Research Paper

Association between QTLs and morphological traits toward sheath blight resistance in rice (*Oryza sativa* L.)

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Sheath blight is considered the most significant disease of rice and causes enormous yield losses over the world. Breeding for resistant varieties is the only viable option to combat the disease efficiently. Seventeen diverged rice genotypes along with 17 QTL-linked SSR markers were evaluated under greenhouse conditions. Pearson's correlation showed only the flag leaf angle had a significant correlation with sheath blight resistance under greenhouse screening. Multivariate analysis based on UPGMA clustering and principal component analysis (PCA) indicated that the flag leaf angle, flag leaf length, and plant compactness were significantly associated with the following SSR marker alleles: RM209 (116,130), RM202 (176), RM224 (126), RM257 (156), RM426 (175), and RM6971 (196), which are linked to the SB QTLs: *QRlh11*, *qSBR11-3*, *qSBR11-1*, *qSBR9-1*, *qShB3-2*, and *qSB-9*. A Mantel test suggested a weak relationship between the observed phenotypes and allelic variation patterns, implying the independent nature of morphological and molecular variations. Teqing and Tetep were found to be the most resistant cultivars. IR65482-4-136-2-2, MR219-4, and MR264 showed improved resistance potentials. These results suggest that the morphological traits and QTLs which have been found to associate with sheath blight resistance are a good choice to enhance resistance through pyramiding either 2 QTLs or QTLs and traits in susceptible rice cultivars.

Key Words: sheath blight, resistance, association, QTLs, morphological traits, SSR markers.

Introduction

Sheath blight (SB) is a fungal disease of rice caused by a necrotrophic soil-borne fungus *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris* (Frank) Donk) which was first identified as a parasite of potato in 1898 by Kuhn (Almasia *et al.* 2008). SB in rice was first reported in 1910 in Japan (Ou 1985). This disease can cause up to 50% yield losses under favorable environmental conditions (Marchetti and Bollich 1991, Rush and Lee 1992). It has been reported that in China 15 to 20 million hectares of rice field affected by SB instigates yield losses of 6 million tons every year (Chen *et al.* 2012). In 2012, SB affected about 491,932 ha of rice in Japan (JPPA 2013). In the USA, crop losses due to rice SB have been recorded as up to 50% in susceptible cul-

tivars (Prasad and Eizenga 2008). A crop loss of up to 40% has been recorded in Bangladesh (Shahjahan *et al.* 1986). These losses could be reduced by application of chemical fungicides, but these are of limited utility, primarily due to problems related to timing and cost of application, weather dependencies, and a potential damaging environmental impact (Mew *et al.* 2004, Tang *et al.* 2002, Xie *et al.* 2008). Due to these circumstances, development and use of resistant genotypes may be a highly effective method to control the disease.

This disease is characterized by infection of plants at the late tillering stage, especially at internode elongation time. Accordingly, the disease develops an oval-shaped lesion appearing on the leaf sheath at the water line, often at the junction of the leaf and sheath at the early stage. Gradually, lesions turn pale green to off-white with a narrow purple-brown to brown border. At the severe stage of the disease, lesions amass and the disease moves upward, leaves are attacked, and irregular spots develop. SB resistance in rice might be due to variations in morphological and ecological characters (Groth and Nowick 1992) such as plant height

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(Li *et al.* 1995a, Pinson *et al.* 2005, Zou *et al.* 2000), days to heading (Pan *et al.* 1999, Pinson *et al.* 2005, Zou *et al.* 2000), tiller angle (Pinson *et al.* 2005, Zou *et al.* 2000, Zuo *et al.* 2014), plant compactness (Pinson *et al.* 2005, Zou *et al.* 2000), width of flag leaf and leaf (Pinson *et al.* 2005, Zou *et al.* 2000), and leaf morphology (Pinson *et al.* 2005, Zou *et al.* 2000). Environmental factors, such as sunlight, humidity, temperature (Rush and Lee 1992), soil fertility (Rush and Lee 1992), and silicon level in the soil (Rodrigues *et al.* 2003), influence plant growth which indirectly affects disease infestation. As such, correlations between morphological traits and SB resistance have been frequently observed in many findings (Channamalikarjuna *et al.* 2010, Groth and Nowick 1992, Li *et al.* 1995a).

Besides morphological traits, more than 60 quantitative trait loci (QTLs) have been found to have links with SB resistance (Zeng *et al.* 2011). Among the identified QTLs, a few are independent of plant morphology, for example, the QTLs *qSB-9* and *qSB9-1*, which have been mapped on chromosome 9, and *qSBR11-1*, which has been mapped on chromosome 11, are linked to the SSR (simple sequence repeat) markers RM205 (Han *et al.* 2002), RM257, and RM224 (Channamalikarjuna *et al.* 2010), respectively. Moreover, Taguchi-Shiobara *et al.* (2013) reported that the QTL *qSBR-9* showed a stable resistance reaction to SB under 3 years of field trials of backcross introgression lines (BILs). The QTLs *qSB-9* (Han *et al.* 2002) and *qSB3-2* (Liu *et al.* 2009) were also mapped on chromosome 9 and 3 in Teqing and in Jasmine85, respectively, which are linked to the SSR markers RM205 and RM426, respectively. These findings indicate that there is a potential link between SSR markers and sheath blight resistance QTLs in rice. Complete resistance through introgression of these QTLs alone has not yet been reported. Very recently, the tiller angle gene *TAC1* has been pyramided with *qSB-9^{TQ}* and a sufficient quantity of positive influence on SB resistance has been recorded (Zuo *et al.* 2014). This report indicates that pyramiding of QTLs and morphological traits could indeed bring added value to SB resistance. Moreover, the selected QTLs showed higher additive effects suggesting the possible pyramiding of 2 or more QTLs in a single genotype might be a strategy to improve SB resistance in rice.

Nevertheless, identification of SB resistance becomes challenging because disease pathogenicity is highly influenced by physiological traits, variation of disease pressure with location and years, lack of appropriate method to evaluate the disease precisely, variations in the rating system among scientists, variations in experimental conditions in various studies, lack of resistant adapted germplasm, the limited efficiency and effectiveness of available screening methods, and the polygenic nature of the resistance phenotype (Gonzalez *et al.* 2006). Hence, the multitude of factors influencing SB epidemics makes the evaluation of the disease incidence difficult. This is why developing SB resistant genotypes through conventional breeding has reached only limited success (Jia *et al.* 2007). Previous studies on SB

resistance indicated that there are no rice varieties or lines with resistance to this disease in rice landraces (Jia *et al.* 2007, Liu *et al.* 2009, Zeng *et al.* 2011). Hence, detailed study on the association of QTLs with morphological traits which are controlling SB resistance necessitates this study to find out the key responsible elements for quantitative SB resistance for pyramiding them in a single genotype to further improve resistance.

Therefore, the present study was undertaken to: i) identify and validate the reported QTL effects on local *R. solani* field isolates and discover their association with morphological traits, ii) identify desirable morphological traits and SSR marker alleles which contribute to SB resistance, iii) discover new SB resistance sources against local *R. solani* field isolates in local genotypes.

