Chiang Mai J. Sci. 2018; 45(6) : 2312-2321
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Development of Aromatic Glutinous Rice for Rainfed Lowland Areas by Marker Assisted Selection

Srisawat Khanthong [a,c,d], Sureeporn Kate-ngam** [b,d], Kamonwan Riabroy [a], Jonaliza Lanceras-Siangliw [a] and Theerayut Toojinda* [a]**

[a] Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Kasetsart University, Kamphaengsaen, Nakhon Pathom 73140, Thailand.

[b] Department of Agronomy, Faculty of Agriculture, Ubon Ratchathani University, Warin Chamrap, Ubon Ratchathani 34190, Thailand.

[c] Plant Breeding Program, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand.

[d] These authors contributed equally to this work.

* Author for correspondence; e-mail: toojindatheerayut@gmail.com

Received: 9 February 2017
Accepted: 7 November 2017

ABSTRACT

Glutinous rice is important in producing glutinous rice flour, festival foods, desserts and staple food in Southeast Asia regions. However, this rice crop is cultivated under rainfed condition. Blast disease and flash flooding which are major constraints in the production areas. Therefore, breeding rice with resistance to blast disease and tolerance to submergence are needed to maintain rice production. Marker assisted and stringent plant type selections (MAS and PTS) were employed to develop new glutinous rice genotypes with supreme cooking quality, high level of blast resistance and submergence tolerance by crossing RGD334-3-11-1-2-34 and RGD07334-34-16. MAS and PTS were carried out in F_2 to F_4 generations. Foreground selection for genes/QTL namely *wx, badh2, SUB1, qBL1* and *qBL11* was performed using gene specific and tightly linked markers. Twenty one superior F_4 plants with combinations of five desirable traits including glutinous, blast resistance, submergence tolerance and grain aroma were selected and advanced to F_5 generation. Phenotypic evaluation revealed that all F_5 lines are glutinous having high level of blast resistance, outstanding submergence tolerance and strong grain aroma. This study confirmed that conventional and marker assisted selection approaches are effective in providing opportunities for breeders to pyramid genes/QTL in developing better rice cultivars.

Keywords: marker-assisted selection, rice, grain quality, blast disease, submergence tolerance

1. INTRODUCTION

Glutinous rice is indispensable in the production of glutinous flour, festival foods and desserts although it also serves as the staple food in regions of Southeast Asia [1] including Thailand and Laos PDR. In Thailand, glutinous jasmine rice RD6 is the premium

quality rice and most popular. However rice cultivation these areas are largely cultivated under rainfed lowland in which blast disease, flooding and drought have caused severe yield loss every year [2]. Therefore, breeding for blast resistance, submergence and drought tolerance are required to maintain rice production in these areas.

Marker assisted selection (MAS) approach have enormous potential for rice breeding by providing efficient and precise selection for traits related to yield stability and sustainability [2-3]. MAS has been proven to speed up the selection process [3-4] and provides opportunity in selecting proper genotypes that contain many traits especially appropriate for traits with low heritablility. However, MAS required DNA marker-trait association in which the information are limited in many traits related to adaptation and productivity in the target environments thus the utilization of MAS alone may not deliver successful product to the farmers. The proper way is to combine conventional and MAS in breeding program to select target traits and agronomic characters that fit the target environment and farmer's preference [5]. In this study,four traits including blast resistance (BR), submergence tolerance (SubT) and two traits for grain qualities, such as grain aroma (Aro) and glutinous (Glu) were combined into new aromatic glutinous rice using MAS and plant type selection.

DNA marker-trait associations of BR, SubT, Aroand Gluare well documented [6-7]. In Thailand, blast resistance QTL including *qBL1* and *qBL11*which showed broad spectrum resistance to rice blast fungus (*Magnaporthegrisea*) [8-9] and had been deployed to improve blast resistance in RD6 [10]. *SUB1*, a major gene for SubT was originally identified in rice variety FR13A [11-13] and cloned [14]. *SUB1* was deployed to improve several Thai rice varieties [12-13, 15]. The recessive gene '*badh2'* determing grain aroma was identified [16] and cloned [17]. It was sucessfully deployed to improve grain aroma in rice breeding program worldwide [15, 18-19]. The *WX* gene, encoding a granule-bound starch synthase, has been reported to be a major contributor determining amylose content (AC) and gel consistency (GC)[20] in rice. The 23 bp insertion in the *WX* gene yield the glutinous phenotype in rice[7]. DNA markerswere used in MAS forline conversions and great success in improving poor cooking-quality genotypes into desirable cooking-quality genotypes were noted [15, 18-19, 21].

