of *Xanthomonas oryzae* pv. *oryzae* were tested against three different Basmati cultivars of rice under local conditions. These tests were conducted on three different growth stages of rice plants at seedling stage, tillering stage and at leaf flag stage. At seedling stage i.e., after 28-30 days of sowing, Super Basmati was susceptible to all exotic strains of *Xoo* with maximum percentage disease incidence of 89.5% for PXO 340. PXO 112 showed minimum percentage of disease incidence which was 55% Basmati 2000 which was resistant to all exotic strains was less susceptible to PXO 61 which was 15.1%. Basmati 385 was moderately resistant and only susceptible to PXO 61 and PXO 340 (Table 1, Figs. 1-5).

The results of the pathogenicity test of 8 strains of *Xoo* against 3 different Basmati rice cultivars after 58-60 days of sowing (8 week) at maximum tillering stage are given in Table 2. Super Basmati was susceptible to all the 8 strains of *Xoo* with maximum disease incidence for PXO 61i.e, 84.54%. Basmati 385 was highly resistant only susceptible to PXO339 with the maximum disease incidence of 58.18%. on the other hand in the case of Basmati 2000, reaction of all eight exotic bacterial strains was variable against three rice varieties. PXO 145 showed resistance against Basmati 2000, but this variety was susceptible to all strains with maximum disease incidence for PXO 280 i.e., 75.96%,

Table 1. Reaction of eight different strains of *Xanthomonas oryzae* pv. *oryzae* on Basmati 385, Basmati 2000 and Super Basmati at seedling stage.

Strains	Total length of inoculated leaves (cm)			Total lesion length (cm) from point of inoculation			% Disease incidence			Host response		
	Bas. 385	Bas. 2000	Sup. Bas	Bas. 385	Bas. 2000	Sup. Bas	Bas. 385	Bas. 2000	Sup. Bas	Bas. 385	Bas. 2000	Sup. Bas
PXO 145	39.0	86.00	59.7	5.00	0.00	50.1	12.82	0.00	83.9	R	HR	S
PXO 86	57.1	76.00	32.5	22.2	0.00	18.5	38.8	0.00	74.00	MS	HR	S
PXO 339	35.75	112	39.7	19.6	0.00	20.5	54.82	0.00	51.6	S	HR	S
PXO 280	45.5	79.00	49.5	3.00	1.2	40.00	6.59	1.5	80.00	R	HR	S
PXO 112	61.00	88.00	22.00	0.00	5.00	12.1	0.00	5.6	55.00	HR	R	S
PXO 340	57.00	115.5	75.5	37.00	2.5	67.5	64.9	2.16	89.5	S	HR	HS
PXO 61	75.00	40.1	58.7	49.00	6.00	48.7	65.33	15.1	82.9	S	MR	S
PXO 341	88.00	51.00	29.00	5.00	3.00	25.5	5.6	5.8	87.09	R	R	HS

HR=Highly Resistant, MR= Moderately Resistant, R= Resistant, HS= Highly Susceptible, MS= Moderately Susceptible, S= Susceptible

