Pak. J. Agri. Sci., Vol. 53(2), 365-370; 2016
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/16.5357
http://www.pakjas.com.pk

APPRAISAL OF RESISTANT GENES AND GENE PYRAMID LINES OF RICE AGAINST INDIGENOUS PATHOTYPES OF *Xanthomonas oryzae* pv. *oryzae* IN PUNJAB, PAKISTAN

Hafiz Muhammad Imran Arshad[1], Shahbaz Talib Sahi[2,*], Muhammad Atiq[2] and Waqas Wakil[3]

[1]Plant Protection Division, Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad, Pakistan; [2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan; [3]Department of Entomology, University of Agriculture, Faisalabad, Pakistan.
[*]Corresponding author's e-mail: iagill_1@yahoo.com

The studies were carried out to identify the resistant potential of 26 IRBB lines (10 Near Isogenic Lines (NILs) and 16 pyramids) provided by the IRRI, Philippine, against 29 prevailing pathotypes of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) under artificial inoculation field conditions. The inoculation was done at pre-panicle emergence stage through clipping method and IR24 was used as a susceptible check. The experiment was carried out in a split plot manner during 2013 & 2014. Disease scoring was carried out 21 days after inoculation. None of the NILs or gene pyramids provided complete protection against all the pathotypes. However, *Xa21* expressed resistance against 93% *Xoo* pathotypes followed by *xa13*, *Xa7*, Xa10 and *Xa14* conferring resistance to 79, 72, 72 and 72% pathotypes respectively. Among gene pyramids, IRBB-57 (*Xa4*, *xa5*, *Xa21*) exhibited incompatible reaction against 93% *Xoo* pathotypes followed by IRBB-64 showing resistance against 86% *Xoo* pathotypes including pathotype-1. The rest of the Xa genes either alone or in pyramid were moderately susceptible to susceptible. Based on the current study, IRBB-21, IRBB-13, IRBB-7, IRBB-10 and IRBB-14 as a single gene and IRBB-57, IRBB-64, IRBB-53 and IRBB-54 as pyramid are recommended to mitigate the BLB severity. The temptation of newly identified *Xa* gene evaluation is required for achieving complete resistance against all the *Xoo* pathotypes. Thus, the resistant genotypes could be considered a potential source for disease resistance and could be used further in the breeding programme for development of Bacterial leaf blight resistant rice varieties.
Keywords: Host resistance, bacterial leaf blight, disease management, bactericide.

INTRODUCTION

The Bacterial Leaf Blight (BLB) disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the main reasons limiting production of rice in Asia ensuing to an average of 20 to 30% and in some Asian countries, up to 50% rice yield losses .The disease, when occurs in severe condition on susceptible cultivars, causes 74 to 81% yield losses (Srinivasan and Gnanamanickam, 2005). It was reported that yield losses can be 100% in Basmati rice at tillering stage (Mannan *et al*., 2009). Cultural practices, chemical control or resistant varieties are optional strategies for the control of plant diseases. The chemical control of BLB is impractical in the monsoon climatic conditions of Asia (Agrawal *et al*., 2005). Furthermore, no effective bactericide is commercially available for disease control. Therefore, the preferred strategy for disease management is through varietal resistance (Naveed *et al*., 2010). The manipulation of host resistance has been shown to be the only and most reliable method to manage this disease. Globally, to date, 39 *Xa* genes (28 dominant and 11 recessive) conferring resistance against *Xoo* have been identified (Chen *et al*., 2011; Zhang *et al*., 2014). These identified genes were evaluated in different countries against their indigenous *Xoo* populations to deploy in local germplasm for BLB resistance. The response of 23 lines like 10 NILs and 13 pyramids was evaluated against BLB under field conditions in Vietnam. The resistance gene *xa13* and *Xa14* were susceptible, *Xa4* and *xa5* were moderately susceptible and the rest were moderately resistant to BLB. Among the pyramids, *Xa4+xa5+xa13+Xa21*, *Xa4+Xa7+Xa21* and *xa5+Xa7+xa13* had short lesion length and low diseased leaf area (Loan *et al*., 2006). Three gene pyramid (*xa5+xa13+Xa21*) in variety Sawarna was transferred to Jalmagna, a popular deep-water variety in India, which exhibited a high level of resistance against BLB (Pradhan *et al*., 2015). However, the durability of resistance depends upon the prevalence of pathogen races in time and space.

