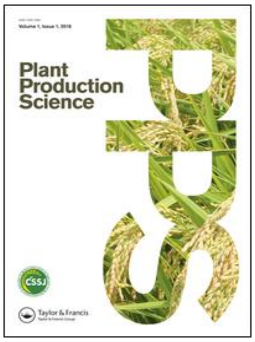

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Molecular Breeding for Rainfed Lowland Rice in the Mekong Region

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Abstract : In the past 20 years, the rice-breeding program in Thailand had little success in developing new cultivars to replace Kao Dawk Mali 105 (KDML105) and Kao Khor 6 (RD6) for the rainfed lowland rice environments. The main reason for the poor adoption of new cultivars by farmers is the susceptibility to diseases and unacceptable grain qualities. The conventional breeding program also takes at least 15 years from initial crossing to the release of new cultivars. A new breeding strategy can be established to shorten the period for cultivar improvement by using marker-assisted selection (MAS), rapid generations advance (RGA), and early generation testing in multi-locations for grain yield and qualities. Four generation of MAS backcross breeding were conducted to transfer genes and QTL for bacterial blight resistance (BLB), submergence tolerance (SUB), brown plant hopper resistance (BPH) and blast resistance (BL) into KDML105. Selected backcross lines, introgressed with target gene/QTL, were tolerant to SUB and resistant to BLB, BPH and BL. The agronomic performance and grain quality of these lines were as good as or better than KDML105.

Key words : Grain quality, Marker-assisted selection, Molecular breeding, Rice.

The Mekong region of the Southeast Asia comprises Myanmar, Thailand, Cambodia and Laos and is known as the primary origin of domesticated rice and for high quality rice production. More than 60 % of total rice growing areas in the region is rainfed; drought and submergence and poor soils and biotic stresses are major constrains limiting yield and grain quality. Among them drought stress and blast disease are the most serious constrains. In the past, genetic improvement for such traits has been hampered due to lack of clear understanding of the genetics and of the genotype by environment (G × E) interactions. Biotechnology is now a powerful tool to discover genes governing important traits and for understanding their functions. Establishing the association between such traits and genes or molecular marker can facilitate genetic manipulation via marker-assisted selection (MAS). The rice-breeding program of the Rice Gene Discovery Unit (RGDU) of the National Center for Genetic Engineering and Biotechnology, Thailand was established in 1998. One of the main objectives is to enhance the development of rice cultivars that adapt well to harsh environments of the_rainfed lowlands in North and Northeast Thailand and_that are acceptable by farmers in the region. Marker assisted selection (MAS) in backcross breeding has been used to transfer favorable alleles of genes/QTL for biotic and abiotic stress resistance/tolerance into the genetic background of KDML105 and RD6. We demonstrate here a successful implementation of MAS in the rice-breeding program to improve KDML105 for biotic and abiotic stress resistance/tolerance.

Materials and Methods

Backcross breeding was carried out in four rice populations using KDML105 as the recurrent parent (Fig. 1). The lines FR13A, IR1188, Abhaya and IR68835 were used as donors for submergence tolerance, bacterial leaf blight (BLB) resistance, brown planthopper (BPH) resistance and blast (BL) resistance, respectively. The quantitative trait loci (QTL) approach identified QTL controlling these traits in donor cultivars and the cooking quality in KDML105 as shown in Table 1 and Fig. 1. DNA markers flanking these QTLs were identified and developed (Table 1).T_

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Table 1. DNA markers linked to QTL for traits of interest for the improvement of rice cultivars for the rainfed lowlands of Thailand. The DNA markers were used to select the promising backcross lines in crosses with the recipient parent KDML105.

