ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. VII: 07-12: 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.in

PYRAMIDING OF THREE BACTERIAL BLIGHT RESISTANCE IN DUBRAJ RICE CULTIVAR USING MARKER-ASSISTED SELECTION

Kadu Tanvi Pradiprao *et al.,*

KEYWORDS

Bacterial blight
Gene pyramiding
Marker assisted selection

Proceedings of National Conference on Harmony with Nature in Context of Bioresources and Environmental Health
(HORMONY - 2015)
November 23 - 25, 2015, Aurangabad,
organized by
Department of Zoology,
Dr. Babasaheb Ambedkar Marathwada University
Aurangabad (Maharashtra) 431 004
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

KADU TANVI PRADIPRAO*, KALE SONAM SURESHRAO, CHAVAN NARENDRA RAMESHSING, TOSHY AGRAWAL AND S. B. VERULKAR
Department of Plant Molecular Biology and Biotechnology,
College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur - 492 006 C. G. INDIA
e-mail: tanvi.kadu20@gmail.com

ABSTRACT

Dubraj is a high yielding medium slender grain aromatic indica rice variety that is very popular with farmers and consumers across India. However, the variety is susceptible to several diseases and pests, including bacterial blight (BB). The pathogen, *Xanthomona s oryzae* pv. *oryzae*, causing BB disease is highly virulent to rice crop and is capable of evolving new races. The incorporation of single BB resistance gene leads to resistance breakdown within a short period to overcome these we have to introgressed three bacterial blight resistance genes *Xa21, xa5*and *xa13* into Dubraj from rice cultivar RP-Bio226 through MAS. The linked SSR marker pTA28, *xa*5R/S and RM6765 for *Xa21, xa5*and *xa13* were used to identify the genes in the parents and in F_2 pyramided population. This study demonstrated that Breeding lines with two or three resistance genes were developed and tested for resistance to the bacterial blight. They showed a wider spectrum and a higher level of resistance as compared to lines with only a single gene. All three genes in homozygous condition (*xa21Xa2*/*xa13xa13*/*xa5xa5*) showed resistance disease reaction whereas all these three gene in heterozygous condition (*Xa*21*xa*21/*Xa*13*xa*13/*Xa*5*xa*5) show MR reaction. Similarly two gene combinations gave more resistance reactions than single gene.

Abbreviations: BB Bacterial blight, Xoo: *Xanthomonas oryzae* pv. *Oryzae*, MAS Markerassisted selection, SSR Simple sequence repeats

***Corresponding author**

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important world food crops, serving as staple food for (Deepti Davia *et al.,* 2013) about 90 % rice in the world is grown and consumed by the population of the Asian countries which constitute 58 % population of the World (Samanta A. *et al.,* 2014).To ensure the global food security for increasing population growth, it is vital to control the various insect pests and diseases that damage rice (Normile, 2008). Bacterial blight disease is a systemic disease and can cause severe yield loss up to 50 % depending on growth stage, geographic localization and season (Nino-Liu *et al.,* 2006). Since Chemical control for bacterial diseases is not effective (Devadath, 1989). Therefore, host plant resistance offers the most effective, economical and environmentally safe option for management of BB (Khush *et al.,* 1989). Till date 40 BB resistance genes (*Xa* genes) conferring host resistances against various strains of *Xoo*have been identified (Bhasin*et al.*, 2012; Natrajkumar *et al.,* 2012; Zhang *et al.*, 2014; Suk Man Kim *et al.*, 2015). The recessive R gene, *xa*13 was first characterizedin the rice variety BJ1 and fine-mapped to agenomic region < 4 cM on the long arm of ricechromosome 8 (Zhang *et al.*, 1996; Sanchez *et al.,* 1999). The *xa*5 was mapped to the telomeric region on short arm of chromosome 5 localized to a 8.1 kb region revealing that a general transcription factor IIA gamma subunit (TFIIAc) was encoded by the *xa*5 gene(Iyer A.S. 2004). The another broad spectrum bacterial blight resistancegene *Xa*21 was introgressed from a wild species *O. longistaminata* onto *O. sativa* chromosome 11 (Khush *et al.*, 1989) which have been found to confer resistance to diverseBB pathotypes (Khush *et al.,* 1990; Ikeda *et al.,* 1991).

