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The application of Gene Function Markers for identification of high quality of Vietnamese traditional rice varieties

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ABSTRACT

In today's growing economy, consumer demand for rice is evolving, with a preference for high-quality varieties that are soft and aromatic when cooked. We conducted a study to identify high-quality rice varieties that meet these consumer preferences. Molecular markers were used to find genes linked to desirable traits in rice. These genes are DRR-GL for grain length (gene GS3), Wxin1 for amylose content (gene Wx), and BADH2 for aroma. The findings revealed that the molecular marker DRR-GL, used with primers EFP, IRSP, ERP, and IFLP, accounted for 90% of the expression of the long-grain phenotype. Similarly, the Wxin1 marker, with primers GF, TR, GR, and TF, explained 90% of the variation in amylose content. The BADH2 marker, using primers ESP and IFAP, INSP, and EAP, accounted for 100% of the aroma phenotype expression across all tested varieties. Among the 20 traditional rice varieties examined, the variety Nep Trong Vo exhibited superior qualities, including an elongated grain length of 7.13 mm, an average amylose content of 17.58%, a soft gel consistency graded at 3, and high heat resistance also graded at 3. These characteristics make Nep Trong Vo a promising candidate for meeting the high-quality standards demanded by consumers.

1. INTRODUCTION

Rice is an important crop in the Mekong Delta. Rice cultivation is not only the main source of food supply but also the livelihood source for the majority of the people. The Mekong Delta region produces more than 50% of national product and more than 90% rice export volume of the country. According to Raghavan et al. (2019), Viet Nam is warned as one of the countries that will be most affected by climate change, and the Mekong Delta is expected, as a key food production area of the country will bear the heaviest consequences when climate change occurs. According to Stanford University, by 2030, food production in Asia will decrease 10% or more, especially rice productivity and output are always threatened by natural disasters, pests, and environmental factors (Toriyama et al., 2005). According to Viet Nam 2021 statistics, the rice-growing area by the end of the first quarter (mid-March) is estimated at 891.5 thousand hectares, down 27,5% over the same period; average yield is estimated at nearly 66.9 quintals/ha, an increase of about 1.0 quintal/ha, the output is 5.964 thousand tons, down 26.4% (General Statistics Office Viet Nam, 2021). However, low-quality rice and rice varieties have limited exports, so Vietnamese quality rice brands have not been popular on the international market. As a result, in order to create new, high-quality rice varieties, it is

essential to look for traditional rice germplasm for high-quality rice variations.

Especially in recent years, the two varieties ST24 and ST25 respectively have achieved high prize in international competitions. ST24 made an impressive achievement when it won the World Top 3 Best Rice award held in Macao, China by The Rice Trader Organization in 2017, creating the premise for the title of World's Best Rice at the 11th World's Best Rice Contest held in Manila (Philippines) in 2019 of ST25 rice variety. These two rice varieties shared the characteristics of high-quality cooked rice, including a low amylose content, long grains, and a pleasant aroma. But in fact, rice production is heavily dependent on agrochemicals, degraded soil, disease development. Therefore, the output quality of this rice variety products is not high, so Viet Nam's quality rice brand is still precarious in the international market.

In recent years, advancements in technology have significantly enhanced the identification of genes through sequencing and genetic mapping via the application of molecular markers. These methods have elucidated much of the genetic expression in various plant species, particularly in rice (Oryza sativa L.). One commonly used technique is the application of Simple Sequence Repeats (SSR), also known as microsatellites. SSRs are short DNA sequences, typically ranging from 2 to 6 nucleotides in length, with consecutive repeat sequences that can vary from 2 to 40 repeats (Raza et al., 2023). This method has proven to be highly effective in genetic studies and breeding programs for rice. To be more precise, gene function markers have been developed to be applied to crop breeding programs. According to the research of Zhou et al. (2017), the length of grain rice, which was regulated by gene *GS3*, was long in grain size with the mutation C > T in exon 2 of gene *GS3*. In addition, the content of amylose and the grade of long-grain rice were two characteristics that determined rice quality (Cai et al., 1998). According to the results, seven positions in the gene *GBSSI* were altered, which affected the amylose content of rice (Zhang et al., 2019).

In addition, the aroma of rice was one of the criteria for selecting a quality variety. Compound 2-acetyl-1-pyrroline (2-AP) accumulated in the grain of rice will give off a smell like popcorn, and this smell is controlled by the gene *OsBADH2*. This gene lost its ability when it had a mutation in the coding regions that helped in the synthesis of 2-AP (Bradbury et al., 2005). Following practical needs combined with the development of science, in this research, genes *GS3*, *Waxy*, and *OsBADH2* were three genes related to quality characteristics used to find quality traditional rice varieties for the purpose of breeding quality varieties.

2. MATERIALS AND METHODS

2.1. Materials

For this experiment, we used seeds of twenty traditional rice varieties, which are stored in a gene repository at the Agricultural College of Can Tho University. The majority of these samples, which have been collected in the Mekong Delta since the 1980s, are currently being discontinued. In order to provide genetic material for rice breeding programs, this study reevaluated the genetic resource. In this study, ST25 was used as a control for aromatic rice varieties.