A lot of research has been done on the evaluation of rice genotype (Khan *et al*., 2009), virulence reaction of local *Xoo* isolates (Mannan *et al*., 2009), molecular screening of local germplasm against a specific R gene (Abbasi *et al*., 2011) and evaluation of resistance genes in rice against local isolates of *Xoo* (Khan *et al*., 2012) in Pakistan. But for the development of durable resistant cultivar, it is of prime importance to screen all available resistant genes either single or in combination against all prevailing 29 pathotypes of *Xoo*

(personal communication). This study is an effort to appraise genes and their pyramids which may be used for evolving new varieties with genes conferring resistance to the BLB.

MATERIALS AND METHODS

Sowing of rice lines: The seeds of 26 International Rice Bacterial Blight (IRBB) lines in the genetic background of IR24 comprising 10 NILs carrying single bacterial blight resistance gene, 16 pyramids having 2-5 resistant (Xa) gene combinations and IR-24 as parent/susceptible check were received from the International Rice Research Institute (IRRI), Philippines (Table 1). The nursery was prepared at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. At the age of 35 days, the seedlings of each line were triplicated in the split plot manner. The IRBB lines were planted in the main plot, and the *Xoo* pathotypes were used in sub-plots (Gomez and Gomez., 1984) having 9 inches plant to plant and 18 inches row to row distance.

Inoculum preparation: The isolates, representatives of the 29 prevailing pathotypes of *Xoo*, isolated from the diseased samples of rice collected from the 17 districts of Punjab were sub-cultured in peptone sucrose agar (PSA) petri plates and mass cultured in PS liquid medium at 28^{o}C for 48 hours in a shaker incubator. The 2 days old cultures were then used to prepare inoculum by suspending separately the bacterial cells of each isolate in 10 ml of sterile distilled water and adjusting the concentration up to 10^8 colony-forming units (CFU)/ml with the absorbance of 590 nm (Verdier *et al*., 2012; Yang, 2010).

Inoculation of plant materials: Split plot design was used to test the virulence of *Xoo* pathotypes on the near-isogenic rice lines in which the isogenic lines were considered as the main plot and bacterial isolates as subplots (Gomez and Gomez, 1984). The experimental unit consisted of three plants per isolate inoculation (Adhikari *et al*., 1999). About 10 flag leaves of three plants of each isogenic line were inoculated with each of the *Xoo* pathotype using a clip inoculation method at maximum tillering stage (Kauffman *et al*., 1973). Three plants of IR-24 were also inoculated with each isolate as a susceptible check.

Disease assessment: The lesion length of inoculated leaves was measured after 21 days of inoculation in centimeters (cm) and host-pathogen interaction in terms of lesion development on IRBB lines was marked both on the quantitative and qualitative typescript of the lesions by following disease rating scale (Cottyn and Mew, 2004; Lore *et al*., 2011).

Lesion length >5cm = Resistant (R)
Lesion length 5–10cm = Moderately resistant (MR)
Lesion length 10–15cm = Moderately susceptible (MS)
Lesion length >15 cm = Susceptible (S).

RESULTS

Reactions of near isogenic lines against *Xoo* pathotypes: The Behavior of 10 NILs against 29 pathotypes representing the 17 districts of Punjab was significantly different in their reaction. None of the NILs exhibited complete resistance to all the pathotypes so resistant to moderately resistant reaction considered as resistant behavior. IRBB-21 and IRBB-13 carrying *Xa21* and *xa13* genes exposed high level of resistance against 93 and 80% pathotypes respectively especially against pathotype-1 (highly virulent pathotype on a set of IRRI differentials) (Table 2). The response of these single *Xa* genes against disease showed that the pathotypes, lines and their interaction was significant at $P<0.05$. Three lines namely IRBB-21, IRBB-13 and IRBB-7 carrying *Xa21*, *xa13* and *Xa7* genes revealed a high level of resistance with

Table 1. Rice lines harboring resistance genes used to evaluate for resistance against *X. oryzae* pv. *oryzae* pathotypes in Punjab, Pakistan.

