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Research Article

Marker-assisted selection for sheath blight resistance in rice (*Oryza sativa* L.)

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Abstract
Sheath blight is one of destructive diseases of rice, causing substantial loss to rice production. Developing resistant cultivars to sheath blight is quite difficult due to lack of effective resistant source in the available germplasm. Pyramiding of gens/QTLs for different biotic stresses gives broad-spectrum resistance to multiple diseases for any crop.The present study was carried out to introgress sheath blight resistant QTL, *qSBR11*-1, into the backgrounds of CB14004 and CB14002 from the donor parent Tetep. CB14004 and CB14002 are the two bacterial blight resistant genotypes, pyramided with three bacterial blight resistant genes *viz.,xa5*, *xa13* and *Xa21*. The positive plants of BC_3F_1 individuals for sheath blight QTL i.e., *qSBR11-1*were self-pollinated to generate BC_3F_2 individuals. The BC_3F_2 individuals were screened with linked molecular marker of *qSBR11-1* i.e., RM 224 and the confirmed plants were assessed for morphological traits to identify the superior segregates.

Keywords
Rice, marker-assisted selection, sheath blight resistance

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major staple food crops for more than 50% of world population (Sharma *et al.,* 2012). The population may increase to 9 billion by the end of 2050 and food production is sufficient to meet the requirements of only 60% of the population (FAO, 2018). China is the leading producer of rice (142.3 million tonnes) followed by India (110.4 million tonnes) (FAO, 2018), thereby plays a major role in meeting the rice demand. In India, rice shared by 48% of total food grain production and it is main source of income to many people for meeting their daily requirements (Kiruthikadevi *et al*., 2020). Rice production is affected by several pathogens, which are major threats for food security. This threat can be addressed and production can be enhanced by introgressing the resistant genes/QTLs, which are available in the wild species and germplasm accessions into cultivated varieties. Recent advances in biotechnology, enhances the application of molecular markers for precise introgression of resistant genes/QTLs into the targeted varieties. Many varieties and improved lines have been developed for the benefit of farmers for different stresses through marker-assisted selection (Sundaram *et al.,* 2008; Ramalingam *et al*., 2017; Chithrameenal *et al*., 2018).

Rice sheath bight, caused by*Rhizoctonia solani* Khun, is considered as one of the important diseases of rice and it causes a yield loss up to 45% (Margani *et al*., 2018). Sheath blight is the second most important disease of rice next to blast (Susmita Dey *et al*., 2020). *R. solani* is a necrotic soil borne pathogen, survives in the crop residues of rice as sclerotia or in the form of mycelium. The sclerotia of the infected plant floats on the water surface and germinates on the rice sheath, forms appressorium (Richa *et al.,* 2016). The appressorium causes the initial infection by colonizing the entire plant through the surface hyphae and causes necrotic damage in the sheath region (Ou*,* 1985). The disease aggressive at the time of panicle differentiation stage and inhibits grain filling. Till date, none of the varieties having resistance to ShB were reported (Channamallikarjuna *et al.,* 2010). Sheath blight is challenging to control because of its wide host ranging capacity and persistence of sclerotia in harder climatic conditions (Pooja Singh *et al*., 2019). Many QTLs have been identified in the background of Tetepand linked to molecular markers through QTL mapping approach. Among them, *qSBR11-1* (linked to RM 224), providing a moderate resistance to ShB across the years and locations (Channamallikarjuna *et al.,* 2010). In the present study, *qSBR11-1*was introgressed in the backgrounds of CB14004 and CB14002 for moderate resistance to sheath blight disease.

MATERIALS AND METHODS

CB14004 and CB14002 are the two bacterial blight resistant genotypes, pyramided with *xa5, xa13* and *Xa21*, developed at TNAU, Coimbatore (Perumalsamy *et al.,* 2009). Both CB14004 and CB14002 were used as recurrent parents for introgression of sheath blight resistance. Tetep, a Vietnamese*indica* landrace conferring moderately resistance to sheath blight (Channamallikarjuna *et al.,* 2010) was used as donor parent for targeted introgression of sheath blight resistant QTL i.e., *qSBR11-1*into the backgrounds of CB14004 and CB14002 to combine sheath blight resistance with bacterial blight resistance in the recurrent parents.

