Estimation of yield and grain qualities of marker assisted backcross derived lines of submergence rice against sheath blight disease

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Abstract: Sheath blight caused by *Rhizoctonia solani* is one of the most devastating diseases of rice (*Oryza sativa*) and causes enormous yield losses over the world after blast, the disease can cause yield loss upto 50 per cent in advanced stage and adversely affects quality of straw. Breeding for resistant varieties is the only viable option to combat the disease efficiently. In this study, our findings showed a significant increase in number of spikelet's per panicle (3.45 %), test weight (0.62 %) and grain yield (0.72 %) compared to recurrent parent Swarna sub-1. The range of mean performance of 18 BC_2F_1 selected improved lines varied for per cent disease severity from 26.75 to 43.58 at 16 days after inoculation. Among the 18 improved lines, only four lines (Swarna sub-1-6, Swarna sub-1-32, Swarna sub-1-13 and Swarna sub-1-29) showed resistance score of 1-3. The remaining fourteen lines showed moderate resistance with a score of 3-5. Hence, the resistance line could be exploited in sheath blight resistance breeding programme and the same line can also be released as a variety against sheath blight of rice after testing over multilocation trails.

Keywords: Per cent disease severity, Sheath blight, Rice, Yield per plant

INTRODUCTION

Soil borne pathogen *Rhizoctonia solani* is endemic to areas where temperature and relative humidity are high and reduced yield upto 54.3 per cent in advanced stage and also adversely affects quality of straw (Chahal *et al.,* 2003; Savary *et al.,* 2006). Losses depend on the growth stage of plant at which infection occurs, level of resistance in a particular genotype and prevailing environmental conditions (Joshi *et al.,* 2016). Rice is India's pre-eminent crop, and is the vital food for people in the eastern and southern parts of the country (Ghritlahre *et al.,* 2016). Though its productivity fluctuates significantly from region to region; season to season due to various biotic factors such as pest and diseases. Rice genetic resources have not been comprehensively exploited for improvement of sheath blight resistance, although many cultivars and lines have been reported as promising sources of resistance (Srinivasachary *et al*., 2011). The host resistance is the most economical and environmentally sound strategy in managing sheath blight, but substantial differences in the levels of susceptibility to the sheath blight pathogen among rice cultivars have been observed under field conditions. Sheath blight resistance is believed to be controlled by polygenic quantitative trait loci (QTLs)). Li *et al*., (1995) first identified Sheath blight-QTLs using restricted fragment length polymorphism (RFLP) markers and field evaluations. Subsequently, a number of QTLs (around 50 QTLs) associated with sheath blight resistance have been reported spanning over all 12 chromosomes in cultivated varieties, deep-water varieties and wild species (Jia *et al.,* 2009; Zuo *et al.,* 2010; Wang *et al.,* 2012; Xu *et al.,* 2010) by using segregating F_2 populations, double haploid (DH), and recombinant inbred line (RIL) populations under field conditions, although the mechanisms of resistance of these QTLs are poorly understood. Among these, *qSBR11-1* is a major QTL that has been found to be effective against the sheath blight pathogen consistently over time and at different locations (Channamallikarjuna *et al.,* 2010). 'Tetep', an indica rice cultivar from Vietnam, is the source of resistance
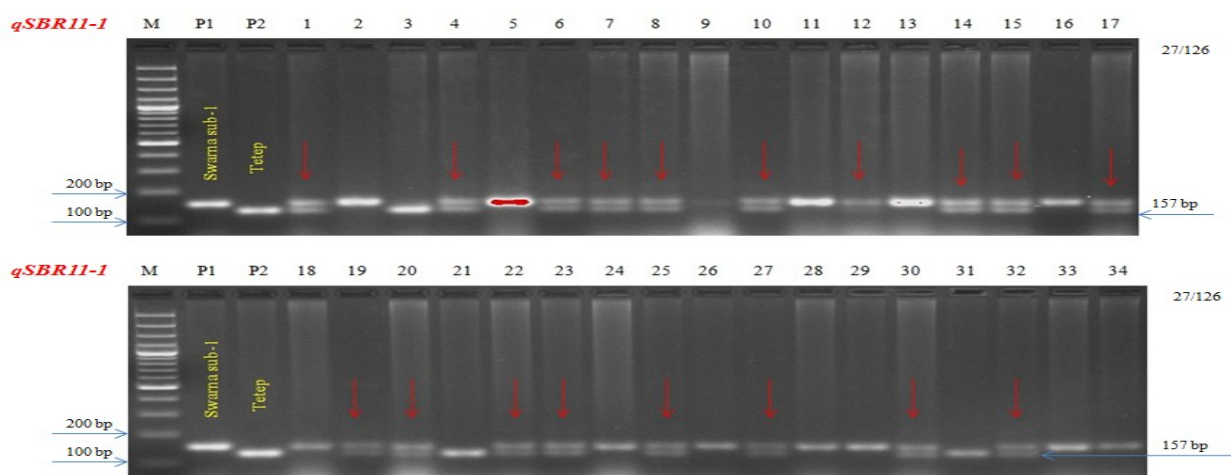
Plate 1. *Foreground selection for sheath blight disease resistance QTL/genes qSBR11-1 in BC$_1$F$_1$ generations. Where P$_1$ is recurrent parent, P$_2$is donor parent and M is 100 bp DNA size markers.*

