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SCREENING OF RICE (*Oryza sativa* L.) GENOTYPES WITH LOW AMYLOSE CONTENT BY USING MOLECULAR MARKERS

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SUMMARY

The grain quality improvement of rice is considered as an antecedent research area of rice breeding programs in Vietnam. Amylose content is a key determinant of rice cooking quality. A total of 87 rice germplasm were collected from Cuu Long Delta Rice Research Institute, Vietnam, and clustered on the basis of the phenotypic diversity of amylose content. By using the amylose content of the materials as a quality index, two rice populations (F_2) were selected for molecular analysis. Two SSR markers, namely, WxF-R and RM42, showing an association with amylose content in previous reports were selected. This study demonstrated the feasibility of using primers WxF-R and RM42 for the detection of polymorphisms associated with the amylose content of OM5991/OM4900 and OM5992/OM4900 populations. Based on the quality aspect of the amylose content of the tested populations, three and seven lines belonging to OM5991/4900 and OM5992/OM4900, respectively, showed associations with low amylose content in terms of phenotype and genotype. Therefore, the SSR markers WxF-R and RM42 may be useful in marker-assisted breeding for improving rice genotypes with intermediate and low amylose content.

Keywords: Waxy gene, amylose content, SSR markers, *Oryza sativa* L.

Key findings: A total of 87 rice germplasm were collected from Cuu Long Delta Rice Research Institute, Vietnam, and clustered for the phenotypic diversity of amylose content. By using the amylose content of the materials as a quality index, two F_2 rice populations were selected for molecular analysis. Two SSR markers, namely, WxF-R and RM42, were selected from previous reports. In this study, they showed association with amylose content. Three and seven lines belonging to OM5991/4900 and OM5992/OM4900, respectively, were associated with low amylose content on the basis of phenotype and genotype.

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INTRODUCTION

Rice (*Oryza sativa* L.) is not only one of the most important crops, it is also an essential source of energy for half of the world's population. More than 90% of rice is produced and consumed in Asia (Bhattacharjee *et al.,* 2002). The strategy to improve rice varieties requires the food safety and high quality of agricultural products (Buu, 2004). Quality is considered as the main factor determining the commercial value of agricultural products (Takane *et al.,* 1997, Tong *et al.,* 2019). In recent years, Vietnam's rice production has continuously increased as typically exemplified by the increase in rice exports. However, the value of rice products is low due to the low quality of rice varieties. The goal of improving grain yield and quality, especially rice and nutritional quality, is crucial. Among rice quality properties, the amylose content of rice grain emulsions is the key to high-quality rice and decides the softness or firmness of cooked rice (Pang *et al.,* 2016). Normally, amylose content is affected during seed maturation. Kumar and Khush (1986) observed that rice cultivars are commonly categorized as glutinous/waxy (0%–5% amylose) cultivars or cultivars with low (<20%), intermediate (21%–25%) and high (>25%) amylose contents. The ratio of starch in rice grains also varies depending on variety. The amylose content of *japonica* type varies from 10% to 22% and that of *indica* type usually varies from 20% to 30% (Lang, 2002).

Biotechnology helps improve plant and animal varieties by enabling genome analysis, locating genes on genetic maps, and determining the location of markers associated with genes. The molecular marker selection strategy (marker-assisted selection [MAS]) is effective and is based on the use of markers and polymerase chain reaction (PCR) products, which can be quickly detected. As shown by numerous published results, genetic variation among rice varieties has been detected by using the polymorphism of PCR products (Lang, 2002). Earlier works on rice genetics identified major genes and quantitative trait loci (QTL) responsible for the amylose content of rice endosperm (Zhang *et al*., 2019). These QTL are present on chromosomes 5 and 6, and *waxy*, the major gene that accounts for 91.9% of the total variation of amylose content, is located on chromosome 6 (He *et al.,* 1999). Recently, generating new *indica* or hybrid parent lines with intermediate or low amylose content has become very important. The results of research and the reality of phenotypes have shown that gel consistency is highly related to amylose content (Jenning *et al.,* 1979). Zhou *et al.* (2003) reported the use of MAS to improve the eating and cooking quality of Zhenshan 97 by penetrating the wax gene region from Minghui 63 (Wx-MH), a recovery line with average amylose content. Conventional methods for measuring quality traits are time-consuming and costly. DNA markers provide a useful,

Table 1. Varieties used in this study.