Rice lines	Xa genes	Cross	Rice lines	Xa genes	Cross
IRBB1	*Xa1*	IR24*5/KOGYOKU	IRBB54	*xa5, Xa21*	-
IRBB3	*Xa3*	IR24*5/CHUGOKU 45	IRBB55	*xa13, Xa21*	-
IRBB4	*Xa4*	IR24*5/IR20	IRBB57	*Xa4, xa5, Xa21*	AY4+5/IR66700-4-2-9-5-2
IRBB5	*xa5*	IR24*5/IR1545-339	IRBB58	*Xa4, xa13, Xa21*	NH11-35/NH9-53
IRBB7	*Xa7*	IR24*5/DV85	IRBB59	*xa5, xa13, Xa21*	NH11-35/NH9-53
IRBB10	*Xa10*	IR24*5/CAS209	IRBB60	*Xa4, xa5, xa13, Xa21*	NH11-35/NH9-53
IRBB11	*Xa11*	IR24*5/IR8	IRBB61	*Xa4, xa5, Xa7*	IR-BB7/IR-BB60
IRBB13	*xa13*	BJ1/5*IR24	IRBB62	*Xa4, Xa7, Xa21*	IR-BB7/IR-BB60
IRBB14	*Xa14*	Taichung Native 1/5*IR24	IRBB63	*xa5, Xa7, xa13*	IR-BB7/IR-BB60
IRBB21	*Xa21*	IR24*8/O BARTHII	IRBB64	*Xa4, xa5, Xa7, Xa21*	IR-BB7/IR-BB60
IRBB50	*Xa4, xa5*	-	IRBB65	*Xa4, Xa7, xa13,Xa21*	IR-BB7/IR-BB60
IRBB51	*Xa4, xa13*	IRBB4/IR66699-9-1-1-5-2	IRBB66	*Xa4,xa5, Xa7, xa13, Xa21*	IR-BB7/IR-BB60
IRBB52	*Xa4, Xa21*	IRBB4/66700-3-3-3-4-2	IR 24	No. *Xa* gene	-
IRBB53	*xa5, xa13*	IRB4/IR66699-9-1-1-5-2			

Table 2. Reaction of near isogenic lines to pathotypes of X. oryzae pv. oryzae.

Pathotypes	Xa1	Xa3	Xa4	xa5	Xa7	Xa10	Xa11	xa13	Xa14	Xa21	IR-24
Pt-1	MR	MS	MS	S	MS	MS	MS	MR	MS	MR	S
Pt-2	MR	MS	MR	MS	MS	MS	MS	MR	MS	MS	S
Pt-3	R	MR	MR	S	MS	MS	MS	MS	MS	MR	S
Pt-4	MR	R	MS	S	MR	MR	MR	MR	MS	MR	S
Pt-5	S	MR	MS	MS	MS	MR	MS	MR	MR	MR	S
Pt-6	MR	R	MR	MS	MR	MR	MS	MR	R	MR	MS
Pt-7	MR	R	MS	MR	MR	MR	MR	MR	MR	R	MS
Pt-8	MR	MS	MR	MS	MR	MR	MR	MR	MR	R	S
Pt-9	MS	MR	MS	MR	MS	MS	MS	MS	MS	MR	S
Pt-10	MR	MR	MS	MR	MR	MR	MS	MR	MR	R	S
Pt-11	S	S	MS	MS	MS	MS	S	MR	S	MS	S
Pt-12	S	MR	MS	MS	MS	MS	MS	MR	MR	MR	S
Pt-13	S	MS	MS	MR	MR	MR	MS	MS	MR	MR	S
Pt-14	MS	MS	MR	MR	R	MR	MR	MR	MR	MR	S
Pt-15	MS	MS	MS	MS	MS	MR	MS	MR	MR	MR	S
Pt-16	MS	MR	MR	MR	MR	MS	MR	MS	MR	MR	S
Pt-17	MR	MS	MR	MS	MR	MR	MR	MR	R	R	S
Pt-18	MR	MS	MR	MR	MR	MR	MR	MS	MR	MR	S
Pt-19	MS	MS	MR	MR	MR	MR	MS	MS	MS	MR	S
Pt-20	MR	MR	R	MS	MR	MS	MR	MR	MR	MR	S
Pt-21	MR	MR	MR	MR	MR	MR	MR	MR	R	MR	S
Pt-22	MR	MR	MS	R	MR	MR	R	R	MS	R	MS
Pt-23	R	MR	R	R	R	MR	R	R	MR	R	MS
Pt-24	R	MR	R	R	R	MR	R	R	R	R	MS
Pt-25	MS	MR	MR	MS	MR	MR	MR	MR	R	R	MS
Pt-26	MR	R	R	R	R	MR	R	R	R	R	MS
Pt-27	MR	R	R	MR	R	R	MR	R	R	MR	MS
Pt-28	MR	MR	R	MR	R	R	MR	R	R	MR	MS
Pt-29	R	MR	R	R	R	R	R	R	R	R	MR

