Marker-assisted selection to improve submergence tolerance, blast resistance and strong fragrance in glutinous rice

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ABSTRACT

Glutinous rice is popularly grown for consumption in Lao PDR. Most of the Laotian glutinous rice varieties grew nowadays are intolerant to flash flooding, susceptible to major diseases such as blast and bacterial blight and lacking of good grain and cooking quality. Our goal of this study is to develop new glutinous rice varieties that have good cooking quality, tolerance to flash flooding and resistance to blast disease by combining *Sub1*, *badh2*, *qBl1* and *qBl11* loci from the glutinous elite lines IR85264 (*Sub1*), TDK303 (*badh2*) and RGD07529 (*qBl1*+*qBl11*) through marker-assisted foreground selection (MAS) strategy. Three-way crossing was made to develop a breeding population in which MAS was applied in the series to select for individuals carrying submergence tolerance (*Sub1*), blast resistance (*qBl1*, *qBl11*) and fragrance (*badh2*). According to the marker genotypes, twenty eight F_5 homozygous lines carrying *Sub1*, *badh2*, *qBl1* and *qBl11* loci were finally selected and evaluated for submergence tolerance (SUBT), blast resistance (BLR) and fragrance (FR). All F_5 selected lines are fragrance and showed high level of SUBT and BLR. This study provides further support on the effective of MAS to improve FR, SUBT and BLR in rice breeding program.

Key words: marker-assisted; submergence; blast resistance; fragrance; glutinous rice

INTRODUCTION

Glutinous or waxy rice is the most popularly growing and consuming in Lao PDR. It is considered as one of the traditional Laotian lives. Waxy rice contains 85.5% of rice genetic diversity found in Lao PDR (Appa-Rao *et al*., 2002). Currently, global warming and climate change become more and more serious threatening the rice production in which rice cultivation in Lao PDR is under a rainfed condition. Frequent flooding and severe water deficit occur frequently and unpredictably due to uncertain and uneven distribution patterns of the rainfall during the monsoon season (Dey and Upadhyaya, 1996). The fluctuation of temperature also leads to an outbreak of disease and insect pest. Blast disease caused by *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*) is also the most serious disease reducing yield substantially in the rainfed lowlands (Teng and Revilla, 1996; Gnanamanickam, 2009).

Most of traditional and improved waxy rice varieties are intolerant to submergence and susceptible to blast disease (Schiller *et al*., 2001; Douangboupha *et al*., 2006). In Lao PDR, national and international market currently demand and prefer fragrance and soft texture waxy rice, therefore grain and cooking qualities will be significant factors determining farmers income in the future.

Genetic information of submergence tolerance, blast resistance and cooking quality traits such as fragrance, amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) through various studies using QTL mapping and map-based cloning approaches is well documented (Kumar and Khush, 1986; He *et al*., 1999; Tan *et al*., 1999; Li *et al*., 2003; Zhou *et al*., 2003). Fragrance is one of cooking quality traits of the waxy rice preferred by Lao consumers. Fragrance gene, *badh2*, was identified from various rice cultivars namely KDML105, Basmati, Pathein Nyunt, Yangon Saba and Kyet Paung (Wanchana *et al*., 2005; Bradbury *et al*., 2005b; Myint *et al*., 2012). The expression of *badh2* leads to the production of 2-acetyl-1-pyrroline (2AP), the major active compound in fragrant rice (Buttery *et al*., 1982; Lorieux *et al*., 1996). The *Sub1* gene located on rice chromosome 9 was identified as a major gene conferring submergence tolerance in tolerant rice cultivar FR13A and its derived progenies such as IR49830, IR57514, IR85264 ect (Xu and Mackill, 1996; Xu *et al*., 2000; Siangliw *et al*., 2003; Toojinda *et al*., 2003; Xu *et al*., 2004). *Sub1* contains three related ethylene response factor-like genes named as *Sub1A*, *Sub1B* and *Sub1C* in which the *Sub1*A and *Sub1*C are robustly induced in the tolerance cultivars in response to submergence (Mackill, 2006; Ruanjaichon *et al*., 2008). The *qBl1* and *qBl11*, two QTL for broad spectrum blast resistance, were identified in rice cultivar Jao Hom Nin. These QTLs confer high resistance against blast isolates from Thailand and Lao PDR (Wongsaprom *et al*., 2010; Korinsak *et al*., 2011).