to both blast (Pi54) and sheath blight (*qSBR11-1*) diseases. In addition, 'Tetep' is an invaluable donor of several other resistance genes for blast resistance (Singh *et al.,* 2012); and 10 other QTLs for sheath blight resistance (Channamallikarjuna *et al.,* 2010). Marker-assisted backcross breeding (MABB) coupled with phenotypic selection for agronomic, grain quality traits has been used to incorporate sheath blight resistance QTL qSBR11-1 into 'Improved Pusa Basmati 1' (Singh *et al.,*2012a) and QTLs qSBR11-1, qSBR11-2 and qSBR7-1 into 'Pusa 6B' CMS line (Singh *et al.,* 2015). In our study, in order to incorporate of ShB disease resistance QTL qSBR11-1 into Swarna sub-1, a backcross approach was used in which backcross was restricted to two generations and foreground and background marker assisted selection was also done at the early segregating generations such as BC$_1$F$_1$ and BC$_2$F$_1$. Further, the generations were handled as in pedigree model.

MATERIALS AND METHODS

Plant material: Swarna sub-1, submergence tolerance variety, was used as the recurrent parent in the present study. Tetep, a Vietnamese indica rice variety has been used as the donor for the sheath blight resistance QTLs, *qSBR11-1*. Tetep was crossed with Swarna sub-1; and the single F$_1$ plant was backcrossed with Swarna sub-1, to generate the BC$_1$F$_1$. Marker assisted foreground selection among BC$_1$F$_1$ plants initially targeted the SSR marker RM224 linked for *qSBR11-1*were done which was coupled with selection for phenotypic similarity with the recurrent parent. Selected plants that were heterozygous for RM224 marker were further backcrossed with Swarna sub-1 to generate BC$_2$F$_1$. Among BC$_2$F$_1$ plants were again marker assisted foreground selection with SSR marker RM224 linked for *qSBR11-1* done which was coupled with selection for phenotypic similarity with the recurrent parent. Selected plants that were heterozygous for

RM224 marker in BC$_2$F$_1$ generation were artificially screened against *Rhizoctonia solani* and the resistant lines were subjected for qualitative and quantitative traits estimation.

Molecular marker analysis: Total genomic DNA was extracted by the micro-extraction protocol of Prabhu *et al.,* (1998). Polymerase chain reaction (PCR) was performed in a thermal cycler (G-Storm, Somerset, UK) using a 10 mL total reaction volume as described previously (Basavaraj *et al.,* 2010). This contained 30 ng mL21 of template DNA, 5 pmol of each primer (synthesized from Sigma Inc., St. Louis, MO, USA), 1.5 mM MgCl2, 0.2 mM dNTPs (MBI, Fermentas, Vilnius, Lithuania) and 0.5 U of Taq polymerase (Bangalore Genei, Bangalore, Karnataka, India). Polymerase chain reaction comprised one cycle of denaturation at 95 8C for 5 min, followed by 35 cycles at 95 8C for 30 s, 55 8C for 30 s and 72 8C for 1 min, with a final extension of 72 8C for 7 min. The amplified products were resolved on 3.5 % MetaphorTM gel (Lonza, Rockland, ME, USA) containing 0.1 mgmL21 of ethidium bromide (Amresco, Solon, OH, USA) along with a DNA size standard ladder (MBI, Fermentas) and documented in a Gel Documentation System (Biorad, Hercules, CA, USA).

Screening for sheath blight resistance: The screening for sheath blight resistance in the backcross derived lines was carried out through artificial inoculation under field conditions at the experimental farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. A virulent isolate of *R. solani* was obtained from Indian Institute of Rice Research, Hyderabad (wild type strain-Rajendranagar). The inoculum of isolate was multiplied following the procedure described by Bhaktavatsalam *et al.* (1978). Shoots of water sedge (*Typha angustata*) were cut into pieces of 4-5 cm long washed thoroughly and soaked in Typha medium (peptone: 10.0 g, Sucrose: 20 g, K$_2$HPO$_4$: 0.1g, MgSO$_4$: 0.1 g, Distilled water: 1 L) for 5 min.
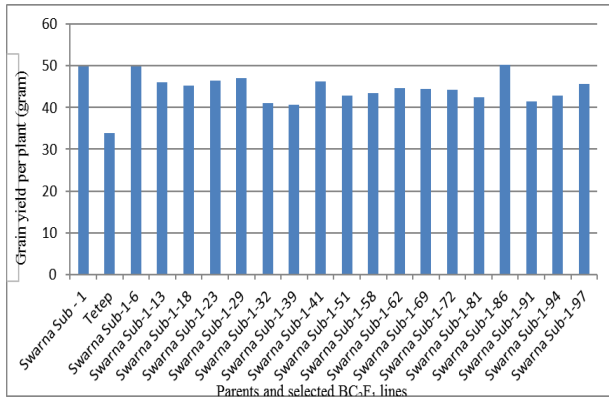
Fig. 1. *Mean performance of grain yield per plant of parents and 18 BC₂F₁ selected backcross derived lines.*