lowest mean lesion length across all the pathotypes to the tune of 6.6 cm, 7.6 cm and 7.9 cm, respectively against the susceptible check (15.2 cm). This accounted for more than 50% reduction in lesion length as compared to control. The three lines showed maximum resistance (LL ≤5 cm) against specific pathotypes having varying varietal preferences with details as under:

i. IRBB-21 with lesion length in the range of 3.59cm≤X≤4.81cm against nine pathotypes viz. 25<29<26<8<24<22<10<23<7.
ii. IRBB-13 with lesion length in the range of 3.59cm≤X≤4.96cm against seven pathotypes viz. 24<26<27<29<28<23<22.
iii. IRBB-7 with lesion length in the range of 3.85cm≤X≤5.0cm against six pathotypes viz. 26<14<29<24<27<23.

Reactions of pyramid lines against *Xoo* pathotypes: In the case of 16 pyramiding lines containing 2-5 resistant (*Xa*) gene combinations and IR-24 as parent/susceptible check, the high level of resistance was observed in IRBB-57 carrying three resistant genes *Xa4*, *xa5* and *Xa21*. IRBB-57 has 93% resistant potential against tested pathotypes, including pathotype-1 with the mean lesion length of 6.6 cm. Among the six rice pyramids having a combination of two genes, IRBB-53 (*xa5*, *xa13*) and IRBB-54 (*xa5*, *Xa21*) were significantly more resistant against 83% *Xoo* pathotypes followed by IRBB-52 (*Xa4*, *Xa21*), IRBB-50 (*Xa4*, *Xa5*) and IRBB-51 (*Xa4*, *xa13*). In three gene combinations of six rice pyramids, the performance of IRBB-57 was found best, followed by IRBB-58 (*Xa4*, *xa13*, *Xa21*), IRBB-63 (*xa5*, *Xa7*, *xa13*), IRBB-59 (*xa5*, *xa13*, *Xa21*), IRBB-62 (*Xa4*, *Xa7*, *Xa21*) and IRBB-61 (*Xa4*, *xa5*, *Xa7*). Among the three rice pyramids having combination of four genes, IRBB-64 (*Xa4*, *xa5*, *Xa7*, *Xa21*) was better with resistance against 86% *Xoo* pathotypes including pathotype-1. The pyramids with four gene combinations IRBB-65 and IRBB-60 as well as five genes combination IRBB-66 carrying *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21* genes expressed resistance against 76 and 72% pathotypes but they were susceptible to pathotype-1. Maximum susceptibility to all pathotypes was observed in IR-24 which was used as susceptible parent check having no resistance gene.

The significant results were observed among the pathotypes, 16 pyramids and their interaction at P<0.05 (Table 3). The minimum disease symptoms (mean lesion length ≤2.52 cm) was produced by pathotype-29 while highest disease (LL >10.0 cm) was recorded with 9 pathotypes namely: 11<1<5<3<12<4<15<13<6 with mean lesion length in the range of 10.09 cm≤X≤12.41 cm. In case of control treatment disease was at its peak to the tune of 15.18 cm lesion length.

Table 3. Reaction of pyramid lines to pathotypes/races of *X. oryzae* pv. *oryzae*.

