

Screening of rice (*Oryza sativa* L.) germplasm against *Xanthomonas oryzae* pv. *oryzae* (Xoo) in green house condition

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Abstract:

Bacterial leaf blight (BLB) is one of the apex devastating diseases of the rice crop around the world. It continuously reduced the yield and quality of rice. Host plant resistance is an important component of IPM for this disease. It is easy to use, viable, durable, effective, and long term method as compared to any other control measure. In this investigation effort has been made to screen the rice germplasm against Xoo pathogen under greenhouse condition. For this purpose most aggressive strain PKXOO4 was used. The fresh culture of most aggressive strain was prepared approximately 10⁹ CFU/ml and inoculated the rice germplasm by clip method. Among 28 Pakistani germplasm screened, none was found immune, resistant or moderate resistant against Xoo. However six genotypes RICC 101, RICC 115,

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RICC 116, RICC 119, RICC 138 and RICEF 166 were found moderately susceptible, twenty were susceptible and three were highly susceptible.

Key words: Rice, bacterial blight, *Xanthomonas oryzae* pv. *oryzae*, screening, germplasm

Introduction

Rice is life. Rice is one of the prime staple food crop of world especially in south east asia , and at same time it attain the 2ed position on creal road map of world after wheat crop. Approximately 90% rice is produced and consumed in Asia (Salim *et al.*, 2003).In Pakistan rice is also an important food crop after wheat. In world Pakistan has a unique identity due to his God gifted aroma basmati rice .It is planted an area of 2.7 million hectares and annual production of 6.7 million tones. Its account for 4.4 percent of value added in agriculture and 0.7 % in GDP (Economics Survey of Pakistan 2013-2014).Despite much development in rice research, the rice yield in Pakistan is almost half as compared to other rice growing countries. One of the yield limited factor is rice diseases. Among them bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is of economic importance and cuse substantial yield loss every year in rice growing countries including Pakistan (Swing *et al.*,1990).This disease was first reported by Mew (1977) followed by Ahmed and Majid (1980). Disease incidence increased day by day especially in kaller belt which is public figure due its producing premium quality of scented basmati rice. Yield reduction under mild BLB infection is 10-20% (Mew *et al.*, 1993). Whereas severely infected field may show 50 % loss (Ou, 1985).

Actually, there is no approach or method wich is considered as an effective, efficient, environment friendly and economical to control bacterial leaf blight in rice crop. The varietal resistance is the only way to consider as a key tool

under any environment conditions to control disease in rice crop. This method is more economical, convenient and most suitable on large scale as compared to other control method.

Materials and Methods

Isolation of causal organism:

The disease affected leaves were collected from rice fields of Punjab during 2011. Disease symptomatic leaves were cut in to small pieces. The small pieces were placed in 1% Clorox for one minute and then in 70% ethanol for one minute .finally they were washed with autoclave d distal water at least three times and placed on modified Wakimoto,s Agar media in petri plates at 28 in an incubator. On the basis of morphology, those bacteria which have yellow circular colonies with entire margins, smooth and shiny surface were isolated .These selected pure isolated yellow colonies were shifted on Peptone Sucrose Agar (PSA) medium (Ou, 1985).

Preservation of Xoo

After the purification these isolates were store in 20% glycerol at -20 for further studies. (Fig: 1)

