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Pyramiding of Three Bacterial Blight Resistance Genes in Rice Cultivar Using Marker Assisted Selection

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Abstract

Bacterial leaf blight (BLB) is a major disease of rice, limiting production throughout the world. *Xanthomonas oryzae* pv.*oryzae* is highly virulent pathogen, causing major losses in Rice. Present scenario needs development of disease resistant variety through marker assisted selection is the most promising way to improve their performance. Safri-17 is high yielding most popular rice variety of Chhattisgarh but it is highly susceptible to BLB disease. In the present investigation molecular markers were used to introgressed three major BLB resistance genes i.e *xa5, xa13* and *Xa21* into Safri-17 from a donor line RP-Bio226. Genetic analysis was used to confirm the F_2 population for the presence of BLB resistance genes with the help of SSR and STS markers (RM6765, RM13, and *pTA*248, *xa*5R/S respectively). Breeding lines with two or three resistance genes were developed and tested for resistance to the BLB. They showed a wider spectrum and a higher level of resistance as compared to lines with only a single gene. The BLB resistance genes in homozygous condition (*Xa*21*Xa*21/*xa*13*xa*13/*xa*5*xa*5) showed resistance disease reaction whereas in heterozygous condition (*Xa*21*xa*21/*Xa*13*xa*13/*Xa*5*xa*5) showed moderate resistance reaction. Similarly two gene combinations gave more resistance reactions as compare to progenies have single gene.

Keywords: Bacterial leaf blight, Markers assisted selection, *Oryza sativa* L., Pyramiding

1. Introduction

Rice (*Oryza sativa* L.), the most important staple food crop which serves as a major carbohydrate source for half of the world's population. Bacterial leaf blight is caused by the *Xanthomonas oryzae pv. oryzae* and is one of the devastating diseases of rice causing yield losses ranging from 74% to 81% (Srinivasan and Gnanamanickam, 2005). The bacterium invades the xylem tissue, either through wounds or stomata, leading to systemic infection. Therefore, host plant resistance offers the most effective, economical and environmentally safe option for management of BLB (Khush et al., 1989). Till date 40BLB resistance genes (*Xa* genes) conferring host resistances against various strains of *Xoo* have been identified (Bhasin et al., 2012; Natrajkumar et al., 2012; Suk et al., 2015). Among total identified BLB resistance genes most of the genes are dominant in nature (e.g. *Xa4, Xa7, Xa21, Xa23, Xa26* and *Xa27*), while some are recessive (e.g. *xa5, xa8, xa13* and so on). Some of them have been tagged and mapped by closely linked molecular markers (Rao et al., 2002). Due to ineffectiveness of chemicals and antibiotics in controlling the disease, development of disease resistant cultivars is the best way for management of this destructive disease. The deployment of rice cultivars that have multiple BLB resistance genes is expected to lead to more durable resistance. Fortunately, the availability of molecular markers closely linked with each of the resistance genes makes the identification of offspring with two or more genes possible, i.e. marker-assisted selection (MAS). However, using conventional breeding methods, plants with multiple resistance genes cannot be selected based on phenotype alone, because of epistasis effect of genes, wherein the action of a gene conferring resistance to many races of the pathogen may mask the action of another resistance gene. Though, using DNA markers it would be possible to select plants having multiple resistance genes without actual disease inoculation if markers linked with resistance genes are available.

DNA fingerprinting and pathotype analysis have indicated that there is a significant amount of diversity within populations of *Xoo* in India and other rice growing countries (Shanti et al., 2001; Gupta et al., 2001; Singh et al., 2003). A number of near-isogenic rice lines with genetic background of IR24 having single resistance gene either *Xa21, xa13* or *xa5* were inoculated with Indian strains of *Xoo* were found to provide moderate to strong levels of resistance (Goel et al., 1998; Joseph et al., 2004). Different gene pyramid combinations containing two or three gene combination have been observed to possess high

level of resistance against multiple isolates of *Xoo*. In order to enhance the durability of resistance, molecular markers that are tightly linked to each of these three resistance genes have been developed (Zhang, 1996) and used to pyramid them into the genetic background of different rice varieties (Singh et al., 2001; Joseph et al., 2004).

The present study was undertaken to develop a BLB resistant variety by introgression of three BLB resistance genes viz., *xa*5, *xa13* and *Xa21* from RP-Bio 226 into the genetic background of Safri17. The SSR and STS markers viz., RM 6765, RM13 and *pTA*248, *xa*5R/S were validated in the resistant parent and the parental polymorphism was studied between susceptible and resistant parents. The genotypic and phenotypic segregation was analyzed to determine the inheritance pattern of these genes in the single hybrid derived F_2 population.