The deployment of rice cultivars that have multiple BB resistance genes is expected to lead to more durable resistance (Raman M. *et al.,* 2008). MAS has also been employed for moving genes from pyramided lines into new plant type (Sanchez *et al.*, 2000) as well as into improved varieties grown in India (Singh *et al.,* 2001). However, this strategy is difficult to accomplish by conventional breeding methods since the effect of individual resistance gene cannot be easily identified or measured in the presence of other resistance genes in a specific background.(jiandi Xu 2013). Singh *et al.* (2001) pyramided three BB resistancegenes *xa*5, *xa*13 and *Xa*21 in PR106 cultivar using MAS and during testing with 17*X anthomosnas oryzae* pv. *oryzae (Xoo)* isolates under artificial inoculation and field conditions in Punjab found that the combination of genes provided wider spectrum of resistance to the pathogen populations prevalent in the region. Molecular markers are widely applied in agriculture, and their application in rice improvement has been recently reviewed (Mackill and McNally, 2004, Jordan *et al.*, 2004, Toojinda*et al.*, 2005, Liu *et al.*, 2006 and Mackill, 2007). The markers tightly linked with gene used for selection have relatively high selection efficiency (Yunbi, 2010) and they can be used to indirectly select the trait that are expressed late in the life of the plant or that require progeny testing due to their recessive nature of a lack of heritability (Jung-Pil Suh*et al.,* 2009).This study determined the association of BB resistance genes with disease reaction caused by the *Xoo* isolate collected from Dhamtari regionand analyzed the gene expression levels of *Xa21*, *xa 5* and *xa13*genes for resistance to *Xoo* isolate

in F_2 segregating populations.

MATERIALS AND METHODS

Dubraj aromatic popular rice cultivar was taken as the recurrent parent and RP-Bio 226 was used as the donor parent for three bacterial blight resistance genes *Xa21, xa5* and *xa13*. Cross was made between Dubraj(Medium slender grain, aromatic and BB susceptible cultivar) and RP-Bio 226 to obtained F_1 plants then generation advancement was done.

DNA extraction

Total genomic DNA was extracted from the leaf tissue for each rice cultivar/genotype following the miniPrep method described by Doyle and Doyle, 1987. The DNA samples were quantified using Nanodrop Spectrophotometer (ND 100) and the absorbance ratio (A260/A280) was recorded for each sample to find out the purity of DNA. Further concentration of DNA was adjusted to 40 ng/μL with TE buffer and stored at 4ºC. The diluted DNA was subsequently used for PCR amplification.

DNA markers

Three gene specific markers for *Xa*21, *xa5* and *xa13* (Table 1)were used to monitor the presence of each gene and their different combinations. The plants homozygous for all three genes, and their homozygous and heterozygous combinations were identified.

Polymerase chain reaction (PCR)

PCR amplification was performed in 20 ìlreactionvolume containing 40 ng template DNA, 10 μM of forward and reverse primers, 1mM dNTPs, 10X PCR buffer and 1 unit/μl of *Taq* polymerase. The template DNA was initially denatured at 94ºC for 5 min followed by 30 cycles of PCR amplification under the following parameters for *Xa21* ,*xa5*and *xa13*each cycle with 30 Sec denaturation at 94ºC, 30 Sec primer annealing at 55ºC and 1min primer extension at 72ºC. A final 7 min incubation at 72ºC was allowed for completion of primer extension. The amplified product was resolved on 5% PAGE and visualized under UV light after staining with ethidium bromide.

Development and Confirmation of F_1 plants

F_1 seeds were raised in the main field by planting single seedling per hill at a spacing of 10 x10cm during Rabi 2014. DNA isolated from all F_1 plants were used for genotyping of target genes. The seeds harvested from single hybrid plant carrying *Xa21, xa5* and *xa13* genes (Dubraj x RP-Bio226) were selfed and advanced to F_2 generation during Kharif, 2014. These segregating populations were screened by using gene linked SSR markers for three BB resistance genes viz., *Xa21, xa 5 and xa13*.