In this study, efforts were undertaken to develop new glutinous rice lines with good cooking quality, SubT and BR from two improved rice lines, RGD334-3-11-1-2-34 (RGD334), a glutinous jasmine rice line with BR and RGD07334-34-16(RGD07334), a non glutinous jasmine line with SubT. Gene specific and tightly linked markers were adopted to identify plants with combinations of genes or QTL determining the target traits. As a result, F_5 recombinant inbred lines were developed. The present study reports the cooking quality tests including fragrance, glutinous grain, gel consistency (GC) and alkali spreading value (ASV) and the evaluation of SubT and BR. Field evaluation for important agronomic traits was also reported.

2. MATERIALS AND METHODS

2.1 Rice Materials and Breeding Scheme

RGD334, a glutinous breeding line with good cooking quality and broad spectrum BR (*qBL1, qBL11*)[10] and RGD07334, the new jasmine breeding line with jasmine cooking quality and SubT[22] were crossed to generate the population. RGD334 was crossed with RGD07334 to obtain F_1 seeds (Figure 1.) and undergone self-pollination to generate the F_2 population which were

subjected to phenotypic selection (PTS) using good plant type, good tillering and grain size as criteria and MAS. The selected plants were self-pollinated and PST and MAS were applied until F_4 generation (Figure 1). Finally, twenty one F_4 plants were selected and used in this study and were advanced to F_5 for phenotypic validation. Parents and germplasm generated are all available in Rice Gene Discovery, BIOTEC.

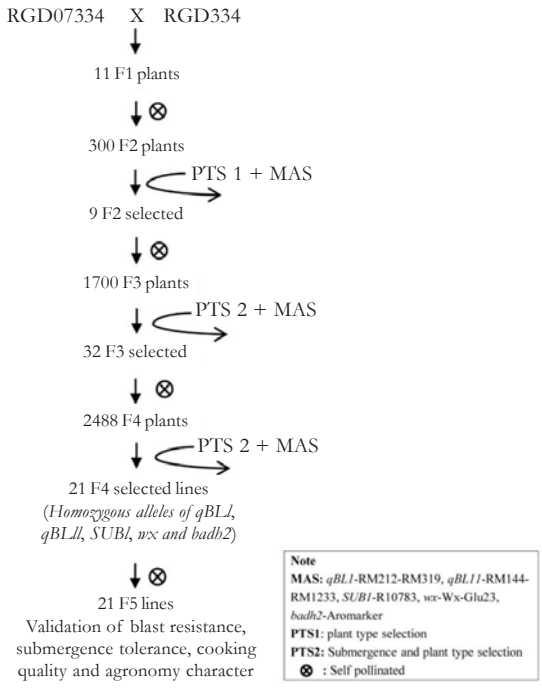

Figure 1. Breeding scheme in developing new aromatic glutinous rice highly resistant to blast diseases, tolerant to submergence and with good grain qualities. The cross was made between RGD07334 with RGD334 and the population was generated by selfing method. PTS and MAS were employed from F_2 to F_4 generations. Twenty one F_4 plants with favorable alleles were selected and advanced to F_5 for validation and agronomic character evaluation.

2.2 Molecular Marker Analysis

Seven DNA markers including R10783 (for *SUB1*), Aromarker (for *badh2*), Wx-Glu23 (for *wx*) and RM212-RM319 (for *qBL1*) and RM144-RM1233 (for *qBL11*) were used for foreground selection (Table 1).