2. Materials and Methods

2.1. Plant materials

The experimental material comprised Safri17 popular rice cultivar which was taken as a recurrent parent and RP-Bio 226 (Released By (DRR), Hyderabad, India) was used as the donor parent containing three bacterial blight resistance genes (*xa*5, *xa13* and *Xa21*). In *kharif*, 2013 crosses were made, each F_2 progeny was planted by maintaining 15cm plant to plant and 20cm row to row distance along with respective parents.

2.2. DNA extraction

The young leaves of Rice were collected from three week of transplanted cultivar during *kharif*, 2014. Approximately 1.0 g leaves were taken from each progeny of F_2 population placed in 2.0 ml collection microtubes (Qiagen Tissue Lyser II, Qiagen, U.S.A.) and in each microtube 3 mm 2 tungsten beads were dispensed and tube containing block dipped in liquid N_2 for 4-5 min. Tissues were disrupted and homogenized with qiagen tissue lyser to a fine powder at frequency of 30 vibrations/ seconds for 3 min. Fine powdered leaf samples were used for isolation of genomic DNA using CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1987). The DNA samples were quantified spectrophotometrically (ND 100) by measuring at wavelength A260/A280 nm. A ratio between 1.7 and 1.9 was considered as relatively pure DNA sample. After checking the concentration of the DNA based samples were diluted to 50ng μl^{-1} for further polymerase chain reaction (PCR) analysis.

2.3. DNA markers

To monitor the presence of each gene and their different combinations gene specific markers for three genes i.e *xa*13, *Xa*21 and *xa5* (Table 1) were used. The plants homozygous for all three genes, and their homozygous and heterozygous combinations were identified.

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*13	8	RM6765	F: 5'TCGGAGAAAGCTGCAAGC 3'	-
			R: 5'TCGTTGACTACGTTTGCAGC 3'	
*Xa*21	11	pTA248	F: 5'AGACGCGGAAGGGTGGTTCCCGGA3'	Huang et al., 1997
			R: 5'AGACGCGGTAATCGAAGATGAAA3'	
*xa*5	5	xa5R	F: 5'TGGTAAAGTAGATACCTTATCAAACTGGA3'	Sundaram et al., 2011
			R: 5'TGACTTGGTTCTCCAAGGCTT 3'	
		xa5S	F: 5' GTCTGGAATTTGCTCGCGTTCG 3'	Sundaram et al., 2011
			R: 5'TGGTAAAGTAGATACCTTATCAAACTGGA3'	
		RM-13	F: 5'TCCAACATGGCAAGAGACAG3'	Panaud O et al., 1996
			R :5' GGTGGCATTCGATTCCAG3'	

2.4. Polymerase chain reaction (PCR)

PCR reaction mixture contained 50ng of template DNA, 1mM dNTPs, 1 µM of forward and reverse primers, 10X PCR buffer and 1 unit/µl of *Taq* polymerase. The template DNA was initially denatured at 94 °C for 5 min followed by 30 cycles of PCR amplification under the following parameters for *xa*5, *xa*13 and *Xa*21 each cycle

with 30 Sec denaturation at 94 °C, 30 Sec primer annealing at 55 °C and 1 min primer extension at 72 °C. Final 7 min incubation at 72 °C 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.

2.5. Disease evaluation in F_2 population

Each F_2 seedlings were inoculated with *Xanthomonas oryzae* pv. *oryzae* isolate of Dhamtari (BLB culture was isolated and provided by Dr. A.S. Kotasthane (Professor and head of deparment of plant pathology) IGKV, Raipur) at maximum tillering stages with the leaf clipping method as described by Kauffman et al., (Kauffman et al., 1973). The inoculum was prepared by suspending bacteria, grown on it is Wakimoto's medium for 2-3 days at 28 °C, 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. After 14–21 days of inoculation the disease was scored by measuring the lesion length. The scoring and the plant reaction were rated according to standard evaluation system i.e SES (1980) developed at the International Rice Research Institute, the Philippines. From each progeny five leaves/plant were taken for scoring purpose. 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).

2.6. Statistical analysis

For the studies of BLB resistance inheritance in F_2 segregating population, the goodness of fit of expected genetic ratios were tested by means of χ2 –test (Singh and Chaudhary, 1977). The chi-square analysis for genotypic and phenotypic ratio was calculated by using following formula: $\chi 2=\Sigma (O-E)\ 2 / E$, where, O is observed value, E is expected value, and Σ is Summation.