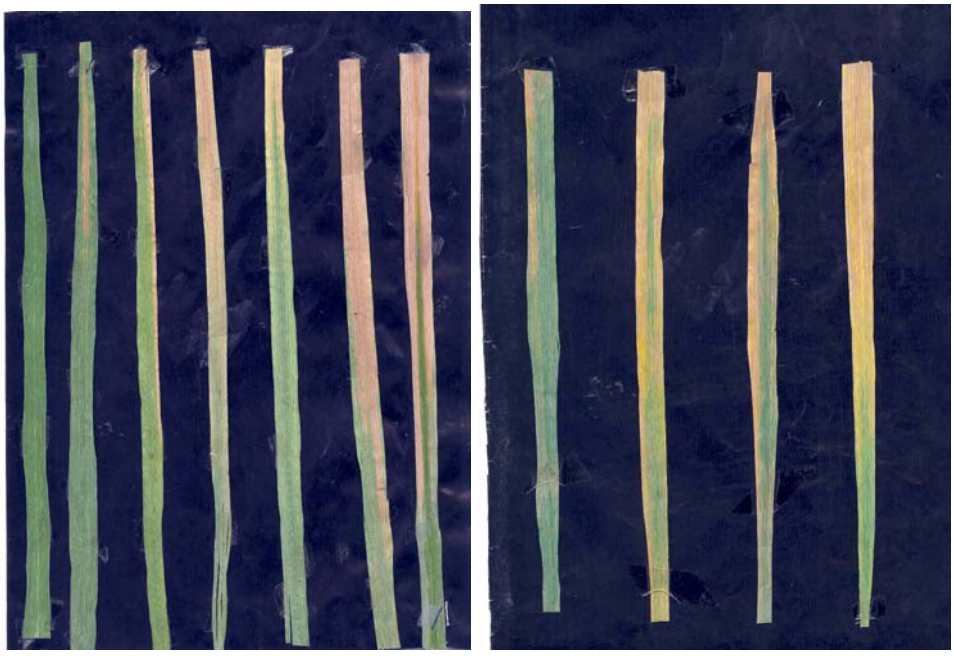

Fig. 1& 2. After inoculation with clip method, bacterial blight of rice showing mild to severe symptoms

Table 2. Reaction of eight different strains of *Xanthomonas oryzae* pv. *oryzae* on Basmati 385, Basmati 2000 and Super Basmati at maximum tillering stage.

Strains	Total length of inoculated leaves (cm)			Total lesion length (cm) from point of inoculation			% Disease incidence			Host response		
	Bas. 385	Bas. 2000	Sup. Bas	Bas. 385	Bas. 2000	Sup. Bas	Bas. 385	Bas. 2000	Sup. Bas	Bas. 385	Bas. 2000	Sup. Bas
PXO 145	88.00	168.5	259.70	20.00	0.00	250.1	22.7	0	83.9	MR	HR	S
PXO 86	115.5	159.0	295.0	0.00	110.00	265.0	0.00	69.18	80.45	HR	S	S
PXO 339	55.00	111.0	243.0	32.00	70.5	200.0	58.18	63.5	82.30	S	S	S
PXO 280	120.00	129.0	301.5	0.00	98.0	250.0	0.00	75.96	83.0	R	S	S
PXO 112	115.5	88.0	275.0	2.50	20.5	210.0	2.16	22.72	76.36	HR	MR	S
PXO 340	86.00	98.00	283.5	0.00	70.1	219.5	0.00	71.53	77.42	HR	S	S
PXO 61	95.00	110.00	295.7	18.00	50.2	250.3	18.94	45.63	84.54	MR	MS	S
PXO 341	86.00	170.00	259.8	16.50	92.5	206.5	19.18	54.41	79.29	MR	S	S

HR=Highly Resistant, MR= Moderately Resistant, R= Resistant, HS= Highly Susceptible, MS= Moderately Susceptible, S= Susceptible

Fig. 3. Photograph showing 21 days after inoculation (DAI), Vascular bundles in the meristem region filled with bacteria and the plant begins to wilt and after 30 days (DAI) plant become died.

Fig. 4. Bacterial blight disease reaction of rice cultivars to *Xoo* strains.

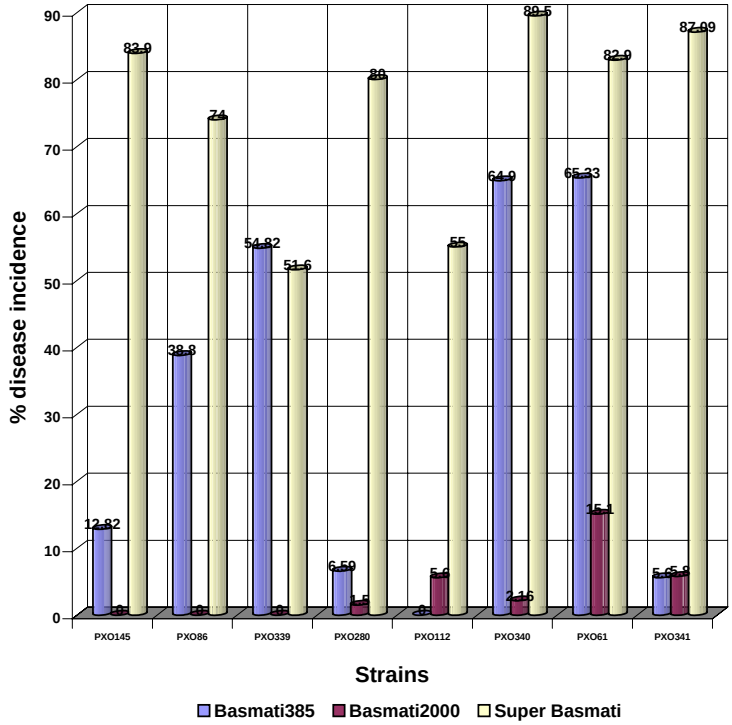

Fig. 5. Reaction of eight different strains of *Xanthomonas oryzae* pv. *oryzae* on Basmati 385, Basmati 2000 and Super Basmati at seedling stage.