Total genomic DNA were isolated from 0.5 g young leaf tissue according to the DNA trap® kit developed by DNA Technology Laboratory, Kasetsart University, Kamphaeng SaenCampus, Thailand. PCR reaction for R10783, Wx-Glu23, Aromarker, RM212, RM 319, RM144 and RM1233 markers were performed in a 10 µl reaction mixture containing 50 ng of DNA template, 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each forward and reverse primers and 1U Taq DNA polymerase. The volume was raised to 10 µl with distilled water. PCR reaction was initiated by denaturation at 94°C for 3 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min. Final 7 min at 72°C was allowed for the completion of primer extension. Polymorphism was detected by 4.5% polyacrylamide gel electrophoresis.

Table 1. DNA markers for foreground selection of QTL and genes for blast resistance, glutinous, fragrance and submergence tolerance.

Gene/ QTL	Marker	Sequence (5'-3')	PCR product (bp)	Chromosome	Type of marker	Reference
qBL1	RM212	F- CCACTTTCAGCTACTACCAG	130	1	SSR	Wongsaprom *et al.* [10]
		R- CACCCATTTGTCTCTCATTATG				
	RM319	F- ATCAAGGTACCTAGACCACCAC	130	1	SSR	Wongsaprom *et al.* [10]
		R- TCCTGGTGCAGCTATGTCTG				
qBL11	RM144	F- TGCCCTGGCGCAAATTTGATCC	240	11	SSR	Wongsaprom *et al.* [10]
		R- GCTAGAGGAGATCAGATGGTAGTGCATG				
	RM1233	F- GTGTAAATCATGGGCACGTG	175	11	SSR	www.gramene.org
		R- ATTGGCTCCTGAAGAAGG				
wx	Wx-Glu-23	F- TGCAGAGATCTTCCACAGCA	196	6	Gene specific	Wanchana *et al.* [7]
		R- GCTGGTCGTCACGCTGAG				
badh2	Aromarker	F- TGCTCCTTTGTCATCACACC	392	8	Gene specific	Jantaboon *et al.* [15]
		R- TTTCCACCAAGTTCCAGTGA				
SUB1	R10783	F- CTGCTCCGACGACCTGATGG	358	9	Gene specific	Jantaboon *et al.* [15]
		R- ATTAAATGGAACATTCGAGAA C				

2.3 Phenotypic Evaluation of the F_5 Recombinant Inbred Lines

Twenty one superior F_5 recombinant inbred lines (RILs) obtained from the selected F_4 plants were evaluated for submergence tolerance (SubT), blast resistance (BR), cooking quality and agronomic characteristics.

2.3.1 Evaluation for submergence tolerance

F_5 RILs were evaluated for submergence tolerance. FR13A and IR57514 were used as submergencetolerant checks and RD6 as intolerant check. The evaluation was conducted in an outdoor lagoon at Agronomy Field, Kasetsart University, Kamphangsaen Campus, Thailand following the screening protocol described by Siangliw *et al.*[13]. Twenty day-old seedlings were completely submerged for 11 days and the water level was maintained at 60-100 cm above the tallest seedlings to prevent leaf tips from emerging into the air. Plant height (cm) was measured before and after submergence based on 10 randomly selected plants for each line. The measurement was taken as the distance from the soil surface to the tip of the longest leaf. Number of plants were counted before submergence and 7 days after desubmergence. Percent plant survival (PPS) and percent plant elongation (PPE) were calculated as described by Siangliw *et al.* [13].

2.3.2 Evaluation for BR

Four most virulent Thai blast isolates (THL) collected from rice production areas in Thailand including THL41, THL855, THL211 and THL244 [9] were used to assess resistance to blast disease at seedling stage. About 50 mL of the spore suspension containing gelatin (0.5%) were sprayed onto two week-old seedlings. Inoculated seedlings were kept in chamber at dark with temperature maintained at 25°C. Distilled water was sprinkled four times a day to maintain high humidity. The disease reaction was recorded 7 days after inoculation using a 0-6 disease scoring scale. Plants exhibiting reactions that score 0-2 were considered resistant (R), 3-4 as moderately resistant (MS) and 5-6 as susceptible (S).

