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Molecular Marker-Assisted Selection: A Tool for Insulating Parental Lines of Hybrid Rice Against Bacterial Leaf Blight

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Abstract: Bacterial leaf blight is a major disease in all rice countries and a constraint in hybrid rice production. This study was undertaken to insulate the most popular parental lines of hybrid rice KMR3 and PRR78 (restorer lines) and IR58025B and Pusa 6B (maintainer lines) against the disease by pyramiding bacterial blight resistant genes into each background. Molecular markers are vital components in breeding programs involving gene pyramiding. Using marker-assisted selection in a-breeding program, four bacterial leaf blight resistant genes namely *Xa4, xa5, xa13* and *Xa21* have been introgressed into the restorer and maintainer lines. Foreground selection was done using markers. Conventional breeding strategy was adopted for background selection. The pyramids showed very high level of disease resistance to 10 highly virulent isolates of *Xoo*. Grain quality parameters of the pyramids were on par with that of the original genotype. This work demonstrates the successful application of marker-assisted breeding for pyramiding four BB resistant genes into multiple backgrounds, simultaneously. These lines can be used directly or as donors of Bacterial Blight (BB) resistance breeding.

Key words: Bacterial leaf blight, rice, gene pyramiding, marker-assisted breeding, foreground selection, resistance genes

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INTRODUCTION

Rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a very serious plant disease in rice growing regions of the world. It causes leaf wilting, affects photosynthesis, reduces 1000-grain weight and generally results in yield losses by 20-30% (Ou, 1985) and upto as high as 80% (Singh *et al*., 1997) and even 100% under very severe conditions (Zhai and Zhu, 1999). The disease being systemic there are no effective ways of protecting rice, other than development of resistant cultivars (Sanchez *et al*., 2000; Singh *et al*., 2001; Zhang, 2005; Sundaram *et al*., 2008).

The genetic basis of host plant resistance to bacterial blight has been studied in depth and 30 resistant genes conferring resistance against various races of *Xoo* have been identified from cultivated and wild species (Lin *et al*., 1996; Nagato and Yoshimura, 1998; Zhang *et al*., 1998; Khush and Angeles, 1999; Chen *et al*., 2002; Lee *et al*., 2003; Tan *et al*., 2004; Xiang *et al*., 2006).

Molecular Marker Assisted Selection (MAS) has been advocated as a highly efficient breeding method, because of its rapid and precise selection of the targeted gene. Gene pyramiding using conventional breeding alone is difficult to achieve because of linkage with

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some undesirable traits that is very difficult to break even, after repeated backcrossings (Tanksley *et al*., 1989). Single gene resistance is short-lived and we have to depend on gene pyramiding for longer lasting and durable resistance (Shanti *et al*., 2001; Shanti and Shenoy, 2005; Nayak *et al*., 2008). When two or more genes are introgressed, phenotypic evaluation is unable to distinguish the effect of individual gene precisely since each gene confers resistance to and combats multiple races of the pathogen. Moreover, in the presence of a dominant and a recessive allele, the effect of the recessive gene is masked. The advent and easy availability of molecular markers closely associated with each of the resistance genes makes identification of plants with multiple genes possible.

In rice production hybrid rice often displays lower level of resistance to bacterial blight. The susceptibility period for the disease is longer than that of varieties. During grain filling, hybrid rice management practices such as leaf cutting and rope pulling cause wounds increasing susceptibility to pests and diseases.

KMR3 is one of the best restorers for hybrid rice breeding programs and is the male parent of KRH2 one of the most popular public bred non-aromatic hybrids. It has a maturity period of 130-135 days. PRR78 is the male parent of Pusa RH10 aromatic hybrid. This hybrid is very popular for its superfine grain and aroma. It has a maturity period of 125 days. These two popular lines are highly susceptible to BB and recorded yield losses as high as 40%. IR58025A is one of the most popular CMS line and many hybrids have been developed both in public and private sector using this as the female line. KRH2 is one such example. IR58025A/KMR3 = KRH2. Pusa 6A is another important CMS line in the aromatic types and is the female parent for Pusa RH10 hybrid. Pusa 6A/PRR78 = RH10.