No.	Varieties	No.	Varieties	No.	Varieties	No.	Varieties
1	Khao 105	23	OM6874	45	HG2	67	OM6380
2	IR64	24	OM6625	46	OM6600	68	OMCF39(D)
3	OM5993	25	OMCS2009	47	OM5629	69	OM6035
4	OM6607	26	OM5633	48	OM6063	70	OM6382
5	OM5992	27	OM6625	49	OM6677	71	OM6389
6	OM5338	28	JASMIN85	50	OM6062	72	OM5936
7	OM6619	29	OM6614	51	OM5628	73	OM4668
8	OM5981	30	OMCS2007	52	OM5637	74	OM5799
9	OM6878	31	OM5934	53	OM6379	75	OM5625
10	OM6611	32	OM6624	54	OM6162	76	OM5900
11	OM6064	33	OM6623	55	OM5239	77	HG1
12	OM5991	34	OM2008	56	OM5626	78	OM2395
13	OM6055	35	OM6621	57	OM6381	79	OM5930
14	OM5798	36	OM5756	58	OM5634	80	OM6683
15	OM6613	37	OM6616	58	OM6599	81	OM4244
16	OM5240	38	OM6864	60	OM6378	82	OM6878
17	OM6615	39	OM6620	61	OM5636	83	OM4900
18	OM6608	40	OM5790	62	OMCS2000	84	OM5240
19	OM5704	41	OM576	63	OM2718	85	OM5636
20	OM6843	42	OM5703	64	OM6877	86	OM6035
21	OM6612	43	OM6599	65	OM6073	87	OM5981
22	OMCS2007	44	OM6879	66	OM6074		

easy, and effective way to identify plants with the desired amylose content (Tabkhkar *et al.,* 2012). Therefore, this study was conducted to detect suitable DNA markers that are closely linked to amylose traits to facilitate rice breeding.□

MATERIALS AND METHODS

Plant material

The first trial was conducted with 87 rice varieties from Cuu Long Delta Rice Research Institute, Vietnam, in a randomized complete block design with three replications (Table 1). The trait of amylose content was used for cluster analysis via the UPGMA method.

Scheme for crossing

Five rice parents, namely, OM5951, OM5952, OMCS2000, OM2718 (high yielding), and OM4900 (low amylose content) were crossed to obtain four single crosses by following the scheme OM 5951/OM 4900; OM 5952/OM 4900; OMCS 2000/OM 4900; and OM 2718. The first generation of F1 progeny was self-pollinated to produce populations of 280 F2 of each combination.

Estimation of genetic advance

Genetic advance (GA) was estimated by using the formula $GA = i \times h^2 \times s^2p$, where i (selection indexes), s^2p (phenotypic variance), and h^2 (broad-sense heritability) are in accordance with the values reported by Singh and Chaudhary (1985).

Analysis of amylose content

Amylose content was estimated by using the procedure of Sadasivam (1992). A total of 100 mg of rice flour was extracted overnight in a solution of 1 mL of absolute ethanol and 10 mL of 1 N NaOH. This suspension was heated in a boiling water bath for 10 min, followed by cooling to room temperature. Samples were diluted to 100 mL with distilled water. A total of 2.5 mL of the sample suspension was transferred to a 100 mL flask, and 1 mL of 1 M acetic acid along with 1 mL of idionic solution (0.2% I2 in 2% KI) was added to acidify the sample. The final volume was made up to 100 mL with distilled water, and the suspension was mixed thoroughly and then kept for 15 min. As a control, 5 mL of 1N NaOH solution was used to replace the sample suspension. Absorbance was read at 590 nm with a spectrophotometer. Samples with known high, medium, and low amylose values were used to draw standard amylose curves

DNA extraction

DNA from rice leaves was extracted in accordance with the modified CTAB method described by Lang (2002). DNA quality was checked through 0.9% agarose gel electrophoresis in TAE 1×.