In the first half of the 20th century, breeding methodology is a simple process in which selection is based upon the phenotypic performance of individuals under circumstance that allows gene or genes controlling the trait being fully expressed. Gene-pyramiding to combine several traits together in a single genotype is very difficult by conventional breeding (Babu *et al*., 2004; Basavaraj *et al*., 2010). Advanced in DNA marker technology has proved itself as powerful tools for genetic manipulation in putting together beneficial gene complexes (Toojinda *et al*., 2005; Wan *et al*., 2005; Jena and Mackill, 2008). Marker-assisted selection (MAS) has been advocated as a highly efficient breeding method. It makes possible rapid and precise selection of the targeted gene (Hospital, 2005). The most widespread use of MAS to date is to assist introgression of major genes into already proven, elite cultivars by backcrossing and to assist pyramid of the major genes (Siangliw *et al*., 2003; Xu *et al*., 2004; Neeraja *et al*., 2007; Jairin *et al*., 2009; Septiningsih *et al*., 2009; Yi *et al*., 2009; Win *et al*., 2012; 2013).

In Lao PDR, since the green revolution, new rice varieties were bred through conventional breeding program and few varieties such as TSN1 and TDK1 have been popularly cultivated (Inthapanya *et al*., 2006). However, the new improved varieties are either intolerant to submergence, susceptible to blast disease or lack of good cooking quality. In this study, we reported a success story of combining the submergence tolerance (*Sub1*), blast resistance (*qBl1/qBl11*) and fragrance (*badh2*) genes using marker-assisted selection (MAS) for Laos rice breeding program within three years.

MATERIALS AND METHODS

Plant materials and breeding

Three glutinous rice varieties including TDK303-140-3-93 (TDK303), IR85264-34-141 (IR85264) and RGD07529-1-1-MAS-38-1-B (RGD07529) were intercrossed to develop three-way population. TDK303 carrying *badh2* gene was developed by marker-assisted backcrossing (MAB) at NAFRI, Lao PDR through the Mekong breeding program. It derived from the cross between Thadokkham 1 (TDK1) and Hom Nang Nuan. IR85264 carrying *Sub1* gene was developed by MAB at IRRI (Septiningsih *et al*., 2009). RGD07529 carrying two blast resistance QTLs (*qBl1* and *qBl11*) was developed by MAB at Rice Gene Discovery Unit (RGDU), BIOTEC, Thailand (Wongsaprom *et al*., 2010).

DNA marker analysis

Six molecular makers including R10783indel (for *Sub1*) (Siangliw *et al*., 2003); RM212-RM319 (for *qBl1*) and RM224-RM144 (for *qBl11*) (Noenplab *et al*., 2006) and Aromarker (for *badh2*) (Vanavichit *et al*., 2008) were used for all DNA analysis to identify progenies carrying *Sub1*, *badh2*, *qBl1* and *qBl11*. DNA of the rice plants was extracted from young leaves using DNA Trap kit (http://dnatec.kps.ku.ac.th). The PCR reaction, detection and scoring were performed using the protocol described by Yi *et al*. (2009).

Pyramiding of *Sub1*, *badh2*, *qBl1* and *qBl11*

Marker-assisted selection (MAS) and plant type selections (PS) were performed in each of the F_1 to F_4 generations. The rice plants carrying a combination of the genes/QTL in each generation were selected as showed in Figure 1. In F_2 generation, only two plants carrying positive alleles at *Sub1*, *badh2*, *qBl1* and *qBl11* and having very good plant type were selected and self-pollinated to generate F_3. At F_3 generation, 90 plants carrying homozygous positive alleles at *Sub1*, *badh2*, *qBl1* and *qBl11* were selected. The selected F_4 plants were planted following a plant to row design. Finally, twenty-eight F_4 homozygous pyramid lines (PLs) were selected for this study. Submergence tolerance (SUBT), blast resistance (BLR) and fragrance (FR) including other cooking quality were evaluated in this material.