Fig. 2. *Mean performance of area under disease progress curve of parents and 23 BC₂F₁ selected backcross derived lines.*

The pieces were drained for excess water and later these were filled loosely to one third volume of 250 ml conical flask and sterilize in autoclaved at 1.05 kg/cm² for 20 min each for two consecutive days. The sterilized typha flask was inoculated with 5 mm diameter disc of actively growing mycelium of the isolate and incubated for 15 days at 28±2 °C. These colonized typha pieces were used as inoculum.Plants were inoculated at the maximum tillering stage (30-35 days after sowing) with colonized typha pieces. Two pieces of typha were placed between tillers in the central region of rice hills, just above the water level. Water level (5-10 cm) was maintained constantly for ensuring enough humidity to promote disease development. The inoculated plants were regularly examined for appearance of symptoms starting from 48 hours after inoculation and number of lesions and their length on the rice sheath around the inoculation point will be recorded from 96 hours after inoculation. The data on disease intensity was recorded on four different dates at four day intervals i.e. 4th, 8th, 12th, and 16th day after inoculation (Kumar *et al.*, 2008).

PDI = (Sum of all ratings ×100) / (Total number of observations × Maximum rating scale)

$$AUDPC = \sum_{i=1}^{n-1} \{[\,(X_{i+1} + X_i)\,/2] * (t_{i+1} - t_i)\}$$

Where;

X_i is the disease index expressed as a proportion at the ith observation.

t_i is the time (days after planting) at the ith observations and

n is the total number of observations.

Evaluation of quantitative and qualitative traits for statistical analysis: The derived lines in BC₂F₁ generation together with parental lines were evaluated for quantitative and qualitative traits performance in a randomized complete block design with three replications with 20 × 15 cm spacing at the experimental farm of Institute of Agricultural Sciences, BHU, Varanasi during *Kharif* 2013. The data of 10 plants were recorded for various quantitative traits such as number of reproductive tillers per plant (NRT), plant height (PH),
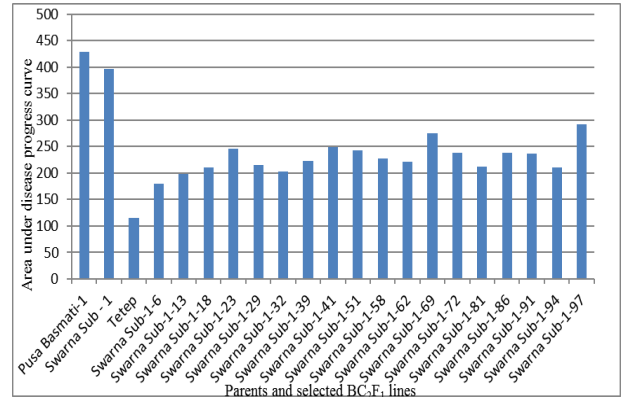
days to maturity (DM), days to heading (DH), panicle length (PL), Weight of panicle (WP), number of spikelet's per panicle (SP), test weight (TW) and yield per plant (YP) using the standard evaluation system of rice (IRRI 2002). The qualitative traits (grain quality) were determined by length/breath before cooking (L/B ratio BC), length/breath after cooking (L/B ratio AC), Amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) (Gopalakrishnan *et al.,* 2008).

RESULTS AND DISCUSSION

MAS for QTLs governing sheath blight resistance: In the present study, 77 F₁ plants were raised from a cross (Swarna sub-1 × Tetep) and their leaf samples were taken at 21 days after transplanting, DNA was isolated from these samples and each and every plant was tagged. Foreground selection was done with RM 224 primer for *qSBR11-1* QTL linked with sheath blight resistant. The 68 plants showed presence of *qSBR11-1*QTL in the F₁ plants while remaining 9 plants showed bands corresponding to recurrent parent indicating they were selfed. All 68 plants that contained *qSBR11-1 QTL*/gene, showed the 10-15 days earlier maturity than the recurrent parent Swarna sub-1. It means all 68 F₁ plants had *qSBR11-1* QTL/gene bands from the donor parent (Tetep). The plant characters of Swarna sub-1 and Tetep were distinctly different and the true F₁ plants were easily recognised. Out of 68 F₁ plants, only 17 F₁ plants were exactly similar to their recurrent parent Swarna sub-1 and these were tagged and backcrossed with Swarna sub-1 to produced BC₁F₁ seeds.