Table 1. The list of 20 traditional rice varieties was used in research

No	Varieties	Group	No	Varieties	Group
1	Nang dum	Rice	11	Nep than 6	Glutinous rice
2	Nang dum chanh phu	Rice	12	Nep tam sac	Glutinous rice
3	Nang dum do 2	Rice	13	Ngu	Rice
4	Nang nhen 1	Rice	14	Nho thom	Rice
5	Nang ray	Rice	15	Soc sau 2	Rice
6	Nang tay muon	Rice	16	Bang tay nau	Rice
7	Nep mau luon 5	Glutinous rice	17	Huyet rong 4	Rice
8	Nep mua	Glutinous rice	18	Nep trong vo	Glutinous rice
9	Nep ruoi moc	Glutinous rice	19	Nep vo do	Glutinous rice
10	Nep than 3	Glutinous rice	20	Nang co do 2	Rice
			21	ST25 (+ control)	Rice

2.2. Methods

2.2.1. Length grain rice and rice shape

The samples were dehulled. The length, width, and ratio of length to width of ten granules of brown rice (avoid broken samples) were measured and averaged by the electronic ruler. Classifying rice grains according to the IRRI (2013) scale (Table 2).

Table 2. Separated size and shape of rice follow standard of IRRI (2013)

Group	Length grain rice (mm)	Group of grains	Length/width ratio	Shape
1	>= 7,50	Extra long	>3,0	Slender
2	6,61-7,50	Long	2,1-3,0	Medium
3	5,51-6,60	Medium	1,1-2,0	Bold
4	<=5,51	Short	<1	Round

2.2.2. Method of quantitative amylose content

The quantitative measurement of amylose, as initially determined by Juliano (1971) and later refined by Khoomtong and Noohohm (2015), has significantly improved over time. Samples of rice husks were peeled, ground up into a fine powder, measured for moisture content, converted to 12% standard moisture, and weighed. Place 25 mg of finely ground powder (12% moisture) in a 15-mL test tube (100 x 13 mm) and weigh it. Add 0.25 mL of 95% ethanol, shake it well, then add 2.25 mL of 1M NaOH and stir the solution. Boiling the solution mixture in boiling water for 10 minutes, then cool at room temperature. Put all the solution in the test tube into the volumetric flask 25 mL. After filling the solution to the mark, keeping it overnight, 1.25 mL of starch solution is absorbed into a volumetric vial. Adding 0.25 milliliter of 1M acid acetic and 0.5 ml of iod, vigorously shake the mixture. To the volumetric mark, titrate distilled water, agitate vigorously, and store at room temperature for 20 minutes. Collect samples: At 620 nm, absorbance was measured with a spectrophotometer. Based on the resulting standard curve, the absorbance value was converted to amylose content. Evaluation of amylose content using the scale developed by Juliano and Villareal (1993). Finally, document and assess the results. The evaluation of amylose content adheres to the scale developed by Juliano and Villareal (1993) (Table 3).

Table 3. The rating scale amylose content of (IRRI, 2013)

Group	Ratio (%) amylose
Waxy	0-5%
Very low	5.1-12%
Low	12.1-20%
Medium	20.1-25%
High	>25%

2.2.3. Methods of gelatinization temperature of rice

The gelatinization temperature of rice was measured using Graham's (2002) method. Prepare the samples: each sample consists of 18 grains of rice evenly divided into three repetitions; all rice grains must be whole, undamaged, and devoid of husk and embryo. Adding 6 grains of rice to each petri dish (each variety has 3 dishes), 10 ml of 1.7% KOH to each variety, and waiting 23 hours at ambient temperature. Lastly, the gelatinization temperature of rice was evaluated using the IRRI (2013) rating scale (Table 4).

Table 4. Separated level of heat resistance follow standard of IRRI (2013)

Level	Spreading	Heat resistance
1	Kernel not affected	High
2	Kernel swollen	High
3	Kernel swollen, collar complete and narrow	High/Medium
4	Kernel swollen, collar and wide	Medium
5	Kernel split or segregated, collar complete and wide	Medium
6	Kernel dispersed, merging with collar	Low
7	Kernel completely dispersed and intermingled	Low

2.2.4. Method for gel consistency

Evaluated gel consistency (Cagampang et al., 1973). All grains of rice were peeled, then crushed to soft powder, then measured the moisture content. Sample Weighing (100 mg with 12% moisture) put in test tube (100 x 13 mm), then add 0.2 mL ethanol 95% has 0.025% thymol blue. Adding 2 mL KOH 0.2N, after that stir with machine Vortex. Cover the

test tube and place it in a bain-marie at a temperature of 100°C for 8 minutes. Remove it and let it sit for 5 minutes, then cool it using ice water for 20 minutes. Finally, read and record the result by placing the test tube horizontally on the flat surface of the checkerboard. After 60 minutes, measure the length of the gel (from the bottom to the upper consistency of the gel) in millimeters. Evaluated gel consistency followed by the rating scale of IRRI (2013).