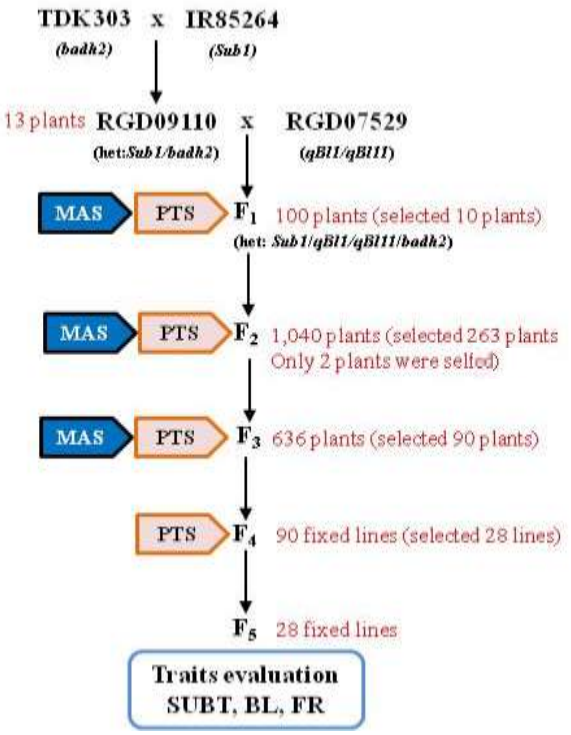

Figure 1 Breeding scheme showed the three-way cross and marker assisted selection (MAS) to combine a submergence tolerance gene (*Sub1*), two blast resistance QTLs (*qBl1*/*qBl11*) and a fragrance gene (*badh2*). Numbers of plants that were developed and selected in each generation were illustrated. Plant-type selection based on apparent agronomic characteristics was performed in each generation.

Phenotyping

Evaluation for Submergence tolerance

Two experiments were conducted at paddy field, Kasetsart University, Kamphangsean Campus, Thailand in dry season 2013. The submerge condition experiment (SC) was conducted under complete submergence in the outdoor lagoon and the normal condition experiment (NC) was conducted under normal irrigation (control) in the experimental field located near by the outdoor lagoon. Both experiments were arranged in a randomized complete block design (RCBD) with three replications. The 28 PLs, parental lines (TDK303, IR85264 and RGDU07529) and three check varieties (TDK1, RD6 and FR13A) were assigned into the plots based on RCBD. FR13A (*Sub1*) and IR85264 (*Sub1*) were used as standard checks for tolerance to submergence while TDK1, RD6, RGDU07529 and TDK303 were used as intolerant checks. All PLs and checks were direct-seeded in three-row plots with 1.25 m in length and 0.25 m between rows. At four weeks, number of seedlings was counted for each plot in both SC and NC and the average plant height of the seedling was taken. Then completely submerged for 10 days at 150 cm depth of the water in the lagoon was implemented in the SC. In the 10 days of submerged period, the water level was maintained at 100-120 cm above the leaf tip of the seedlings. Subsequently, the water was drained out from the lagoon. The seedlings were allowed to recover for 10 days. Then number of seedlings and average plant height were taken again in both experiments. In SC, percentage of surviving seedlings (PSS), percentage of plant elongation (PSE) and number of effective tillers per plant (NETP) were calculated for each plot following the protocol described by Siangliw *et al.* (2003).