The 126 BC₁F₁ seeds were sown to raise BC₁F₁ generation and each and every plant was tagged at seedling stage. Again marker assisted selection has been applied for the foreground selection among 126 BC₁F₁ plants. Out of them, only 27 plants showed positive bands for QTL*qSBR11-1*, while remaining plants carried the negative QTL/gene to the marker RM224 (Plate 1). The resistance of these 27 plants were also confirmed by exposing them to disease inoculation with *Rhi-*

zoctonia solani strain *R. nagar* wild type. The per cent disease severity of these 27 plants were significantly lesser than the recurrent parent Swarna sub-1 as well as susceptible check Pusa Basmati-1 (PB-1), while closer to the resistance parent Tetep as these plants had resistance QTL*qSBR11-1*. Out of 27 plants, only 9 BC_1F_1 plants were exactly similar to recurrent parent Swarna sub-1carring the resistant QTL/gene and these were backcrossed with Swarna sub-1 to produced BC_2F_1 seeds. The 93 BC_2F_1 generation seed sown, each and every plant was tagged further foreground selection for the sheath blight resistant QTL/gene *qSBR11-1*. On the basis of foreground selection, among 93 BC_2F_1 plants, only 32 plants showed *qSBR11-1 QTL*. The 32 plants showed the QTL/gene for resistance and among them only 18 plants were phenotypically similar to recurrent parent in respect to morphological traits. Therefore, only 18 plants (selected backcross derived lines) subjected for quantitativeand qualitativetraits estimation.

Evaluation for disease resistance of improved Swarna sub-1 lines: Evaluation of Swarna sub-1 and Tetep along with the improved Swarna sub-1 lines for sheath blight resistance confirmed susceptible reaction of Swarna sub-1 tohighly virulent isolate *R. nagar* wild type under field conditions, Tetep showed resistance with a score of 1-3 and the recurrent parent Swarna sub-1 showed susceptible reaction with score of 5-7. Among the 18 improved lines, only four lines (Swarna sub-1-6, Swarna sub-1-32, Swarna sub-1-13 and Swarna sub-1-29) showed resistance score of 1-3. The remaining fourteen lines showed moderate resistance with a score of 3-5. Overall, two improved Swarna sub-1 lines (Swarna sub-1-6 and Swarna sub-1-32) advanced for further generation.

Agronomic performance of selected backcross derived lines for quantitative traits: The agronomic performance for 9 quantitative traits of parents and selected 18BC_2F_1 backcross derived lines showed that most of the agronomic traits were similar to the recurrent parent (Table 1). The number of reproductive tillers of the selected 18BC_2F_1 linesranged from 9.0 to 15.0 with an average of 12 similar to that of Swarna sub-1. The average plant height was 98.33 cm and with a range of 91.33–105.33.1 cm similar to that of Swarna sub-1 (98.00 cm). The days to heading ranged from 94.0 to 98.0 days with an average of 96 days which was lesser than Swarna sub-1 (102 days). Similarly, the days to maturity ranged from 130.0 to 138.0 days with an average of 134 days lesser than recurrent parent (140 days). However, the maximum panicle weight of 4.38 was observed in Swarna sub-1-62 which was higher than Swarna sub-1(4.10). Similarly, number of spikelets per panicle (S/P) and test weight (TW) of some of the backcross derived lines (232 S/Pand 230S/P in Swarna sub-1-81and Swarna sub-1-18) and TW of 19.25 g and 19.24 g in Swarna sub-1-18 and Swarna sub-1-72) were better that of Swarna sub-1 (S/P224 and TW 19.13 g). The grain yield per plant ranged from 39.98 g to 50.17 g with anaverage of 45.8 g which was lower than Swarna sub-1 (49.81 g). However, the maximum grain yield per plant of 50.17 g was observed in Swarna sub-1-86 which was higher than Swarna sub-1(49.81 g). Mean performance of grain yield per plant of parents and 18BC_2F_1 selected back-

Table 1. Mean performance for 9 quantitative traits of parents and 18 BC_2F_1 selected backcross derived lines.

Lines number	NRT	PH	DM	DH	PL	PW	S/P	TW	Y/P
Swarna Sub - 1	13	96.00	140	102	27.00	4.10	223.67	19.13	49.81
Tetep	7	129.67	104	72	25.38	3.71	165.67	21.56	33.85
Swarna Sub-1-6	13	101.33	131	97	27.05	4.20	218.33	18.84	49.78
Swarna Sub-1-13	11	99.33	132	97	26.30	3.83	202.67	18.92	46.09
Swarna Sub-1-18	13	100.33	135	98	25.85	4.13	230.00	19.25	45.33
Swarna Sub-1-23	14	103.67	130	95	22.02	3.96	190.67	18.80	46.49
Swarna Sub-1-29	15	100.00	137	96	26.97	4.19	211.67	19.08	47.08
Swarna Sub-1-32	11	105.33	136	98	23.03	3.74	216.67	19.1	41.05
Swarna Sub-1-39	9	103.00	131	96	26.67	3.93	209.33	18.69	40.62
Swarna Sub-1-41	14	105.33	132	94	25.20	4.22	188.61	19.09	46.17
Swarna Sub-1-51	11	103.00	138	97	24.04	3.87	216.67	19.16	42.78
Swarna Sub-1-58	10	102.00	136	97	24.29	3.88	216.00	19.02	43.39
Swarna Sub-1-62	11	104.67	132	94	22.26	4.38	206.00	18.97	44.60
Swarna Sub-1-69	12	103.33	130	95	25.42	3.75	207.33	19.10	44.51
Swarna Sub-1-72	13	104.67	132	95	25.63	3.77	205.33	19.24	44.34
Swarna Sub-1-81	10	103.33	134	96	26.45	4.16	231.67	18.91	42.49
Swarna Sub-1-86	15	101.67	134	97	24.84	3.77	226.00	19.12	50.17
Swarna Sub-1-91	13	103.67	131	94	26.00	4.23	213.67	18.9	41.53
Swarna Sub-1-94	9	102.33	135	97	26.14	3.85	209.33	18.9	42.79
Swarna Sub-1-97	12	104.67	136	98	25.82	3.80	190.33	19.16	45.74
SE	1.85	3.09	0.99	0.75	1.86	0.28	17.56	0.1	4.24
SD	1.97	6.49	7.32	5.71	1.50	0.21	15.87	0.59	3.75