Materials and Methods

Plant materials

A total of 17 rice genotypes (Table 1) were selected from diverse geographic origin based on their importance regarding SB resistance potentialities, high-yielding attributes, and sources of resistance elements against other major diseases. Six of the 17 genotypes, Teqing, Tetep, Jasmine85, Pecos, Azucena, and Taducan, were selected based on previous reports for their moderate resistance capabilities to SB. Teqing was reported as a major source of SB resistance having QTLs such as *qSB-9* and *qSB-9-2* (Pan *et al.* 1999, Pinson *et al.* 2005) as well as *QRlh6*, *QRlh7a*, *QRlh7b*, and *QRlh11* (Xie *et al.* 2008). Tetep was found to have substantial resistance potential with SB QTLs like *qSBR7-1*, *qSBR9-1*, *qSBR11-1*, *qSBR11-2*, and *qSBR11-3* (Channamalikarjuna *et al.* 2010). Moreover, the QTL *qSBR11-1* was also fine mapped to the 0.85 Mp region on chromosome 11 (Channamalikarjuna *et al.* 2010). Sato *et al.* (2004) reported that the rice line

Table 1. Basic information on the 17 rice genotypes used in this study

SL No.	Genotype	Source	Origin
1	Teqing	IRRI	China
2	Jasmine85	IRRI	USA
3	Tetep	IRRI	Vietnam
4	Azucena	IRRI	Philippines
5	Taducan	IRRI	unknown
6	MR264	MARDI	Malaysia
7	UKMRC2	UKM	Malaysia
8	UKMRC3	UKM	Malaysia
9	UKMRC9	UKM	Malaysia
10	UKMRC11	UKM	Malaysia
11	MR219	MARDI	Malaysia
12	MR219-4	MNA	Malaysia
13	MR219-9	MNA	Malaysia
14	IR65482-4-136-2-2	IRRI	Philippines
15	BR11	BIRRI	Bangladesh
16	BRRIdhan28	BIRRI	Bangladesh
17	BRRIdhan29	BIRRI	Bangladesh

IRRI = International Rice Research Institute, MARDI = Malaysian Agricultural Research and Development Institute, UKM = Universiti Kebangsaan Malaysia, MNA = Malaysian Nuclear Agency, BIRRI = Bangladesh Rice Research Institute.

WSS2, derived from Tetep, displays a highly partial resistance to sheath blight. On the other hand, the QTLs *qShB3-2*, *qShB-9-1*, and *qShB-9-2* conferring resistance to SB were also mapped on chromosome 9 of Jasmine85 (Liu *et al.* 2009). Resistance QTLs to SB in Pecos (Bollich *et al.* 1985, Sharma *et al.* 2009) were also reported. However, the three cultivars Tetep, Jasmine85 were used in our experiment as standard QTL donors by which we can make comparisons with our unknown genotypes and predict the resistance potentialities based on the presence or absence of these resistance QTLs with validation under greenhouse screening. On the other hand, MR219 is a high-yielding mega variety of Malaysia, whereas BRRIdhan28, BRRIdhan29, and MR11 are high-yielding mega varieties of Bangladesh. UKMRC2, 9, and 11 are newly developed high-yielding varieties in Malaysia. The remaining materials are also promising with high-yield potentials. Hence, the main objective of selecting these 17 materials was to find out resistant donors and susceptible recipients against local *R. solani* field isolates from previously reported sources and local unknown popular varieties, respectively.

Plant growth in greenhouse

Germination of seeds

Seeds of 17 rice genotypes were germinated on moist filter paper in petri dishes. Fifty seeds of each entry were treated with Bavistin DF (Carbendazim 0.5% w/w, BASF, Germany) for 24 h to control seed-borne pathogens. After treatment, seeds were thoroughly cleaned with distilled water several times and put on moist filter paper in the petri dishes. These petri dishes with about 0.5 cm water were placed on a lab bench at 30°C, and incubated at 12 h dark and 12 h light conditions for 7 days. Every day, seed germination and seedling growth were monitored. A necessary amount of water was added to the petri dishes for normal growth of seedlings.

Transplanting of seedlings

A ratio of 4:1 of top soil and cow-dung compost were filled into an autoclave bag. Soil was autoclaved at 121°C and 1.05 kgcm⁻² for 15 min. A total of 51 plastic pots (15-cm-diameter) were filled with steam-sterilized soil, which ensured the absence of soil-borne *R. solani* inocula. To decompose soil, pots were soaked with water for 7 days. Fifty

mg of Furadan 3G (Carbofuran 3% w/w) insecticide was used per pot during pot preparation to control soil-borne nematodes. Five 7-day-old seedlings of each entry were transplanted into a prepared pot 10 cm apart.

Application of fertilizers

Urea (N), triple super phosphate (P₂O₅), and potassium chloride (K₂O) were applied at 850 mg, 650 mg, and 575 mg per pot, respectively. Fertilizers were applied with 4 equal portions during pot preparation, 21 days and 42 days after planting, and 1 day before inoculation. Plants were grown on the greenhouse bench (Fig. 1A) until inoculation.

Artificial plant inoculation

Preparation of fungal inoculums

Collected diseased plant tissue of rice stems were dipped in 70% ethanol and cut into 1.5- to 2.0-cm pieces. The pieces were then surface-sterilized with 10% Clorox solution for 30 s. The tissues were cultured on rose bengal agar (RBA) to facilitate fungal growth while inhibiting bacterial contaminants. After 1 week of incubation in the dark at 28°C, the petri dishes were examined for *R. solani* growth, which was then confirmed by observation under an Olympus TH4-200 compound microscope (Olympus, Australia). Verified isolates of *R. solani* were sub-cultured by transferring a 5- to 7-mm RBA block onto PDA containing tetracycline (0.005%, w/v). Culture media were incubated in the dark for 3 days at 28°C. Single tips of mycelia were then transferred onto fresh PDA and maintained at 27 to 30°C (Jia *et al.* 2007).

Pathogen inoculation

Three tillers of each plant, i.e. 9 tillers in each pot per replication were inoculated, at 60 days old. Five- to 7-mm PDA blocks were placed at the base of each stem and covered with aluminum foil (Fig. 1B). A single pot with 3 plants of each genotype was used as a control. Pots were arranged following the completely randomized design (CRD), with 3 replications. The aluminum foil was removed after 3–5 days of inoculation when disease lesions appeared. The inoculated plants were kept for 2 weeks inside the walk-in humidity chamber (Fig. 1C), and returned to the greenhouse bench for 1 week to maintain the normal growth conditions of the plants. Pots were again placed in the walk-in humidity chamber for another week to provide favorable conditions

Fig. 1. (A) Plant growth on greenhouse bench (B) Plant inoculation with *R. Solani* (C) Incubation of inoculated rice genotypes in mist-chamber.

for infection. The average day-night temperatures and relative humidity in the screening chambers were maintained from 28 to 34°C and >80%, respectively. The average temperature and humidity in the greenhouse was 35°C and 85%, respectively, during experimental periods.

Disease evaluation

Plants were evaluated after 4 weeks of inoculation. Relative lesion height (RLH) based disease scores were used to assess SB severity. RLL was calculated using the following formula: (lesion length/plant height)*100 (Sharma *et al.* 1990). RLH was then converted into the SB disease index based on a 0 to 9 scale, where 0 = no lesion and 9 = lesion visible up to the tip of the longest leaf (Prasad and Eizenga 2008). Visual ratings (VRT) were also assigned to the infected plants based on the actual visible damage of the plant tissues. VRTs were also scored according to the SB disease index on a 0 to 9 scale, where 0 = no lesion, 9 = complete damage of the plant, and <4 was considered moderately resistant (Jia *et al.* 2007, Prasad and Eizenga 2008).

Data recording on morphological traits

Data from the following parameters were collected according to the guidelines described in standard evaluation systems for rice (IRRI 2002).

Plant height (PHT)

The average height of 5 plants from the ground level to the tip of the main panicle was measured in centimeters (cm) at maturity.

Days to heading (DTH)

The number of days required for the plants to show panicle emergence or blooming from the date of sowing.

Flag leaf length (FLL)

Flag leaf length was measured from the collar joint to the tip of a flag leaf in centimeters using a ruler.