Table 5. The rating scale gel consistency of IRRI (2013)

Length of gel consistency (mm)	Type of gel consistency	Level
81-100	Very soft	1
61-80	Soft	3
41-60	Medium	5
35-40	Hard	7
<35	Very hard	9

2.2.5. Aroma analysis using alkali method

The aroma of rice was evaluated by organoleptic rating; a procedure is performed according to the following steps. Firstly, 10 grains of rice of each variety were husked, whitened and crushed. After that, the power of rice was put into a test tube of 100 ml containing 500 µl KOH 1.7%, then covered and kept for 10 minutes at room temperature. Finally, aroma was evaluated by the organoleptic rating method with 10 people and evaluated, followed by IRRI (2013) (Table 6). Compared to the rice of ST 25 controls (level 2) very fragrant, Dai thom 8 controls (level 1) light fragrant and IR50404 controls (level 0) flavorless.

Table 6. Evaluated aroma by organoleptic rating (IRRI, 2013)

Level	Aroma
0	Flavorless
1	Light fragrant
2	Very fragrant

2.2.6. Genic markers for quality traits

Young leaves samples of 20 varieties in this research were extracted according to the process of Doyle and Doyle (1990). The obtained DNA was further genotyped based on the synthesized primers whose sequences are listed in Table 7 by PCR method. PCR products was electrophoresis with gel agarose 2% (w/v) such as gene *GS3*, *Waxy* and *BADH2*, standard bar 1 kb plus used to estimate the size band.

Table 7. Sequence of primers used to identify genotypes for quality traits

Gene	Primer	Sequence of primers (5' to 3')	Product	Size (bp)
Gene *Wx* (Cai et al., 2015)	GF	TACAAATAGCCACCACA	GF-TR	387
	TR	GATCAGCCTAACCAAACA		
	GR	GGGAAACAAAGAATTATAAACATATATGTACAC	GF-GR	207
	TF	CATCAGGAAGAACATCTGCAAGT	TF-TR	235
Gene *BADH2* (Bradbury et al., 2005)	ESP	TTGTTTGGAGCTTGCTGATG	ESP-EAP	577
	EAP	AGTGCTTTACAAAGTCCCGC		
	INSP	CTGGTAAAAAGATTATGGCTTCA	INSP-EAP	355
	IFAP	CATAGGAGCAGCTGAAATATATACC	IFAP-ESP	257
Gene *GS3* (Ramkumar et al., 2010)	EFP	AGGCTAAACACATGCCCATCTC	EFP-ERP	365
	ERP	CCCAACGTTCAGAAATTAAATGTGCTG		
	IRSP	AACAGCAGGCTGGCTTACTCTCTG	ERP-IFLP	262
	IFLP	ACGCTGCCTCCAGATGCTGA	EFP-IRSP	147

2.2.7. Data analysis

All of the parameters were processed on Microsoft Excel 2013. Besides that, using statgraphics 18 (Statgraphics, 1988) to evaluate for descriptive statistical analysis (mean, standard deviation), comparison of treatment means by variance (one-way ANOVA) and combined comparison of treatment mean pairs by LSD test, F value and the coefficient of variation CV (%) were calculated through the analysis of variance and descriptive statistics, and using Origin 2018 software (OriginLab, 2018) to plot the graph.

3. RESULTS AND DISCUSSION

3.1. Length grain rice traits and shape of grain rice

Specifically determined based on the combination of length, width, and the length-to-width ratio, it is one of the crucial quality characteristics influencing the commercial value of rice grains for domestic consumption or export (Somrith, 1974; Unnevehr, 1992). This is a crucial agricultural characteristic for artificial selection in rice reproduction (McKenzie & Rutger, 1983).

3.1.1. Length of grain rice

Length-grain rice was an important criterion for classifying rice for export, and it also depends highly on the preferences of consumers in each country (Jenning et al., 1979). The IRRI (2013) rating scale for rice grain length classifies rice into four categories: Level 1 for very long grains (>7.5 mm), Level 3 for long grains (6.61–7.5 mm), Level 5 for medium grains (5.5–6.6 mm), and Level 9 for short grains (<5.5 mm). The results (Figure 1) revealed that there was 1% statistically significant difference in the length of rice grains. Seven varieties in level 3 (Nep mua, Nep than 3, Huyet rong 4, Nang tay muon, Nep mau luon 5, Nep vo do, and Nep trong vo) varied between 6.61 and 7.13 mm, whereas all other varieties in level 5 varied between 5.66 and 6.13 mm. In addition, three of the 20 rice varieties had grain lengths between 7.01 and 7.13 mm. According to Nguyen Thi Lang and Bui Chi Buu (2000), rice varieties with grain lengths greater than seven mm can meet the needs and preferences of global consumers.

According to Ramkumar et al. (2010), the *GS3* gene on chromosome 3 is the most important factor for determining rice grain length by 80–90% compared to other genes. Thus, a mutation in the second exon of this gene changed the length of the rice grain. Based on this mutation, Ramkumar et al. (2010) developed primers DRR-GL to identify gene GS3, which is a length grain control. In this genic marker, pair of primers EFP/ERP amplify a region at 365 bp including both dominant and recessive alleles. Primer pairs specific to each allele EFP/IRSP produce a region at 147 bp for cultivars/varieties have length grain rice lower than 6.4 mm, and pairs of primers ERP/IFLP amplify a region 262 bp identification for varieties with longer rice grain length 6.4 mm. According to the results of the amplification of the PCR product by primer DRR-GL (Fig. 2), The length of the fragment is very clearly found in 13 of the 20 traditional rice varieties had a band size of 147 bp, indicating that the length grain rice was short (less than 6.4 mm), whereas 7 of the varieties got a band size of 262 bp, indicating that the length grain rice was long (greater than 6.4 mm). However, phenotypic and genotypic have differences between Nep than 6, Nho thom, Nang dum, Nep than 3, Huyet rong 4, Nep mua, Nep trong do. With the use of molecular markers, which accurately accounted 90% of phenotypic variation, compared to a manual technique, it was shown that the Nang tay muon variety had different genotypes and phenotypes (Table 8).