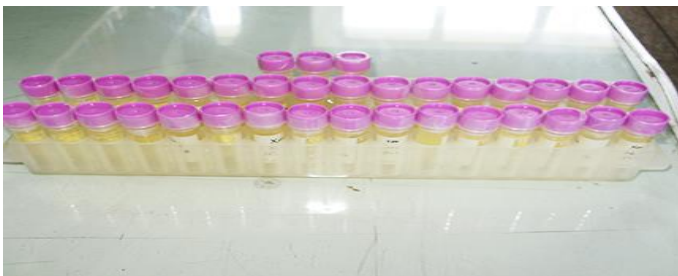


Fig 1: Glycerol Preservation of *Xanthomonas oryzae* pv. *oryzae* culture

Pathogenicity test

Seeds of elite commercial cultivar Super Basmati were sown in wooden trays and after one month seedling were transplanted to 25 cm diameter plastic pots. Rice plants were grown under greenhouse conditions. For pathogenicity test, inoculation suspension was prepared in 5ml of sterile distilled water @ 10^9 CFU/ml. For pathogenicity confirmation, plants with fully fresh and expanded leaves were inoculated by leaf cutting method (Kauffman *et al.*, 1993). Plants were inoculated before panicle initiation. The autoclave scissors was dipped in the inoculum and clipped the 3 leaves per plant. Approximately 2-3 cm from tip of each leaf. BLB lesions on clipped leaves were observed 15 days after inoculation.

Inoculation of rice germplasm under greenhouse condition

Twenty eight rice germplasm (*Oryza sativa* L.) Including eighteen course candidate varieties, nine fine candidate varieties and one elite commercial variety were screened. The seeds of all rice germplasm were collected from Rice Program, Crop Research Institute (CSI), National Agricultural Research Centre (NARC) Islamabad.

All of germplasm were tested against Xoo, a causal agent of BLB in rice crop. Seed of rice germplasm were sown in plastic tray containing fertile soil. The plastic was watered with sprinkler to keep the most condition till seedling emerges. Evaluation was done after 21 days of sowing. For the inoculation autoclaved distilled water approximately (10ml) poured into culture plates of most aggressive Pakistani strain PKXOO 4 and maintains the inoculum concentration @ 10^9 CFU/ml. Before the inoculation all plants were sprayed with fresh water to create humidity which is required for successful disease development. Clipped method was used for inoculation. All the plants were observed after 24 hours on daily basis.

The following rating scale will be used for showing disease infection of inoculated leaves. (Standard Evaluation System for Rice, IRRI. 1996)

Results and Discussion

Twenty eight rice genotypes including eighteen course candidate varieties, nine fine candidate varieties and one elite commercial variety were screened against *Xanthomonas oryzae* pv. *oryzae* under green house condition at Insectary Biocontrol Lab, National Agriculture Research Centre, Islamabad. Rice genotypes were classified into three classes based on degree of reaction and genotypes were falling in particular class are present in table 1.

S.no	Rice germplasm	Disease %	Score	Response
1	RIC 121	68.4	6	S
2	RIC 129	67.27	6	S
3	RIC 134	74.00	6	S
4	RIC 135	62.00	6	S
5	RIC 138	46.86	5	MS
6	RIC 115	36.00	5	MS
7	RIC 116	43.00	5	MS
8	RIC 119	54.00	6	S
9	RIC 122	74.06	6	S
10	RIC 101	49.50	5	MS
11	RIC 102	83.50	7	S
12	RIC 103	89.00	8	HS
13	RIC 104	72.65	6	S
14	RIC 105	73.00	6	S
15	RIC 108	91.59	8	HS
16	RIC 109	87.00	7	S
17	RIC 111	81.50	7	S
18	RIC 114	77.60	7	S
19	RIC 160	54.90	6	S
20	RIF 163	61.66	6	S
21	RIF 166	49.40	5	MS
22	RIF 169	82.25	7	S
23	RIF 152	91.00	8	HS
24	RIF 153	68.66	6	S
25	RIF 154	85.75	7	S
26	RIF 157	65.40	6	S
27	RIF 159	53.60	6	S
28	Bas. 385	85.40	7	S