Table 3. Reaction of eight different strains of *Xanthomonas oryzae pv. oryzae* on Basmati 385, Basmati 2000 and Super Basmati at Leaf flag stage

Strains	Total length of inoculated leaves (cm)			Total lesion length (cm) from point of inoculation			% Disease incidence			Host response		
	Bas. 385	Bas. 2000	Sup. Bas	Bas. 385	Bas. 2000	Sup. Bas	Bas. 385	Bas. 2000	Sup. Bas	B. 385	B 2000	S. B
PXO 145	220.5	217.00	224.5	0.00	0.00	111.50	0.00	0.00	49.66	HR	HR	MS
PXO 86	210.0	241.6	181.5	0.00	0.00	90.00	0.00	0.00	49.58	HR	HR	MS
PXO 339	219.0	281.00	247.0	14.50	8.50	100.00	6.62	3.02	40.48	R	HR	MS
PXO 280	252.0	239.0	194.5	151.1	0.00	85.00	60.11	0.00	43.70	S	HR	MS
PXO 112	239.5	263.5	205.5	0.00	0.00	110.00	0.00	0.00	53.52	HR	HR	S
PXO 340	232.0	252.0	241.5	0.00	0.00	135.00	0.00	0.00	56.01	HR	HR	S
PXO 61	217.0	298.0	185.0	110.0	0.00	104.00	50.69	0.00	56.21	MS	HR	S
PXO 341	199.5	258.0	179.5	151.0	10.5	98.00	75.68	4.06	54.50	S	R	S

HR=Highly Resistant, MR= Moderately Resistant, R= Resistant, HS= Highly Susceptible, MS= Moderately Susceptible, S= Susceptible

The results of pathogenicity test of *Xanthomonas oryzae pv*. oryzae against three rice cultivars after 80-90 day of sowing (12-weks) at leaf flag stage are summarized in Table 3, Fig. 6. Like two previous stages i.e., seedling stage and maximum tillering stage, Super Basmati was also susceptible against all the 8 exotic strains of *Xoo* at leaf flag stage and the maximum % ercentage disease incidence was still high in this stage which was 56.21% with PXO61. As compared to this, Basmati 385 showed highly resistance to four exotic strains of *Xoo* susceptible to PXO 280 and PXO 341 with % disease incidence 60.11 % and 75.68% respectively. Basmati 2000 was very resistant to all exotic strains of *Xoo*

All the five varieties were inoculated at three different growth stages, seedling stage, maximum tillering stage and at leaf flag stage i.e. after 30, 60 and 90 days of germination respectively as the age of the host plant influence development of bacterial blight. Similarly Zhang & Mew (1985) also tested 13 cultivars for the resistance to four races at three different growth stages. Ou *et al*., (1971a) reported that the testing at seedlings and flag leaves stage would be sufficient for preliminary screening of a large number of rice cultivars against BB disease.

At the seedling stage the most susceptible variety was Super Basmati which exhibited susceptible reaction to all the 8 strains of *Xoo* tested. The maximum disease incidence was 89.5% against PXO 340. Khan *et al*., (2000) also reported that super basmati showed susceptible reaction against the indigenous strain of *Xoo*.86 and PXO 341. Akhtar *et al*., (2003) also reported that Super basmati was the main cultivar in the Punjab and it was highly susceptible to bacterial blight.

On the other hand Basmati 385 was resistant to susceptible. On seedling stage it showed completely resistant to PXO 112 i.e. 0%, but showed susceptible resistance to PXO 61 and PXO 340 i.e. 65.33% and 64.9%, respectively. Our findings are contrary to Bhutta & Ahmad (1994) who reported Basmati 385 showed a resistant reaction, whose disease incidence on Basmati 385 was 10 - 20 %. But Khan *et al*., (2000) reported a susceptible response of Bas. 385 against indigenous strains of Xoo.