Trait	Chromosome	QTL name	DNA marker	Reference
Submerge tolerance	9	QTL Sub$_{\text{chr9}}$	RasSAP-R10783Indel	Siangliw et al. (2003)
Bacterial leaf blight resistance	11	Xa 21 locus	PB7/PB8	Chungwongse et al. (1993)
Brown plant hopper resistance	6	QTLBph$_{\text{chr6}}$	RM50	Toojinda et al. (2004)
	12	QTLBph$_{\text{chr12}}$	RM277-RM511	Toojinda et al. (2004)
Blast resistance	2	QTLneckblast$_{\text{chr2}}$	RM211,RM6	Unpublished
Blast resistance	4	QTLneckblast$_{\text{chr4}}$	RM273-RM303	Unpublished
Amylose content	6	Waxylocus	4F-3R	Wanchana et al. (2003)
Gel temperature	6	GT	GT11-RM121	Lanceras et al. (2000)
Gel consistency	6	GC	RM00-RM204	Lanceras et al. (2000)
Aroma	8	Aroma	RGD_Aroma	Vanavichit et al. (2004)

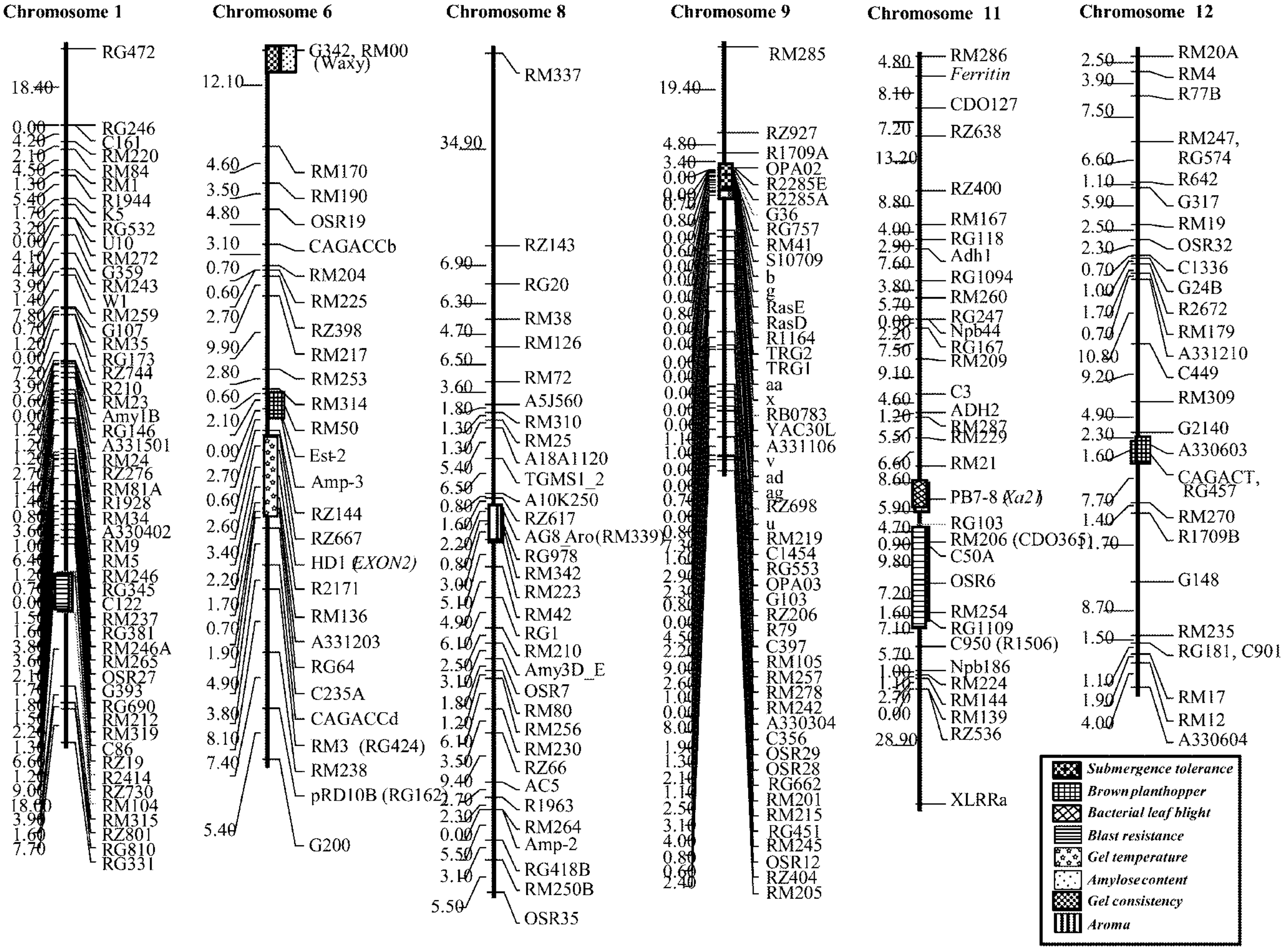

Fig. 1. The genomic location of QTLs corresponding with submergence tolerance, bacterial leaf blight resistance, blast resistance, brown plant hopper resistance, aroma, amylose content (*waxy*), gel consistency and gel temperature in rice.