Table 1: Response of rice germplasm against *Xanthomonas oryzae* pv. *Oryzae*

None of them were found to immune or resistant or moderately resistant against *Xanthomonas oryzae* pv. *oryzae*. However six genotypes (RICC 101, RICC115 ,RICC116 RICC 119, RICC 139 and RIF 166 were found to be moderately susceptible with grade 5 and twenty genotypes were found susceptible with grade 6 and 7 and three genotypes were found highly susceptible with grade 8. Commercial variety basmati 385 was screened against *Xanthomonas oryzae* pv. *oryzae* and showed susceptible reaction. Similarly results were also reported by Shah (2008), evaluated the basmati 385 against *Xanthomonas oryzae* pv. *oryzae* in Pakistan and he showed maximum lesion length against Xoo, which clearly showed susceptible character. Khan *et al.*, (2000) screened basmati 385 against *Xanthomonas oryzae* pv. *oryzae* under field condition and found that basmati 385 was susceptible against BLB. Cheema *et al.*, (1998) also tested basmati 385 against *Xanthomonas oryzae* pv. *oryzae* and showed that variety failed to show resistant against Xoo. (Ali *et al.*, (2009) also screened the basmati 385 against BLB, he also supported that this variety is also susceptible (Akhtar *et al.*, (2008) reported that basmati 385 was showed moderately susceptible reaction against *Xanthomonas oryzae* pv. *oryzae* during study the effect of different methods of inoculation.

REFERENCES

- Ahmad, W. and A. Majid. 1980. Incidence of bacterial leaf blight of rice in Punjab (Pakistan) IRRN 5:5.
- Akhtar, M. A., A. Rafi and A. Hameed. 2008. Comparisons of methods of inoculation of *Xanthomonas oryzae* pv. *oryzae* in rice cultivars. Pak. J. Bot. 40(5): 2171-2175.
- Ali, A., M.H. Khan, R. Bano, H. Rashid, N.I. Raja and Z. Chaudhary. 2009. Screening of Pakistani rice (*Oryza sativa*) cultivars against, *Xanthomonas oryzae* pv. *oryzae*. Pak. J. Bot., 41 (5) : 2595 - 2604.

- Cheema, A.A., M. A. Awan and Y. Ali.1998. Screening of Basmati rice mutant against prevalent disease in the Punjab province. *Pak. J. Phytopathol.*, 10(1): 39-41.
- Economics survey of Pakistan. 2013-2014. Ministry of Food, Agriculture and Livestock. Govt. of Pakistan, Islamabad.
- Kauffman, H.E., A.P.K. Reddy, S.P.Y. Hsieh and S.D. Merca. 1973. An improved technique for evaluating resistance of rice varieties to, *Xanthomonas oryzae* in rice. *Plant Disease Report*, 57: 537 - 541.
- Khan, J.A., F.F. Jamil and M.A. Gill. 2000. Screening of rice varieties/lines against Bakanae and Bacterial leaf blight (BLB). *Pak. J. Phytopathology*, 12 (1): 6 - 11.
- Mew, T.W. 1977. Bacterial blight of rice in Pakistan. *IRRN*, 2: 5.
- Mew, T.W., A.M. Alvarez, J.E. Leach and J. Swings. 1993. Focus on Bacterial leaf blight of rice. *Plant Disease*, 77: 5 - 12.
- Ou, S.H. 1985. Rice Diseases. Second edition Common Wealth Mycobiological Institute, Kew, Surrey, England, 61 - 96.
- Salim, M., M. Akram, M. Ehsan and M. Ashraf. 2003. Rice a production handbook PARC, Pakistan.
- Shah, M.A. 2008. Morphological, molecular and resistance (bacterial blight and leaf folder) characterization of wild species of rice and F1 hybrids. Ph.D. Thesis submitted to NWFP Agriculture University, Peshawar.
- Standard Evaluation System for Rice. 1996. INGER Genetic Resources Center, IRRI, Manila, Philippines. 4th edition, 20 - 21.
- Swings, J., M.V.D. Mooter, L. Vauterin, B. Hoste, M. Gillis, T.W. Mew and K. Kerestes. 1990. Reclassification of the causal agents of bacterial blight (*Xanthomonas compestrise* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzacola*) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. rev. *Int. J. Syst. Bacteriol.*, 40 (3): 309 - 311.