NRT- Number of reproductive tillers per plant, PH-Plant height, DM-Days to maturity, DH-Days to heading, PL-Panicle length, PW-Panicle weight, S/P-Total no of spikelet per panicle, TW-Test weight, Y/P-Yield per plant

cross derived lines are presented in Fig 1. The spikelet's per panicle, test weight and grain yield were increased up to 3.45 %, 0.62 % and 0.72 %, in Swarna sub-1-81, Swarna sub-1-18 and Swarna sub-1-86 respectively over recurrent parent Swarna sub-1.

Agronomic performance of selected backcross derived lines for qualitative traits (grain quality), disease severity and AUDPC: The grain quality of selected backcross derived lines (Table 2) showed that all of them were almost similar in grain and cooking quality with that of the recurrent parent. The length/breadth ratio before cooking (L/B Ratio BC) ranged from 2.66 mm to 2.74 mm as against 2.66 mm of Swarna sub-1, while the length/breadth ratio after cooking (L/B Ratio AC) of the backcross derived lines ranged from 2.81 mm to 2.90 mm for which Swarna sub-1 recorded an average of 2.87 mm. The average amylase content (AC) was 29.91 % and with a range of 28.70–31.11 % slightly higher to that of Swarna sub-1 (27.69 %). All of the selected backcross derived lines was found to have an ASV score of 2 and gel consistency (GC) ranged from 125.67 mm to 129.68 mm with an average of 127.68 mm similar to that of Swarna sub-1. The per cent disease severity ranged from 26.75 to 43.58 % with an average of 35.17 % which was significantly lower than the recurrent parent (59.43 %) at 16 days after inoculation. The areas under disease progress curve (AUDPC) of parents, susceptible check (PB-1) and $18BC_2F_1$ selected backcross derived lines are presented in Fig 2. The AUDPC ranged from 179.22 to 292.24 with an average of 235.73 which was significantly lower than the recurrent parent (395.87).

Marker assisted selection (MAS) has been proved to be an efficient selection tool for those traits that are difficult and expensive to evaluate in a shorter time period (Tanksley *et al.*, 1989) such as rice blast and sheath blight resistance owing to its advantages of ease and precision in selection of target traits even before their expression. MAS coupled with stringent phenotypic selection can provide rich dividends in breeding success (Collard and Mackill, 2008). Marker assisted backcross breeding (MABB) is widely demonstrated to be the most effective way of transferring specific gene(s)/QTLs into rice varieties/parental lines of hybrids such as resistance to sheath blight (Singh *et al.*,2012b). Introgression of major resistant QTL/genes in a single variety and the use of varietal mixtures with different resistance QTL/genes are suggested as way to prevent or delay the breakdown of resistance.

The sheath blight disease is managed agronomically by the use of chemical agents (Jia *et al.,* 2009). Development of sheath blight resistance cultivars remained elusive by conventional approaches because, identification of complete sheath blight resistance was difficult due to conditioning of the resistance by quantitative genes (Zuo *et al.,* 2008). Recently, eight QTLs for sheath blight resistance were mapped on six chromosomes such as 1, 3, 7, 8, 9 and 11 in the genotype Tetep using QTL mapping strategy

Table 2. Mean performance for 4 qualitative traits, disease severity and AUDPC of parents and $18BC_2F_1$ selected backcross derived lines.