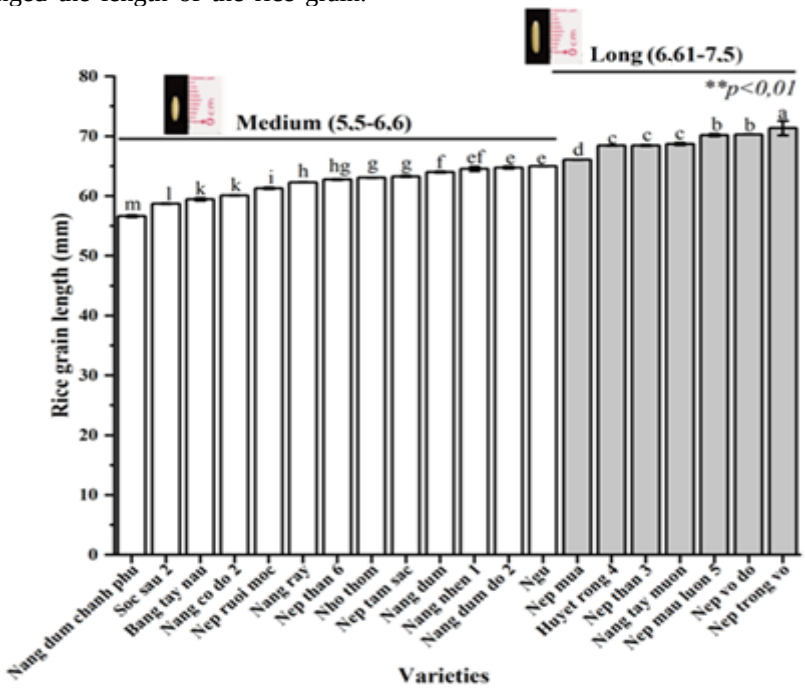

Figure 1. The chart shows the length grain rice of 20 traditional rice varieties, gray columns indicate the length grain ≥ 6.61 mm

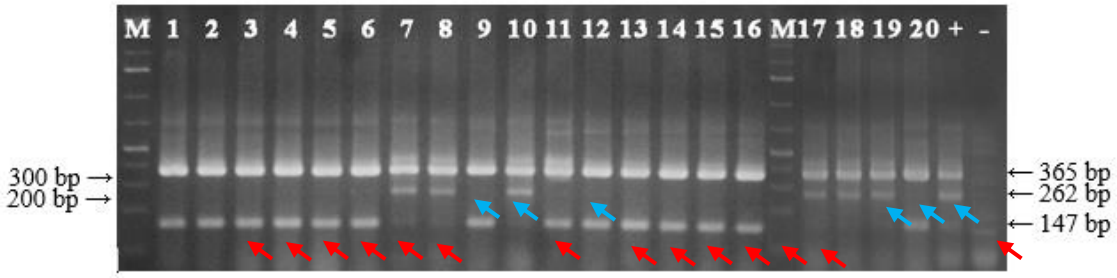

Figure 2. PCR products profile using primer *GS3* for 20 traditional rice varieties on 2% gel agarose (v/w)

The red arrow shows a suspected have length grain rice lower than 6.4 mm amplified at ± 147 bp. The blue arrow shows a suspected the length grain rice was greater than 6.4 mm amplified at ± 262 bp. (M: DNA ladder 1kb plus (Inivtrogen, USA), 1-20: corresponding with ordinal numbers of the traditional rice varieties presented in Table 1).

Table 8. Comparing results between genotypic (*GS3*) and phenotypic grain length traits

STT	Variety	Genotypic		Phenotypic
		147 bp (Short grain)	262 bp (long grain)	
1	Nang dum	-		Medium
2	Nang dum chanh phu	-		Medium
3	Nang dum do 2	-		Medium
4	Nang nhen 1	-		Medium
5	Nang ray	-		Medium
6	Nang tay muon	-		Medium
7	Nep mau luon 5		+	Long
8	Nep mua		+	Long
9	Nep ruoi moc	-		Medium
10	Nep than 3		+	Long
11	Nep than 6	-		Medium
12	Nep tam sac	-		Medium
13	Ngu	-		Medium
14	Nho thom	-		Medium
15	Soc sau 2	-		Medium
16	Bang tay nau	-		Medium
17	Huyet rong 4		+	Long
18	Nep trong vo		+	Long
19	Nep vo do		+	Long
20	Nang co do 2	-		Medium

3.1.2. Shape of grain rice

Based on the length/width ratio and the IRRI (2013) rating scale, grain shape is categorized into four levels: level 1 for slender seeds >3, level 3 for medium seeds 2.1-3, level 5 for bold seeds 1.1-2, and level 9 for round seeds 1,1. The length/width ratio of rice grains had a statistically significant difference of 1%, as shown by the graph (Fig. 3). Nep tam sac had the maximum length/width ratio (3.43), while Nang ray had the lowest (2.13). Five out of twenty varieties (Nep mau luon 5, Nep trong vo, Nep vo do, Huyet rong 4, and Nep tam sac) had rice grains ranging in length/width ratio from 3.01 to 3.43. The remaining varieties contained medium-sized seeds of level 3 with a range of 2.13 to 2.95.