Pyramiding resistant genes in the restorer lines alone is not enough as the hybrid will have these genes in heterozygous condition and the level of resistance imparted will be reduced. Since, *xa5* and *xa13* are recessive genes, so they have to be introgressed in the female line also. Presently most of the BB resistance genes are available in partial restorer backgrounds, thus making it impossible to transfer them directly into the A lines. There are not enough molecular markers to detect the *Rf* genes and hence we have to depend on conventional breeding and test cross evaluation. This necessitates first to transfer them to the maintainer background and after stabilization; they can be transferred with ease to the male sterile background. Once transferred to the maintainer and the CMS line base, transferring multiple resistance to any other CMS line will be easier without having to face the problem of fertility restoration. Hence, it is necessary to insulate both the parents to be able to get resistance in the hybrid. This necessitates the introgression of BB resistant genes in the backgrounds of maintainers as well as restorers. Earlier efforts to improve Minghui 63, a restorer line with *Xa21* and *xa7* using MAS were met with success (Zhang *et al*., 2006).

Studies conducted to identify the best gene combinations conferring broad spectrum resistance showed that the four-gene combination was the most effective and did not show any sign of breakdown of resistance to various strains of the pathogen from different parts of the country (Shanti and Shenoy, 2005; Nayak *et al*., 2008).

We report here the pyramiding of four BB resistance genes *Xa4, xa5*, *xa13* and *Xa21* through marker-assisted selection (MAS) in the BB susceptible parental lines of hybrid rice viz. maintainers IR58025B and Pusa 6B and the restorer lines KMR3 and PRR78, simultaneously. The results demonstrate increased and wide spectrum resistance to the pathogen populations from different parts of India. This work is the first successful example of the use of molecular markers in foreground selection in conjunction with conventional breeding for simultaneous introgression of genes of interest into multiple backgrounds.

MATERIALS AND METHODS

Plant Materials

IRBB60, a near isogenic line in the background of IR 24, carrying the four resistant genes *Xa4, xa5, xa13* and *Xa21* served as the donor for all the crosses attempted. The recipient parents were the restorer lines KMR3 and PRR78 and the maintainer lines IR58025B and Pusa 6B respectively for each cross. The parental crosses were initiated in *kharif* 2004 and every season crossing as well as marker assisted selection were attempted. The restorer lines pyramiding was direct (Fig. 1), whereas the maintainer lines pyramiding was tedious (Fig. 2), since there was a need to do away with the fertility restoration genes.

Marker-Assisted Selection

Miniscale DNA isolation for PCR analysis of the parents and backcross progenies was carried out following (Dellaporta *et al*., 1983). Three STS markers Npb 181, RG 136 and pTA 248, tightly linked to *Xa4, xa13* and *Xa21* and one SSR marker RM 122 tightly linked to *xa5* were used to confirm the presence of each gene and the different combinations. The PCR mixture contained 50 ng of template DNA, 5 picomoles of each primer, 0.05 mM dNTPs, 1X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg mL^{-1} gelatin) and 1 U Taq DNA polymerase in a reaction volume of 25 μL. Template DNA was initially denatured at 94°C for 5 min followed by 35 cycles of PCR amplification with the following parameters: a 30 sec denaturation at 94°C, a 30 sec annealing at 55°C and a 1 min primer extension at 72°C. A final extension was done at 72°C for 5 min the amplified product of pTA 248 and Npb 181 was electrophoretically resolved on 1.4% agarose gel for *Xa21* and 2.5% for *Xa4* and visualized under UV.

For the amplified products of RG136, 5 μL of PCR product was used for gel electrophoresis to determine the success of the PCR. The remaining product was used for restriction digestion. The reaction mixture consisted of 0.5 μL (10U μL^{-1}) of restriction