2.3.3 Evaluation for cooking quality

Four grain quality traits were measured using the bulk seeds of the F_5. For alkali spreading value (ASV) to determine gelatinization temperature (GT), six milled rice grains were taken in petri plates and added with 10 ml of 1.7% of KOH and kept in room temperaturefor 23 hours. Then the alkali spreading value was scored as follows, score of 1-3 was classified as high GT (74.5-80°C), 4-5 as intermediate GT (70-74°C), 6-7 as low GT (<70°C). Amylose content (AC) was measured using the procedure of Juliano[23] with minor modification. One hundred mg of rice flour, 1 ml of 95% ethanol and 9 ml of 1.0 N NaOH were mixed well and then incubated at room temperature over night to gelatinize the starch. Samples were diluted to 100 ml with distilled water. From this suspension, 5 ml of sample was taken and 1 ml of acetic acid (57.75 ml in one liter water) was added to acidify the sample along with 1.5 ml of iodine solution (0.2% iodine + 2% potassium iodide) and the volume was made to 100 ml with distilled water. The samples were incubated at room temperature for 20 min. The absorbance was measured at 620 nm using spectrophotometer. As a control, NaOH solution was used. The AC of different varieties was calculated in comparison with standard graph [23].

For gel consistency (GC) 100mg of rice powder in 13 × 150 mm test tube was added with 200 µl Xylene solutions in each sample. Tubes were mixed and 2.0 ml of 0.2 N KOH was added immediately and

mixed for 1 min. Tubes were covered with glass marbles and placed for 8 min in boiling water bath and kept in room temperature for 5 min then allowed to cool down on ice cold water for 15 min. The tubes were laid down horizontally over a graphing paper with mm grids. The lengths of the gel were classified as 61-100 mm for soft, 41-60mm for medium, 36-40mm for medium-hard, 24-35mm for hard [23]. For aroma, five brown rice seeds from each linewere added with 200 µl of distilled water in mirco tubes. Tubes were incubated for 3 hours at 65°C and then allowed to cool down. The lids were opened one by one and samples were sniffed by three panelist. Scores were given as strongly scented (++), mild scented (+), non scented (-) [7].

2.3.4 Evaluation for agronomic traits

Parentals and F_5RILs were evaluated for essential agronomic traits by growing in the paddy field at Agronomy Field, Kasetsart University, Kamphaengsaen Campus, Thailand in 2012. Treatments were arranged following a randomized complete block design (RCBD) with three replications. Data on germination to 50% flowering (day: DF) in a plot, number of grains per panicle (NGP) using three randomly selected panicles per plot were collected. Number of tillers per plant (TN) and number of panicles per plant (NPP) were collected fromthree randomly selected plants per plot. Grain size of paddy rice were measured by randomly selecting 15 seeds per panicle per plant fromthree plants per plot.

2.4 Statistical Analysis

All measurments in each experiment were subjected to statistical analysis using CROPSTAT Version 7.2. The means were compared by Least Significant Difference (LSD) if the F value was significant.

3. RESULTS AND DISCUSSION

3.1 The Performance of the F_5 RILs under Flash Flooding Condition and Blast Disease Infection

The mean of PPS of the F_5 RILs was 73.4±3.4% and ranged from 40.9 to 87.4 % (Table 2). All F_5 RILs exhibited higher level of PPS than the susceptible parent RGD334 (0%). The PSS of FR13A and RD6 were 87.7% and 6.7%, respectively. Sixteen F_5 RILs exhibited higher PPS than the tolerant parent RGD07334 (48.0%). The mean of PPE of the F_5 RILs was 19.4±1.9% with a range of 11.9 to 31.5 %. All F_5 RILs exhibited lower level of PPE than the susceptible parent RGD334 (43.4%) but higher than tolerant parent RGD07334 (10.3 %).In this study, several F_5 RILs exhibited higher level of submergence tolerance (PPS) than RGD07334. This might result from the application of the phenotypic selection at the F_3 and F_4 generations before following up by MAS. The superior F_5 RILs for SubT might contain minor QTL that support the function of *SUB1* in promoting higher PPS after desubmerged. Siangliw *et al.* [13] reported minor QTL for submergence tolerance on chromosomes 2and9 while Toojinda *et al.* [12] reported on chromosomes 1, 2, 5, 7, 10 and 11. Moreover, Septiningsih *et al.* [24] reported minor QTL for submergence tolerance on chromosomes 1, 2, 9 and 12 (*qSUB1.1, qSUB2.1, qSUB9.1* and *qSUB12.1*) and reports from Jantaboon *et al.* [15] were found on chromosomes 1, 2, 6 and 9.