Flag leaf width (FLW)

Flag leaf width was measured from the middle across the width of a flag leaf in centimeters using a ruler.

Flag leaf angle (FLA)

Flag leaf angles were recorded from the angle produced between the stem and flag leaf using a protractor.

Second leaf length (SLL)

Second leaf length was measured from the collar joint to the tip of a flag leaf in centimeters using a ruler.

Second leaf width (SLW)

Second leaf width was measured from the middle across the width of a flag leaf in centimeters using a ruler.

Second leaf angle (SLA)

Second leaf angles were recorded from the angle produced between the stem and flag leaf using a protractor.

Tiller number per hill (TNH)

The number of tillers was counted from the primary and secondary culms of a hill.

Plant compactness (PCP)

Plant compactness was calculated according to the following formula: PCP = (total number of tillers per plant)/(sum of the total angles among the tillers of a hill).

SSR marker genotyping

A total of 17 SSR markers were selected from the previously reported 60 QTLs conferring resistance to SB. Points were considered in selection of markers especially on consistencies of effectiveness of QTLs which were frequently authenticated and validated, LOD scores (>3.0), percent phenotypic variances, and successful uses in previous breeding programs.

Isolation of total DNA

Leaf samples were collected from 40-day-old rice plants. Fifteen- to 20-cm-long second leaves were cut from 5 plants and put into a plastic bag. An ice box was used to collect leaf samples. Leaf samples were stored at -20°C for immediate further use. DNA was extracted following the step-by-step procedures using Qiagen DNeasy mini kit (Qiagen, Germany) as described in the manufacturer's guidelines. Two µL of DNA was pipetted on the pedestal stand of the Nanodrop-ND1000 spectrophotometer system (Thermo Scientific, USA). Absorption at 260/280 and 260/230 nm wavelengths was recorded. The purity of the extracted DNA was also checked on 1% agarose gel.

Polymerase chain reaction (PCR)

Each polymerase chain reaction (PCR) was conducted in 10 µL reaction volume containing 1.0 µL 10x reaction buffer (10 mM) (MgCl₂ included), 0.2 µL dNTPs (200 µM), 0.2 µL Taq polymerase (1 U/L), 1 µL of each primer (5 pM), 1 µL of template DNA (10 ng), and 5.6 µL of ddH₂O. The following temperature profiles and cycles were maintained using Eppendorf Nexus Gradient Thermocycler: 1 cycle at 94°C for 4 min, followed by 35 cycles of at 94°C for 45 s, annealing at 55°C or 61°C (depending on primers) for 45 s, 1 cycle at 72°C for 1 min, a final extension at 72°C for 10 min, and storage at 10°C. A list of primers is given in **Table 2**.

Visualization of DNA bands

Fifty mL of 8% polyacrylamide gel was prepared according to the procedures described by Sambrook *et al.* (1989). CBS mini vertical gel electrophoresis system (CBS Scientific, USA) was used to run polyacrylamide gel electrophoresis (PAGE). After completion of the electrophoresis, the gel was removed from the glass plates very carefully to avoid any tears in the gel. DNA was stained by soaking the gel in Sybr-safe DNA gel-stain solution (10 µL/100 mL H₂O) for 15 to 20 min. Subsequently, gels were visualized under UV transilluminator and images were documented using the Alphaimager gel documentation system (Alpha Innotech, USA).

Allele scoring of 17 SSR genotypes

Alleles of 17 SSR markers were genotyped based on 2 scoring systems using the presence or absence of respective alleles. PCR bands correspond to the size of the base-pair (bp). Bands' sizes were estimated using AlphaEase software (Alpha Innotech, USA). The band sizes were categorized into different groups with 5 bp intervals. The bands which differed at least 5 bp from each other were taken into separate

Table 2. Detailed information on alleles detected in rice genotypes for 17 SSR markers and their linked SB QTLs

SSR marker	Alleles detected (bp)	Number of genotypes for the allele detected	Rice genotypes for the allele detected	QTLs associated with SB resistance	Mapping population	Reference
RM209	110 116 125 130	8 3 5 1	MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, IR65582-4-136-2-2, BR11 Teqing, Jasmine85, Azucena Taducan, MR219-4, MR219-9, BRRIdhan28, BRRIdhan29 Tetep	<i>QRh11</i> , <i>qSBR11-2</i>	Teqing/Lemont, Tetep/HP2218	Liu <i>et al.</i> 2009 Channamalikirjuna <i>et al.</i> 2010
RM245	110 116 125	1 6 10	Tetep Azucena, MR219-4, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29 Teqing, Jasmine85, Taducan, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, MR219-9	<i>qShB9-2</i> <i>qSB9-2</i>	Jasmine85/Lemont Teqing/Lemont	Liu <i>et al.</i> 2009 Wang <i>et al.</i> 2011
RM257	136 141 156	4 12 1	Tetep, Taducan, MR219-4, MR219-9 Teqing, Jasmine85, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29 Azucena	<i>qSBR9-1</i>	Tetep/HP2218	Channamalikirjuna <i>et al.</i> 2010
RM434	144 151	14 3	Jasmine85, Tetep, Taducan, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29 Teqing, UKMRC2, UKMRC3	<i>qShB9-1</i>	Jasmine85/Lemont	Liu <i>et al.</i> 2009
RM439	246 282	16 1	Teqing, Jasmine85, Tetep, Taducan, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, MR219-4, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29 Azucena	<i>QRh6</i>	Teqing/Lemont	Xie <i>et al.</i> 2008
RM3691	112 124 130	1 2 14	BRRIdhan28 Azucena, UKMRC3 Teqing, Jasmine85, Tetep, Taducan, MR264, UKMRC2, UKMRC9, UKMRC11, MR219, MR219-4, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan29	<i>qSBR7-1</i>	Tetep/HP2218	Channamalikirjuna <i>et al.</i> 2010
RM1339	122 134 148	2 14 1	Tetep, Taducan Teqing, Jasmine85, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, MR219-4, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29 Azucena	–	Pecos/Lemont	Sharma <i>et al.</i> 2009
RM478	234 253	8 9	Teqing, Tetep, Azucena, Taducan, MR219-4, MR219-9, UKMRC9, IR65582-4-136-2-2 MR264, UKMRC2, UKMRC3, UKMRC11, MR219, BR11, BRRIdhan28, BRRIdhan29	<i>QRh7b</i>	Teqing/Lemont	Xie <i>et al.</i> 2008
RM205	102 114	8 9	MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, MR219-4, MR219-9 Teqing, Jasmine85, Tetep, Taducan, MR219-4, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29	<i>qSB9</i>	Teqing/Lemont	Xie <i>et al.</i> 2008

Table 2. (continued)