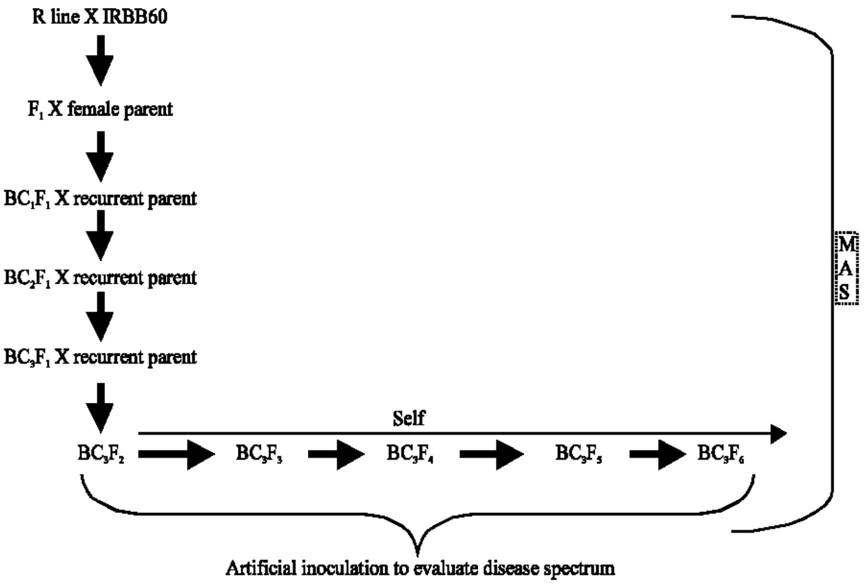

Fig. 1: Flow chart for pyramiding of restorer lines

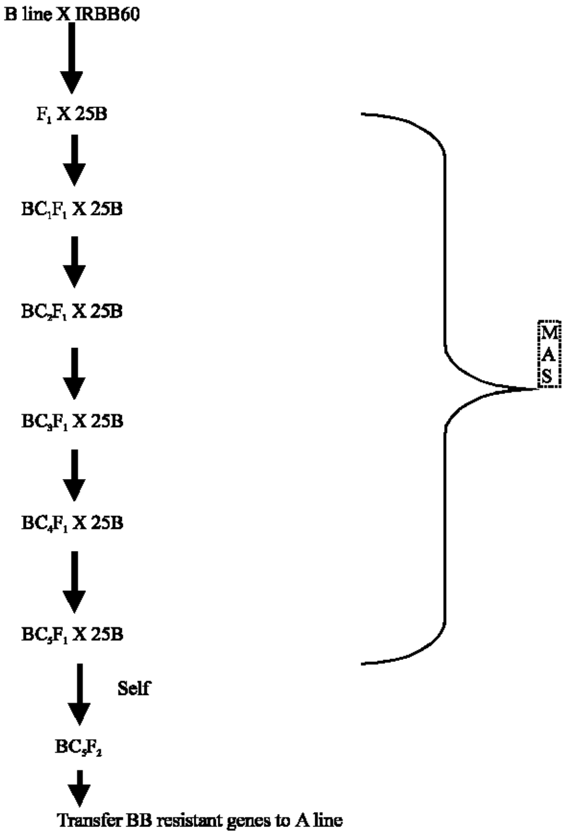

Fig. 2: Flow chart for pyramiding of restorer lines

enzyme (*HinfI*), 2.0 μL of 10X PCR buffer, 2.5 μL of sterile distilled water and 15 μL of PCR product. The reaction mixture was incubated for 4 h at 37°C and the products of restriction digestion were separated by gel electrophoresis (1.4% agarose) and visualized under UV after staining with ethidium bromide.

For *xa5* PCR was carried out using 20 ng DNA as template for amplification, 5 picomoles of each primer, 0.05 mM dNTPs, 1X PCR buffer and 1U of Taq polymerase in a total volume of 15 μL. Template DNA was initially denatured for 94°C for 5 min followed by 34 cycles of PCR amplification with the following parameters: a 30 sec denaturation at 94°C, a 30 sec primer annealing at 55°C and a 1 min primer extension at 72°C. A final extension was done at 72°C for 5 min. The PCR products were resolved in 3% agarose gel.

Screening for Resistance

The pyramided lines in the backgrounds of KMR3 and PRR78 were evaluated for their reaction to BB under glass house conditions using 10 very virulent strains of *Xoo* from Maruteru (a hotspot for bacterial leaf blight), West Godavari Andhra Pradesh, India. The cultures were maintained on modified Wakimoto's semi-synthetic medium (Karaganilla *et al*., 1973) (per liter: 20 g sucrose, 5 g peptone, 0.5 g calcium nitrate, 1.82 g disodium hydrogen phosphate, 0.05 g ferrous sulphate, 18 g agar, pH 6.8-7).

For long-term storage, the cultures were maintained as glycerol stocks at -70°C. The stored cultures were revived and grown on modified Wakimoto's medium for inoculation and

DNA experiments. The strains used for inoculation were passed through the susceptible cultivar TN1 and re-isolated in the lab before use in inoculation experiments.