3. Results and Discussion

Rice (*Oryza sativa* L.) is one of the most important staple food crop of the world. Therefore resistance breeding with Marker assisted selection (MAS) has been employed to develop broad spectrum durable disease resistance in rice. The present study was carried out with the objective to improve BLB resistance through marker assisted selection. The primer pairs viz., *pTA*248, RM6765*,* RM13*,xa*5S/R were used for BLB resistance genes viz., *Xa21* , *xa13 and xa5* in marker assisted selection. 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. SSR Markers can detect a significantly higher degree of polymorphism in rice (Okoshi et al., 2004). Similarly, McCouch et al. (1997) and Olufowote et al. (1997) also used SSRs to study the polymorphism in rice varieties. Hybrid combinations of Safri 17×RP-Bio226 was raised along with their respective parents to fix true hybrids. The true hybrids were identified using all three markers based on the presence of heterozygous bands. Two plants were found to possess DNA fragments of both the parents. The selected true F_1 plants alone were self and advanced to F_2 generation. Two plants showed homozygous for all the three loci in the F_2 generation. However, four plants were homozygous resistance for two loci and heterozygous for one loci show difference in disease reactions.

In the present study, the primer pair, RM6765 amplified a clear band of 290bp in Safri17 while another band of 300bp was amplified in the resistant parent, RP-Bio226. Similarly, polymorphism was observed between RP-Bio226 (1200bp) and Safri17 (800bp) when *pTA248* primer pair was used for amplification of *Xa21* gene. For *xa*5gene two linked markers were used among them RM13 amplified a clear band of 150bp in Safri17 while 140bp for RP-Bio226 and two STS (*xa*5S and *xa*5 R)gene linked markers gave dominant marker type of band *xa*5 S amplified a band of 310bp only in Susceptible parent(Safri17) whereas *xa*5R amplified 160bp band only in Resistant parent(RP-Bio226).The clear polymorphism existed between the parents, Safri17 and RP-Bio226 for *xa13, Xa21* and *xa*5 genes when amplified with RM6765, *pTA248 and* RM13 primer pairs, respectively ,While *xa5*S 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 BLB 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 populations were subjected to PCR amplification using gene linked markers to study the co-segregation of the three disease resistant genes viz., *xa13, Xa21* and *xa*5. One twenty four F_2 progeny developed from cross of Safri17 × RP-Bio226 were analyzed with linked markers. 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 plants having bands from both parents 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 χ2 value for the segregation data (Table 2). In Safri17×RP-Bio226 Chi square (χ2) value for RM6765 was non-significant. While in case of *pTA*248, 34 plants were found to be homozygous resistant, 59 in heterozygous and 31 in homozygous susceptible conditions and which are significant.

Table 2: Segregation ratio of the marker genotypes in the F_2 population of Safri17×RP Bio226

Markers	Observed frequency				
	homozygous dominant	hetero-zygous	homozy-gous recessive	Total	x^2 (1:2:1)
RM6765	73	18	33	124	31.3
pTA248	34	59	31	124	0.14
RM13	30	71	23	124	1.41
*xa*5R/S	30	64	30	124	0.82

Similarly RM13 and *xa*5S+R have computed χ2 value was 1.41 and 0.82, less than the critical value. The data for *Xa*21 and *xa*5 showed significance at both the level of 5% ($^*p$<0.05) and 1% ($^{**}p$<0.01) as the computed value was less than 5.99 ($^*p$<0.05) and 9.21 ($^{**}p$<0.01).

3*.1. Selection of plants with bacterial blight resistance gene combinations*

The Pairwise combination of bacterial blight resistance

genes identified in Safri17×RP-Bio226 derived F_2 population is presented in Table 3. Plants homozygous for the resistant allele and remaining susceptible loci were also identified. The selected above plants were selfed and forwarded to next generation for phenotypic evaluation. Figure 1 shows PCR analysis of the F_2 population using RM6765, *pTA*248, RM13 and *xa*5S/R linked to *xa*13, *Xa*21, and *xa*5 respectively.