SSR marker and PCR amplification

Two SSR markers, namely, Wx F-R and RM42, were used in this study (Lang and Buu, 2004; Gour *et al*., 2017). All markers have been previously mapped to chromosome 6 and are closely linked to the QTL controlling amylose content (Table 2). PCR was performed in a volume of 20 µL with the final dNTP concentration of 100 µM, primer concentration of 0.25 µM, Taq polymerase concentration of 2.5 unit/20 µL, and 1 µL of DNA (1.25 ng/µL). PCR reactions were denatured at 94 °C for 5 min and then subjected to 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The final extension was set at 72 °C for 5 min. PCR products were electrophoresed on 3% agarose gel for 1 h and 15 min on the basis of the procedure given by Lang (2002).

Statistical analysis

Orthogonal comparisons of parental genotypes, parental versus population lines, and simple and combination variance analysis (ANOVA) were performed by using SAS 9.0 software. Broad-sense heritability was calculated by following the method given by Wang *et al*. (2007). Cluster analysis was carried out to derive the genetic distance matrix based on amylose content via the UPGMA clustering method in NTSYS program (Rohlf, 1997).

RESULTS

Phenotypic clustering of rice germplasm

The 87 rice varieties used in this study clustered into three main groups (Figure 1). Only one variety (OM2008) in Group I had very low amylose content. Varieties in Group II were divided into four subclusters. The majority of the varieties belonged to subclusters II-1 (16 varieties), II-2, and II-3 with high amylose contents (>25%). In the group II subcluster,