Disease Evaluation in F_2 Population

To determine segregation patterns of BB resistance genes, F_2 seedlings were inoculated with *X. oryzae* pv. *oryzae* (*Xoo*) isolate collected from Dhamtari. F_2 population was inoculated with bacterial culture at maximum tillering stages by using the leaf clipping method described by Kauffman *et al.* (1973). The inoculum was prepared by suspending bacteria, grown on potato Dextrose agar media for 2-3 days at 4ºC, in sterile distilled water at a final concentration of approximately 10^8 cells/ml. and plant inoculation was carried out by clipping the tip (about 1 to 2 cm) of the fully expanded uppermost leaf with scissors that had been dipped into the inoculum.

The reactions/disease score of individual F_2 plants to the pathogen were evaluated 14-21 days after inoculation by measuring the lesion length. Five leaves per plant were taken for scoring and the plant reaction was rated according to standard evaluation system i.e SES (1980) developed at the International Rice Research Institute, the Philippines. The scale has six classes (coded as 0, 1, 3, 5, 7 and 9) with the standard of 0–1 as resistant (R), 3–5 as moderately resistant (MR) and 7–9 as susceptible (S).

Statistical analysis

For inheritance studies of BB resistance in the segregating population, the goodness of fit of expected genetic ratios were tested by means of χ^2 -test (Singh *et al.,*1977). The chi-square analysis for genotypic and phenotypic ratio was calculated by using following formula: $\chi^2 = \Sigma(O\text{-}E)2/E$, where, Σ is observed value, E is expected value, and Σ- Summation.

RESULTS AND DISCUSSION

The present study was carried out with the objective to improve BB resistance through marker assisted selection.The primer pairs viz., *pTA 248* (Huang *et al.,* 1997) ,*RM6765 and* xa5*S/R* were used for BB resistance genes viz., *Xa21 , xa13 and xa5* in marker assisted selection. SSR Markers can detect a significantly higher degree of polymorphism in rice (Okoshi*et al.,*2004). The true hybrids were identified selfed and advanced to F_2 generation. Study of parental polymorphism is a prerequisite to begin marker assisted selection. Unless the parents are polymorphic for the traits of interest, the further selection of plants carrying the traits of interest is not possible in the progenies.

Pyramiding of BB resistance genes into Dubraj background through MAS

The Dubraj (BB susceptible) rice variety was crossed to RP-Bio226 (donor of the BB resistance genes *Xa21 ,xa5*and *xa13*). The F_1 plants were confirmed for their heterozygosity for the'R' gene linked PCR based markers. For *Xa21,* pTA248 an STS marker amplified a resistant parent specific 1200bp fragment. In case of xa13 RM6765 amplified a 300 bp fragment while for *xa5* linked *marker* xa5R amplified a 160bp band in resistant parent (RP-Bio226). The clear polymorphism existed between the parents, Dubraj and RP-Bio226 for*Xa21, xa13* ,and xa5genes when amplified with *pTA 248 ,* RM6765*and* xa5*R/S* primer pairs, respectively ,While xa5S and *xa5* R give presence/absence of band for susceptible and resistance genotype. The present investigation clearly stated that three resistance genes viz., *xa13,Xa21*and *xa5* for BB were present in RP-Bio226. Since the polymorphism was very clear among the parents for the targeted genes, these markers were selected for selection in the segregating generations.