These markers were used for genotypic fingerprinting of individual plants of the populations. The BC plants containing the target QTL were selected and backcrossed to KDML105 until the BC4F1 was obtained. Selected BC4F1 plants were then self-pollinated to generate BC4F2 and again DNA markers were used for genotypic fingerprinting individual plants. The individuals containing the target QTL and with cooking quality of KDML105 were evaluated for performance of the target abiotic and biotic trait by growing in appropriate environments. The breeding scheme is shown in Fig. 2.

Results

A number of backcross lines, introgressed with the target QTL, were tolerant to submergence (Table 2)

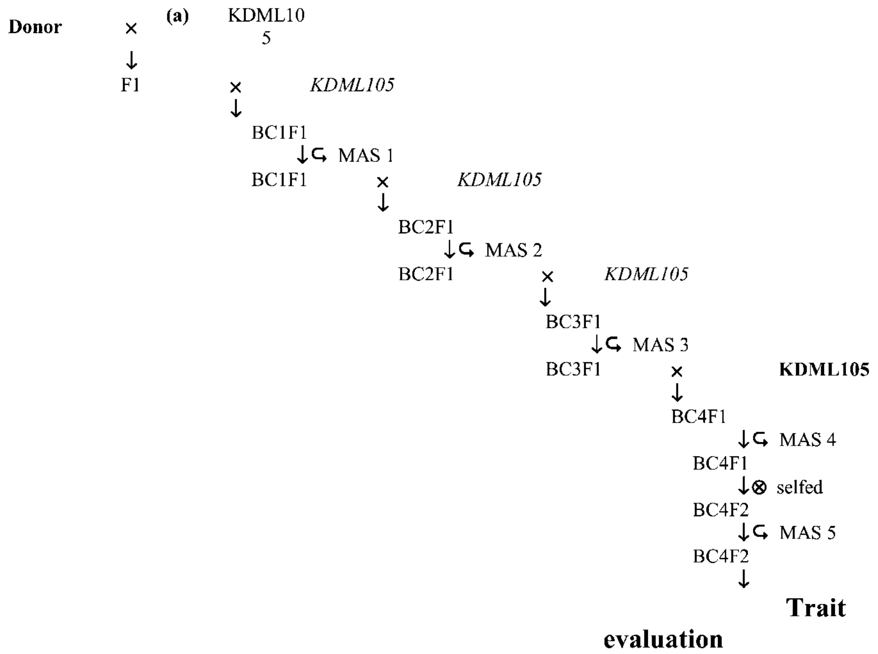

Fig. 2. The breeding and selection scheme using DNA marker assisted selection (MAS) to introduce abiotic and biotic traits into the recipient rice line KDML105.

Table 2. The submergence tolerance (shown as percentage of survived individuals under controlled submergence conditions) and the agronomic performances of backcross lines_selected with QTLSub$_{chr9}$ relative to the agronomic performance and grain quality of KDML105. The "a" – "d" were the DNA marker profile of_the backcrossed introgressed lines as described in Table 1. FR and KD stand for FR13A and KDML105 alleles, respectively.