RGD07334 and RGD334 showed susceptible reaction (S or MS) and resistance reaction (R) against all isolates of blast disease, respectively. All F_5 RILs carrying the *qBL1* and *qBL11* exhibited high level of resistance to blast disease same as donor

parent RGD334 (Table 2). The results were similar to those reported by Sriwongchai *et al.* [8], Manivong *et al.* [9], Wongsaprom *et al.* [10], and Ruengphayak *et al.* [21] with the same QTL introgressed into different genetic backgrounds through MAS. These results indicated the high accuracy of MAS when used with tightly linked markers.

Table 2. Submergence tolerance, blast resistance and grain quality evaluation in the F_5 lines and parents.

Accession	Submergence tolerance[a,b]		Blast resistance					Cooking quality[a,b]			
	PPS	PPE	THL41	THL211	THL244	THL855	ASV	AC	GC	Aro
F_5	73.4	19.4	R	R	R	R	7	5.6	120.2	++
	[40.9-87.4]	[12.1-31.1]	[0-1]	[0-1]	[0-2]	[0]	[6.5-7.0]	[5.1-6.1]	[100.6-135.7]	[+-++]
RGD07334	48.0	9.5	S	S	S	MS	7	17.2	67.7	++
RGD334	0.0	43.4	R	R	R	R	7	5.7	129.3	-
P-value	**	**					ns	**	**	
Mean	64.0	22.1					6.7	7.7	114.9	
Standard error (SE)	3.4	1.9					0.02	0.1	2.2	
5%LSD	15.7	9.4					0.2	1.2	25.1	
% CV	18.1	21.1					1.7	9.7	13.2	

[a] Percentage plant survival (PPS), Percentage plant elongation (PPE), alkaline spreading value (ASV), amylose content (AC), gel consistency (GC), aroma or fragrance (Aro) and number in [ ] represents minimum and maximum data

[b] *, ** significant difference at 0.05 and 0.01 respective, ns not significant difference

3.2 The Grain Qualityof the F_5 RILs

There were distinct differences between the parents for AC, GC and aroma and no difference was observed for ASV. All F_5 RILs and parents had a score of 7 for ASV indicating low gelatinizing temperature. All F_5 RILs are glutinous rice andthe mean AC and GC were 5.6±0.1%and 120±2.2 mm respectively, indicating similar characters with the glutinous parent RGD334 (AC: 5.7% and GC: 129.3 mm). All F_5RILs are aromatic. Twelve F_5 RILsexhibited strong aroma (++) and eight F_5 RILs were slighty aromatic (Table 2.).These results indicated high accuracy of using gene markers [15, 19, 21]. The gene specific marker Wx-Glu23 was used for the first time in rice breeding program to select for glutinous genotype [7, 25]. It showed very high accuracy in selection. All F_5 RILs are glutinous with similar AC and GC to that of the glutinous parent. GC was found closely linked with *WX* gene on chromosome 6 [20, 26] that probably explain the GC obtained in all F_5 RIL are similar to RGD334.

3.3 Agronomic Performance of the F_5 RILs

The eight measured agronomic traits of the parents and F_5 RILs are shown in Table 3. Significant differences among the tested lines were observed for the NGP, GL, GTN and GL/W. The NGP, GL, GTN and GL/W of the F_5 RILs ranged from 61 to 115, 9.7 to 10.5 mm, 1.80 to 1.96 mm and 3.49 to 4.04 respectively.GL and NGP are important characteristics for rice improvement in which these characters easily benefit from the PTS in this study. Most of the F_5 RILs have higher values for GL and NGP than the parents. The GL and NGP are higher than parents because PTS was done three times and the RILs were homozygous for these traits. GL were reportedly controlled by many genes/QTLs such *GS3, qGL3, qGL10 qGL11* and *TGW6*[27]. It could be that the combination of these genes increased GL in F_5 RILs compared with the parent.

Table 3. Agronomic performance and yield components of the elite F_5 lines and check varieties.