Lines number	Kernal L/B Ratio Before cooking (mm)	Kernal L/B Ratio after cooking (mm)	AC (%)	GC	Disease severity (%)				AUDPC
					4 DAI	8 DAI	12 DAI	16 DAI	
Pusa Basmati-1	-	-	-	-	8.79	28.18	41.50	66.41	429.16
Swarna Sub – 1	2.66	2.87	27.69	129.18	8.89	26.70	38.11	59.43	395.87
Tetep	2.84	3.01	33.53	121.26	4.47	7.24	10.56	17.85	115.83
Swarna Sub-1-6	2.71	2.89	28.92	126.37	6.28	9.49	18.80	26.75	179.22
Swarna Sub-1-13	2.70	2.81	29.33	127.66	5.54	9.81	22.16	29.25	197.48
Swarna Sub-1-18	2.73	2.90	29.06	127.98	4.77	10.49	23.53	32.22	210.05
Swarna Sub-1-23	2.66	2.84	29.03	129.25	6.75	15.39	26.14	32.89	245.43
Swarna Sub-1-29	2.73	2.90	29.88	126.55	5.41	14.59	21.85	29.37	215.32
Swarna Sub-1-32	2.68	2.85	28.81	128.36	5.64	13.65	20.41	27.64	202.81
Swarna Sub-1-39	2.72	2.89	28.77	129.41	4.62	14.74	22.92	31.43	222.73
Swarna Sub-1-41	2.72	2.89	28.73	128.31	5.25	16.27	24.52	37.54	248.73
Swarna Sub-1-51	2.74	2.90	28.70	127.55	5.22	15.50	25.66	33.49	242.07
Swarna Sub-1-58	2.67	2.84	28.72	126.55	5.72	14.56	23.25	32.52	227.70
Swarna Sub-1-62	2.73	2.90	28.78	129.68	5.57	14.69	22.51	30.46	220.85
Swarna Sub-1-69	2.74	2.90	31.11	125.67	5.52	17.12	27.14	43.58	275.25
Swarna Sub-1-72	2.73	2.90	28.83	126.51	5.54	14.53	25.07	34.55	238.60
Swarna Sub-1-81	2.73	2.90	28.73	127.54	4.74	14.96	20.05	30.92	211.35
Swarna Sub-1-86	2.70	2.86	29.05	128.50	4.78	14.48	24.51	36.34	238.19
Swarna Sub-1-91	2.72	2.89	28.80	129.00	5.93	14.73	25.24	32.40	236.55
Swarna Sub-1-94	2.70	2.86	28.74	127.99	4.77	12.65	21.21	32.39	209.75
Swarna Sub-1-97	2.72	2.88	30.22	125.89	6.00	19.88	29.42	41.53	292.24
SE	0.02	0.02	0.18	0.28	0.44	0.73	1.26	2.11	13.06
SD	0.04	0.04	1.22	1.90	1.19	4.93	6.35	10.66	67.28

AC- Amylose content, GC-Gel consistency, AUDPC-Area Under Disease Progress Curve

(Channamallikarjuna *et al.,* 2010). Singh *et al.,* (2015) validated all the eight QTLs using respective tightly linked peak markers and had chosen three QTLs (qSBR11-1, qSBR11-2 and qSBR7-1) for introgression into Pusa 6B line. Among these QTLs, qSBR11-1 was mapped across two locations and seasons and qSBR7-1 was mapped under two locations, while qSBR11-2 was detected in one location (Channamallikarjuna *et al.,* 2010).

While transferring sheath blight resistance QTLs, notably, one line (Pusa 1604-05-3-5) possessing single QTL qSBR11-1 showed comparable disease reaction score as that of the donor parent, Tetep while Pusa1604-05- 45-1 possessing two and Pusa1604-05-43-1 with three ShB resistance QTLs, showed moderate resistance only (Singh *et al.,* 2015). This could be attributed to several reasons such as QTL-marker recombination (Moreau *et al.,* 1998), QTL-background interactions (Wang *et al.,* 2009; Chen *et al.,* 2014), presence of un-known QTLs in the improved lines derived from donor (Tinker and Mather 1995) and/due to the competitive effectiveness of qSBR11-1 than the other two QTLs (Pandian *et al.,* 2012). Further, disease resistance variations among the lines positive for all QTLs, can be attributed to epistatic effects and QTL-background interaction, a well-recognized component of natural genetic variation (Malmberg and Mauricio 2005). In our study, after validation of the donor parent Tetep for single major QTL (qSBR11-1) using tightly linked peakmarker (RM 224), introgression of QTL (qSBR11-1) was carried out into Swarna sub-1 by marker assisted backcross breeding.

The marker assisted backcross breeding QTLs has improved the resistance to sheath blight in all theimproved backcross derived lines while maintaining the quantitative and qualitative traits are almost similar with Swarna sub-1. This observation was similar to the resistance reaction reported against blast and sheath blightamong the MAS improved lines of Pusa Basmati 1 (Singh *et al.,* 2012a) and to the resistance reaction reported against blast and sheath blight among MAS improved lines of Pusa 6B (Singh *et al.,* 2015).