Evaluation of Resistance

Individual plants were grown in plastic pots under flooded conditions with a mixture of soil and farmyard manure (3:1 ratio). The pots were fertilized with N:P at 100:50 kg ha^{-1} with P applied as basal dose and top dressing at 25 days after sowing. Approximately 40 day old plants were clip inoculated (Kauffman *et al*., 1973). Top three or four leaves of plants at maximum tillering stage were clip-inoculated with a cell suspension of 10^8 cfu mL^{-1} prepared from 48 h old cultures. For each culture-strain combination, five leaves of a plant were inoculated per replication. Each test was replicated thrice. Observations were recorded 15 days after inoculation and lesion lengths were measured to the nearest centimeter for classification of disease response. Each plant was classified as resistant (0-4 cm) and susceptible (>4 cm).

Grain Quality Parameters

Grain quality parameters were tested adopting standard methods at the Grain Testing Laboratory at Central Rice Research Institute, Cuttack, India.

RESULTS

MAS for BB Resistant Genes into Different Backgrounds

Nine F_1 plants from each of the crosses between KMR3/IRBB60 (IRBB60 is the donor for the four BB resistant genes), PRR78/IRBB60, IR58025B/IRBB60, Pusa 6B/IRBB60 were tested for their heterozygosity for the R gene linked markers and were backcrossed using the female parent. The resulting BC_1F_1 lines were first checked for presence of the *Xa21* resistance allele. All plants carrying the resistant allele were checked for the presence of *xa5* allele in heterozygous condition. Plants containing resistant alleles for both the genes were further screened for *Xa4* gene using Npb 181. Finally, the triple positives were screened for the presence of *xa13* allele using the CAPS marker and subjecting them to digestion (Fig. 3). Phenotyping of these target plants was done at the field level and only those showing the maximum similarity to the recurrent parent and showing high yield were selected. This was continued up to BC_3F_1 generation. BC_3F_2 that were screened using the R gene linked markers to identify plants that were homozygous for different R genes or their combinations.

Disease Resistance

The gene pyramids in the backgrounds of KMR3 and PRR78 showed a very high degree of resistance as compared to their parents to all the 10 isolates of *Xoo* inoculated upon (Table 1). There were varying degrees of resistance to each of the isolates, but no isolate could break down the resistance of any of the four-gene pyramids. In KMR3 background isolates 4, 6 and 10 showed almost same levels of virulence and the least virulent was isolate 3 (Fig. 4). In case of PRR, isolates 6, 7 and 10 showed a high degree of virulence as compared to isolate 9, which was the least virulent (Fig. 5). This is indicative that the isolates 6 and 10 showed a similar pattern of virulence in the restorers. The isolates showed a varying degree of disease spectrum to all the genotypes. Our earlier studies with these isolates proved that they were highly virulent and even broke down the three gene combination in IR 24 near isogenic lines (Shanti and Shenoy, 2005). This was one of the main reason to pyramid four