	Agronomic performance [a, b]							
Accession	DF	TN	NPP	NGP	GW	GL	GTN	GL/W
F_5	84	11	6	89.1	2.60	9.88	1.86	3.82
	[73-93]	[8-13]	[5-8]	[61-115]	[2.47-2.82]	[9.18-10.50]	[1.80-1.96]	[3.49-4.08]
RGD07334	83	12	5	89.3	2.55	9.44	1.85	3.71
RGD334	79	9	5	72.4	2.67	9.68	1.87	3.62
P-value	ns	ns	ns	*	ns	**	*	*
5%LSD	7	5	4	26.3	0.29	0.36	0.08	0.35
Mean	84	11	6	88.4	2.61	9.82	1.86	3.77
Standard error (SE)	1.7	0.5	0.4	4.7	0.03	0.1	0.01	0.1
% CV	5.0	27.6	41.4	17.7	6.5	2.2	2.6	5.5

[a]Data on germination to 50% flowering (DF), Number of tillers per plant (TN), Number of panicles per plant (NPP), number of grains per panicle (NGP), Grain width (GW), Grain length (GL), Grain thickness (GTN), Grain length-width ratio (GL/W) and number in [ ] represents minimum and maximum data

[b] ** represent significant difference at 0.01, ns represent not significant difference

The development of glutinouse rice varieties with multiple traits including submergence tolerance, blast resistance and cooking quality is achieved through the use of MAS in this study. It is the first report on a successful MAS technology in developing new aromatic glutinous rice variety with multiple traits in Thailand. There are several reports on the use of MASin assembling new rice varities with multiple traits, such as Ruengphayak *et al.* [21], Luo and Yin [28], Wan *et al.* [29], and Luo *et al.*[30]. All of these success breeding efforts support the potential of MAS in achieving a substaintial impact on rice improvement that will play key role in increasing world food production. However, most of the published success use of MAS in plant breeding relates to the introgression or pyramiding of large QTL. Minor QTL are most of the time affected by the environment and therefore their effects has not been combined realistically by means of marker assisted selection.

4. Conclusions

Intergrated MAS and PTS increased the chance for progenies to carry target genes/QTL along with minor effect QTL controlling the traits. All F_5 RIL lines are glutinous having a high level of blast resistance, outstanding submergence tolerance and strong grain aroma. This study confirmed that the intergrated MAS and PTS approach effectively provide opportunities for breeders to pyramid genes/QTL in developing better rice cultivars. The new aromatic glutinous rice lines in this study may be released as a new glutinous variety suitable for the rainfed lowland in Thailand.

Acknowledgments

We are grateful to Cluster Program Management of the National Science and Technology Development Agency and the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand and Generation Challenge Program for funding this project.The Thailand Graduate Institute of Science and Technology (TGIST), NSTDA and the Faculty of Agriculture, Ubon Ratchathani University are acknowledged for the scholarship rendered to the author. This research was conducted at the Rice Gene Discovery Laboratory a researh unit of BIOTEC at Kasetsart University, Thailand. We thank the staff and graduate students of Rice Gene Discovery Laboratory for their technical support in the laboratory, greenhouse and field activities. We are also thankful to Faculty of Agriculture at Kamphaeng Saen, Kasetsart University.

References

[1] Olsen K.M. and Purugganan M.D., *Genetics*, 2002; **162**: 941-950.

[2] Toojinda T., Tragoonrung S., Vanavichit A., Siangliw J.L., Pa-In N., Jantaboon J., Siangliw M. and Fukai S., *Plant Prod. Sci.*, 2005; **8**: 330-333.

[3] Jena K.K. and Mackill D.J., *Crop Sci.*, 2008; **48**: 1266-1276.

[4] Mackill D.J., Nguyen H.T. and Zhang J.X., *Field Crops Res.*, 1999; **64**: 177-185.

[5] Hu J., Cheng M., Gao G., Zhang Q., Xiao J. and He Y., *Pest Manag. Sci.*, 2013; **69**: 802-808.

[6] Tan Y.F., Li J.X., Yu S.B., Xing Y.Z., Xu C.G. and Zhang Q., *Theor. Appl. Genet.*, 1999; **99**: 642-648.