Evaluation for Blast resistance

Assessment of blast resistance was done at seedling stage using an artificial inoculation in the greenhouse at Rice Gene Discovery Unit, Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand. Fifteen Lao PDR blast isolates (H08 and X09) and forty-two Thai blast isolates (THL) in which they represent the genetic diversity of blast pathogen found in the rainfed lowland of Lao PDR and Thailand respectively were used for blast screening in the parental lines (TDK303, IR85264 and RGDU07529) (Table 1).

Table 1 List of blast isolates used for evaluation of blast resistance in this study.

Source	Group	Isolate
Laos		H08-040-1, H08-269-1, H08-025-1, H08-044-1, H08-259-1, H08-245-1, H08-243-1, H08-234-1, H08-190-1, H08-184-1, ta403, H08-158-1, H08-171-1, H08-027-1, X09-42-1
Thailand	Mix1	THL832, THL710, THL282
	Mix2	THL137, THL906, THL122, THL757, THL603
	Mix3	THL191, THL266, THL456, THL653, THL658
	Mix4	THL730, THL810, THL838, THL838, THL967, THL985
	Mix5	THL144, THL1023, THL303, THL906
	Mix6	THL690, THL41, THL855, THL949, THL1003, THL1003, THL1009
	Mix7	THL458, THL831, THL943
	Mix8	THL186, THL190, THL486, THL634, THL868
	Mix9	THL211, THL244, THL374, THL734, THL759, THL1066

For Thai isolates, individual THLs were mixed to create 9 mixed isolates (Mix1 to Mix9) for parental screening. The mixed isolates were grouping by genetic diversity cluster analysis using AFLF technique. The preparation of rice plants and inoculation were followed the protocol described by Korinsak *et al*. (2011). Lesion score (LS) was recorded at 7 days after inoculation (DAI) and scored based on infection type or disease severity index using a scale of 0-6. The classification of resistance and susceptible was followed as described by Roumen *et al.* (1997).

Four Laos isolates, H08-040-1, H08-269-1, H08-025-1 and H08-044-1 showing a clear different reaction among the parents were used for the assessment of blast resistance in the PLs. The inoculation and scoring were following the protocol described above.

Evaluation for cooking quality

The 28 PLs, parental lines (TDK303, IR85264 and RGDU07529) and two standard checks (TDK1 and RD6) were planted in the two-row plot (30 cm between rows and 3 m in length) at RGDU in 2012. Rice grains of each line were harvested at maturity stage and dried naturally in a greenhouse. Grain samples of 100 g mechanically dehulled and polished by a minipolisher. Milled rice samples were divided into three samples and used for the grain quality test. All PLs, parental lines and checks were tested for amylose content (AC), Gel consistency (GC), Alkali spreading value (ASV) following the procedure as described by Lanceras *et al*. (2000). Fragrance and non-fragrance were determined by a sensory test according to Wanchana *et al*. (2005) and Yi *et al*. (2009).

Data analysis

Statistical analysis was performed for each parameter studied based on their statistical design using STATGRAPHICS plus 3.0 software. Means were compared by LSD test if the *F* value was significant. Fragrance, AC, GC and GT of the PLs were compared with TDK303 and RGD07529 while their NETP, PH, PSE, NP and PSS were compared with IR85264.

RESULTS

Submergence tolerance

In this study, submergence was clearly limited the plant growth as seen in the significant difference of the NETP (69 days) of PLs (Table 2) between control (NC) and submerged experiments (SC). Average NETP at 69 days was 2 and 21 (tillers) for SC and NC, respectively, while their average PH (69 days) was not significantly difference (62.6 cm). Ten days after the water was drained from the submerging ponds, intolerance checks (TDK1, RD6, TDK303 and RGD07529) showed typical symptoms such as high PSE and low PSS while tolerant checks (FR13A and IR85264) showed opposite phenotypes (low PSE and high PSS).

