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MOLECULAR BREEDING FOR IMPROVEMENT OF BLAST AND SHEATH BLIGHT RESISTANCE IN SRI LANKAN RICE CULTIVAR 'POKURU SAMBA'

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EXTENDED ABSTRACT

Rice, being the staple food crop, provides about 1.6% of Gross Domestic Products of Sri Lankan economy. Rice blast and the rice sheath blight (SB) are the two most devastating diseases affecting the productivity of rice in Sri Lanka. Rice blast, caused by *Magnaporthe oryzae*, could result in loss of about one million hectares of paddy in Asia annually, depending on the varieties grown. The disease SB caused by *Rhizoctonia solani* Kühn is another destructive rice disease worldwide, and it can lead to severe losses in rice productivity. It is noted that some of the Sri Lankan rice varieties have lost their blast resistance after few years of cultivation. Therefore, improving disease resistance in rice is crucial for stable rice production.

Almost 100 blast resistance genes have been identified worldwide. All these genes have been mapped on all rice chromosomes except for chromosome 3. Rice cultivar 'Tetep' is one of the sources of resistance genes. It is a donor of blast resistance genes, *Pita, Pi1(t), Pi4a(t), Pi4b(t), Pi3(t), Pi-tp*(t), and *Pi54*. Among these, *Pi54* is a major dominant gene. Major genes for blast resistance *Pi-1(t)* and *Pi 54* have been mapped on chromosome 11 and *Pi-tp (t)* on chromosome 1. SSR marker *RM 206*, is tightly linked to the *Pi54* gene and it produces DNA fragment of 130 bp in Tetep. The cultivar '*Tetep*' also possesses a major QTL for SB resistance, *qSBR11-1*, on the arm of chromosome 11, in which *Pi54* is also located. The SSR marker *RM 224* is flanking the SB resistance QTL *qSBR11-1*.

'*Pokuru Samba*' is a high yielding popular rice cultivar in Sri Lanka but not recommended as a rice variety due to its susceptibility to blast disease. The objective of this study was to develop marker-assisted selection in backcross breeding programmes for improving disease resistance in popular rice varieties. In this study, the cultivar '*Tetep*' was used as donor for incorporating the blast resistance gene, *Pi54* and sheath blight resistant QTL *qSBR11-1* into local susceptible cultivar *Pokuru Samba*, through marker-assisted foreground selection in combination with fungal inoculation and

selection for blast disease in the blast screening nursery, followed by phenotypic selection for recovery of agronomic, morphological traits.

The recurrent parent, *Pokuru samba* was hybridized with *'Tetep'*, the donor for the blast and SB resistance gene *Pi54* and *qSBR11-1* QTL, respectively. BC_2F_1seeds were obtained by back crossing the BC_1F_1 with recurrent parent *Pokuru Samba.* All the seeds were planted in trays and inoculated with *Pyricularia oryzae* in the blast screening nursery at Rice Research and Development Institute, Bathalagoda in the 2014/15 Maha cropping season in comparison with blast susceptible traditional rice cultivar 'Puchchaperumal'. Plants were selected when the blast disease was developed and susceptible cultivar 'Pachchaperumal' died completely. At that stage, all the plants in backcrossed population were scored using the standard scoring system developed in International Rice Research Institute. Plants which did not show any blast symptom and scored 'Resistant' category were subjected to molecular marker assisted foreground selection.

Total DNA was extracted from leaf samples by using the KCl DNA extraction protocol. The PCR reactions were carried out with in a final volume of 15 µl containing, 3 µl of DNA sample, 3.0 µl 5x PCR buffers with 0.9 µl $MgCl_2$ (25 mM), 0.15µl of 10 mM dNTP (Promega, U.S.A.), 1.0 µl of primer (RM 206 *and* RM 224 at the concentration of 20 µM), and 0.075 µl of 5 u/µl Taq DNA polymerase (Promega, U.S.A.) in a thermal profile of initial denaturation (5 minutes at 95^0C) followed by 35 cycles of denaturation (at 95 ^{0}C for 1 minute), Primer annealing (55 ^{0}C for 30 seconds), extension (at 72^0C for 1 minute) and final extension at 72 ^{0}C for 2 min. Two simple sequence repeat markers used in previous studies, *RM 206* and *RM 224* for gene *Pi54* and *qSBR11-1* QTL, respectively were selected for PCR amplification. The PCR amplicons were visualized in 2% agarose gel. Heterozygous plants for both markers were further selected for recovery of recurrent parent phenotype and used for developing BC_3F_1. Similarly, the plants of BC_3F_1 population were selected following the same procedure.

Seventy two plants of BC_2F_1 population were screened for blast resistance at the blast screening nursery at RRDI. A total of 27 plants were grouped in 'Resistant' category and were subjected to genotypic screening. In BC_3F_1 population, 66 plants were screened, and 12 plants were included in 'Resistant' category. Out of 27 plants, six plants carried RM 206 (130bp) molecular maker which is tightly linked to *Pi54* gene. Marker-assisted selection for SB showed 21 plants with heterozygous molecular band for flanking marker for SB QTL, *qSBR11-1*, RM 224. Out of five plants selected as pyramided lines of both *Pi54* and *qSBR11-* genotypes only 2 plants were selected from

BC_2F_1 for recurrent parent phenotypic recovery. Similarly, out of 66 plants in BC_3F_1 population 2 gene pyramided plants were selected. Instead of marker aided background selection, phenotypic selection was the method used to have maximum recovery of recurrent phenotypes such as plant height, grain shape, and number of panicles, because use of large number of molecular markers for back ground selection is a costly, laborious and labour consuming task although it is more accurate and reliable.

This study proved that marker assisted back cross breeding approach can be successfully used for introgression of one or more genes into a desired rice variety. It accelerates the improvement of rice varieties with disease resistance. The breeding lines selected in this study will be an important source for developing disease resistant rice varieties. The breeding approach used would be guidance for application of MABB in developing disease resistant varieties with higher reliability and accuracy.

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