SSR marker	Alleles detected (bp)	Number of genotypes for the allele detected	Rice genotypes for the allele detected	QTLs associated with SB resistance	Mapping population	Reference
RM3685	106	10	Teqing, Jasmine85, Azucena, Taducan, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219	–	Pecos/Lemont	Sharma <i>et al.</i> 2009
	112	7	Tetep, MR219-4, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29			
RM6971	176	16	Teqing, Jasmine85, Tetep, Taducan, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, MR219-4, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29	<i>qSB-9</i>	Teqing/Lemont	Han <i>et al.</i> 2002
	196	1	Azucena			
RM182	392	13	Jasmine85, Tetep, Azucena MR264, UKMRC2, UKMRC3, UKMRC9, MR219-4, MR219-9, BR11, BRRIdhan28, BRRIdhan29	<i>QRlh7a</i>	Teqing/Lemont	Xie <i>et al.</i> 2008
	412	1	Taducan			
	436	3	Teqing, UKMRC11, MR219			
RM201	182	1	Azucena	<i>qSB-9</i>	Teqing/Lemont	Han <i>et al.</i> 2002
	196	16	Teqing, Jasmine85, Tetep, Taducan, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, MR219-4, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29			
RM202	176	2	Tetep, Taducan	<i>QRlh11</i>	Teqing/Lemont	Xie <i>et al.</i> 2008
	204	7	Teqing, Azucena, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11	<i>qSBRR11-3</i>	Tetep/HP2218	Channamalikarjuna <i>et al.</i> 2010
	216	1	Jasmine85			
	220	7	MR219, MR219-4, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29			
RM224	126	2	Tetep, Taducan	<i>qSBRR11-1</i>	Tetep/HP2218	Channamalikarjuna <i>et al.</i> 2010
	142	6	MR219-4, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29			
	150	9	Teqing, Jasmine85, Azucena, Taducan, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219			
RM215	156	1	MR219-4	<i>qShB9-2</i>	Jasmine85/Lemont	Liu <i>et al.</i> 2009
	168	14	Teqing, Azucena, Taducan, MR264, UKMRC2, UKMRC3, UKMRC9, UKMRC11, MR219, MR219-9, IR65582-4-136-2-2, BR11, BRRIdhan28, BRRIdhan29	<i>qSB9-2</i>	Teqing/Lemont	Wang <i>et al.</i> 2011
	174	2	Jasmine85, Tetep			
RM426	156	1	Azucena	<i>qShB3-2</i>	Jasmine85/Lemont	Liu <i>et al.</i> 2009
	175	4	Taducan, MR264, BRRIdhan28, BRRIdhan29			
	190	12	Teqing, Jasmine85, Tetep, Azucena, UKMRC3, UKMRC9, UKMRC11, MR219, MR219-4, MR219-9, IR65582-4-136-2-2, BR11			
	200	1	UKMRC2			

groups. Then the bands in the different groups were scored as 'A/A', 'B/B', 'C/C' or 'D/D' across the whole entries. Missing bands were scored as '0/0'. The markers' alleles were also scored as '1' or '0' based on the presence or absence of the respective allele, and missing bands were scored as '9'.

Statistical analysis

Disease evaluation data recorded from 9 individual plants (tillers) were averaged as a single replication from mist-chamber screening. Morphological data were measured from 5 individual plants for each replication. RLL and VRT scores were recorded from 9 individuals and averaged into a single replication. Data of each of 3 replications were subjected to statistical analysis. Analysis of variance (ANOVA) and least significance differences (LSD) were calculated using the ANOVA procedure using SAS version 9.1 for Windows (SAS, Cary, NC). Simple Pearson's correlation coefficients were estimated between morphological traits and sheath blight disease scores. Standardized data of morphological traits were used to calculate the Euclidean distance coefficient (**Supplemental Table 1**) using SIMINT program (NTSYS pc version 2.21q, Rohlf 2008). For SSR markers, each genotype was scored manually as 'A/A', 'B/B', 'C/C', 'D/D', or '0/0' for missing data. Genetic distance (**Supplemental Table 2**) was estimated based on Nei and Chesser (1983) using PowerMarker v4.3 (Liu 2003). SIMQUAL program of NTSYS-pc version 2.21q was used to calculate allelic distances among the genotypes. Cluster analysis was performed separately for morphological and molecular data. UPGMA clustering was performed based on Euclidean distance and the Sequential Agglomerative Hierarchical and Nested (SAHN) method (Sneath and Sokal 1973). Principal component analysis (PCA) and scattered plot diagrams were also performed for both morphological and molecular data using Multivariate Statistical Software Package (MVSP) version 3.1 (Kovach 2007). To understand the association between SSR marker alleles and morphological traits, Mantel test (Mantel 1967) was performed using both morphological and molecular distance matrices (**Supplemental Tables 1, 2**) at 250 permutations followed by the determination of a significant level of correlation (MXCOMP program of NTSYS-pc, Rohlf 2000).

Results

Analysis of variance and mean performances

Analysis of variance (mean sum of squares) and related statistics for 11 morphological traits of 17 rice genotypes are presented in **Table 3**. Results indicate that all the genotypes showed highly significant differences from each other ($p < F$, 0.0214 to 0.0001). The mean performance of the morphological traits (**Table 4**) revealed that UKMRC9 was the latest maturing genotype. IR65482-4-136-2-2 had the lowest growth duration, and 'Tetep' possessed the highest plant height followed by 'Azucena'. 'Jasmine 85' was the shortest cultivar. The longest flag leaf was recorded in MR219-4, whereas BR11 had the shortest one. The widest flag leaf was observed in BRRIdhan29 followed by MR219-9, whereas the narrowest was in 'Tetep' and 'Taducan'. 'Teqing' had the most erect flag leaf, but 'Azucena' produced the droopiest flag leaves followed by UKMRC2. MR219-9 had the longest second leaves, whereas the shortest second leaves were found in MR264 next to BR11. The widest second leaves were possessed by MR219-9, but the narrowest by 'Tetep'. 'Teqing', MR264, and UKMRC9 produced the most erect second leaves, whereas the droopiest second leaves were in BR11 next to BRRIdhan28. The most compact tillering patterns were observed in UKMRC11 followed by MR219-4 and BR11. The most highly spreading type plants were recorded from IR65482-4-136-2-2. The lowest relative lesion height (RLH) was observed in 'Tetep' followed by IR65482-4-136-2-2, MR264, MR219-4, Teqing, and UKMRC11. The highest RLH was recorded from UKMRC2. The most resistant cultivars, Teqing (**Fig. 2A**) and Tetep (**Fig. 2B**), had the lowest VRT (3.5). UKMRC2 (**Fig. 2C**) was the most susceptible cultivar among the 17 tested genotypes.

Correlation coefficients among morphological traits

Pearson's correlation coefficients were estimated among all the 11 morphological traits (**Table 5**). Only the trait FLA was found to be significantly correlated with both SB scores, RLH (0.550) and VRT (0.553). Signification correlation (0.651) was also detected between RLH and VRT. However, among the morphological traits, significant correlations were also detected between PHT and FLW (−0.492),

Table 3. Analysis of variance (ANOVA) for 11 morphological traits of 17 rice genotypes

Source of variation	DTH ^a	PHT ^b	FLL ^c	FLW ^d	FLA ^e	SLL ^f	SLW ^g	SLA ^h	PCP ⁱ	RLH ^j	VRT ^k
Genotype	336.00	651.08	151.79	0.282	1355.24	504.69	0.146	515.10	31.15	0.884	2.70
Error	155.6	14.78	8.837	0.0018	8.83	3.41	0.0013	15.39	5.03	0.18	0.65
F	2.16	44.04	17.18	150.27	153.48	147.89	105.9	33.48	6.19	4.89	4.16
p < F	0.020	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
LSD	20.62	6.36	4.91	0.072	4.91	3.05	0.06	6.48	3.71	0.70	1.33
Mean	99.57	112.77	40.34	1.655	41.76	52.52	1.42	46.72	6.92	4.10	5.03
% CV	17.25	7.23	0.41	22.22	12.74	0.25	13.67	2.48	0.68	1.04	10.29

Footnote: ^aDays to heading, ^bPlant height, ^cFlag leaf length, ^dFlag leaf width, ^eFlag leaf angle, ^fSecond leaf angle, ^gSecond leaf width, ^hSecond leaf angle, ⁱPlant Compactness, ^jRelative lesion height, ^kVisual ratings.