Complete submergence decreased PSS in both tolerance and intolerance varieties but the decrease was significantly less in the tolerance. The PSS was 95 and 78.0% for FR13A and IR85264, respectively. The PSS of the PLs carrying the *Sub1* ranged from 39.7% to 92.9% (average of 66.3%) which were slightly lower than that of the tolerant check, FR13A (PSS = 95%). Ten PLs consisted of RGD10033-MAS-77-438-51(PSS = 79%), RGD10033-MAS-77-149-14 (PSS = 80%), RGD10033-MAS-77-149-16 (PSS = 81%), RGD10033-MAS-77-327-45, RGD10033-MAS-77-438-48, RGD10033-MAS-77-438-47 and RGD10033-MAS-77-303-36 (PSS = 83%) and RGD10033-MAS-77-524-75 (PSS = 87%), RGD10033-MAS-77-438-46

Table 2 Comparisons of submergence tolerance performance among parents and PLs using STATGRAPHICS plus 3.0 software.

Traits	Tolerance check				Intolerance check								PLs		LSD (0.05)		F-test		CV (%)	
	FR13A		IR85264		TDK1		RD6		TDK303		RGDU0529		mean							
	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC
$NETP_B$ (tiller)	3 ^{A}	6 ^{a}	3 ^{A}	4 ^{a}	4 ^{A}	5 ^{a}	4 ^{A}	3 ^{a}	3 ^{A}	3 ^{a}	4 ^{A}	3 ^{a}	4 ^{A}	4 ^{a}	1	3	ns	ns	22.3	42.5
$NETP_A$ (tiller)	27 ^{ABC}	8 ^{a}	26 ^{ABC}	2 ^{b}	33 ^{A}	0 ^{b}	21 ^{C}	0 ^{b}	23 ^{ABC}	0 ^{b}	20 ^{C}	0 ^{b}	21 ^{C}	2 ^{b}	5	2	**	**	20.9	61
PH_B (cm)	57.8 ^{A}	66.1 ^{a}	46.7 ^{B}	53.3 ^{b}	47.1 ^{B}	53.9 ^{b}	53.3 ^{AB}	57.3 ^{ab}	54.2 ^{AB}	60.1 ^{ab}	58.8 ^{A}	56.8 ^{b}	49.8 ^{AB}	53.7 ^{b}	4.9	4.9	**	**	7.8	8.9
PH_A (cm)	65.1 ^{ABC}	75.1 ^{abcd}	59.7 ^{C}	55.7 ^{d}	57.7 ^{C}	70.4 ^{bcd}	66.9 ^{ABC}	95.1 ^{a}	66.9 ^{ABC}	78.1 ^{abc}	77.1 ^{AB}	83.2 ^{ab}	62.6 ^{ABC}	62.6 ^{cd}	7.8	10.1	**	**	6.2	9.9
NP_B (plant)		13.7 ^{a}		13.3 ^{a}		5.7 ^{a}		10 ^{a}		11 ^{a}		8.7 ^{a}		13.8 ^{a}		5.1	-	ns		22.6
NP_A (plant)		13 ^{a}		10.3 ^{ab}		0 ^{d}		0.7 ^{cd}		2 ^{bcd}		0.7 ^{cd}		9.3 ^{abc}		4.6	-	**		30.2
PSE (%)	12.8	13.6 ^{ab}	27.7	8.2 ^{a}	22.5	18.1 ^{ab}	31	66.7 ^{a}	23.6	29.9 ^{b}	31.3	46.9 ^{a}	26.4	17.5 ^{abc}		15.2	-	**		53.2
PSS (%)		95 ^{a}		78 ^{a}		0 ^{c}		6.1 ^{c}		16.3 ^{bc}		4.2 ^{c}		66.3 ^{ab}		26.6	-	**		24.6

Notes: NC = normal condition; SC = submerged condition; $NETP_B$ = number of effective tillers per plant before submerged (52 days); $NETP_A$ = number of effective tillers per plant after submerged (69 days); PH_B = plant height before submerged (52 days); PH_A = plant height after submerged (69 days); PSE = percentage of seedling elongation; NP_B = number of plant before submerged; NP_A = number of plant after submerged; PSS = percentage of surviving seedlings, ** = significant at $P < 0.01$; ns = not significant; $^{A, B, C}$ and ^{D} stand for the clustering value on NC which are significant or not significant ; $^{a, b, c}$ and ^{d} stand for the clustering value on SC which are significant or not significant