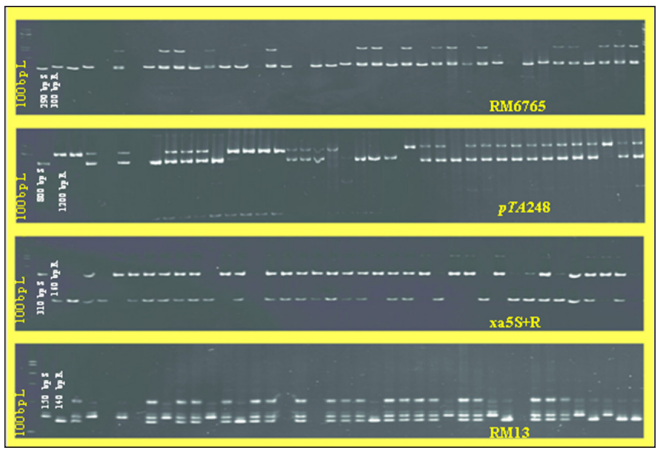

Figure 1: PCR amplification of markers to resistance genes *xa*13, *Xa*21 and *xa*5 using primers a) RM6765, b) *pTA*248, c) *xa*5S/R and d) RM13 of F_2 population. Only 38 samples plus the 2 parents for this marker are shown (L=100bp ladder).

3.2. Disease reaction of pyramided genotypes against BLB pathogen

The parental lines and 124 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 BLB 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 (*xa*5*xa*5+*xa*13*xa*13+*Xa*21*Xa*21) condition showed BLB resistance of Score 1.15, whereas these entire three gene(s) in heterozygous condition (*Xa*5*xa*5+*Xa*13*xa*13+*Xa*21*xa*21) show moderately resistance disease reaction. For two genes homozygous and one gene heterozygous, plants carrying both dominant and recessive genes in the homozygous condition, either *xa*13*xa*13/*Xa*21*Xa*21 or *xa*5*xa*5/*Xa*21*Xa*21 or combinations were resistant. But those plants carrying single dominant (*Xa21Xa21*) resistance gene in homozygous state were more resistant than plants having single recessive resistant gene (*xa13xa13 or xa5xa5*). As expected, both the parents i.e. Safri17 and RP-Bio226 were recorded as susceptible and

Table 3: Phenotype screening of pyramided lines against Dhamtari isolate

Gene combinations	No. of plants	Mean lesion length	SD	Disease reaction
Homozygous for all 3 gene	2	1.15	1 .0	R
Heterozygous for all 3 gene	6	3.33	0.89	MR
2 homozygous+1 heterozygous				
Xa21Xa21+xa13xa13+Xa5xa5	1	2.67	0.58	R
Xa21Xa21++xa5xa5+Xa13xa13	1	3.33	0.58	MR
xa13xa13+xa5xa5+Xa21xa21	2	2.83	0.58	R
2 gene heterozygous+1 gene homozygous				
Xa21Xa21+Xa13xa13+Xa5xa5	8	2.79	1.2	R
xa13Xa13+Xa21xa21+Xa5xa5	5	2.93	1.04	R
xa5xa5+Xa21xa21+Xa13xa13	3	3.89	1.7	MR
Safri17	-	12	1	HS
RP-Bio226	-	1.1	0.25	R

*According to SES scale 2002: R: Resistant; MR: Moderately resistant; HS: Highly susceptible

resistance disease reactions respectively. Segregating lines in F_2 were forwarded to fix the genotypes in F_3. The genotypes with different resistance gene combinations were challenged with Dhamtari *Xoo* isolate under field condition. The level of resistance in the pyramided lines and their derived hybrids were much higher than those of the single resistance gene hosts. When three gene in homozygous condition (*Xa*21*Xa*21/*xa*13*xa*13/*xa*5*xa*5) show resistance disease reaction, While 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 show more resistance reactions than single gene reactions.

4. Conclusion

The use of molecular markers associated with diseases resistance can differentiate rice F_2 progenies from each other.

This in turns provide a base to select the parents variegated to utilize heterosis in hybrid progenies for disease resistance. The phenotypically resistant progenies containing two gene or three gene combinations were further used for crossing in the development of multiple resistance genes containing line. In Safri-17 background the progenies containing three resistance genes including *xa*5, *xa*13 and *Xa*21 developed more resistance towards BLB disease.

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6. Referances

Bhasin, H., Bhatia, D., Raghuvanshi, S., Lore, S.J., Gurpreet, K., Sahi, K.G., Kaur, B., Vikal, Y., Singh, K., 2012. New PCR-based sequence-tagged site marker for bacterial blight resistance gene *Xa*38 of rice. Molecular Breeding 30, 607–611.