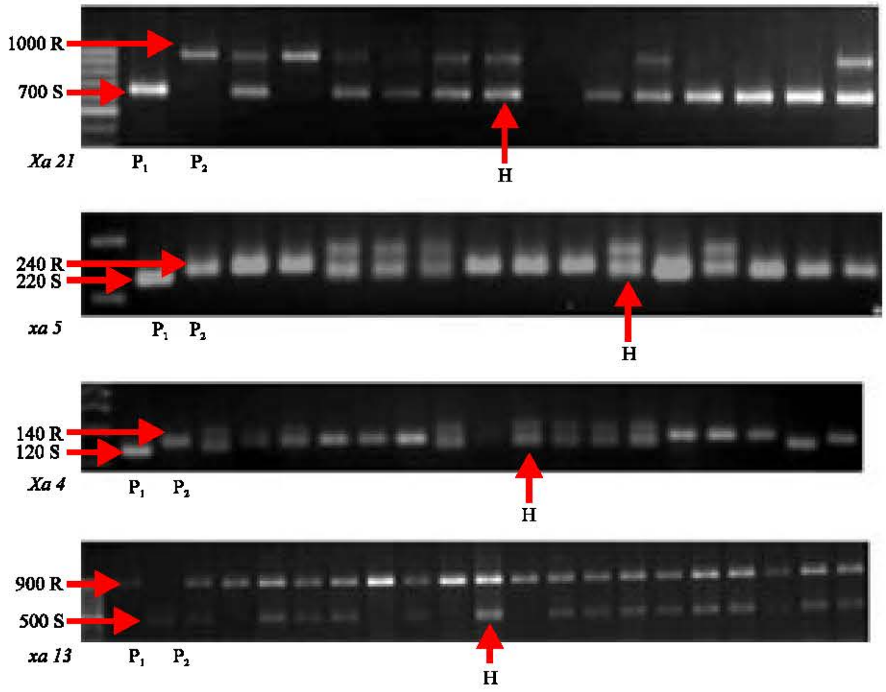

Fig. 3: Foreground selection using R gene linked PCR based markers for the four BB genes at BC_1F_1 stage. Gel photographs showing banding pattern of the markers used in the introgression. P_1-Parent, P_2-IRBB60, R indicates resistant and S indicates susceptible

Table 1: Reaction of the 10 isolates from Maruteru to the parents and pyramids

	Isolates from Maruteru (lesion length in cm)									
BB resistant genes	*Xoo 1*	*Xoo 2*	*Xoo 3*	*Xoo 4*	*Xoo 5*	*Xoo 6*	*Xoo 7*	*Xoo 8*	*Xoo 9*	*Xoo 10*
KMR3 parent	18.0	20.0	20.0	25.0	21.0	15.0	14.0	14.0	15.0	21.0
KMR3 pyramid	2.0	2.0	1.0	3.0	2.5	3.0	1.5	2.0	2.0	3.0
PRR 78 parent	22.0	24.0	22.0	23.0	22.0	25.0	22.0	21.0	22.0	22.0
PRR78 pyramid	1.8	2.0	1.5	2.0	2.0	2.5	3.0	1.5	1.0	2.5
IRBB60	3.2	2.5	2.6	2.2	2.5	2.3	2.0	3.0	2.0	2.0
Malagkit sung song (Resistant check)	2.2	2.0	2.8	3.0	1.2	2.0	2.2	1.3	2.0	3.3
TN1 (Susceptible check)	20.6	18.0	20.0	16.3	20.0	18.0	23.0	17.5	18.2	25.0

genes into all the parents in this study. These four gene pyramids have been inoculated with 20 isolates collected from different parts of the country but have not shown any sign of susceptibility to any one of the isolates indicating that this is the most effective gene combination to combat the ever changing pathogen population.

Grain Quality Parameters

Grain quality parameters between the parents and pyramid were comparable and there were no significant differences between the parents and pyramids (Table 2). This is of prime importance since the pyramid should have the same acceptability as that of the parent. The grain quality parameters of the B lines were not considered at this stage as the population is being stabilized and transfer into the A line is still being attempted. Earlier attempts by scientists on the same lines was met with success (Singh *et al*., 2001; Joseph *et al*., 2004; Sundaram *et al*., 2008).

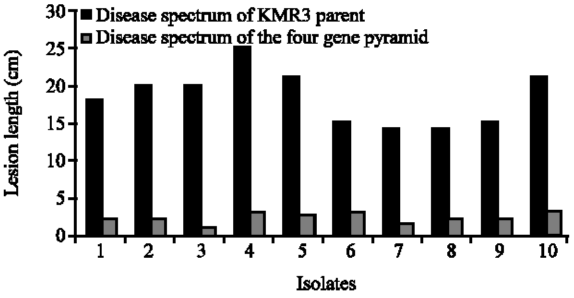

Fig. 4: Disease spectrum of KMR3 pyramid against its parent

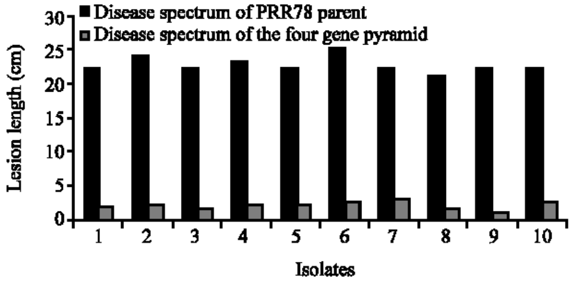

Fig. 5: Disease spectrum of PRR78 pyramid against its parent

Table 2: Grain quality parameters of the parents and the pyramids containing the four BB resistant genes