Patho-types	Xa4, xa5	Xa4, xa13	Xa4, Xa21	xa5, xa13	xa5, Xa21	xa13, Xa21	Xa4, xa5, Xa21	Xa4, xa13, Xa21	xa5, xa13, Xa21	Xa4, xa5,xa13, Xa21	Xa4, xa5, Xa7	Xa4, Xa7, Xa21	xa5, Xa7, xa13	Xa4, xa5, Xa7, Xa21	Xa4, Xa7, xa13, Xa21	Xa4, xa5, Xa7,xa13, Xa21	IR 24
Pt-1	S	MS	S	S	S	MS	MR	MR	MS	MS	S	MS	S	MR	MS	MS	S
Pt-2	MS	MS	MS	MS	MR	MS	MR	MR	MR	MS	MS	MS	MR	MR	MR	MS	S
Pt-3	MS	MR	MS	MS	MS	MS	MR	MS	MR	MR	S	MS	MS	MR	MS	MR	S
Pt-4	MR	MR	MS	MR	MR	S	MR	MR	MR	MR	MS	MR	MR	MR	MS	MS	S
Pt-5	MS	S	MR	MR	MR	MR	MS	MR	MR	MR	S	MS	S	MR	MR	MR	S
Pt-6	MS	MR	MR	MR	MR	MR	MR	MS	MR	MR	MR	MS	MS	MR	MR	MS	MS
Pt-7	MR	MS	MR	MR	MR	MS	R	MR	MR	MS	MS	R	MR	R	MR	MR	MS
Pt-8	MR	MR	R	R	MR	MR	MR	MS	MS	R	MS	MR	R	MR	MR	MS	S
Pt-9	MR	MS	MR	MS	MR	MR	R	MR	MS	MS	MR	MS	R	R	MR	MR	S
Pt-10	MR	MS	MR	R	MR	MS	R	MR	MR	MS	MR	MR	R	R	MR	MR	S
Pt-11	S	S	MS	MS	MS	S	MR	MR	MS	MS	MS	MS	MR	MS	MS	MS	S
Pt-12	MS	MS	MR	MR	MS	MR	MR	MR	MS	MR	MS	MS	MS	MR	S	MR	S
Pt-13	MR	MR	MR	MR	MR	S	MR	MR	MR	MS	MS	MS	MR	MS	MR	MR	S
Pt-14	R	MR	MR	MR	R	MS	R	MR	R	MR	MS	MR	MR	R	MR	S	S
Pt-15	MS	MS	MR	MR	MR	MS	R	MS	MR	MS	MR	MS	MR	MR	MS	MS	S
Pt-16	MR	MR	MR	R	MR	MS	R	MR	MS	MR	MR	S	MR	MR	MR	R	S
Pt-17	MR	R	MS	MR	MR	MS	R	MS	MR	R	MS	MR	MR	MR	MR	R	S
Pt-18	MR	MR	MR	MR	MR	MR	R	MS	MS	MR	MS	MR	MR	MR	MR	MS	S
Pt-19	MS	MS	MR	MR	MR	MR	R	MR	MS	MR	MR	MR	MR	MS	MS	MR	S
Pt-20	MR	MS	MR	MR	MS	MS	MR	MR	MR	MR	MS	R	MS	MR	MR	MR	S
Pt-21	MR	MR	MR	MR	R	MR	MS	MR	R	MR	MR	R	MR	MR	MR	R	S
Pt-22	R	MS	MR	MR	MR	MR	R	R	R	R	MR	MR	S	MR	R	MR	MS
Pt-23	MR	R	R	MR	R	MR	R	R	MR	MR	MR	MR	R	MR	MR	MR	MS
Pt-24	R	R	R	R	MR	MR	R	MR	R	MR	MR	MR	MR	R	MR	MR	MS
Pt-25	MR	R	MR	R	R	MR	MR	MR	MR	R	MR	MR	MR	MR	R	MR	MS
Pt-26	MR	R	R	R	MR	R	MR	R	R	MR	R	R	MR	R	R	R	MS
Pt-27	R	R	MR	MR	MR	MR	R	R	R	R	MR	R	R	R	R	R	MS
Pt-28	MS	MR	R	MR	MR	MR	MR	R	MR	MR	R	MR	R	MR	MR	R	MS
Pt-29	R	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	R	MR

Three lines namely IRBB-57, IRBB-64 and IRBB-60 revealed high level of resistance with lowest mean lesion length across all the pathotypes to the tune of 5.92 cm, 7.19 cm and 7.9 cm respectively against the susceptible check (15.2 cm). Similar to the single gene lines here again three lines showed maximum resistance (LL ≤5 cm) against specific pathotypes having varying varietal preferences with details as under:

IRBB-57 with lesion length in the range of 3.63cm≤X≤4.96cm against 12 pathotypes viz. 27<10<15<14<18<7<29<17<19<23<24<16.

IRBB-64 with lesion length in the range of 2.52cm≤X≤4.93cm against seven pathotypes viz. 29<27<26<28<16<17<21.

IRBB-60 with lesion length in the range of 3.93cm≤X≤5.0cm against six pathotypes viz. 8<27<22<29<25<17.