Table 4. Mean performances and standard deviations from the mean of 11 morphological traits of 17 rice genotypes

Genotype	DTH ^a	PHT ^b	FLL ^c	FLW ^d	FLA ^e	SLL ^f
Teqing	79.0 ± 1.0 cd	110.6 ± 2.9 de	47.3 ± 0.9 b	1.7 ± 0.0 d	15.3 ± 2.3 h	50.2 ± 3.0 e
Jasmine85	97.7 ± 1.5 abc	96.8 ± 1.1 k	38.5 ± 0.8 efg	1.6 ± 0.0 g	57.3 ± 2.3 c	50.1 ± 0.3 e
Tetep	96.7 ± 2.5 abcd	146.0 ± 1.1 a	42.2 ± 8.3 bcde	1.0 ± 0.0 i	34.7 ± 1.2 e	63.7 ± 2.8 b
Azucena	107.7 ± 1.5 ab	140.6 ± 3.0 b	42.8 ± 1.7 cdef	1.9 ± 0.1 c	85.7 ± 3.8 a	57.7 ± 2.3 cd
Taducan	111.3 ± 1.5 ab	145.8 ± 3.8 a	44.2 ± 2.9 bcd	1.0 ± 0.0 i	38.0 ± 1.7 e	58.4 ± 1.4 cd
MR264	107.3 ± 1.0 ab	99.6 ± 1.0 ijk	40.1 ± 4.1 defg	1.6 ± 0.0 ef	16.7 ± 1.2 h	31.1 ± 1.0 i
UKMRC2	105.0 ± 1.0 ab	107.1 ± 7.9 efg	37.7 ± 1.2 efg	1.5 ± 0.0 h	84.7 ± 1.2 a	42.7 ± 0.7 gf
UKMRC3	96.0 ± 1.0 abcd	105.5 ± 2.0 fgh	37.3 ± 1.3 fg	1.5 ± 0.0 h	28.0 ± 3.1 fg	38.4 ± 0.8 h
UKMRC9	115.0 ± 1.2 a	109.0 ± 0.7 def	35.2 ± 5.9 gh	1.6 ± 0.0 e	27.3 ± 2.3 g	39.8 ± 0.5 gh
UKMRC11	111.0 ± 2.0 ab	117.9 ± 6.4 c	40.4 ± 2.4 def	1.7 ± 0.0 d	33.2 ± 2.0 ef	59.5 ± 2.3 cd
MR219	111.0 ± 1.0 ab	116.9 ± 1.2 c	31.7 ± 1.9 h	1.7 ± 0.0 d	28.7 ± 1.4 fg	43.7 ± 0.9 e
MR219-4	98.7 ± 2.1 abc	113.9 ± 1.3 cd	59.0 ± 0.9 a	1.9 ± 0.0 c	29.3 ± 3.1 fg	73.3 ± 1.6 a
MR219-9	107.7 ± 1.5 ab	104.2 ± 2.9 fghi	46.8 ± 2.4 bc	2.1 ± 0.1 b	16.7 ± 1.2 h	74.3 ± 0.5 a
IR65482-4-136-2-2	74.3 ± 0.6 d	113.7 ± 2.2 cd	40.1 ± 1.7 defg	1.5 ± 0.0 h	35.7 ± 6.8 e	56.9 ± 3.6 d
BR11	91.3 ± 3.1 bcd	97.9 ± 2.2 jk	23.9 ± 1.3 i	1.8 ± 0.0 e	62.0 ± 2.0 c	32.0 ± 1.0 i
BRRIdhan28	97.7 ± 1.5 abc	101.9 ± 1. hij	31.5 ± 1.1 h	1.4 ± 0.0 j	68.0 ± 3.5 b	39.5 ± 1.9 h
BRRIdhan29	102.7 ± 1.5 ab	103.1 ± 1.3 ghi	41.2 ± 0.7 def	2.3 ± 0.1 a	48.0 ± 6.0 d	60.2 ± 0.3 e
Grand Mean	99.6	112.8	40.3	1.7	41.8	52.5
SD (±)	10.6	14.7	7.1	0.3	21.3	13.0

Genotype	SLW ^g	SLA ^h	PCP ⁱ	RLH ^j	VRT ^k
Teqing	1.4 ± 0.0 g	30.0 ± 5.3 h	7.1 ± 3.2 ef	3.97 ± 0.0 cde	3.5 ± 1.0 d
Jasmine85	1.4 ± 0.0 fg	59.3 ± 2.3 b	4.9 ± 2.1 de	4.34 ± 0.6 bcde	4.7 ± 1.0 bcd
Tetep	1.0 ± 0.0 i	55.3 ± 3.1 bc	7.5 ± 0.7 b	3.03 ± 0.0 f	3.5 ± 1.2 d
Azucena	1.8 ± 0.0 a	42.7 ± 6.1 ef	10.2 ± 1.9 cd	4.47 ± 0.6 bc	6.5 ± 0.6 a
Taducan	1.0 ± 0.1 i	55.3 ± 2.3 bc	4.3 ± 1.9 fg	4.39 ± 0.6 bcd	5.2 ± 0.6 abc
MR264	1.5 ± 0.1 e	30.0 ± 2.3 bc	11.5 ± 5.3 c	3.63 ± 0.6 ef	4.7 ± 0.6 bcd
UKMRC2	1.4 ± 0.0 ef	44.7 ± 2.0 h	11.0 ± 2.1 cd	5.60 ± 0.6 a	6.3 ± 1.5 a
UKMRC3	1.3 ± 0.0 g	49.3 ± 1.2 ef	5.8 ± 2.1 efg	3.86 ± 0.6 cde	5.3 ± 1.0 abc
UKMRC9	1.6 ± 0.1 bc	30.0 ± 0.0 de	6.1 ± 0.4 efg	4.20 ± 0.6 cde	5.2 ± 1.0 abc
UKMRC11	1.5 ± 0.0 d	45.3 ± 5.0 h	2.6 ± 0.7 g	3.98 ± 0.6 cde	5.7 ± 1.5 ab
MR219	1.6 ± 0.0 c	53.3 ± 5.0 cd	4.7 ± 0.6 g	4.92 ± 0.6 ab	5.7 ± 0.0 ab
MR219-4	1.5 ± 0.1 e	57.3 ± 2.3 bc	3.2 ± 0.4 g	3.67 ± 1.0 def	4.3 ± 0.6 bcd
MR219-9	1.7 ± 0.0 b	34.7 ± 5.0 gh	4.7 ± 1.3 g	4.01 ± 0.6 ef	4.3 ± 1.2 bcd
IR65482-4-136-2-2	1.6 ± 0.0 bc	36.0 ± 3.5 g	14.0 ± 2.5 a	3.31 ± 1.2 ef	4.3 ± 0.6 bcd
BR11	1.3 ± 0.0 g	71.3 ± 4.9 a	3.2 ± 1.6 fg	4.11 ± 1.0 cde	6.2 ± 0.6 a
BRRIdhan28	1.2 ± 0.0 h	70.0 ± 0.0 a	5.9 ± 1.9 cd	4.26 ± 0.0 bcde	4.3 ± 0.6 cd
BRRIdhan29	1.7 ± 0.0 b	34.7 ± 3.1	8.7 ± 2.6 cd	4.14 ± 0.0 cde	4.2 ± 0.6 cd
Grand Mean	1.4	46.7	6.9	4.10.6	5.0
SD (±)	0.2	13.1	3.2	0.60.6	0.9

Footnote: ^aDays to heading, ^bPlant height, ^cFlag leaf length, ^dFlag leaf width, ^eFlag leaf angle, ^fSecond leaf length, ^gSecond leaf width, ^hSecond leaf angle, ⁱPlant compactness, ^jRelative lesion height, ^kVisual ratings. Numbers followed by the same letter/s are not significantly different at $p < 0.05$ level of probability.