The morphology of rice grains is one of the most important characteristics used to evaluate the genetic diversity of seed plants (Lang, 2005). In addition, the size and shape of rice grains were mainly determined by the preferences of consumers in each country, such as people in the United States, China, and South and Southeast Asia, for instance, who prefer grains with a slender shape (Jenning et al., 1979; Juliano & Villareal, 1993).

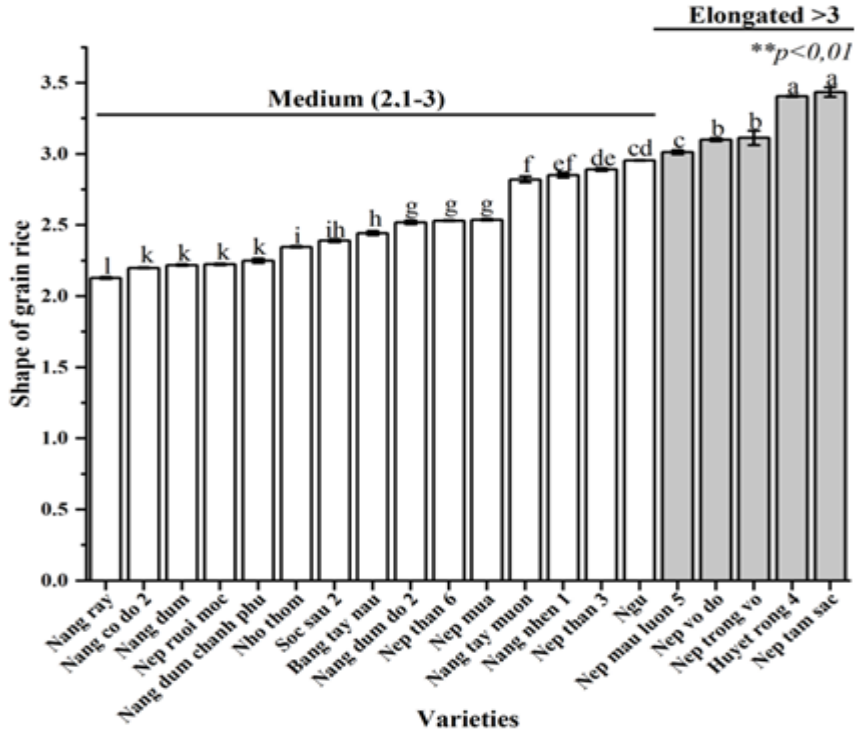

Figure 3. The chart shows length/width ratio grain rice of 20 traditional rice varieties

3.2. Amylose content (AC)

Amylose content (AC) was determined using the Juliano method (Juliano, 1971), and amylose content is divided into five groups: Waxy (0–5%), very low (5–12%), low (12-20%), intermediate (20-25%), and high (>25%). In this study, the result of Fig 3 showed that amylose content (AC) of 20 varieties had statistical difference at the level of significance 1%, ranged from 4.62 to 28.34%. Nang dum do 2, Ngu, Nang nhen 1, Nang ray, Nep ruoi moc, Nho thom, Nang Dum, Nang tay muon, and Huyet rong 4 were among the nine varieties with a high AC, with values comprising from 25.42% to 30.35%. While Bang tay nau, Nep than 3, Nang um chanh phu, Nep than 6, Soc sau 2, and Nang co do 2 had intermediate AC levels ranging from 20.81 to 24.72%, only Nep trong vo had a low AC level of 17.58%. In addition, because the AC ranged from 4.62 to 5.61 percent, the remaining four varieties Nep mau lươn 5, Nep tam sac, Nep mua, and Nep vo đo were categorized as having a very low AC.

The results showed that Nep trong vo variety is preferred by many people in the world over the rest of the rice varieties because they have AC at a low in among 20 traditional rice varieties rice have AC at a low level, when cooking, it becomes mushy, and the rice becomes sticky. On the other hand, rice with AC at a high level had great puffiness and was dry when cooked, whereas rice with a high AC level will make the rice firmer after cooled. In rice-growing countries around the world, consumers often choose varieties of rice with AC at intermediate or low level.