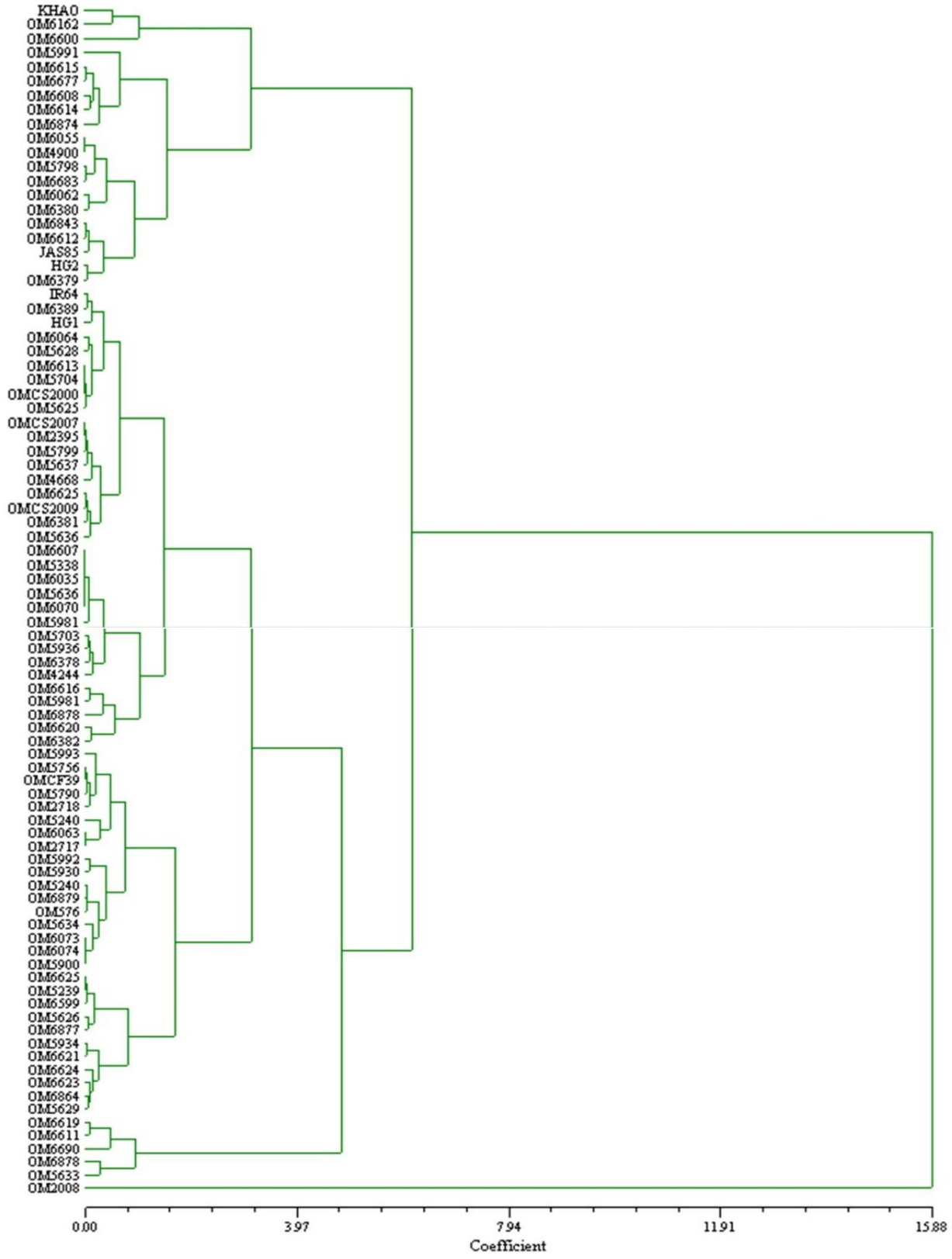

Figure 1. Cluster analysis of the amylose contents of 87 rice varieties.

Table 2. DNA sequence information of the SSR markers used in this study (Lang and Buu, 2004; Gour *et al*., 2017).

No.	Marker	Primer sequence	Chromosome	Size (bp)
1	Wx F-R	CTTTGTCTATCTCAAGACAC-	6	210–220
2	RM42	TTGCAGATGTTCTTCCTGATG- ATCCATCCGCTGACCATGAG TTTGGTCTACGTGGCGTACA	5	200–250

subclusters II-4 and II-5 had average amylose contents of 22%–23% (18 varieties) and 23%–24% (18 varieties), respectively. Group III varieties were found to have medium-to-low amylose contents that ranged from 16% to 20% (20 varieties).

Heritability of amylose content

The genetic variance and the broad-sense heritability estimated from four cross combinations were high and significant (Table 3). The high heritability (broad sense) showed that for this generation of hybrid combinations, the target trait was mainly controlled by internal genetic factors.

Effect of selection for the amylose content of F_2 populations

The estimated genetic parameters of the individuals of the three populations (OM5952/OM4900 [1], OM5951/OM4900 [2], OMCS2000/OM4900 [3]) with low, medium, and high amylose contents are shown in Table 4. Low amylose content was selected with low efficiency and low genetic coefficients in OM5991/OM4900 and OM5992/OM4900 populations, demonstrating that the fluctuation in amylose content was affected by environmental factors. In the group selected for high amylose content (hard), the OM5992/OM4900 population showed a low heritability (H^2bs = 0.39) value, indicating the influence of environmental factors on selection. The high heritability values (H^2bs) of the selected groups ranged from 0.61 to 0.78 for high and medium average amylose contents and indicated that amylose content was also determined by internal genetic factors.

χ^2 test for the genetic traits of the amylose content of F_2 populations

The results presented in Table 5 showed that high and low amylose traits segregated in the F2 generation in accordance with Mendel's law, with low amylose content expressed as a recessive trait. The segregation ratio of the high and low amylose contents of the OMCS2000/OM4900 cross was 2:1, indicating that amylose content was also affected during grain maturation under the effect of weak external environmental factors.□

Evaluation of molecular markers associated with amylose content

Amylose content is regulated by the waxy gene. The linked markers reported in this study were applied to select the amylose trait in breeding programs and to look for signs of strong association with this character. Also, they can be used as a basis for selecting the flanking markers of

Table 3. Heritability estimates of the amylose contents of F_2 populations.

	No. Crossing	σ^2_g	H_{bs}
1	OM 5991/OM 4900	0.813725**	0.929426
2	OM 5992/OM 4900	0.646237**	0.893920
3	OMCS 2000/OM 4900	1.149731**	0.947442
4	OM 2718/OM 4900	0.210541**	0.753317

σ^2_g; genetic variance; $H_{bs:}$ broad-sense heritability

Table 4. Estimates of the genetic parameters of the amylose contents of three populations of rice.