Fig. 2. (A) Sheath blight reaction in resistant cultivar Teqing, and (B) Tetep (C) susceptible genotype UKMRC2.

Table 5. Simple Pearson's correlation coefficient (r) of 11 morphological traits of 17 rice genotypes

Character	DTH ^a	PHT ^b	FLL ^c	FLW ^d	FLA ^e	SLL ^f	SLW ^g	SLA ^h	PCP ⁱ	RLH ^j	VRT ^k
DTH	1.00	0.189	-0.043	0.064	0.058	0.014	0.148	-0.046	-0.314	0.434	0.455
PHT		1.00	0.309	-0.492*	0.064	0.438	-0.299	0.055	0.030	-0.136	0.041
FLL			1.00	0.145	-0.353	0.769**	0.139	-0.345	0.010	-0.306	-0.456
FLW				1.00	-0.004	0.209	0.826**	-0.321	-0.050	0.130	0.087
FLA					1.00	-0.178	-0.011	0.438	0.200	0.550*	0.553*
SLL						1.00	0.170	-0.128	-0.150	-0.285	-0.378
SLW							1.00	-0.573*	0.269	0.163	0.210
SLA								1.00	-0.521	0.107	0.197
PCP									1.00	-0.054	-0.070
RLH										1.00	0.651*
VRT											1.00

Footnote: * and ** indicate a significant difference at $p < 0.1$ and $p < 0.05$ level of probability, respectively. ^aDays to heading, ^bPlant height, ^cFlag leaf length, ^dFlag leaf width, ^eFlag leaf angle, ^fSecond leaf length, ^gSecond leaf width, ^hSecond leaf angle, ⁱPlant compactness, ^jRelative lesion height, ^kVisual ratings.

FLL and SLL (0.769), FLW and SLW (0.862), SLA and SLW (-0.573), and SLA and PCP (-0.521). Days to heading showed a positive correlation with both disease indices.

UPGMA based cluster analysis

Cluster analysis of 17 genotypes using 11 morphological traits revealed 2 major groups, A-I and B-I. The largest cluster, A-I, was composed of 15 genotypes of diverse geographic origin. The cluster further differentiated into 4 sub-clusters, namely A-I-I, A-I-II, A-I-III, and A-I-IV (Fig. 3). The largest sub-group, A-I-III, had a total of 8 genotypes; most of them are susceptible, including BR11 and MR219. Resistant genotypes, such as Tetep and Teqing, were clustered into 2 different sub-groups, B-I and A-I-IV, respectively. UPGMA clustering produced a low cophenetic correlation score (0.717). The mantel test also showed an insignificant and low relationship ($r = 0.253$) between morphological and molecular data sets.

However, UPGMA clustering based on 47 marker alleles

Fig. 3. Dendrogram based on UPGMA clustering using 11 morphological traits data of 17 rice genotypes.

of 17 SSR loci produced 2 broad sub-groups, A-I and B-I (Fig. 4). Only the genotype Azucena was represented into sub-group A-I. The largest sub-group B-I contained the remaining 16 genotypes. The sub-group B-I has further been discriminated into 3 major sub-sub-groups, namely B-I-I, B-I-II, and B-I-III. The dendrogram indicated that the sub-sub-group B-I-I was composed of 8 genotypes with different

Fig. 4. Dendrogram based on UPGMA clustering using 47 alleles of 17 SSR markers for 7 rice genotypes.

levels of SB resistance including the resistant genotypes Teqing and Jasmine85 (sub-branch) and the susceptible genotypes UKMRC2 and MR219. Sub-sub-group B-I-II has been confined with six genotypes of variant environmental origins. Sub-sub-group B-I-III had 2 resistant genotypes, ‘Tetep’ and ‘Taducan’, which were clearly distinguished from the other groups comprising susceptible genotypes.

Principal component analysis (PCA)

Principal component analysis (PCA), performed using 11 morphological traits, is presented in Table 6. The table showed that all 4 of the principal components (PCs) accounted for the maximum variations (78.73%) explained. The first two PCs accounted for 47.28% of the total variations. PCA variable loadings indicated that FLL (0.328) and PCP (0.332) contributed the maximum in the total variation explained by PC1 (24.58%). In the case of PC2, FLW (0.341), SLW (0.455), VRT (0.404) and RLH (0.356) added the highest values and interpreted 22.70% to the total variations. Similarly, FLL and SLL induced 18.38% and FLW contributed 13.07% to the total variations in PC3 and PC4, respectively. PCA scattered plot diagram (Fig. 5) showed a good separation of the genotypes which was high in agreement with the UPGMA clustering. Scattered plot diagram

(Fig. 5) showed significant discrimination of the genotypes into quadrangles. The upper right quadrangle was occupied with 4 genotypes which showed moderate susceptibility. UKMRC2 was located on axis 1, whereas the upper left quadrangles were composed of 3 genotypes, including MR219, susceptible check. BR11 was found on axis 2. The lower left quadrangles contained 4 genotypes UKMRC11, BRRIdhan28, Jasmine85, and Taducan. On the other hand, the lower right quadrangle contained most of the resistant cultivars including ‘Tetep’, ‘Teqing’, IR65482-4-136-2-2, and MR219-4.

Another PCA, performed using 47 alleles at 17 SSR marker loci, constructed the principal components (PCs) with 4 major axes based on Eigenvalues which interpreted about 80% of the total variation explained. The results indicated that PC axis 1 possessed the highest Eigenvalues (13.145), followed by 9.515, 6.724, and 4.488 which accounted for 27.97%, 20.25%, 14.31%, and 9.55%, respectively, of the total genetic variations explained (Supplemental Table 3). However, based on the value of PCA variable loadings from the 4 major axes, the following SSR marker alleles: RM209 (116, 130), RM257 (156), RM202 (176), RM224 (126), RM426 (156, 175), RM6971 (196), RM1339 (122, 148), RM439 (282), RM205 (102), RM245 (125), and RM434 (144) were identified as the major contributors to create the observable discrimination among genotypes into different quadrangles. However, a scattered plot diagram (Fig. 6) showed that only one genotype, Azucena, constituted the upper right quadrangle, whereas the upper left quadrangle was constituted of 8 genotypes, including both resistant (Tetep) and susceptible (BR11) cultivars. The lower left quadrangle contained only 1 resistant genotype, Jasmine85. On the other hand, 3 genotypes, MR264, UKMRC9 and UKMRC11, were located on axis 1. The lower right quadrangle constituted 4 genotypes, including both resistant (Teqing) and susceptible (UKMRC2) cultivars (Fig. 6).