The F_2 population of 120 plants with single resistance gene and with combinations of two and three resistance genes was identified. The presence of BB resistance gene viz *xa13, Xa21* and *xa5* in F_2 segregating population were determined by using

Table 1: Markers used for marker-assisted selection of resistance genes to *Xa*21 *xa*13 and *xa*5]

Gene	Ch	Marker	Primer pair	Reference
*Xa*21	11	pTA28	F:5'AGACGCGGAAGGGTGGTTCCCGGA3' R:5'AGACGCGGTAATCGAAGATGAAA3'	Huang *et al.,* (1997)
*xa*5	5	*xa*5R	F:5'TGGTAAAGTAGATACCTTATCAAACTGGA3' R:5'TGACTTGGTTCTCCAAGGCTT 3'	Sundaram*et al.,* (2011)
		*xa*5S	F:5'GTCTGGAATTTGCTCGCGTTCG 3' R: 5'TGGTAAAGTAGATACCTTATCAAACTGGA3'	
*xa*13	8	RM6765	R:5'TCGTTGACTACGTTTGCAGC 3' F:5'TCGGAGAAAGCTGCAAGC 3'	-

Table 2: Segregation of F_2 plants at three marker loci linked to BB resistance genes

Markers	Observed frequency			Total	χ^2(1:2:1)
pTA248	29	66	25	120	1.46
*xa*5 S/R	30	67	23	120	3.05
RM6765	27	62	31	120	0.82

Table 3: Phenotypic screening of pyramided lines against Dhamtari isolate.

Gene combinations	No. of plants	Mean lesion length	SD	Disease reaction
Homozygous for all 3 gene	1	2	1 .0	R
Heterozygous for all 3 gene	13	2.93	1	MR
2 homozygous + 1 heterozygous				
xa21Xa21+xa13xa13+Xa5xa5	3	2.11	0.6	R
xa21Xa21+ +xa5xa5+Xa13xa13	1	5.67	1.1	MS
xa13xa13+xa5xa5+ Xa21xa21	1	3	1	R
2 gene heterozygous + 1 gene homozygous				
xa21Xa21+Xa13xa13+Xa5xa5	5	3.47	0.8	MR
xa13Xa13+Xa21xa21+Xa5xa5	9	2.37	0.9	R
xa5xa5+Xa21xa21+Xa13xa13	4	2.33	0.7	R
Dubraj	-	20	1	HS
RP-Bio226	-	1.1	0.3	R

*According to SES scale 1980; R, Resistant; MR, Moderately resistant; MS, Moderately susceptible; HS, Highly susceptible.

respective molecular marker (Fig. 1). These markers linked to resistance genes allowed efficient screening of the F_2 population. Scoring was done based on the banding pattern with reference to their parents, that is those bands similar to resistant and susceptible parents were scored as 1 type and 3 type respectively. While the plants having bands from both parents were scored as 2 (Heterozygous).

The observed and expected frequencies of the various marker genotypes were calculated using the chi square test. The homozygotes and heterozygotes were scored and goodness of fit was tested using the χ^2 value for the segregation data (Table 2). In Dubraj x RP-Bio226 Chi square (χ^2) value for pTA 248 , 29 plants were found to be homozygous resistant, 66 in heterozygous and 25 in homozygous susceptible conditions and which are significant. The χ^2-square analysis (Table 2) indicated that the *Xa21* gene segregated in a genotypic ratio 1*Xa21Xa21*: 2*Xa21xa21*:1*xa21xa21* and exhibited a good fit to the expected segregation ratio for single gene model with χ^2-square value 1.46 at 5% level. Such selection of plant carrying desirable gene is possible through MAS using genetic analysis of pTA248 whichclearly exhibited a goodness of fit to the expectedsegregation ratio of 1*Xa21 Xa21*: 2*Xa21xa21*: 1*xa21xa21* for single gene model for *Xa21* gene. Phenotypically it is not possible to differentiate all the genotypes. Similarly xa5S/R andRM6765 have computed χ^2 value was 3.050 and 82 less than the critical value. The data for all three genes *Xa*21 (pT248), *xa5 (xa5R/S)*and *xa13* (RM6765) showed significance at both the level of 5% and 1% as the computed value was less than 5.99 (5% level) and 9.21 (1% level).

Reaction of pyramided genotypes against BB pathogen

The parental lines and 120 plants of F_2 were analyzed using molecular markers having different combinations of the three or two genes and also screened for phenotypic evaluation. The Phenotypic screening of pyramided lines are presented in Table 3.