Traits	Cross	Mean AC (%)	SD	PCV (%)	GCV (%)	H^2bs	GA (%)
Low	1	19.2	0.55	20.11	8.85	0.19	2.998
	2	18.1	0.23	21.60	9.70	0.20	3.237
	3	18.3	0.15	29.08	23.36	0.65	14.130
Medium	1	22.4	0.32	34.98	30.85	0.78	25.660
	2	23.6	0.23	29.10	22.3	0.59	14.650
	3	22.7	0.14	35.81	31.05	0.75	25.570
High	1	26.6	0.20	23.78	18.50	0.61	13.070
	2	26.6	0.12	21.50	13.40	0.39	7.601
	3	26.9	0.11	24.27	19.20	0.62	13.84

PCV: Phenotypic coefficient variation; GCV: Genotypic coefficient variation; H^2bs: broad-sense heritability; GA: Genetic advance; AC: Amylose content. Crosses 1, 2, and 3 are OM 5991/OM 4900, OM 5992/OM 4900, and OMCS 2000/OM 4900, respectively.

Table 5. Segregation of the amylose content phenotype in F_2 populations.

No.	Crossing	Observed		Expected		Ratio	χ2
		High	Low	High	Low		
1	OM5991/OM4900	23	6	21	8	3:1	0.69
2	OM5992/OM4900	40	14	37	17	3:1	0.77
3	OMCS2000/OM4900	100	56	106	50	2:1	1.06

chromosome regions to find markers that are tightly linked with the desirable trait. In this study, two primers designed on the basis of the published nucleotide sequence of the *waxy* gene available from GenBank under the accession number AF031162 were used to amplify the *waxy* gene. Lang and Buu (2004) have reported this pair of primers previously.

The primer pair WxF-R produced amplification products for 90% of the total samples tested from the cross OM5991/OM4900 (Figure 2). Two clear alleles were detected in this marker position, and, on the basis of band size, the alleles were recorded as the A allele (250 bp) and B allele (size 245 bp). Between the parents of this cross, the male variety OM4900, which had low amylose content, expressed allele B, and the female variety OM5991, which had average amylose content, expressed allele A. Lanes 3 and 4 in Figure 2 indicated

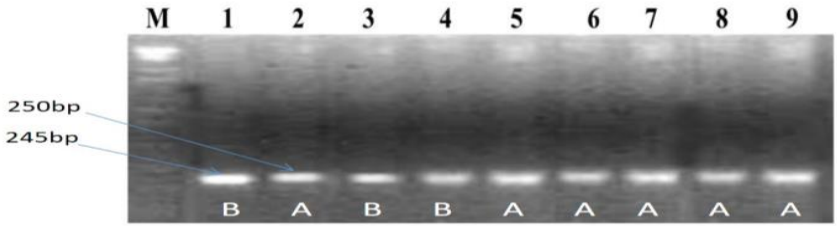

Figure 2. PCR products of the OM5991/OM4900 F_2 population with primer Wx. M: marker (ϕX 174 *Hae* III), lane 1: OM4900 (B), lane 2: OM5951 (A), lanes 3, 4: similar to B, lanes 5–9: similar to A.

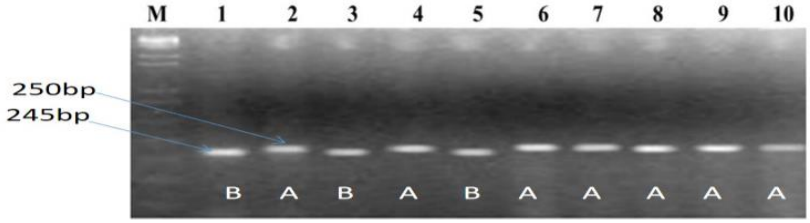

Figure 3. PCR product of the OM5992/OM4900 F_2 population with primer Wx. M: marker (ϕX 174 *Hae* III), lane 1: OM4900 (B), lane 2: OM5992 (A), lanes 3, 5: similar to B; lanes 4, 6, 7, 8, 9, 10: similar to A.

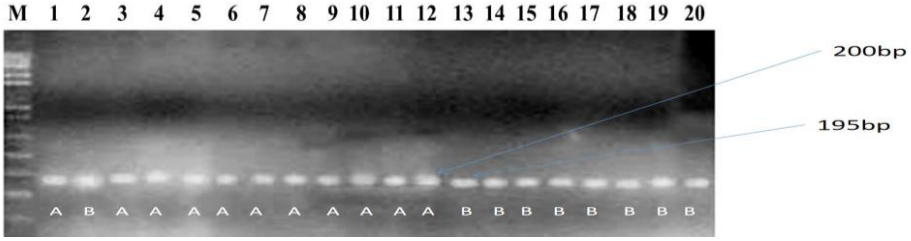

Figure 4. PCR product of the OM5992/OM4900 F_2 population with primer RM 42. M: marker (ϕX 174 *Hae* III), lane 1: OM5992 (A), lane 2: OM4900 (B), lanes 3–12: similar to A, lanes 13–20: similar to B.