DISCUSSION

Induction breeding in rice crop and deployment of resistant cultivars carrying major resistant genes has been the most effective approach for the control of Bacterial leaf blight but for the incorporation of the most efficient gene, it is a pre-requisite to evaluate the resistant sources against existing races or isolates of *Xoo*. In the present study, 26 rice differentials were evaluated against 29 prevailing pathotypes of *Xoo* representing 17 different districts of rice growing areas in the Punjab, Pakistan. The main objective of this study was to develop rice cultivars with effective resistance genes to *Xanthomonas oryzae* pv. *oryzae*. The level of resistance was established to the bacterial blight pathogen conferred by a single gene individually and multiple gene combinations. Previously, five races of *Xoo* PKX1, PKX2, PKX3, PKX4 and PKX5 were identified on the basis of host-pathogen interaction in Pakistan where PK stands for Pakistan and X stand for *X. oryzae* pv. *oryzae* (Mannan *et al*., 2009). To date, 39 BB resistant (R) genes have been identified and mapped (Zhang *et al*., 2014). Disease reaction analysis showed that none of the single gene performance can be expected to control all the isolates of BLB.

In this study, *Xa21* gene performed well as a single gene against 93% *Xoo* isolates. However, the rest of the *Xoo* isolates showed virulence and overcome the *Xa21*. The same results were documented by (Mannan *et al*., 2009) where *Xa21* gene has been overcome by only one race PKX 2 of *Xoo*, a dominant race in Pakistan, but remained effective

against other four races. Moreover, *Xa21* was found to be ineffective against seven Bangladeshi races of *Xoo* (Jalaluddin *et al*., 2005). These results suggest that the resistance conferred by *Xa21* may not be effective against all the isolates in a particular location. Thus, in order to maximize the durability of resistance, it is important to determine the best strategy for the deployment of lines containing *Xa21* in a particular location (Wang *et al*., 1996).

The performance of IRBB-13 carrying *xa13*, a recessive gene located on chromosome number 8, was found effective, and many isolates in Punjab were avirulent to IRBB-13. The *xa13* was reported to confer resistance against three races in Pakistan (Mannan *et al*., 2009), PXO99A the broadly virulent race in the Philippines (Ogawa *et al*., 1987) and seven *Xoo* pathotypes in India (Lore *et al*., 2011). In Vietnam, IRBB-13 was found highly susceptible when evaluated as a single gene line (Loan *et al*., 2006) which indicates the prevalence of *xa13* virulent strain in the Vietnam *Xoo* population. The performance of IRBB14 carrying *Xa14* was also good in Punjab, but in another study, it was found to be susceptible to 81% isolates collected throughout the Pakistan (Mannan *et al*., 2009). The 100% susceptibility of *Xa14* to all prevailing races of *Xoo* was reported in Punjab province of India (Lore *et al*., 2011) and in China (Gao and Sun, 2013). The *Xa7* as a single gene is also an effective gene for deployment in BB resistance in Punjab. It has also been speculated to confer durable resistance in Korea (Jeung *et al*., 2006), in Indonesia (Utami *et al*., 2013) and in Iran (Khoshkdaman *et al*., 2014).

It has been found, in this investigation, that the performance of IRBB-57 (*Xa4*, *xa5*, *Xa21*) was the best in all pyramids. These results are in line with an earlier study which concludes that three gene combinations would be most promising in Pakistan against BLB (Mannan *et al*., 2009), in Korea (Jeung *et al*., 2006) and in India (Mishra *et al*., 2013). Three gene pyramid (*xa5+xa13+Xa21*), in variety Sawarna, was transferred to Jalmagna, a popular deep-water variety in India, which exhibited a high level of resistance against BLB (Pradhan *et al*., 2015). Similarly, a combination of two genes, IRBB-53 (*xa5* & *xa13*) and IRBB-54 (*xa5* & *Xa21*) and three genes (*Xa4*, *xa5*, *Xa21*) showed a high level of resistance against Indian *Xoo* isolates (Shanti *et al*., 2010) and in Cuulong river delta (Dinh *et al*., 2010). IRBB-64 having four gene combinations (*Xa4+xa5+Xa7+Xa21*) showed resistance against 86% *Xoo* pathotypes including pathotype-1 (the most virulent pathotype in this study) followed by IRBB-53 (*xa5*, *xa13*) and IRBB-54 (*xa5*, *Xa21*) which were resistant against 82% *Xoo* pathotypes respectively. The rest of the *Xa* genes either alone or in pyramid were moderately susceptible to susceptible. Based on the current study, IRBB-21, IRBB-13, IRBB-7, IRBB-10 and IRBB-14 as a single gene and IRBB-57, IRBB-64, IRBB-53 and IRBB-54 as pyramid are recommended to mitigate the BLB severity. The temptation of newly identified *Xa* gene evaluation is required for achieving complete resistance against all the *Xoo* pathotypes. The information gained in this study has significant implications for the resistance gene deployment in Punjab region. Developing a sound strategy for deployment of R gene containing lines is most challenging and an important area because of the ability of pathogens to rapidly overcome major R genes. This study also explored the potential of gene pyramiding to develop cultivars with durable resistance to BB disease.