BC_1F_1 and BC_2F_1 hybrids have been developed and fixed for sheath blight resistance in the backgrounds of CB14004 and CB14002 (Vidya *et al*., 2018). BC_3F_1 hybrids were developed by crossing CB14004 × Tetep and CB14002 × Tetep to combine both sheath blight and bacterial blight resistance in the backgrounds of CB14004 and CB14002. RM 224 is a closely linked molecular marker of *qSBR11-1*, explains 14% of phenotypic variation and mapped on the chromosome number 11 (Channamallikarjuna *et al.,* 2010). The BC_3F_1 hybrids were screened with linked molecular marker RM 224 to confirm the targeted QTL in heterozygous condition. The plants showing heterozygous for the targeted QTLwere self-pollinated to generate BC_3F_2 individuals. The BC_3F_2 segregates were screened with linked molecular marker of *qSBR11-1*to fix the plants for sheath blight resistance. The confirmed plants for sheath blight resistance were also assessed for morphological and grain quality traits, to select the segregates similar to recurrent parents.

DNA was extracted from the fresh young leaves of $BC_3F_1$and BC_3F_2 individuals along with parents at maximum tillering stage by CTAB method (Sambrook *et al.,* 1989). DNA quality was checked by 0.8% agarose gel followed by quantified with spectrophotometer and the concentration of DNA was adjusted to 50ng/µl. 10 µl of PCR reaction mixture contains 4 µl of smart prime 2XX red master mix, 4 µl of water, 1 µl of template DNA, 0.5 µl of each forward and reverse primers. PCR was conducted in the Effendorf thermocycler with following protocol: initial denaturation of 94ºC for 4 min, followed by 35 cycles of denaturation of 94ºC for 1 min, annealing at 55ºC for 1 min, initial extension of 72ºC for 1 min and final extension of 72ºC for 7 min. The PCR products were separated by using 3% agarose gel stained with ethidium bromide and visualized on UV light.

RESULTS AND DISCUSSIONS

The present study was carried out to introgress sheath blight resistance into the backgrounds of popular varieties (CB14004 and CB14002) harboring BB resistance genes (*xa5*, *xa13* and *Xa21*) through marker-assisted selection. A total of 143 BC_3F_1 hybrids were produced by crossing with the positive plants of BC_2F_1 hybrids with recurrent parents (CB14004 and CB14002).

Table 1. Agro-morphological characters of selected lines harboring ShB QTL

Genotype	PH (cm)	DFF	NPT	PL (cm)	NGP	SPY (g)
IL # 1-1-3	85.3	82	17	23.2	197	25.6
IL # 1-1-9	79.4	87	18	25.5	190	26.5
IL # 1-2-5	88.8	80	16	22.5	188	24.3
IL # 1-2-8	79.2	88	19	25.1	203	27
IL # 1-1-7	85.4	81	16	22.8	189	24.1
IL # 1-5-4	83.1	81	15	23.5	191	24.2
IL # 2-1-9	87.9	74	16	25.6	177	23.5
IL # 2-1-5	82.3	77	17	23.4	184	25.3
IL # 2-3-7	88.2	79	16	24.3	191	25.7
IL # 2-4-2	82.8	76	18	22.8	193	26
IL #2-6-8	85.3	79	17	24.2	190	25.6
CB14002	84.5	85	16	22.5	191	26.1
CB14004	86.3	84	17	23.9	198	27
Tetep	123	91	20	27.2	155	29.4
Mean	86.91	81.71	17	23.82	188.35	25.57
SD	10.38	4.65	1.26	1.26	10.99	1.44
CV (%)	11.95	5.69	7.44	5.30	5.83	5.64

PH - Plant height (cm), DFF - Days to 50% flowering, NPT - Number of productive tillers, PL - Panicle length (cm), NGP - Number of grains per panicle, SPY - Single plant yield (g)