Amylose content is an important indicator of rice grain quality (Fitzgerald et al., 2009). Gene *Wx* controlled amylose content in rice was substitution mutation G → T at +1 loci in the first intron. From the results of the study on the sequence of the genotype Wx, Cai et al. (2015) successfully developed molecular markers Wx In 1 SNP involving 4 primers are GF, TR, GR and TF to identify AC in grain rice. In details, Primer GF-TR will amplify at the band 387 bp from both varieties have high amylose content and low amylose content, besides that at the band 207 bp primer GF-GR will help to identify varieties have amylose content and reserved that varieties have low amylose content primer TF-TR will identify at the band 235 bp. Through amplification of PCR product in the experiment of 20 traditional rice varieties (Fig 5) showed that 6 varieties have a band size at 235 bp such as Nep mau luon 5, Nep mua, Nep than 6, Nep tam sac, Nep trong vo and Nep vo đo have low amylose content. 14 varieties left have a band size at 207 bp presented high amylose content.

Compared with the results of amylose content measured from Fig 3 we have results using 4 primers GF, TR, and GR, TF were explainted exactly 90% amylose content. Table 9 shows that Nep 6 had a low amylose level due to its T genotype, although having a medium amylose amount.

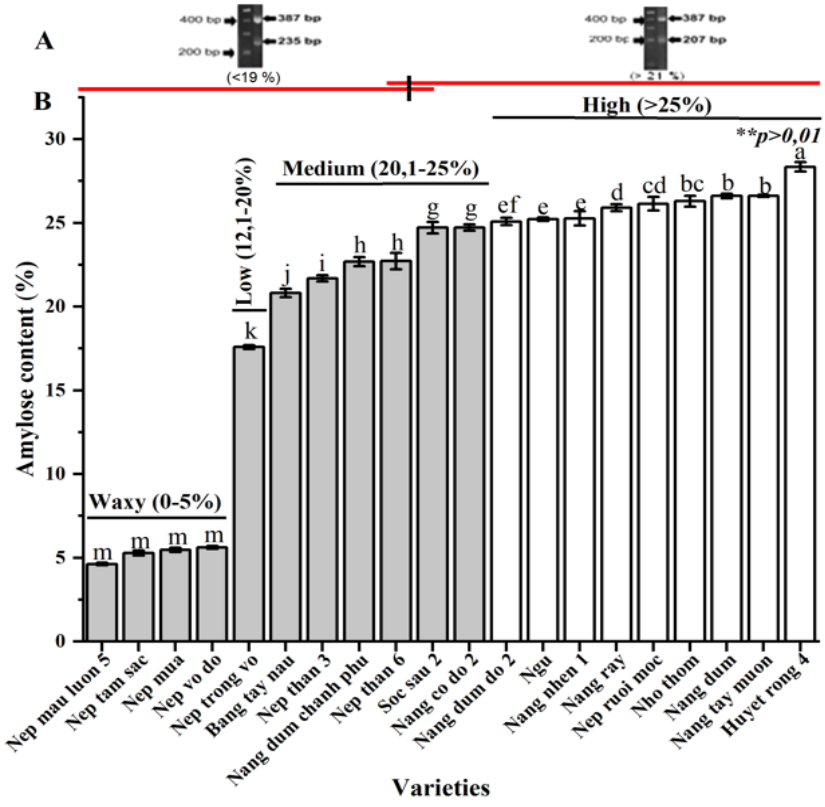

Figure 4. The chart showed amylose content of 20 traditional rice varieties, gray bars have amylose content < 25%

(A: Molecular Indicators Wx identified amylose content in rice; B: The gray bars have amylose content < 25%)

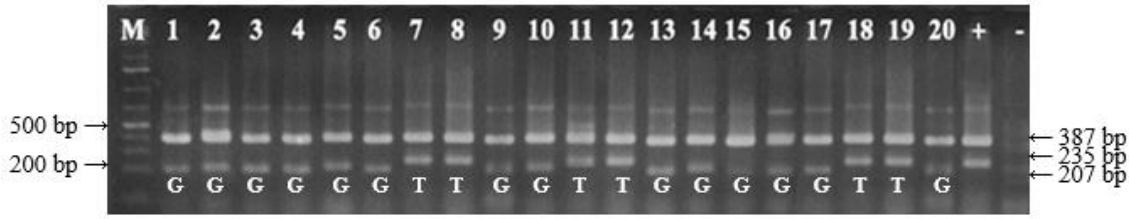

Figure 5. PCR profile with primer Waxy on 2% agarose gel for identification of varieties carrying mutant genes *G → T*

(M: Standard bar 1kb plus, 1-20: corresponding with ordinal numbers of the traditional rice varieties are presented in Table 1). T genotype indicated low amylose content, G genotype indicated medium or high amylose content.

Table 9. Comparing results between genotypic *(Wx)* and amylose content measurement by biochemical

STT	Variety	Genotypic		Phenotypic
		207 bp (high amylose)	235 bp (low amylose)	
1	Nang dum	-		High
2	Nang dum chanh phu	-		Medium
3	Nang dum do 2	-		High
4	Nang nhen 1	-		High
5	Nang ray	-		High
6	Nang tay muon	-		High
7	Nep mau luon 5		+	Waxy
8	Nep mua		+	Waxy
9	Nep ruoi moc	-		High
10	Nep than 3	-		Medium
11	Nep than 6		+	Medium
12	Nep tam sac		+	Waxy
13	Ngu	-		High
14	Nho thom	-		High
15	Soc sau 2	-		Medium
16	Bang tay nau	-		Medium
17	Huyet rong 4	-		High
18	Nep trong vo		+	Low
19	Nep vo do		+	Waxy
20	Nang co do 2	-		Medium