Samples	Hulling (%)	Milling (%)	Head rice recovery (%)	Length (mm)	Breadth (mm)	Length/breadth ratio	Class
KMR3 parent	79.00	69.00	45.00	5.10	2.45	2.08	MS
KMR3 pyramid	79.00	69.00	45.00	5.12	2.40	2.13	MS
PRR78 parent	78.50	67.50	48.00	7.62	1.90	4.01	LS
PRR78 pyramid	79.00	67.00	48.00	7.57	1.86	4.07	LS

Samples	Alkali spreading value	Water uptake	Volume expansion ratio	Kernel length after cooking (mm)	Elongation ratio	Amylose (%)
KMR3 parent	3.50	200	4.25	9.60	1.88	27.30
KMR3 pyramid	4.00	155	4.25	9.50	1.80	26.50
PRR78 parent	7.00	290	4.00	13.50	1.77	23.78
PRR78 pyramid	7.00	335	4.00	13.00	1.72	24.00

MS: Medium slender, LS: Long slender

DISCUSSION

In the present study we have introgressed four bacterial blight resistance genes (*Xa4*, *xa5*, *xa13* and *Xa21*) into the popular restorers KMR3 and PRR78 and the corresponding maintainers IR58025B and Pusa 6B. The pyramided lines exhibited a very high degree of resistance to different races of the pathogen. Inoculation studies across the generations in both the pyramided material as well as the near isogenic line in the background of IR 24 have shown that the four gene combination has shown no sign of breakdown to the different races of the pathogen.

The increased level of resistance of pyramid lines expressed as reduced lesion length has been reported in all previous gene pyramiding programs on BB resistance (Yoshimura *et al*., 1995; Huang *et al*., 1997; Sanchez *et al*., 2000; Singh *et al*., 2001;

Joseph *et al*., 2004; Sundaram *et al*., 2008). This could be due to the fact that there is a complementation wherein the presence of multiple genes has an additive effect on the overall level of resistance. *Xa4* though referred to as a defeated gene, it was found that this gene when in combination with *Xa21* showed higher level of resistance (Jeung *et al*., 2006).

Studies were conducted initially on the different gene combinations (single, two-gene, three-gene and four-gene) of the near isogenic lines in IR 24 background for observing yield penalty, but it was found that under disease free conditions there was no significant difference in yield in any of the combinations as compared to the original parent (data not shown). The yield levels of the four gene pyramids of KMR3 and PRR78 were not significantly different from that of the parents indicating that there is no yield penalty associated with the presence of the resistance genes. The grain quality characteristics of the four gene pyramids (restorer lines) are similar to the original parent.

The main objective of this work was to introgress the four BB resistant genes and insulate against the yield loss without sacrificing the grain characteristics of the parents. Earlier work by scientists to introgress two BB genes in select backgrounds was successful (Joseph *et al*., 2004; Sundaram *et al*., 2008). The complete recovery of yield and grain quality characters is a very significant achievement since these are multigenic traits encoded by loci distributed across the rice genome (Sundaram *et al*., 2008). The maintainer lines are in BC_5F_1 stage. Testcrosses are being attempted this season to evaluate the sterility/fertility of these lines. Once a sterile family is identified conversion of the CMS line will be taken up immediately and the population will be stabilized using MAS and breeding.

Under BB infection there was a ~20% reduction in yield in the original varieties. This proves that cultivation of the four gene pyramids in BB endemic areas would be of a great advantage to reduce the yield loss. These lines are also being used as donors for transfer of these resistant genes to other backgrounds. This study demonstrates that marker assisted backcrossing can be successfully utilized for simultaneous multiple gene introgression.

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REFERENCES

Chen, H., S. Wang and Q. Zhang, 2002. New gene for bacterial blight resistance in rice located on chromosome 12 identified from Minghui 63, an elite restorer line. Phytopathology, 92: 750-754.

Dellaporta, S.L., J. Wood and B. Hicks, 1983. A plant molecular DNA mini preparation. Plant Mol. Biol. Rep., 1: 19-21.

Huang, N., E.R. Angeles, J. Domingo, G. Magpantay and S. Singh *et al*., 1997. Pyramiding of bacterial blight resistance genes in rice: Marker-assisted selection using RFLP and PCR. Theor. Applied Genet., 95: 313-320.

Jeung, J.U., S.G. Heu, M.S. Shin, C.M.V. Cruz and K.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.