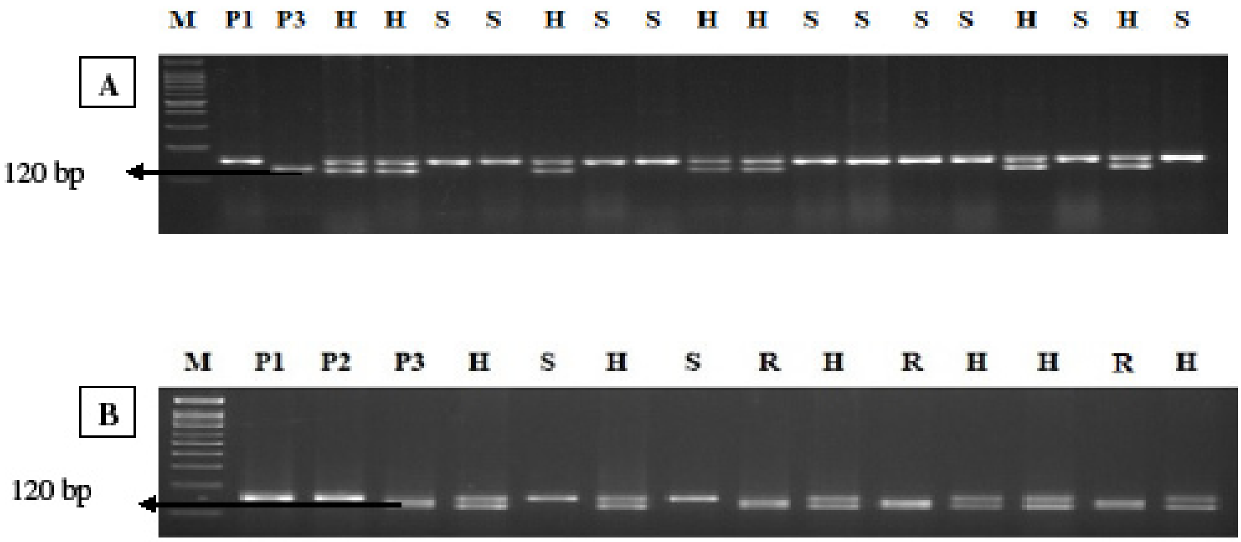

Fig. 1. Agarose gel electrophoresis images illustrating the presence of ShB resistant QTL (*qSBR11-1*) in(A) $BC_3F_1$and (B) BC_3F_2 individuals.P_1 - CB14004, P_2 – CB14002, P_3 - Tetep, M- 100 bp ladder, S – Susceptible, R – Resistant, H – Heterozygous

All the 143 BC_3F_1 hybrids were sown and analyzed with linked molecular marker of *qSBR11-1*i.e., RM 224. Foreground analysis reveals four plants in CB14004 × Tetep combination and seven plants in CB14002 × Tetep combination were showing heterozygous for the targeted QTL i.e.,*qSBR11-1*. The hybrids selected by genotypic screening method were self-pollinated to generate BC_3F_2 individuals. A total of 400 plants in the both combinations (CB14004 × Tetep and CB14002 × Tetep) were first screened linked molecular marker and the positive plants were assessed phenotypically to select the plants having superior agronomic characters. By screening genotypic and phenotypic screening methods, we have found 25 lines in CB14004 background and 21 lines in CB14002 background having presence of targeted sheath blight resistant QTL(*qSBR11-1*). Further, the segregates possessing sheath blight resistance were also assessed for key morphological traits *viz*., plant height (cm), the number of productive tillers, days to 50% flowering, panicle length (cm), the number of grains per panicle, and single plant yield (gm) to identify the lines similar to recurrent parents.The evaluation of morphological traits reveals, most of the selected lines harbouring *qSBR11-1* were have similar morphological traits as recurrent parents (**Table.1**). This indicates, the identified segregants were a result of combination of marker-assisted selection and phenotypic selection to have the recurrent parent alleles and donor parent alleles. Channamallikarjuna*et al*., (2010) have identified *qSBR11-1*, on the chromosome number 11 through composite interval mapping. Among the12 QTLs identified in the recombinant lines of HP2216 (susceptible) and Tetep (resistant), *qSBR11-1*explains 14% of phenotypic variation. The candidate QTL, *qSBR11-1*was also found consistently over the four years and two locations.The present study was focuses on the introgression of*qSBR11-1* QTL for moderate resistance to sheath blight in rice. Pyramiding of two or three major genes/QTLs enhances the resistance levels for different isolates of pathogens than a single gene resistance (Sundaram *et al.,* 2008). Introgression of sheath blight resistance in the background of cultivars harbouring bacterial blight resistance enhances the resistance levels to two different biotic stresses. Singh *et al*., (2012) first time introgressed *qSBR11*-1, in the background of improved Pusa Basmati, to combine the sheath blight resistance along with bacterial blight resistance. The results of Singh *et al*., (2012) were in accordance with the present study results. Further, the lines harbouring ShB resistant QTL need to assessed for physical resistance against the isolates of rice sheath blight pathogen at BC_3F_3 generation. The present study demonstrates precise introgression of targeted QTL for sheath blight resistance in rice.

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