Marker allele analysis of 17 rice cultivars

Together, the 17 SSR markers produced a total of 47 alleles with different sizes across the 17 rice genotypes

Table 6. Principal component analysis of 11 morphological traits of 17 rice genotypes

Factors	Axis-1	Axis-2	Axis-3	Axis-4
Eigenvalue	3.195	2.950	2.389	1.700
Percentage	24.58	22.696	18.378	13.074
Cum. of percentage	24.58	47.276	65.654	78.728
PCA variable loadings				
Days to heading	-0.171	0.238	0.290	-0.275
Plant height	0.014	-0.195	0.037	-0.661
Flag leaf length	0.328	-0.134	0.331	-0.276
Flag leaf width	0.166	0.341	0.292	0.297
Flag leaf angle	-0.264	0.185	-0.278	-0.249
Second leaf length	0.236	-0.173	0.374	-0.303
Second leaf width	0.228	0.455	0.110	0.080
Second leaf angle	-0.449	-0.236	-0.029	0.026
Plant compactness	0.332	0.143	-0.472	-0.094
Visual ratings	-0.313	0.404	-0.160	0.164
Relative lesion height	-0.259	0.356	-0.039	-0.145
PCA case scores				
‘Teqing’	0.514	-0.216	0.029	0.214
Jasmine 85	-0.368	-0.142	-0.019	0.224
‘Tetep’	0.124	-1.082	-0.244	-0.362
‘Azucena’	0.130	0.604	-0.111	-0.633
‘Taducan’	-0.396	-0.421	0.198	-0.643
MR264	-0.266	0.671	-0.486	-0.385
UKMRC2	0.006	0.437	0.099	0.063
UKMRC3	-0.345	0.355	0.169	0.016
UKMRC9	0.468	0.172	-0.310	0.240
UKMRC11	-0.095	-0.032	-0.084	0.211
MR219	-0.303	0.071	0.500	-0.054
MR219-4	0.144	-0.295	0.708	-0.064
MR219-9	0.531	0.060	0.692	0.199
IR65482-4-136-2-2	0.775	-0.177	-0.697	0.057
BR11	-0.866	-0.001	-0.178	0.524
BRRIdhan 28	-0.498	-0.498	-0.353	0.234
BRRIdhan 29	0.443	0.349	0.349	0.156

Fig. 5. Two-dimensional scattered plot based on principal component analysis (PCA) for 11 morphological trait data of 17 rice genotypes.

Fig. 6. Two-dimensional scattered plot diagrams based on principal component analysis (PCA) using 47 alleles of 17 SSR loci for 17 rice genotypes.

investigated in this study (Table 2). Major findings of the allelic variations among the rice genotypes have been described in this section. Marker RM209 produced 4 alleles ranging from 110 to 130 bp in size. Of these 4 alleles, allele 110 was detected in 8 entries. The resistant cultivars Teqing and Jasmine85 produced allele 125, whereas allele 130 was observed in Tetep only for the marker RM209. For the marker RM245, allele 110 was detected in the resistant cultivar Tetep only. Most of the entries produced allele 246 except Azucena (282) for marker 439. For marker RM1339, Tetep and Taducan possessed allele 122 while allele 134 was found in the rest of the cultivars used. In respect of marker RM478, most of the resistant entries, including Teqing and Tetep, possessed allele 234. In the case of marker RM205, allele 114 was observed in most of the cultivars in the resistant group, including Teqing, Jasmine85, Tetep, and Taducan. On the other hand, allele 176 was detected in all the cultivars genotyped for marker RM6971, except Azucena (196). For marker RM201, allele 176 was found only in Azucena, whereas the remaining 16 cultivars harbored allele 196. Four alleles were observed for marker RM202, which ranged from 176 to 220 bp. Resistant cultivars, such as Tetep and Taducan, had allele 176, whereas allele 216 was found in Jasmine85 only. Similarly, allele 126 was detected in Tetep and Taducan only for marker RM224. Marker RM215 produced allele 156 and 174, which were observed in only MR219-4, and in Jasmine85 and Tetep, respectively. Allele 156 and 200 for marker RM426 were only found in Azucena and UKMRC2, respectively.

Discussion

Association between morphological traits and SB resistance

A total of 11 morphological traits of 17 rice genotypes were used to investigate the association between morphological traits and SB resistance. SB resistance in rice may be enhanced due to suitable morphological and ecological parameters (Groth and Nowick 1992) for pathogens, such as reduced solar radiation, high humidity and high temperature

(Rush and Lee 1992), and soil compositions (Rodrigues *et al.* 2003, Rush and Lee 1992), which influence plant growth and indirectly affect disease infestation. Evidence shows that morphological traits, such as plant height, days to heading, tiller angle, plant compactness, flag leaf length and leaf width, impact on SB resistance in rice (Pinson *et al.* 2005, Zou *et al.* 2000). However, from our observations, we found that flag leaf angle significantly correlated with SB resistance. The flag leaf in rice plants contributes greatly to rice grain yield. The influences of the flag leaves are controlled by leaf angle. For example, erect leaf morphology captures more light radiation and produces less shade in the plant canopy, especially at the lower part of the plant, than that of droopy phenotypes. Rice SB pathogens favor hot, humid and shady environments for its pathogenesis. This is why SB is generally more predominant at the lower part of the plant than the upper part. Since erect leaf morphology minimizes the shading effect at the lower part of the plant, this is how rice plants with erect flag leaves evade attacks by sheath blight and show low SB infestation. Similar results were also observed by Loan *et al.* (2004) and Channamallikarjuna *et al.* (2010). Teqing is a proven resistant cultivar with the lowest flag leaf angle (15.3 ± 2.3), and has been distinguished into a separate cluster (Figs. 3, 4). Principal component-1 accounted for the highest proportion (24.58%) of the total morphological variation which was contributed dominantly by flag leaf length (0.328) and plant compactness to create observable discrimination between resistant and susceptible genotypes. For example, MR219-4 with the highest leaf length (59.0) was distinguished into the lower right quadrant (Fig. 5), and showed an improved resistance potential under greenhouse screening (Table 4). Thus, an explainable association between morphological traits and SB resistance was proved by our above findings. Morphological traits associated with SB resistance have been studied frequently. Plant height, days to heading, tiller angle, plant compactness, flag leaf length and flag leaf width have been reported as factors influencing resistance in rice plants (Pinson *et al.* 2005, Zou *et al.* 2000). Plant height, days to heading, and tiller angle were reported as independent to SB resistance (Srinivasachary *et al.* 2011). In our study, positive but insignificant correlations between plant height and SB resistance, and between days to heading and SB resistance were detected. However, Li *et al.* (1995b) mapped 3 SB resistance QTLs, *Qsbr3a*, *Qsbr8a* and *Qsbr9a*, which were at approximately the same locations where the major QTLs, *QPh3a*, *QPh8a* and *QPh9a*, for plant height were mapped (Zou *et al.* 2000). The QTLs, *QHd3a*, *QHd8a*, and *QHd9a*, controlling days to heading corresponded to QTLs for plant height and SB resistance (Zou *et al.* 2000). Flag leaf angle was identified to be significantly correlated with SB resistance in our study. Principal component analysis revealed a good association of flag leaf length with SB resistance in our study. The tiller angle gene *TAC1* has been identified at the region of *qSB-9^{TQ}* associated with SB resistance (Zuo *et al.* 2014).

Marker alleles associated with sheath blight resistance

A total of 47 alleles were detected from 17 SSR markers across the 17 rice genotypes. It has been reported that all the markers were found to have linkage with 19 different putative SB resistance QTLs located on 7 different chromosomes of rice (Table 2). However, our findings indicate that allele 116 of marker RM209 was detected in 3 resistant cultivars, Teqing, Jasmine85, and Azucena (Table 2). Marker RM209 has been reported to be associated with the SB resistance QTL *QRlh11*, and mapped on chromosome 11 using the Teqing/Lemont population (Xie *et al.* 2008). The marker also yielded the unique allele 130 which was observed in another resistant cultivar, Tetep. Four resistant cultivars, Tetep, Taducan, MR219-4, and MR219-9, produced allele 136 of marker RM257 which corroborated the finding of Channamallikarjuna *et al.* (2010), a putative QTL *qSBR9-1* identified and mapped on chromosome 9 using the Tetep/HP2219 mapping population. Marker RM478 produced the potential allele 234 which was identified in 8 cultivars. All these cultivars, including Teqing and Tetep, showed resistant reactions under greenhouse screening. Liu *et al.* (2009) showed that marker RM478 had significant linkage with QTL *QRlh7b* and was mapped on chromosome 7 using the Teqing/Lemont population. In our study, allele 122 of RM1339 was detected in only 2 resistant cultivars, Tetep and Taducan. Sharma *et al.* (2009) reported that marker RM1339, linked to SB resistance QTLs, was mapped on chromosome 1 using the Pecos/Rosemont population. On the other hand, alleles 176 and 126 of markers RM202 and RM224, respectively, were detected in Tetep and Taducan in our study. RM202 has been reported as linked to QTL *qSBR11-3* (Channamallikarjuna *et al.* 2010) and *QRlh11* (Xie *et al.* 2008). Similarly, RM224 was also reported by Channamallikarjuna *et al.* (2010) as a linked marker to the QTL *qSBR11-1* which was fine mapped on chromosome 11 in the 0.85 Mb region using the Tetep/HP2219 mapping population. On the other hand, Han *et al.* (2002) mapped the QTL *qSB-9* on chromosome 9 using the Teqing/Lemont population, and reported that marker RM6971 was linked to the QTL. The alleles discussed above were detected mainly in resistant cultivars and not observed in susceptible high-yielding cultivars. These reports clearly indicate that the described alleles are associated with potential resistance effects in the respective cultivars serving as valuable genetic resources to improve SB resistance in high-yielding susceptible genotypes by marker-assisted selection breeding.