Higher levels of resistance in gene pyramid lines containing multiple BB resistance genes as compared to lines having single (or fewer) resistance genes have been reported earlier (Yoshimura *et al.,* 1996). The plants possessing three genes in homozygous (xa5xa5+ +xa13xa13+ *Xa*21*Xa*21) condition showed BB resistance of Score 2, where as all these three gene in heterozygous condition (*Xa*5xa5+ *Xa*13xa13+ *Xa*21xa21) show moderately resistance disease reaction.

For two genes homozygous and one gene heterozygous, plants

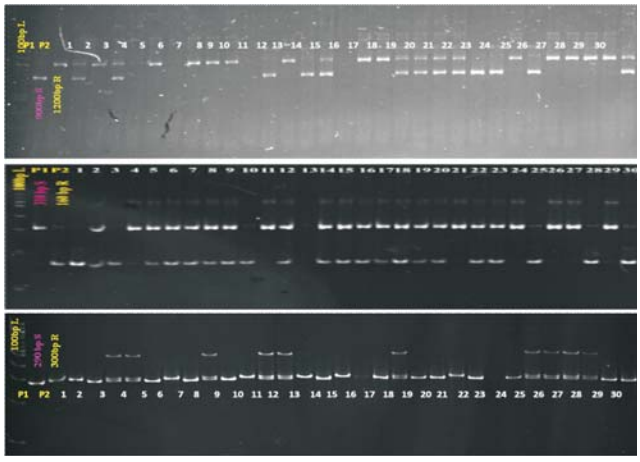

Figure 1: PCR amplification of markers to resistance genes *Xa*21, *xa*5and *xa*13, using primers A) *pAT*248, B) *xa*5S/R and C) RM6765 of F_2 population.

carrying both dominant and recessive genes in the homozygous condition *xa13/Xa21* in homozygous condition show resistance towards BB disease(Table 3), while *xa5/Xa21* in homozygous state were less resistance or moderately susceptible. But those plants carrying single dominant (*Xa21Xa21*) resistance gene in homozygous state were less resistant than plants having single recessive resistant gene (*xa13xa13/xa5 xa5*) along with heterozygous dominant resistant gene (*xa13 xa13 or xa5 xa5 + Xa 21 xa21 + xa13 xa13)*. As expected, both the parents *i.e.* Dubraj and RP-Bio 226 were recorded as susceptible and resistance disease reactions respectively. As above result showed that when three gene present in homozygous condition (*xa21 xa21/xa13 xa13/ xa5 xa5*) show resistance disease reaction, While all these three gene in heterozygous condition (*Xa*21 xa21/xa13 xa13/ *Xa*5xa5) show MR reaction. Similarly two gene combinations show more resistance reactions than single gene reactions.

ACKNOWLEDGEMENT

The financial assistance from Department of Plant Molecular Biology And Biotechnology IGKV, Raipur is gratefully acknowledged

REFERENCES

Bhasin, H., Bhatia, D., Raghuvanshi, S., Lore, S.J., Gurpreet, K., Sahi, K. G., Kaur, B., Vikal, Y. and Singh, K. 2012. New PCR-based sequence-tagged site marker for bacterial blight resistance gene *Xa38* of rice. *Mol Breeding*. **30:** 607-611.

Deepti, D., Sasisharan, N., Macwana, S., Chakraborty, S., Trivedi, R., Ravikiran, R. and Shah, G. 2013. Molecular characterization of rice (Oryza sativa L.) genotypes for salt tolerance using microsatellite markers. *The Bioscan*. **8(2):** 499-502.

Devadath, S. 1989. Chemical control of bacterial blight of rice. In: IRRI (Ed.) Bacterial blight of rice. *IRRI, Manila. Philippines*. pp. 89-98DOI 10.1007/s00122-015-2557-2

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*. **19:** 11-15.

Huang, N., Angels, E. R., Do mingo, J., Mangpantay, G., Singh, S., Zhang, G., Kumar, N., Vadivel, B. J. and Khush, G. S. 1997. Pyramiding of bacterialb lightresistancegenes inrice: marker-assisted selectionusing RFLP and PCR. *Theor. Appl. Genet*. **95:** 313-20.