3.3. Starch gelatinized

The level of starch gelatinized followed by the rating scale of IRRI (2002) was separated into 3 groups: high (level: 1, 2, 3), medium (level: 4, 5) and low (level: 6, 7). The results (Fig 6) showed that in all 20 varieties 1 medium starch gelatinized at level 6 was Nep than 3 and 4 varieties have level 5 starch gelatinized were Nep mau luon 5, Nep mua, Nep tam sac, Nep vo đo. Varieties that have high starch gelatinized at level 3 were Nep ruoi moc, Nho thom, Nang co do 2. The remaining varieties have level 2 starch gelatinized. According to starch gelatinized related to cooking time, high gelatinized starch rice requires more water and takes longer to cook than low or medium gelatinized starch rice.

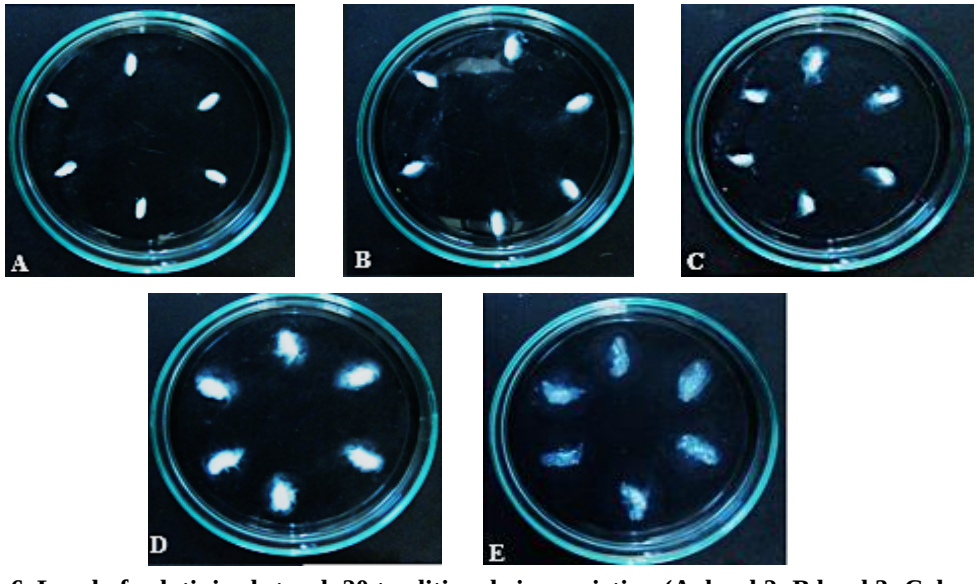

Figure 6. Level of gelatinized starch 20 traditional rice varieties. (A, level 2; B level 3; C, level 4; D, level 5; E, level 6)

3.4. Gel consistency (GC)

The results of gel consistency for 20 traditional rice varieties were performed following the method of Graham (2002), based on the difference in starch cohesion (Cruz & Khush, 2000) and were rated based on the rating scale of IRRI (2002). Fig. 7 shows that in 20 traditional rice varieties were statistically different at the level of significance of 1%, varieties varied from 24.33 to 99.67 mm. 4 varieties have length gel consistency at level 1 (very soft) ranging from 99.00 to 99.67 mm such as Nep mua, Nep tam sac, Nep mau luon 5, Nep vo do. Gel consistencies at level 3 (soft) ranged from 61.33 to 75.33 mm, and consisted of three distinct varieties, including Nep trong vo, Nang co do 2, and Nho thom. Nang dum chanh phu, Bang tay nau, Nang nhen 1, Ngu, Nang ray, and Nang tay muon had length gel consistency at level 5 (medium) that varied from 41.33 to 49.67 mm. Nang dum do 2, Nang dum, Huyet rong 4, and Soc sau 2 were the four varieties that had a gel consistency at level 7 (hard), varying from 35.67 to 40.00 mm. Length gel consistency at level 9 (very hard) ranged from 24.33 to 33.00 mm for the remaining varieties, which included Nep ruoi moc, Nep than 3, and Nep than 6. Consistency of gel was an essential criterion for evaluating the quality of rice, as it determined the rice's cooling tenderness (Nguyen Thi Lang, 2005). When cooking rice, variations within the same group will be preferred over those with a softer gel consistency (Khush & Sidhu, 1979; De, 2008). Therefore, except for thirteen GC varieties belonging to the groups of medium, hard, and very hard GC, the remaining breeding-promising lines belong to the group of soft-very soft GC.