Joseph, M., S. Gopalakrishnan, R.K. Sharma, V.P. Singh, A.K. Singh, N.K. Singh and T. Mohapatro, 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. Mol. Breed., 13: 377-387.

Karaganilla, A., M.P. Natural and S.H. Ou, 1973. A comparative study of culture media of Xanthomonas *Oryzae* pv. *Oryzae*. Phillipines Agric., 57: 141-152.

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.

Khush, G.S. and E.R. Angeles, 1999. A new gene for resistance to race 6 of bacterial blight in rice, *Oryza sativa* L. Rice Genet. Newslett., 16: 92-93.

Lee, K.S., S. Rasabandith, E.R. Angeles and G.S. Khush, 2003. Inheritance of resistance to bacterial blight in 21 cultivars of rice. Phytopathology, 93: 147-152.

Lin, X.H., D.P. Zhang, Y.F. Xie, H.P. Gao and Q. Zhang, 1996. Identifying and mapping a new gene for bacterial blight resistance in rice based on RFLP markers. Phytopathology, 86: 1156-1159.

Nagato, Y. and A. Yoshimura, 1998. Report of the committee on gene symbolization, nomenclature and linkage groups. Rice Genet. Newslett., 15: 13-74.

Nayak, D., M.L. Shanti, L.K. Bose, U.D. Singh and P. Nayak, 2008. Pathogenicity association in *Xanthomonas oryzae* pv. *Oryzae* the causal organism of rice bacterial blight disease. ARPN J. Agric. Biol. Sci. J. Phytopathol., 3: 12-26.

Ou, S.H., 1985. Rice Disease. 2nd Edn., Commonwealth Mycological Institute (CMI), Kew, Surrey, England, pp: 38.

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

Shanti, M.L., M.L.C. George, C.M.V. Cruz, M.A. Bernardo and R.J. Nelson *et al*., 2001. Identification of resistance genes effective against rice bacterial blight pathogen in Eastern India. Plant Dis., 85: 506-512.

Shanti, M.L. and V.V. Shenoy, 2005. Evaluation of resistance genes and their pyramids against rice bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae*. Oryza, 42: 169-173.

Singh, G.P., M.K. Srivastava, R.V. Singh and R.M. Singh, 1997. Variation and qualitative losses caused by bacterial blight in different rice varieties. Indian Phytopathol., 30: 180-185.

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

Sundaram, R.M., M.R. Vishnupriya, S.K. Biradar, G.S. Laha and G.A. Reddy *et al*., 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica, 160: 411-422.

Tan, G.X., X. Ren, Q.M. Weng, Z.Y. Shi, L.L. Zhu and G.C. He, 2004. Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. Yi Chuan Xue Bao., 431: 724-729.

Tanksley, S.D., N.D. Yound, A.H. Paterson and M.W. Bonierbale, 1989. RFLP mapping in plant breeding: New tools for an old science. Biotechnology, 7: 257-264.

Xiang, Y., Y. Cao, C. Xu, X. Li and S. Wang, 2006. *Xa3*, conferring resistance for rice bacterial blight and encoding a receptor kinase–like protein, is the same as *Xa26*. Theor. Applied Genet., 113: 1347-1355.

Yoshimura, S., A. Yoshimura, R.J. Nelson, T.W. Mew and N. Iwata, 1995. Tagging *Xa-1*, the bacterial blight resistance gene in rice, by using RAPD markers. J. Breed., 45: 81-85.

Zhai, W X. and L.H. Zhu, 1999. Rice bacterial blight resistance genes and their utilization in molecular breeding. Adv. Biotechnol., 19: 9-15.

Zhang, Q., S.C. Lin, B.Y. Zho, C.L. Wang and W.C. Yang *et al*., 1998. Identification and tagging a new gene for resistance to bacterial blight (*Xanthomonas oryzae* pv. *Oryzae*) from *O. rufipogon*. Rice Genet. Newslett., 15: 138-142.

Zhang, Q., 2005. Utilization and strategy of gene for resistance to rice bacterial blight in China. Chinese J. Rice Sci., 19: 453-459.

Zhang, J., X. Li, G. Jiang, Y. Xu and Y. He, 2006. Pyramiding of Xa7 and Xa21 for the improvement of disease resistance to bacterial blight in hybrid rice. Plant Breed., 125: 600-605.