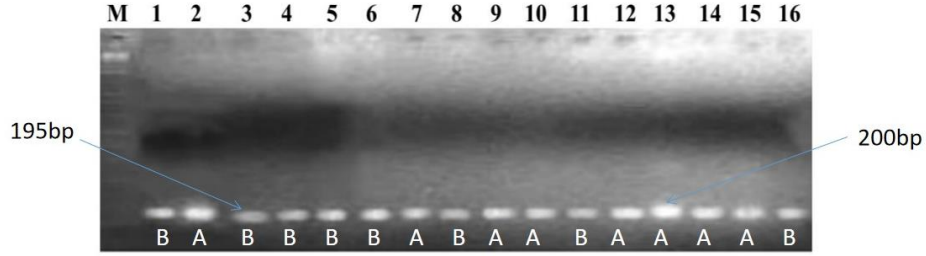

Figure 5. PCR product of the OM5991/OM4900 F_2 population with primer RM 42. M: marker (ϕX 174 *Hae* III), lane 1: OM4900 (B), lane 2: OM5991 (A), lanes 7, 9, 10, 12, 13, 14, 15: similar A, lanes 3, 4, 5, 6, 8, 11, 16: similar B.

the presence of allele B and thus an amylose content that was as low as the amylose content of OM4900. Figure 3 shows a sample of the banding pattern obtained for the cross OM5992/OM49000 for the same two alleles based on the band size of OM4900. Low amylose content corresponded to allele B, and OM5992 with high amylose content corresponded to allele A. Thus, lanes 3 and 5 indicated low amylose content that was similar the amylose content of OM4900.□

The RM42 primer produced amplified products for 90% of the total tested samples. On the basis of the size of the bands (Figure 4), alleles A (195 bp) and B (200 bp) were recorded. OM5992, the parental line with high amylose content, exhibited the A allele, and the parental line OM4900, which had low amylose content, presented the B allele. Thus, similar to OM4900, lanes 13–20 (Figure 4) and lanes 3, 4, 5, 6, 8, 11, and 16 (Figure 5) had low amylose content.

Comparison of the amylose content genotypes and phenotypes of F_2 populations

The relationship between the genotype and phenotype of each individual in the population was compared to determine amylose content accurately. We found that in the OM5992/OM4900 population (Table 6), seven lines with low amylose content (<20%) had the B allele at the RM42 locus of OM4900 (200 bp), whereas three lines had low amylose content and the B allele at the WxF-R locus of OM4900 (245 bp). In population OM5991/OM4900, markers RM42 and WxF-R showed that the same three lines (4, 19 and 34) had low amylose content (Table 7).

DISCUSSION

Rice quality is a complex trait that includes many components, such as milling, appearance, nutrition, cooking, and eating quality. Among these qualitative properties, consumers emphasize appearance and eating quality (Huang *et al*., 1998; Wan *et al.,* 2004). The amylose content of rice grain largely determines the cooking and eating quality of rice (Lin *et al.,* 2011).

The diversity analyses of the germplasm collections of several crop species have revealed considerable variability for a wide range of traits (Yang *et al.,* 1991). The diverse range of phenotypic traits has proven to be a useful tool in the classification of plants, and the information obtained can be utilized by plant breeders in the development of plant species with desirable agronomic and nutritional qualities (Maduakor and Lal, 1989). In this study, the genetic clusters of the rice varieties were distinguished as high, average, and low amylose content groups (Figure 1). OM2718 and OM5992 were clearly included in the high amylose content group, and OMCS2000 and OM5991 were included in the average amylose content group. This result suggested that genetic clustering is a useful basis for selecting and using parents in crossing programs. □

Successful changes in traits in a population are obtained through hybridization only if information about the heritability of these traits is known. The heritability of the amylose content in most populations was high

Table 6. Comparison of amylose content with genotype in selected F_2 populations of OM5992/OM4900.