Association between morphological traits and SSR marker alleles

Principal component analysis for the 17 morphological traits and 47 alleles of the 17 SSR markers revealed measurable discrimination among the genotypes used in this study. The PCA variable loadings for morphological traits indicated that FLL and PCP contributed the maximum value in the total variation explained by PC1. The longest flag leaf was recorded in MR219-4 which was resistant in our greenhouse

screening. On the other hand, morphological traits FLW, SLW, VRT and RLH added the highest values to the total variations explained by PC2. The widest second leaf was possessed by MR219-9 and the narrowest by 'Tetep', 'Teqing', MR264 and UKMRC9. The most resistant cultivars, Teqing (Fig. 2A) and Tetep (Fig. 2B), had the lowest VRT (3.5). The lowest relative lesion height (RLH) was observed in 'Tetep' followed by IR65482-4-136-2-2, MR264, MR219-4, Teqing, and UKMRC11, whereas, the highest RLH was recorded in UKMRC2 (Fig. 2C) the most susceptible cultivar among the 17 tested genotypes. FLL and FLW, and SLL provided considerable contributions to the total variations in PC3 and PC4, respectively. The 47 alleles of the 17 SSR markers indicated a noteworthy partition among the genotypes with the 4 major axes. The following SSR marker alleles: RM209 (116, 130), RM257 (156), RM202 (176), RM224 (126), RM426 (156, 175), RM6971 (196), RM1339 (122, 148), RM439 (282), RM205 (102), RM245 (125), and RM434 (144) were identified as the major contributors to create the observable discrimination among genotypes into the different quadrangles. It can be concluded that the SSR markers that were reported in resistant cultivars were also reported with the same alleles in some improved resistant cultivars from our investigation, implying that the associated morphological traits and SSR markers might generate the potential variations among cultivars.

Association between QTLs and sheath blight resistance

In our study, we analyzed 17 SSR loci linked to 19 QTLs conferring resistance to SB located on different chromosomes (Table 2). More than 60 QTLs (Zeng *et al.* 2011) were mapped on different genetic backgrounds. To understand the association between QTLs and SB resistance in rice, UPGMA-based cluster analysis was performed using 47 alleles at 17 SSR marker loci. The dendrogram showed a clear-cut separation between resistant genotypes (Tetep and Taducan) and susceptible genotypes (UKMRC2 and MR219). The sub-group A-I contained the resistant genotypes, Azucena (A-I-I). Taducan and Tetep (B-I-III), and Teqing (B-I-I) were significantly separated from susceptible genotypes. The following marker alleles: RM209 (116), RM257 (136), RM478 (234), RM202 (176), RM224 (126), RM 426 (175), and RM6971 (196) possessed the higher values of Eigenvectors (Supplemental Table 3) and these alleles were also detected in resistant cultivars. In addition, it has been reported that the above mentioned SSR markers were found to have linkage with the following SB QTLs: *QRlh11* (Xie *et al.* 2008), *qSBR9-1* (Channamallikarjuna *et al.* 2010), *QRlh7b* (Liu *et al.* 2009), *qSBR11-3* (Channamallikarjuna *et al.* 2010), *QRlh11* (Xie *et al.* 2008), *qSBR11-1* (Channamallikarjuna *et al.* 2010), *qShB3-2* (Liu *et al.* 2009), and *qSB-9* (Han *et al.* 2002), suggesting that they contributed significantly to create this measurable variation observed in our study. These results could authenticate that there is some extent of association between genotypes of SSR markers and SB resistance in rice.

Association between morphological traits and QTLs toward SB resistance

The correlation between morphological traits and SB resistance has been frequently observed in many findings (Channamalikkajuna *et al.* 2010, Groth and Nowick 1992, Li *et al.* 1995a). More than 60 QTLs including major and minor effects conferring SB resistance (Zeng *et al.* 2011) have been identified in rice (Table 2). Ten of these 60 QTLs co-localize with QTLs for morphological traits especially, PHT and DTH (Srinivasachary *et al.* 2011). Three QTLs (*qPH-3*, *qPH-4*, and *qPH-11*) controlling plant height and four QTLs (*qHD-2*, *qHD-3*, *qHD-5*, and *qHD-7*) for days to heading were mapped (Zou *et al.* 2000) on different chromosomes in the same mapping population. It has also been reported that the major QTLs *QHd3a*, *QHd8a*, and *QHd9a* controlling days to heading and QTLs *QPh3a*, *QPh8a* and *QPh9a* (Li *et al.* 1995a) controlling plant height were mapped to approximately the same locations which corresponded to the QTLs *QSbr3a*, *QSbr8a* and *QSbr9a* associated with SB resistance. The tiller angle gene *TAC1* has been identified in the region *qSB-9^{TQ}* associated with SB resistance (Zuo *et al.* 2014). For both the cases, findings from the analysis of morphological data and molecular data supported each other strongly. Cluster analysis showed the most common pattern of grouping which indicated that the resistant and susceptible genotypes have been grouped in a similar manner such as the ‘Tetep’ and ‘Teqing’ resistant genotypes were grouped into a separate cluster for both morphological data (Fig. 3) and molecular data (Fig. 4). However, not only cluster analysis, but principal component analysis also produced a similar discrimination (Figs. 5, 6) into components (data not shown).

Association analysis using the Mantel (1967) test indicated a weak relationship between the observed morphological traits and allelic variation pattern and detected low congruence between the two datasets produced in this study. Low correlation between morphological and molecular markers has been reported in many crops (Bushehri *et al.* 2005, Ferriol *et al.* 2004, Koehler-Santos *et al.* 2003) and these authors suggested that it could be as a result of the independent nature of morphological and molecular variations.

This suggests that genotypes like Tetep and Teqing might be selected as donors for improvement of SB resistance in rice. IR65482-4-136-2-2, MR219-4, and MR264 were evaluated and QTLs responsible for SB resistance were mapped. Moreover, QTLs controlling plant height (*qPH-3*, *qPH-4* and *qPH-11*), QTLs controlling days to heading (*qHD-2*, *qHD-3*, *QHd3a*, *qHD-5*, *qHD-7*, *QHd8a*, and *QHd9a*), and those controlling morphological traits, such as FLA, FLL, and PCP, could be good choices with the following SB QTLs: *qSB-9*, *qSBR9-1*, *QRlh6*, and *qShB3-2*, to enhance SB resistance through QTL pyramiding or pyramiding QTLs together with any of these morphological traits. Successful pyramiding of the tiller angle gene *TAC1* and *qSB-9^{TQ}* has been reported by Zuo *et al.* (2014).

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