More resistance QTLs/genes are needed to be identified and introgressing them together into the elite cultivars ensures lifelong sheath blight resistance. Introgression of two or more resistance QTLs/genes should lead to more durable resistance in rice. In this study, introgressing QTL/genes conferring board spectrum and horizontal resistance through MAS were successfully conducted. Hence from the above finding it was concluded that the sheath blight resistance QTL/genes *qSBR11-1* was successfully transferred into background of Swarna sub-1. This result is similar to previous reports on the successful utilization of MAB to transfer sheath blight resistance QTL/genes into Pusa 6B CMS line (Singh *et al.,* 2015) and Improved Pusa Basmati-1 (Singh *et al.,* 2012a). The introgressed lines

obtained in our study can be used as genetic resources for sheath blight resistance in breeding programs that will be paving way for an environmental friendly means to achieve a better disease management. Moreover, the success will facilitate future efforts to transfer combinations of sheath blight resistance QTL/genes into other preferred rice cultivars.

Conclusion

The PCR amplification with RM224 marker for *qSBR11-1* (on chromosome number 11) QTL showed polymorphism between Swarna sub-1 and Tetep, the varieties used as parent in the present study. Among 126BC$_1$F$_1$plants, only 27 plants showed the QTL/gene for resistance and remaining plants showed susceptible for disease due to absence of QTL/genes *qSBR11-1*. The number of spikelet's per panicle, test weight and grain yield were increased up to 3.45 %, 0.62 % and 0.72 %, respectively over recurrent parent Swarna sub-1. The disease severity in selected BC$_2$F$_1$ plants varied from 26.75 to 43.58 at 16 days after inoculation and areas under disease progress curve from 179.22 to 292.24. These broader spectrum and higher levels of resistance in the introgressed line might be due to interaction and / or complementation between the resistance genes. The introgressed lines obtained in our study can be used as genetic resources for sheath blight resistance in breeding programs that will be paving way for an environmental friendly means to achieve a better disease management. Moreover, the success will facilitate future efforts to transfer combinations of sheath blight resistance QTLs/genes into other preferred rice cultivars.

REFERENCES

Basavaraj, S. H., Singh, V. K., Singh, A, Singh, A, Singh, A, Yadav, S, Ellur, R. K., Singh, D., Gopalakrishnan, S., Nagarajan, M,, Mohapatra, T., Prabhu, K. V., and Singh, A. K. (2010). Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. *Mol Breed,* 26: 293–305

Bhaktavatsalam, G., Satyanarayana, K., Reddy, A. P. K., and John, V. T. (1978). Evaluation of sheath blight resistance in rice. *International Rice Research Newsletter,* 3: 9–10

Chahal, S. S., Sokhi, S. S., and Ratan, G. S. (2003). Investigation on sheath blight of rice in Punjab. *Indian Phytopathol,* 56: 22–26

Channamallikarjuna, V., Sonah, H., Prasad, M., Rao, G. J. N., Chand, S., Upreti, H. C., Singh, N. K., and Sharma, T. R. (2010). Identification of major quantitative trait loci qSBR11-1 for sheath blight resistance in rice. *Mol Breed,* 25: 155–166

Chen, Z. X., Zhang, Y. F., Feng, F., Feng, M. H., Jiang, W., Ma, Y. Y., Pan, C. H, Hua, H. L., Li, G. S., Pan, X. B., and Zuo, S. M. (2014). Improvement of *japonica* rice resistance to sheath blight by pyramiding *qSB-9TQ* and *qSB-7TQ. Field Crops Research*, 161: 118-127

Collard, B. C. Y., and Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Phil Trans R Soc B,* 363:557–572

Ghritlahre, S. K., Rao, M., Singh, V., Singh, V.K., Loitongbam, B., Yadav, S. K., Zaidi, N. W, Singh, U. S., and Singh, P. K. (2016). Inheritance of sheath blight disease resistance in submergence rice (*Oryza sativa* L.), *International Journal of Agriculture, Environment and Biotechnology,* 9(4): 507-512

Gopalakrishnan, S., Sharma, R. K., Rajkumar, K. A., Joseph, M., Singh, V. P., Singh, A. K., Bhat, K. V., Singh, N. K., and Mohapatra, T. (2008). Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. *Plant Breed.,*127: 131–139

IRRI. (2002). Standard evaluation system for rice (SES). International Rice Research Institute, Los Banos, Philippines, p 56.

Jia, Y., Liu, G., Costanzo, S., Lee, S., and Dai, Y. (2009). Current progress on genetic interactions of rice with rice blast and sheath blight fungi. *Front Agric China,* 3: 231–239

Joshi, M., Singh, P. K., Waza, S. A., Singh, V., Goswami, S., Pallavi, Singh, P. K., Zaidi N. W., and Singh, U. S. (2016). Establishing an association between molecular markers and sheath blight (*Rhizoctonia solani* Kuhn) resistance in rice. *Plant Omics,* 9(4): 281-288

Kumar, A., Dixit, S., and Henry, A. (2013). Marker-assisted introgressionof major QTLs for grain yield under drought in rice. In: Varshney RK, Tuberosa R (eds) Translational genomics for crop breeding, volume II: abiotic stress, yield and quality. Wiley, New York, Pp. 47–64

Kumar, M., Singh, V., Singh, N., and Vikram, P. (2008). Morphological and virulence characterization of *Rhizoctonia solani* causing sheath blight of rice. *Environ Ecol,* 26 (3): 1158-1166