(PSS = 89%) and RGD10033-MAS-77-149-17 (PSS = 90%) showed higher PSS than the donor parent IR85264-34-141 (PSS = 78%). The rest of the PLs showed PSS equal to or slightly below that of the IR85264. However, the PSS of all PLs was significantly higher than that of intolerant checks, RD6 (PSS = 6%), TDK1 (PSS = 0%), RGD07529 (PSS = 4%) and TDK303 (PSS = 16%).

Shoot elongating (SE) under water when rice plant experiences submergence stress is one of the key traits determining submergence tolerance. The SE of some intolerant varieties increased rapidly underwater. PSE was high in RD6 (66.7%) and RGD07529 (46.9%) but low in TDK1 (18.1%) and TDK303 (29.9%). All PLs showed low PSE which was not significantly different from tolerant parent IR85264 but significant differed from intolerant parents (Table 2).

Blast resistance

Evaluation of blast resistance in parental lines with Laos and Thai isolates

Sariceltik showed high susceptible to all Laos and Thai isolates. TDK1 showed high level of resistance to all isolates from Laos except for H08-044-1 and to all mixed isolates from Thailand. RD6 showed high level of resistance to all isolates from Laos except for H08-269-1 and to most mixed isolates from Thailand except for mixed 3, 4, 8, and 9. All parental lines (IR85264, TDK303 and RGD07529) were resistance to all tested isolates from Thailand and Laos (Table 3). These results were unfortunate and disagreed with those of NAFRI whom had reported on the susceptibility of TDK1 to blast disease in Laos PDR. However, we selected four isolates from Laos (H08-025-1, H08-040-1, H08-044-1 and H08-269-1) in which some parental lines showing MR to evaluate the breeding lines for blast resistance.

Evaluation of blast resistance in breeding lines

RD6, Sariceltik and US2 were susceptible to H08-025-1, H08-040-1, H08-044-1 and H08-269-1 except for RD6 that was resistant to H08-025-1. All 28 PLs carrying *qBl1* and *qBl11* showed high level of resistance (Score 0-1) against all tested isolates (Table 4).

Cooking quality

Gel consistency (GC) of the PLs ranged from 102-118 mm, in which they indicated as soft gel quality. Gelatinization temperature (GT) of the PLs tested with two condition of alkali digestibility. The alkali digestibility values using 1.7% KOH of the PLs were ranged score of 5.4 - 6.8. Gelatinization temperature of the PLs were approximately 55-69^{o}C, indicated as low gelatinization temperature (<70^{o}C). Considering alkali digestibility values 1.3% KOH of the PLs ranged score of 2.3 - 3.9. Gelatinization temperature of the PLs was approximately 70-74^{o}C, in which they indicated as intermediate gelatinization temperature. In case of aroma, all PLs are aromatic.

DISSCUSSION

Breeding superior rice varieties can be achieved through the precision of marker-assisted selection (Siangliw *et al*., 2003; Xu *et al*., 2004; Neeraja *et al*., 2007; Jairin *et al*., 2009; Septiningsih *et al*., 2009; Yi *et al*., 2009; Win *et al*., 2012; 2013; Luo and Yin, 2013; Pinta *et al*., 2013). In this study, combining the submergence tolerance (*Sub1*), blast resistance (*qBl1/qBl11*) and fragrance (*badh2*) genes using MAS for Laos's rice breeding program can be achieved within three years. The gene-specific and tight linkage microsatellite markers were employed to select PLs that combined all favorable alleles of *Sub1*, *badh2*, *qBl1* and *qBl11* loci. All selected PLs showing submergence tolerant, high levels of blast resistance and aroma phenotypes indicated that

Table 3 Pathogenicity test of parental lines and checks against 14 isolates from Laos and 9 mixed isolates from Thailand. Disease scores were rated at seedling stage following the 0-6 scales described by International Rice Research Institute (IRRI).