Ikeda, R., Tabien, R. E. and Khush, G .S. 1991. Chromosomal location of *Xa-21*. *Rice Genet Newsl*. **8:** 102-103.

Iyer, A. S. and McCouch, S. R. 2004. The rice bacterial blight resistance gene xa5 encodes a novel form of disease resistance, Molecular Plant Microbe Interactions. **17:** 1348-1354.

Jiandi, Xu. 2013. Pyramiding of two *BPH* resistance genes and *Stv-bi* gene using marker-assisted selection in *japonica* rice. *Crop Breeding and Applied Biotechnology*. **13:** 99-106.

Jordan, D. R., Tao, Y., Godwin, I.D., Henzell, R.G., Cooper, M., and McIntyre, C. L. 2004. Comparison of identity by descent and identity by state for detecting genetic regions under selection in a sorghum pedigree breeding program. *Molecular Breeding*. **14:** 441-454. DOI: 10.1007/s11032- 005-0901-y

Jung-PilSuh, Tae-Hwan Noh, Ki-Young Kim, Jeong-Ju Kim, Yeon-Gyu Kim, Kshirod, K. J. 2009. Expression levels of three bacterial blight resistance genes against K3a race of Korea by molecular and phenotype analysis in japonica rice (*O. sativa* L.). *J. Crop Sci. Biotech*.**12(3):** 103-108. DOI No. 10.1007/s12892-009-0103-y

Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y., Merca, S. D. 1973. An improved technique for evaluating resistance of rice varieties to Xanthomonasoryzae. *Plant Dis Rep*. **57:** 537-541.

Khush, G. S., Mackill, D. J., Sidhu, G. S. 1989. Bacterial Blight of Rice. Los Banos, Philippines International Rice Research Institute. Breeding of rice for resistance to bacterial blight. pp. 207-217.

Khush, G. S., Bacalangco, E. and Ogawa, T. 1990. A new gene for resistance to bacterial blight from O. longistaminata. *Rice Genetics Newsletter*. **7:** 121-122.

Liu, Q. Q., Li, Q. F., Cai, X. L., Wang H. M., Tang, S. Z., Yu, H. X., Wang, Z. Y. and Gu, M. H. 2006. Molecular marker-assisted selection for improved cooking and eating quality of two elite parents of hybrid rice. *Crop Sciences*. **46:** 2354-2360. DOI: 10.2135/crop. sci. 2006. 03.0180

Mackill, D. J. 2007. Molecular markers and marker-assisted selection in rice. *Genomics assisted crop improvement*. **2:** 147-168.

Mackill, D. J. and McNally, K. L. 2004. A model crop species: Molecular markers in rice. *Molecular marker systems inplant breeding and crop improvement*. **55:** 39-54.

Natrajkumar, P., Sujatha, K., Laha, G. S., Srinivasarao, K., Mishra, B andViraktamath, B. C. 2012. Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomona soryza*epv. *oryzae*. Phytopathology. **102:** 222-228.

Nino-Lui, D. O., Ronald, P. C. and Bogdanove, A. J. 2006. Pathogen profile *Xanthomonasory za*epathovars: model pathogens of a model crop. *Molec. Plant Pathol*. **7(5):** 303-324.

Normile, D. 2008. Reinventing rice to feed the world. *Science*. **321:** 330-333.

Okoshi, M., Hu, J., Ishikawa, R. and Fuimura, T. 2004. Polymorphic analysis of landraces of Japanese rice using microsatellite markers. *Breeding Research*. **6:** 125-133. DOI: 10.1270/jsbbr.6.125

Raman, M. Sundaram, Manne, R. Vishnupriya, Biradar, Sunil K. , Laha, Gouri S., Gajjala, Ashok Reddy Shobha, Rani N., Sarma, Nukala P. and Sonti, Ramesh Venkata 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*. **160:** 411-422. DOI 10.1007/s10681-007-9564-6

Samanta, A., Chakraborti, K., Alam, S. K. F., Das, B. C. and Patra, S. 2014. Pest surveillance in lcc and non-lcc rice plots by Participatory rural folk appraisal. *The Ecoscan*. **8(3&4):** 211-213.