Li, Z. K., Pinson, S. R. M., Marchetti, M. A., Stansel, J. W., and Park, W. D. (1995). Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L.). *Theor Appl Genet,* 91: 374-381

Malmberg, R. L., and Mauricio, R. (2005). QTL-based evidence for the role of epistasis in evolution. *Genet Res* (Camb), 86: 89–95

Moreau, L., Charcosset, A., Hospital, F., and Gallais, A. (1998). Marker assisted selection efficiency in populations of finite size. *Genetics,* 148: 1353–1365

Pandian, R. T. P., Sharma, P., Singh, V. K., Singh, A., Ellur, R. K., Singh, A. K., and Singh, U. D. (2012). Validation of sheath blight resistance derived from Tetep in a Basmati variety and parental lines of rice hybrid. *Indian Phytopathol,* 65: 233–237

Prabhu, K. V., Somers, D. J., Rakow, G., and Gugel, R. K. (1998). Molecular markers linked to white rust resistance in mustard *Brassica juncea. Theor Appl Genet,* 97: 865–870

Savary, S., Castilla, N. P., Elazegui, F. A., McLaren, C. G., Ynalvez, M. A., and Teng, P. S., (1995). Direct and indirect effects of nitrogen supply and disease source structure on rice sheath blight spread. *Phytopathology,* 85: 959–965

Savary, S., Teng, P. S., Willocquet L., and Nutter, F. W. J. (2006). Quantification and modeling of crop losses: a review of purpose. *Ann Rev Phytopathol.,* 44: 89–112

Singh, A., Singh, V. K., Singh, S. P., Ellur, R. K., Singh D., Bhowmick, K., Gopala Krishnan, S., Nagarajan, M., Vinod, K. K., Mohapatra, T., Prabhu, K. V., and Singh, A. K. (2012b). Marker aided improvement of Pusa1460 an elite Basmati rice for resistance to Blast diseases. *AoB Plants pls,* 029. doi:10.1093/aobpla/pls029.

Singh, A. K., Singh, V. K., Singh, A., Ellur, R. K., Pandian, R. T. P., Gopala Krishnan, S., Singh, U.D., Nagarajan, M., Vinod, K. K., and Prabhu, K. V. (2015). Introgression of multiple disease resistance into a maintainer of Basmati rice CMS line by marker assisted backcross breeding, *Euphytica,* 203: 97–107

Singh, V. K., Singh, A., Singh, S. P., Ellur, R. K., Choudhary, V., Sarkhel, S., Singh, D., Gopala Krishnan, S., Nagarajan, M., Vinod, K. K., Singh, U. D., Rathore, R., Prasanthi, S. K., Agrawal, P. K., Bhatt, J. C., Mohapatra, T., Prabhu, K. V., and Singh, A. K. (2012a). Incorporation of blast resistance into ''PRR78'', an elite Basmati rice restorer line, through marker assisted backcross breeding. *Field Crop Res.,* 128: 8–16

Srinivasachary, L., Willocquet, L., and Savary, S., (2011). Resistance to rice sheath blight (*Rhizoctonia solani* Kuhn) [teleomorph: *Thanatephoru scucumeris* (A. B. Frank) Donk.] disease: current status and perspectives. *Euphytica,* 178: 1–22

Tanksley, S. D., Young, N. D., Paterson, A. H. and Bonierbale, M. W. (1989). RFLP mapping in plant breeding: new tools for an old science. *Biotechnology,* 7: 257–264

Tinker, N. A. and Mather, D. E. (1995). Methods for QTL analysis with progeny replicated in multiple environments. *J Agric Genom.,*1: 1–16

Wang, C., Wen, G., Lin, X., Liu, X. and Zhang, D. (2009). Identification and fine mapping of the new bacterial blight gene, *Xa31(t)* in rice, *Eur. J. Plant Pathol.,* 123: 235-240

Wang, Y., Pinson, S. R. M., Fjellstrom, R. G. and Tabien, R. E. (2012). Phenotypic gain from introgression of two QTL, qSB9-2 and qSB12-1 for rice sheath blight resistance. *Molecular Breeding,* 30: 293–303

Xu, Q., Yuan, X. P., Yu, H. Y., Wang, Y. P., Tang, S. X. and Wei, X. H. (2010). Mapping quantitative trait loci for sheath blight resistance in rice using double haploid population. *Plant Breed,* 130: 404-406

Zuo, S. M., Zhang, Y. F., Chen, Z. X., Chen, X J. and Pan, X. B. (2010). Current progress on genetics and breeding in resistance to rice sheath blight. *Sci Sin Vitae,* 40(11): 1014–1023

Zuo, S., Zhang, L., Wang, H., Yin, Y., Zhang, Y., Chen, Z., Ma, Y. and Pan, X. (2008). Prospect of the QTL-qSB-9Tq utilized in molecular breeding program of japonica rice against sheath blight. *J Genet Genom.,* 35: 499–505