Isolate	Blast score					
	IR85264	**TDK303**	**RGD07529**	**TDK1**	**RD6**	**Sariceltic**
LAOS						
H08-190-1	1(R)	0(R)	2(R)	0(R)	3(MR)	6(S)
H08-184-1	0(R)	1(R)	1(R)	0(R)	3(MR)	6(S)
H08-27-1	1(R)	0(R)	0(R)	1(R)	2(R)	6(S)
H08-040-1	0(R)	0(R)	0(R)	0(R)	6(S)	6(S)
H08-245-1	1(R)	0(R)	0(R)	0(R)	0(R)	6(S)
H08-259-1	1(R)	1(R)	3(MR)	1(R)	3(MR)	6(S)
X09-042-1	1(R)	1(R)	3(MR)	1(R)	2(R)	6(S)
H08-158-1	1(R)	1(R)	2(R)	1(R)	2(R)	6(S)
H08-171-1	2(R)	2(R)	2(R)	1(R)	2(R)	6(S)
H08-025-1	1(R)	2(R)	1(R)	0(R)	2(R)	6(S)
H08-044-1	4(MR)	1(R)	1(R)	5(S)	1(R)	4(MR)
H08-269-1	0(R)	0(R)	0(R)	0(R)	S(6)	6(S)
H08-243-1	0(R)	1(R)	1(R)	1(R)	2(R)	3(MR)
H08-234-1	0(R)	0(R)	3(MR)	0(R)	3(MR)	6(S)
Thailand						
Mix1	0(R)	0(R)	0(R)	0(R)	0(R)	5(S)
Mix2	0(R)	0(R)	0(R)	0(R)	4(MR)	6(S)
Mix3, Mix8, Mix9	0(R)	0(R)	0(R)	0(R)	5(S)	6(S)
Mix4	1(R)	0(R)	0(R)	1(R)	5(S)	6(S)
Mix5	0(R)	0(R)	0(R)	0(R)	0(R)	6(S)
Mix6	0(R)	0(R)	0(R)	0(R)	4(MR)	6(S)
Mix7	0(R)	0(R)	0(R)	0(R)	0(R)	6(S)

MAS is highly effective tool for selection in this study. The successful of MAS pyramiding for multi-traits or multi-gene has been reported in rice (Siangliw *et al*., 2003; Xu *et al*., 2004; Neeraja *et al*., 2007; Jairin *et al*., 2009; Septiningsih *et al*., 2009; Yi *et al*., 2009; Win *et al*., 2012; 2013; Luo and Yin, 2013; Pinta *et al*., 2013).

The *Sub1* locus is a major gene contributing to a high plant survival through the reduction of plant growth (Xu and Mackill, 1996; Nandi *et al.,* 1997; Sripongpangkul *et al.,* 2000; Kamolsukyunyong *et al.,* 2001; Siangliw *et al.,* 2003; Toojinda *et al*., 2003). All PLs have higher PSS and lower PSE comparing to TDK303 and RGD0529, intolerant parents. A significantly improved submergence tolerance through the reduction of the PSE was observed (the PSE of most PLs was significantly different from RD6 and TDK1) indicating that the presence

Table 4 Pathogenicity test of the PLs (showed only 10 out of 28 lines) and their parents inoculated with 4 isolates from Laos. Disease scores were rated at seedling stage following the 0 -6 scales described by International Rice Research Institute (IRRI).