Lines	RM42	Wx	Phenotype	Amylose content	Lines	RM42	Wx	Phenotype	Amylose content
1	A	A	25.9	High	41	A	A	26.6	High
2	A	A	25.6	High	42	A	A	25.2	High
3	-	A	24.2	Medium	43	A	A	25.8	High
4	A	A	26.8	High	44	A	A	27.7	High
5	A	A	26.8	High	45	A	A	26.4	High
6	A	A	26.8	High	46	A	A	27.0	High
7	A	A	27.7	High	47	A	-	27.0	High
8	A	A	26.3	High	48	A	A	27.7	High
9	A	A	26.8	High	49	A	A	27.0	High
10	A	A	25.9	High	50	A	A	27.6	High
11	A	A	28.8	High	51	-	A	23.0	Medium
12	B	B	18.5	Low	52	B	B	17.4	Low
13	-	A	23.8	Medium	53	-	A	22.7	Medium
14	A	A	27.7	High	54	A	A	27.0	High
15	A	A	27.1	High	55	A	A	22.7	Medium
16	A	A	21.5	Medium	56	A	A	26.5	High
17	A	-	25.8	High	57	A	A	25.9	High
18	A	A	24.8	Medium	58	A	A	25.5	High
19	-	A	21.7	Medium	59	-	A	24.6	Medium
20	A	A	24.8	Medium	60	A	A	27.7	High
21	A	A	27.7	High	61	A	A	26.7	High
22	A	A	22.8	Medium	62	B	B	18.9	Low
23	A	A	24.7	Medium	63	A	A	24.4	Medium
24	-	A	23.8	Medium	64	A	A	25.8	High
25	A	A	26.0	High	65	-	A	23.8	Medium
26	A	A	26.8	High	66	A	-	27.0	High
27	A	A	26.3	High	67	A	A	22.6	Medium
28	A	A	24.4	Medium	68	A	A	25.6	High
29	A	A	24.4	Medium	69	B	B	17.7	Low
30	B	B	19.6	Low	70	A	-	25.0	High
31	B	B	17.5	Low	71	A	A	26.9	High
32	A	-	22.4	Medium	72	A	A	27.4	High
33	A	A	24.8	Medium	73	B	B	18.5	Low
34	A	A	23.9	Medium	74	A	A	23.8	Medium
35	A	-	26.0	High	75	A	-	25.3	High
36	A	A	25.4	High	76	A	A	24.9	Medium
37	A	A	27.0	High	77	A	A	27.0	High
38	A	A	25.3	High	78	A	A	25.9	High
39	A	A	27.7	High	79	A	A	25.5	High
40	A	A	26.0	High	80	B	-	18.7	Low

Note: A: homozygous recipient allele; B: homozygous donor allele

Table 7. Comparison of amylose content with genotype in selected F_2 populations of OM5991/OM4900.

Lines	RM42	Wx	Phenotype	Amylose content	Lines	RM42	Wx	Phenotype	Amylose content
1	A	A	21.6	Medium	28	A	A	23.6	Medium
2	A	A	27.6	Medium	29	A	A	23.6	Medium
3	A	A	21.6	Medium	30	B	-	18.6	Low
4	B	B	18.7	Low	31	B	-	28.6	High
5	A	A	26.6	High	32	A	A	21.6	Medium
6	A	A	22.6	Medium	33	A	A	26.6	High
7	A	A	25.6	High	34	B	B	18.6	Low
8	A	A	21.6	Medium	35	A	A	27.9	High
9	A	A	25.6	High	36	A	A	20.6	Medium
10	A	A	23.6	Medium	37	A	A	20.6	Medium
11	A	A	26.6	High	38	A	A	20.6	Medium
12	A	A	20.6	Medium	39	A	A	20.6	Medium
13	A	A	24.6	Medium	40	A	A	27.7	High
14	-	A	28.6	High	41	A	A	21.7	Medium
15	A	A	20.6	Medium	42	A	-	26.3	High
16	A	A	28.6	High	43	A	A	24.6	Medium
17	A	A	18.6	Low	44	A	A	25.7	High
18	A	A	22.6	Medium	45	-	A	25.1	High
19	B	B	19.6	Low	46	A	A	25.2	High
20	A	A	21.6	Medium	47	-	A	26.7	High
21	A	A	21.6	Medium	48	A	-	27.6	High
22	-	A	26.3	High	49	A	A	24.6	Medium
23	A	A	22.6	Medium	50	A	A	25.7	High
24	A	A	26.6	High	51	A	-	26.7	High
25	A	A	23.6	Medium	52	A	A	25.7	High
26	A	A	24.8	Medium	53	A	A	24.8	Medium
27	A	A	25.6	High					