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

Goel, R.K., Kaur, L., Saini, R.G., 1998. Effectiveness of different *Xa* genes against *Xanthomonas oryzae* pv. *oryzae* population causing bacterial blight of rice in Punjab (India). Rice Genetics Newsletter 15, 131–133.

Gupta, V.S., Raje Bhosale, M.D., Sodhi, M.S., Singh, S., Gnanamanickam, S.S., Dhaliwal, H.S., Ranjekar, R.K., 2001. Assessment of genetic variability and strain identification of *Xanthomonas oryzae* pv. *oryzae* using RAPD-PCR and IS1112 based PCR. Current Science 80, 1043–1049.

Huang, N., Angels, E.R., Domingo, J., Mangpantay, G., Singh, S., Zhang, G., Kumar, N., Vadivel, B.J., Khush, G.S., 1997. Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theoretical and Applied Genetics 95, 313–20.

Joseph, M., Gopalakrishnan, S., Sharma, R.K., Singhm, V.P., Singhm, A.K., Singhm, N.K., Mohapatra, T., 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker assisted selection in rice. Molecular Breeding. 13, 377–387.

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 *Xanthomonas oryzae*. Plant Disease Report 57, 537–541.

McCouch, S.R., Chen, X., Panaud, O., Temnykh, S., Xu Y., Cho, Y.G., Huang, N., Ishii, T., Blair, M., 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Molecular Biology 35, 89-99.

Natrajkumar, P., Sujatha, K., Laha, G.S., Srinivasarao, K., Mishra, B., Viraktamath, B.C., 2012. Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. Phytopathology. 102, 222–228.

Okoshi, M., Hu J., Ishikawa, R, Fuimura, T., 2004. Polymorphic analysis of landraces of Japanese rice using microsatellite markers. Breeding Research 6, 125–133.

Olufowote, J.O., Xu, Y., Chen, X., Park W.O., Beachell, H.M., Dilday, R.H., Goto, M., McCouch, S.R., 1997. Comparative evaluation of within cultivar variation in rice (*Oryza sativa* L.) using microsatellite and RFLP markers. Genome 40, 370–378.

Panaud, O., Chen, X., McCouch, S.R., 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (Oryza sativa L.). Molecular and General Genetics 252, 597–607.

Rao, K.K., Lakshminarasu, M., Jena, K.K., 2002. DNA markers and marker-assisted breeding for durable resistance to bacterial blight disease in rice. Biotechnology Advances 20, 33–47.

Shanti, M.L., George, M.L.C., Vera Cruz, C.M., Bernardo, M.A., Nelson, R.J., Leung, H., Reddy, J.N., Sridhar, R., 2001. Identification of resistance genes effective against bacterial leaf blight pathogen in eastern India. Plant Disease 85, 506–512.

Singh, R.K., Chaudhary, B.D., 1977. Biometrical methods in quantitative genetic analysis. Kalyani Publishers. New Delhi, 575–578.

Singh, S., Sidhu, J.S., Huang, N., Vikal, Y., Li, Z., Brar, D.S., Dhaliwal, H.S., Khush, G.S., 2001. Pyramiding three bacterial blight resistance genes (*xa*5, *xa*13 and *Xa*21) using marker-assisted selection into indica rice cultivar PR106. Theoretical and Applied Genetics 102,1011–1015.

Singh, S., Sodhi, M., Vikal, Y., George, M.L.C., Bala, G.S., Mangat, G.S., 2003. DNA fingerprinting and virulence analysis of *Xanthomonas oryza* pv. *oryzae* isolates from Punjab, northern India. Euphytica 130, 107–115.

Srinivasan, B., Gnanamanickam, S., 2005. Identification of a new source of resistance in wild rice, *Oryzae rufipogon* to bacterial blight of rice caused by Indian strains of *Xanthomonas oryzae* pv. *Oryzae*. Current Sciences 88, 25.

Standard Evaluation System for Rice, 2002. Manila, Philippines: The International Rice Research Institute (IRRI), 36–37.

Suk-M. K., Jung, P.S., Yang, Q., Tae, H.N., Russell, F.R., Kshirod, K.J., 2015. Identification and fine-mapping of a new resistance gene, *Xa*40, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 17, 1933–43.

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., 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., 154–182.

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

Zhang, G., Angeles, E.R., Abenes, M.L.P., Khush, G.S., Huang, N., 1996. RAPD and RFLP mapping of the bacterial blight resistance gene *xa*13in rice. Theoretical and Applied Genetics 93(1–2), 65–70.