[7] Wanchana S., Toojinda T., Tragoonrung S. and Vanavichit A., *Plant Sci.*, 2003; **165**: 1193-1199.

[8] Sreewongchai T., Toojinda T., Thanintorn N., Kosawang C., Vanavichit A., Tharreau D. and Sirithunya P., *Plant Breed.*, 2010; **129**: 176-180.

[9] Manivong P., Korinsak S., Korinsak S., Siangliw J.L., Vanavichit A. and Toojinda T., *Thai J. Genet.*, 2014; **7**: 110-122.

[10] Wongsaprom C., Sirithunya P., Vanavichit A., Pantuwan G., Jongdee B., Sidhiwong N., Siangliaw J.L. and Toojinda T., *Field Crops Res.*, 2010; **119**: 245-251.

[11] Xu K. and Mackill D.J., *Mol. Breed.*, 1996; **2**: 219-224.

[12] Toojinda T., Siangliw M., Tragoonrung S. and Vanavichit A., *Ann. Bot.*, 2003; **91**: 243-253.

[13] Siangliw M., Toojinda T., Tragoonrung S. and Vanavichit A., *Ann. Bot.*, 2003;**91**: 255-261.

[14] Xu K., Xu X., Fukao T., Canlas P., Maghirang R.R., Heuer S., Ismail A.M., Serres J.B., Ronald P.C. and Mackill D.J., *Nature*, 2006; **442**: 705-708.

[15] Jantaboon J., Siangliw M., Im-mark S., Jamboonsri W., Vanavichit A. and Toojinda T., *Field Crops Res.*, 2011; **123**: 206-213.

[16] Ahn S.N., BollichC.N. and Tanksley S.D., *Theor. Appl. Genet.*, 1992; **84**: 825-828.

[17] Bradbury L.M.T., Fitzgerald T.L., Henry R.J., Jin Q.and Waters D.L.E., *Plant Biotechnol. J.*, 2005; **3**: 363-370.

[18] Shi W., Yang Y., Chen S. and Xu M., *Mol. Breed.*, 2008; **22**: 185-192.

[19] Yi M., Nwe K.T., Vanavichit A., Chaiarree W. and Toojinda T., *Field Crops Res.*, 2009; **113**: 178-186.

[20] Lanceras J.C., Huang Z.L., Naivikul O., Vanavichit A., Ruanjaichon V. and Tragoonrung S., *DNA Res.*, 2000; **7**: 93-101.

[21] Ruengphayak S., Chaichumpool E., Phromphan S., Kamolsukyunyong W., Sukhaket W., Phuvanartnarubal E., Korinsak S., Korinsak S. and Vanavichit A., *Rice*, 2015; **8**: DOI 101186/s12284-014-0035-0.

[22] Kate-ngam S., Riabroy K., Toojinda T.and Kotchasatit U., *Proceedings of BIT's 2nd World DNA and Genome Day-2011: Reopen Bio-Gateway in Green Economy Era*, Dalian World Expo Center, Dalian, China, 25-29 April 2011; 286.

[23] Juliano B.O., *Rice: Chemistry and Technology American Association of Cereal Chemists*, 1985; St Paul, MN.

[24] Septiningsih E.M., Sanchez D.L., Singh N., Sendon P.M., Pamplona A.M., Heuer S. and MackillD.J., *Theor. Appl. Genet.*, 2012; **124**: 867-874.

[25] Prathepha P., *Pak. J. Biol. Sci.*, 2007; **10**: 261-266.

[26] Tian R., Jiang G.H., Shen L.H., Wang L.Q. and He Y.Q., *Mol. Breed.*, 2005; **15**: 117-124.

[27] Gao X., Zhang X., Lan H., Huang J., Wang J. and Zhang H., *BMC Plant Biol.*, 2015; **15**: 156. DOI 101186/s12870-015-0515-4.

[28] Luo Y. and Yin Z., *Mol. Breed.*, 2013; **32**: 709-721.

[29] Wan B., Zha Z., Li J., Xia M., Du X., Lin Y. and Yin D., *Euphytica*, 2014; **195**: 129-142.

[30] Luo W., Guo T., Yang Q., Wang H., Liu Y. and Zhu X., *Mol. Breed.*, 2014; **34**: 805-815.