Note: A: homozygous recipient allele (OM5991); B: homozygous donor allele (OM4900)

(Table 3). Breeding for traits with low h^2 (<0.2) is difficult because a low h^2 value indicates that the phenotype is not highly correlated with the genotype. In other words, the contribution of environmental conditions to these traits is relatively high (Singh, 2005). The low h^2 value of the OM5992/OM4900 population showed the high environmental effect on this trait affected the efficiency of selection for amylose content.

The results of the analysis of the selective efficiency of amylose traits indicated that the genetic parameters of the characteristics of non/low amylose content were affected by genetic and external environmental factors (Table 4). In addition, the trait of high amylose content is not completely determined genetically against that of low amylose content because it is controlled by a gene with several modifiers (an auxiliary gene with an improved nature). This observation is consistent with the conclusions of Seetharaman (1959), Kahlon (1965), and Heu and Park (1976).

Amylose content is known to be regulated secondarily by the *waxy* gene. Recently, generating new *indica*

rice varieties with intermediate or low amylose contents has become crucial. In this study, we used previously developed SSR markers (Wx F-R), which are linked to the Wx-T allele of good-quality rice cultivars with intermediate amylose contents, to derive the key maintenance lines of the OM5992/OM4900 and OM5991/OM4900 populations. The linked markers reported in this study were applied to select the amylose trait in breeding programs and to look for signs of strong association with this character. Also, they could be used as the basis for selecting the flanking markers of chromosomal regions to find markers that are closely associated with the desired trait.

Several groups have mapped a major QTL for amylose content to the Wx locus region of chromosome six (Li *et al.,* 2004; Fan *et al.,* 2005; Tian *et al.,* 2005). Lang and Buu (2004) analyzed chromosome 6 of parental genotypes with 20 SSR markers. The primer pair for the WxF-R locus used in this study showed an association with amylose content. The amylose content of grain endosperm and pollen is mainly determined by the *Wx* gene (Shure *et al*., 1983). Myint *et al.* (2009) found that the quality traits of 'Manawthukha', which has high amylose content, were improved by the incorporation of the *Wx* gene from Basmati 370. Zhou *et al.* (2003) used MAS in a backcross program to introduce the Wx-MH fragment from Minghui 63 into Zhenshan 97B. The derivation of important maintenance lines by identifying the *Wx* allele conferring low amylose content by using the WxF-R marker from crosses between OM5991/OM4900 and OM5992/OM4900 was the target of the present study.

The polymorphism of the RM42 marker between parents was noted, and crosses were linked to markers showing high and low amylose contents, i.e., high amylose content was linked with a 200 bp allele, and low amylose content was linked with a 195 bp allele (Figure 5). However, similar to other quantitative traits, amylose content varies greatly with the environment and is a multigene trait. Therefore, further analyzing the association of the SSR markers used in this study with amylose content is necessary for the development of QTL markers. RM42 showed that the quality traits of two lines of OM5992/OM4900 and seven lines of OM5992/OM4900 with low amylose content improved. Gour *et al*. (2017), Singh *et al*. (2014), and Lang and Buu (2004) also found that RM42 is associated with amylose content.

Measuring the quality traits (amylose content) of rice line varieties is time-consuming and expensive. Rice breeding programs involve numerous lines, especially lines in the initial generations. However, only a few lines are collected from each strain. Thus, directly measuring the cooking and eating quality of the lines is difficult. Therefore, alternative methods must be suggested. The results of the present research indicated that the use of microsatellite markers linked to grain quality traits, especially markers that are tightly linked to the *Wx* gene, is a possible option for the selection of this character.

CONCLUSION

The results of the selective efficiency analysis of amylose content showed that the two SSR markers used in this study can be applied for the efficient selection of a major locus for amylose content. The microsatellite markers WxF-R and RM42 can be used to trace the flow of genes or quantitative traits that determine rice quality and predict the results of crossing programs. At the same time, the selections identified in this study will increase the efficiency of cultivar development in the future.

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