Variety/Pedigree	Blast resistance gene	Blast isolates			
		H08-025-1	H08-040-1	H08-044-1	H08-269-1
RGD10033-MAS-77-43-2	*qBl1,qBl11*	0(R)	1(R)	0(R)	0(R)
RGD10033-MAS-77-43-3	*qBl1,qBl11*	0(R)	1(R)	0(R)	0(R)
RGD10033-MAS-77-149-14	*qBl1,qBl11*	0(R)	0(R)	0(R)	0(R)
RGD10033-MAS-77-149-16	*qBl1,qBl11*	0(R)	0(R)	1(R)	0(R)
RGD10033-MAS-77-149-17	*qBl1,qBl11*	0(R)	0(R)	0(R)	0(R)
RGD10033-MAS-77-149-18	*qBl1,qBl11*	0(R)	1(R)	1(R)	0(R)
RGD10033-MAS-77-291-20	*qBl1,qBl11*	0(R)	1(R)	0(R)	0(R)
RGD10033-MAS-77-291-21	*qBl1,qBl11*	0(R)	0(R)	0(R)	0(R)
RGD10033-MAS-77-291-22	*qBl1,qBl11*	0(R)	0(R)	0(R)	0(R)
RGD10033-MAS-77-291-23	*qBl1,qBl11*	0(R)	0(R)	1(R)	0(R)
RGD10046-MAS-592-13	*qBl1*	0(R)	0(R)	4(MR)	1(R)
RGD10046-MAS-609-2	*qBl1*	0(R)	0(R)	0(R)	0(R)
RGD10046-MAS-576-40	-	1(R)	0(R)	2(R)	0(R)
RGD10046-MAS-592-5	-	0(R)	0(R)	0(R)	0(R)
RD6	-	0(R)	6(S)	0(R)	6(S)
TDK1	-	0(R)	0(R)	4(MR)	0(R)
IR85264	-	0(R)	1(R)	4(MR)	0(R)
TDK303	-	0(R)	0(R)	0(R)	0(R)
RGD07529	*qBl1,qBl11*	0(R)	0(R)	0(R)	0(R)
Sariceltik	-	6(S)	6(S)	0(R)	6(S)
US2	-	6(S)	6(S)	2(R)	6(S)

of *Sub1* inherited from IR85264 clearly reduced the elongation of plant under the submergence event. However, the significant variations among the individual PLs observed for PSS and PSE indicated a quantitative nature of such trait. To date, there have been a number of reports on major and minor QTLs associated with submergence tolerance (Xu and Mackill, 1996; Nandi *et al*., 1997; Sripongpankul *et al*., 2000; Siangliw *et al*., 2003; Toojinda *et al*., 2003; Angaji, 2008).

Parental lines and PLs showed broad-spectrum resistance to blast pathogen collected from Thailand and Lao PDR. Two QTLs, *qBl1* and *qBl11*, were identified to confer broad spectrum resistance to blast disease (Wongsaprom *et al*., 2010; Korinsak *et al*., 2011). In this study, contribution of the QTLs on blast resistance cannot be confirmed because of the existing of other resistant genes in the genetic background as showed in RGD10046-MAS-576-40 and RGD10046-MAS-592-5 (PLs without *qBl1* and

qBl11). Our validation indicated that all PLs are highly resistant to blast disease. Several reports showed that the use of MAS to transfer QTL for broad spectrum blast resistance are effective (Wang *et al*., 1994; Zhou *et al*., 2003; Wan *et al*., 2005; Neeraja *et al*., 2007; Liu *et al*., 2008; Wongsaprom *et al*., 2010; Sreewongchai *et al*., 2010; Jiang *et al*., 2012; Singh *et al*., 2012).

The *badh2* is major locus determining the presence of 2AP in rice grains (Wanchana *et al*., 2003; Bradbury *et al*., 2005a). All PLs carrying the positive allele of *badh2* are aromatic. The means of values of the AC, GC, GT and FR of PLs were not significantly different from those of the parents. In this study, all PLs have acceptable cooking and eating qualities. This result confirms that molecular markers can rapidly assist the development of new varieties that possess submergence tolerance, broad spectrum blast resistance and cooking quality characters with considerable saving in time. The new PLs will help Lao farmers to prevent their yield losses due to submergence and blast disease in the future.

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