Sanchez, A. C., Ilag, L. L. , Yang, D., Brar, D. S., Ausubel, F. , Khush, G. S., ' Yano, T. Sasaki, M. and Huang, N. 1999. Genetic and physical mapping of *xa*13 , a recessive bacterial blight resistance gene

in rice. *Theor. Appl. Genet.* **98:** 1022-1028.

Sanchez, A. C., Brar, D. S., Huang, N, Li, Z. and Khush, G. S. 2000. Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop Sci.* **40:** 792-797.

Singh, R. K. and Chaudhary, B. D. 1977. Biometrical methods in quantitative genetic analysis. *Kalyani Publishers.* New Delhi.

Singh, S., Sidhu, J. S., Huang, N., Vikal, Y., Li Z., Brar, D. S., Dhaliwal, H. S. and Khush, G. S. 2001. Pyramiding three bacterial blight resistance genes (*xa5, xa13 and Xa21*) using marker-assisted selection into indica rice cultivar PR106. *Theor. Appl. Genet.* **102:** 1011-1015.

Standard Evaluation System for Rice. 1980. Manila, Philippines: *The International Rice Research Institute (IRRI).* pp. 36-37.

Suk Man Kim, Jung PilSuh, Yang Qin, Tae Hwan Noh, Russell F., Reinke and Kshirod, K. J.[1] 2015. Identification and fine mapping of a new resistance gene, *Xa40*, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.). *Theor. Appl. Genet.*

Sundaram, R. M., Laha, G. S., Viraktamath, B. C., Sujatha, K., Natarajkumar, P., Hari, Y., Srinivasa Rao, K., Reddy, C. S., Balachandran, S. M., Madhav, M. S., Hajira, S. K., Rani, N.S., Vishnupriya, M. R. and Sonti, R. V. 2011. Marker Assisted Breeding For Development Of Bacterial Blight Resistant Rice. In: Muralidharan K, Siddiq EA (eds) Genomics and Crop Improvement: Relevance and Reservations, Institute of Biotechnology, Acharya NG Ranga Agricultural University, Hyderabad 500 030 India., pp 154-182.

Toojinda, T. S., Tragoonrung, A., Vanavichit, J. L., Siangliw, N., Pa-In J., Siangliw, M. J. and Fukai, S. 2005. Molecular breeding for rainfed lowland rice in the Mekong region. *Plant Production Science.* **8:** 330-333. DOI: 10.1626/pps.8.330

Xu, Y. B., Beachell, H. and McCouch, S. R. 2004. A marker-based approach to broadening the genetic base of rice in the USA. *Crop Science.* **44:** 1947-1959. DOI: 10.2135/cropsci2004.1947

Yoshimura, A., Lei, J. X., Matsumoto, T., Yoshimura, S., Iwata, N., Baraoidan, M. R., Mew, T. W. and Nelson, R. J. 1996. Analysis and pyramiding of bacterial blight resistance genes in rice by using DNA markers. *In: Khush GS (ed) Rice GeneticsIII, Proceedings of the Third International Rice GeneticsSymposium. International Rice Research Institute, P.O.Box 933, Manila, Philippines.* pp 577-581.

Yunbi, Xu 2010. Molecular Plant Breeding. *CAB International.*

Zhang, F., Zhuoa, D. L., Zhang, F., Huang, L. Y., Wang, W. S., Xu, J. L., Vera, Cruz, C., Li, Z. K. and Zhou, Y. L. 2014. *Xa39*, a novel dominant gene conferring broad-spectrum resistance to *Xanthomonasoryzae*pv. *Oryzae in rice. Plant Pathol.* **64:** 568-575. doi:10.1111/ppa.12283.

Zhang, G., Angeles, E. R., Abenes, M. L. P., Khush, G. S. and Huang, N. 1996. RAPD and RFLP mapping of the bacterial blight resistance gene xa13in rice. *Theor. Appl. Genet.* **93:** 65-70.