***Conclusion*:** The behavior of different resistant (*Xa*) genes exposed the existence of diverse *Xoo* population in Punjab province of Pakistan. Although many resistance genes as a single or in combinations/pyramid were found to be useful against the small proportion of pathogen population, none of the gene or gene combination has the capacity to combat against all the pathotypes tested in this study. Based on our results, *Xa21* and *xa13* resistance genes were found to be most effective followed by *Xa7* and *Xa14* while R gene pyramid of *Xa4*, *xa5* & *Xa21* was found to be resistant and would be the most effective for improving rice cultivars against BLB disease. In this study, only 26 R genes/gene combinations were evaluated; so, there is a dire need to evaluate new combinations of genes or newly identified R genes and rice germplasm which can combat against all the *Xoo* isolates not only from Punjab but throughout the Pakistan. It is also desirable to deploy different resistant genes in different location to avoid any epidemic. The generated information was applied in breeding programs by rice breeders of Nuclear Institute for Agriculture & Biology (NIAB), National Institute of Biotechnology & Genetic Engineering (NIBGE) and Rice Research Institute, Kala Shah Kaku to develop rice cultivars with durable resistance against BLB pathogen.

Acknowledgements: The technical along with lab facilities of NIAB and financial support received from the Punjab Agricultural Research Board (PARB) in the Project No. 49 is highly acknowledged for the execution of the Ph.D. studies of the corresponding author.

REFERENCES

Abbasi, F.M., R. Masood, H. Ahmad, U. Khan, M. Afzai, M. Rehman, M. Khan, K. Akbar and M. Khan. 2011. Molecular screening of Pakistani rice germplasm for *xa5* gene resistance to bacterial blight. Afr. J. Biotech. 10:2833-2837.

Adhikari, T.B., T.W. Mew and J.E. Leach. 1999. Genotypic and pathotypic diversity in *Xanthomonas oryzae* pv. *oryzae* in Nepal. Phytopathology 89:687-694.

Agrawal, P., G. Sidu and S. Gosal. 2005. Induction of bacterial blight resistance in elite Indian rice (*Oryza sativa* L.) cultivars using gamma irradiation and ethyl methane sulfonate. Mutat. Breed. Newsl. Rev. 1:17-18.

Chen, S., X. Liu, L. Zeng, D. Ouyang, J. Yang and X. Zhu. 2011. Genetic analysis and molecular mapping of a novel recessive gene *xa34* (t) for resistance against *Xanthomonas oryzae* pv. *oryzae*. Theor. Appl. Genet. 122:1331-1338.

Cottyn, B. and T.W. Mew. 2004. Bacterial blight of rice. In: R.M. Goodman (ed.), Encyclopedia of Plant and Crop Science. Abingdon, UK: Taylor and Francis.

Dinh, H.D., P.V. Du and L.C. Loan. 2010. Study on the use of combination resistance genes in rice lines against *Xanthomonas oryzae* pv. *oryzae* in Cuulong River Delta. Omonrice 17:147-151.

Gao, D. and L. Sun. 2013. In-vitro Screening and Molecular characterization of a bacterial blight resistance gene in rice. J. Rice Res. 1:2.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for Agricultural research, 2nd Ed. John Wiley and Sons, New York.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research John Wiley & Sons, New York.

Jalaluddin, M., T. Yamamoto, H. Nakai and S. Tsuyumu. 2005. Pathogenic variability and DNA fingerprinting of *Xanthomonas oryzae* pv. *oryzae* from Bangladesh. Sabrao J. Breed. Genet. 37:1-10.

Jeung, J., S. Heu, M. Shin, C. Vera Cruz and K. Jena. 2006. Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. Phytopathology 96:867-875.

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*. Plant Dis. Rep. 57:537-541.