Pedigree	QTL Sub[a]	Survival (%)	Aroma allele[nb]	Amylose content allele[c]	Gel temperature allele[d]	Days of flowering (days)	Plant height (cm)	Number of tillers m^{-2}	Number of panicles m^{-2}	Spike lets per panicle	Sterility %	Grain yield ($t ha^{-1}$)
873-B-21-12-B	FR	80	KD	KD	KD	90	105	277	96	173	17	6.18
873-B-73-2-B	FR	85	KD	KD	KD	92	118	235	94	180	20	5.79
873-B-1-10-B	FR	70	KD	KD	KD	90	116	259	94	138	12	5.52
873-B-59-6-B	FR	75	KD	KD	KD	92	112	295	97	181	12	5.00
1012-B-37-10-B	FR	85	KD	KD	KD	93	121	333	94	141	11	4.85
1012-B-37-7-B	FR	90	KD	KD	KD	93	122	360	96	139	10	4.64
1012-B-2-9-B	FR	90	KD	KD	KD	90	118	300	95	118	11	4.56
1012-B-53-B-B	FR	75	KD	KD	KD	89	125	263	90	125	13	4.28
1012-B-11-13-B	FR	70	KD	KD	KD	92	119	285	94	143	15	4.26
779-B-53-1-B	FR	80	KD	KD	KD	93	107	267	95	164	17	4.26
FRI 3A (FR)	FR	95	FR	FR	FR	106	112	437	93	127	22	5.75
KDML105 (KD)	KD	0	KD	KD	KD	98	119	304	95	139	16	4.20
PTT (CHECK 1)	-	-	-	-	-	98	96	507	95	146	12	5.37
SPI (CHECK	-	-	-	-	-	98	117	296	92	172	10	6.05

Table 3. The performance of selected backcross lines using QTLBlast$_{chr2}$ and QTLBlast$_{chr4}$ for blast resistance and for agronomic and grain quality traits relative to KDML105 and other check rice varieties.

Blast QTL allele Pedigree	Chromosome 2(RM6)	Chromosome 4	Neck blast infection (%)	Amylose content (%)	Aroma score^a	Days of flowering (days)	Plant height (cm)	Number of panicles m^{-2}	Spike lets per panicle	Sterility %	Grain yield (t ha^{-1})
IR77955-32-66	KD	IR	1	17.73	2	71	119.6	9	9	9.3	4.5
IR77955-8-66	IR	IR	2	17.09	3	69	108.1	10	10	8.7	3.2
IR77955-25-19	KD	IR	2	16.04	3	71	72.3	19	18	12.0	3.4
IR77955-9-56	IR	IR	3	17.27	2	71	118.8	13	13	10.6	3.3
IR77955-26-22	KD	IR	3	16.63	3	71	111.6	17	17	8.6	3.1
IR77955-26-37	KD	IR	3	16.86	3	69	64.0	20	19	15.1	3.2
IR77955-27-12	KD	IR	3	16.18	3	68	115.1	14	13	9.4	4.6
IR77955-12-8	KD	IR	4	16.04	2	70	108.2	13	13	8.6	3.8
IR77955-18-11	H	IR	4	17.27	2	68	104.4	14	14	11.1	3.5
IR77955-5-89	H	IR	4	17.37	3	68	110.3	14	14	9.4	3.8
KDML105 (KD)	KD	KD	46	16.00	3	66	111.5	11	10	14.6	3.7
PTT (CHECK 1)	-	-	0	16.04	-	-	83.1	21	20	11.2	4.5
SPI (CHECK	-	-	0	17.27	-	-	104.3	11	11	14.4	4.5

IR, H and KD stand for IR68835, heterozygous and KDML alleles, respectively.

[a] – aroma score (2- moderate fragrance and 3- strong fragrance)

and resistant to BLB, BPH and BL (Table 3). The agronomic performance and grain quality of these lines were as good as, or better than, KDML105.

Conclusions

Marker aided selection (MAS) can be a powerful tool for breeders to improve the traits that are lacking in the widely grown KDML105 and at the same time maintain the grain quality of KDML105. Since selected lines have the same genetic background, pyramiding all QTLs can be done using the MAS approach. This work is being used as a “proof of breeding strategy” for the Mekong Regional project which aims to use MAS for grain quality and drought tolerance. Approximately 15 populations based on crosses between donor lines selected for drought tolerance and recipient lines selected for yield and quality traits of acceptance to farmer/markets are being developed in Thailand, Laos and Cambodia.

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