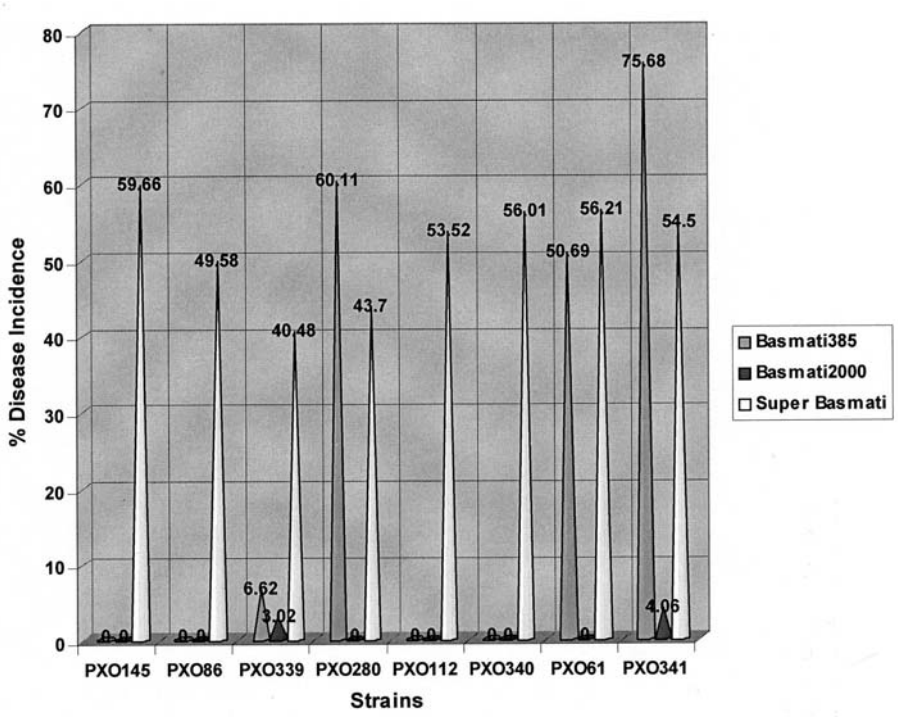

Fig. 6. Reaction of eight different strains of *Xanthomonas oryzae* pv. *oryzae* on Basmati 385, Basmati 2000 and Super Basmati at seedling stage.

Webster & Gunnell (1992), reported that bacterial blight, the more common syndrome, generally occurs from tillering stage to onward, but in our research seedling stage was very susceptible to BB. As compared to maximum tillering stage and leaf flag stage. On the other hand Mew et al., 1993 reported that infection at the tillering stage can lead to total crop losses, more commonly however, plants are affected at the maximum tillering stage.

When the plants were inoculated at the maximum tillering and leaf flag stage, it was found that the young plants were more susceptible than the older ones. The disease incidence of all the three-basmati varieties was less at maximum tillering stage than the seedling stage and most of varieties, which were susceptible at seedling stage gained resistance from tip to leaf flag stage against different bacterial strains. Cho (1975) also reported that the disease incidence was higher in younger plants than older ones.

There was a direct relationship between the resistance and the age of host plant. Plant age greatly influenced the varieties that were susceptible at seedling stage and these varieties showed more pronounced resistant response in the later stages. Super basmati had the highest disease incidence against PXO 340 with 89.5% that reduced to 56.21 at leaf flag stage. On the other hand response of varieties which were resistant to different bacterial strains at seedling stage were not greatly influenced by the host age. Zhang *et al*., (1984) and Mazzola *et al.*, (1993) reported similar results that plants gained resistance with the age against BB disease. Contrary to our observation Mariappan *et al*., (1980) reported that a cultivar ASD 5, was resistant until the tillering stage, but became infected

at the boot leaf stage (60 - 80 days). In contrast to this report, Sahu (1987) stated from IRRI that plants resistant at the seedling stage remained resistant at later growth stages of plant, but reverse was not true.

From these results it is evident that the bacterial strains which induced susceptible reaction to one variety of rice was not necessarily able to induce the similar reaction in other varieties and reaction of a bacterial strain was variable to different rice varieties as PXO 145 induced susceptible on to Super Basmati, however, Basmati 2000 showed complete resistance to this strain.

To confirm that the symptoms showed by the inoculated rice plants were due to presence of *Xanthomonas oryzae* pv.*oryzae*, diseased leaves were plated on Wakimoto's media. Plates were monitored between 72-98 h for bacterial colonies. Our results showed that the colonies appeared were similar to the bacterial colonies used for inoculation i.e., yellow smooth and viscous. Bogdanove & Martin (2000) isolated the bacterium by plating inoculated rice plants using 10^{-3} dilution and reported that yellow colonies appeared on the media which confirmed the presence of *Xanthomonas oryzae*.

From our results it was obvious that the super basmati was susceptible to all the exotic strains *of Xanthomonas oryzae* pv.oryzae tested, on the other handBasmati 385 and Basmati2000 were susceptible to only couple of exotic strains showing resistance to most of them. Among the bacterial strains PXO 61 was most virulent strain at seedling stage, PXO 339 at maximum tillering stage and PXO 341 at leaf flag stage.

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