Khan, J.A., H.M.I. Arshad, F.F. Jamil and S. Hasnain. 2009. Evaluation of rice genotypes against bacterial leaf blight (BLB) disease. Pak. J. Phytopathol. 21:26-30.

Khan, J.A., R. Siddique, H.M.I. Arshad, H.S. Anwar, K. Saleem and F.F. Jamil. 2012. Chemical control of bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*. Pak. J. Phytopathol. 24:97-100.

Khoshkdaman, M., A.A. Ebadi, F. Majidi-Shilsar, and S. Dariush. 2014. Preliminary evaluation of resistance genes in rice against bacterial leaf blight in Guilan Province Iran. Agric. Sci. 5:94-98.

Loan, L.C., V.T.T. Ngan, and P.V. Du. 2006. Preliminary evaluation on resistance genes against rice bacterial leaf blight in Can Tho Province – Vietnam. Omonrice 14:44-47.

Lore, J.S., Y. Vikal, M.S. Hunjan, R.K. Goel, T.S. Bharaj and G.L. Raina. 2011. Genotypic and pathotypic diversity of *Xanthomonas oryzae* pv. *oryzae*, the cause of bacterial blight of rice in Punjab State of India. J. Phytopathol. 159:479-487.

Mannan, S., S.A. Malik, I. Ahamad, J.I. Mirza and M.A. Akhtar. 2009. Studies on virulence reactions of local isolates of *Xanthomonas oryzae* pv. *oryzae*. Pak. J. Bot. 41:391-402.Mew, T. 1987. Current status and future prospects of research on bacterial blight of rice. Ann. Rev. Phytopathol. 25:359-382.

Mishra, D., M.R. Vishnupriya, M.G. Anil, K. Konda, Y. Raj and R.V. Sonti. 2013. Pathotype and genetic diversity amongst Indian isolates of *Xanthomonas oryzae* pv. *oryzae*. PloS one 8:e81996.

Naveed, S.A., M. Babar, A. Arif, Y. Zafar, M. Sabar, I. Ali, M. Chragh and M. Arif. 2010. Detection of bacterial blight resistant gene *xa5* using linked marker approaches. Afr. J. Biotech. 9:3549-3554.

Ogawa, M., T. Kumamaru, H. Satoh, N. Iwata, T. Omura, Z. Kasai and K. Tanaka. 1987. Purification of protein body-I of rice seed and its polypeptide composition. Plant Cell Physiol. 28:1517-1527.

Pradhan, S.K., D.K. Nayak, S. Mohanty, L. Behera, S.R. Barik, E. Pandit, S. Lenka and A. Anandan. 2015. Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. Rice 8:19.

Shanti, M.L., C.M.K. Varma, P. Premalatha, G.L. Devi, U. Zehr and W. Freeman. 2010. Understanding the bacterial blight pathogen combining pathotyping and molecular marker studies. Int. J. Plant Pathol. 1:58-68.

Srinivasan, B. and S. Gnanamanickam. 2005. Identification of a new source of resistance in wild rice, *Oryza rufipogon* to bacterial blight of rice caused by Indian strains of *Xanthomonas oryzae* pv. *oryzae*. Curr. Sci. 88:1229-1231.

Utami, D.W., P. Lestari and S. Koerniati. 2013. A relative expression of *Xa7* gene controlling bacterial leaf blight resistance in Indonesian local rice population (*Oryza sativa* L.). J. Crop Sci. Biotech. 16:1-7.

Verdier, V., L.R. Triplett, A.W. Hummel, R. Corral, R.A. Cernadas, C.L. Schmidt, A.J. Bogdanove and J.E. Leach. 2012. Transcription activator-like (TAL) effectors targeting OsSWEET genes enhance virulence on diverse rice (*Oryza sativa*) varieties when expressed individually in a TAL effector-deficient strain of *Xanthomonas oryzae*. New Phytologist 196:1197-1207.

Yang, C.M. 2010. Assessment of the severity of bacterial leaf blight in rice using canopy hyperspectral reflectance. Precision Agriculture 11:61-81.

Zhang, F., D.L. Zhuo, L.Y. Huang, W.S. Wang, J.L. Xu, C. Vera Cruz, Z.K. Li and Y.L. Zhou. 2014. *Xa39*, a novel dominant gene conferring broad spectrum resistance to *Xanthomonas oryzae* pv. *oryzae* in rice. Plant Pathology 64:568-575.