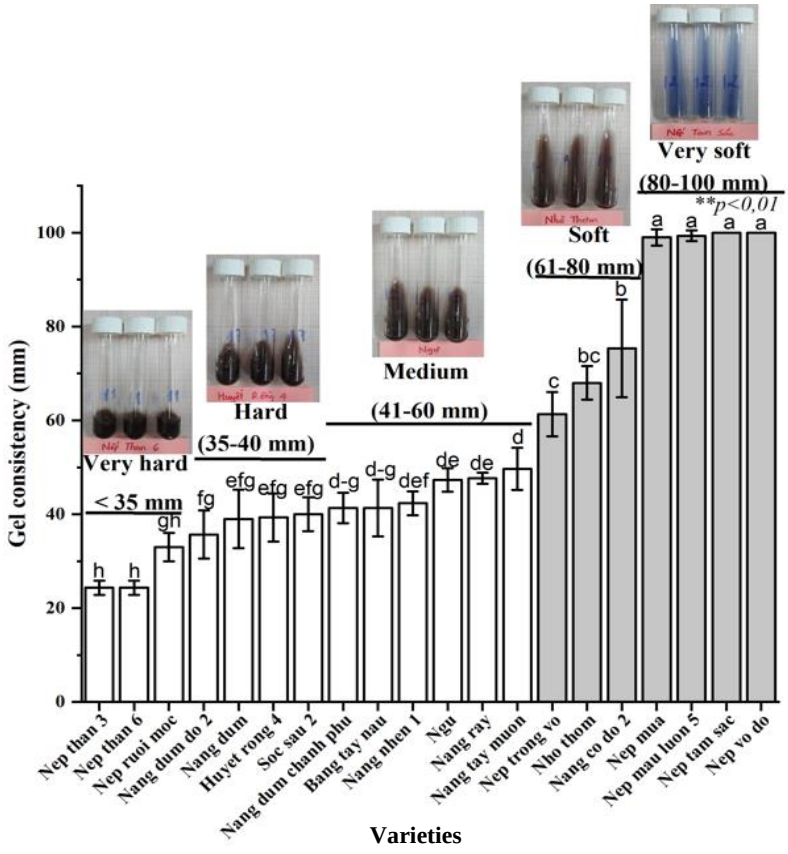

Figure 7. Gel consistency of 20 traditional rice varieties. The corresponding gray bar represents soft rice of length gel > 60 mm

3.5. Aroma analysis

The aroma of cultivars was identified using the KOH method of Yoshihashi et al. (2004) and the IRRI (1996) rating scale. In this investigation, IR50404 lacked aroma (level 0), RVT rice possessed aroma (level 1), and ST 25 possessed aroma (level 2). Through the results, 20 season varieties were non aroma (level 0). Bradbury et al. (2005) designed 4 primers ESP, EAP, INSP and

IFAP. In detail, the pair of primer ESP-EAP will amplify a segment DNA at 580 bp for 20 varieties that have aroma and nonaroma. A pair of primer ESP-IFAP will help identify aromatic genes if PCR products have size band at 257 bp and pair of primer INSP-EAP will help identify non-aromatic genes, when PCR products have size band at 355 bp. If PCR products have both of size band 257 bp and 355 bp, then that variety will have a heterozygous aromatic genotype. In fact, there is an 8-nucleotide deletion on exon 7 of the aroma variety's gene betain aldehyde dehydrogenase 2 (BADH2), which is found on chromosome 8. In non-aromatic rice, there is no deletion. The results demonstrated that none of the 20 varieties of traditional rice lost seven nucleotides at exon 7 on the BADH2 genes when loaded on an agarose 2% gel (Fig. 8). Since there are no variations with aromatic genes, the genotype-phenotype agreement for this molecular marker is 100%.

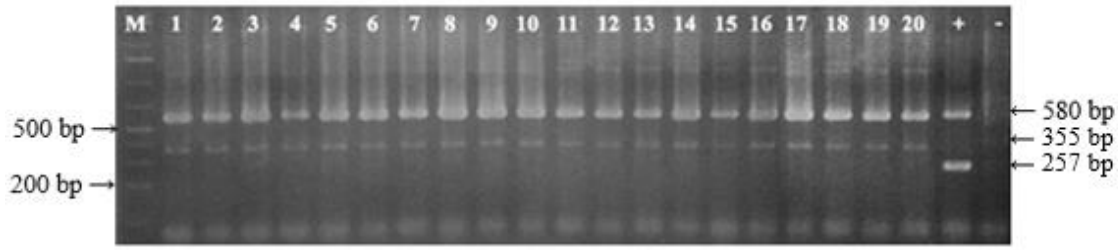

Figure 8. PCR profile for detection of BADH2 mutation gene on gel agarose 2% of 20 traditional rice varieties

(M: DNA ladder 1kb plus, 1-20: corresponding with ordinal numbers of the traditional rice varieties are presented in Table 1)

Table 10. Comparison of BADH2 aromatic genotype with sensory assessment of aroma

STT	Variety	Genotypic		Phenotypic
		257 bp(fragrant)	355 bp (flavorless)	
1	Nang dum	-		flavorless
2	Nang dum chanh phu	-		flavorless
3	Nang dum do 2	-		flavorless
4	Nang nhen 1	-		flavorless
5	Nang ray	-		flavorless
6	Nang tay muon	-		flavorless
7	Nep mau luon 5	-		flavorless
8	Nep mua	-		flavorless
9	Nep ruoi moc	-		flavorless
10	Nep than 3	-		flavorless
11	Nep than 6	-		flavorless
12	Nep tam sac	-		flavorless
13	Ngu	-		flavorless
14	Nho thom	-		flavorless
15	Soc sau 2	-		flavorless
16	Bang tay nau	-		flavorless
17	Huyet rong 4	-		flavorless
18	Nep trong vo	-		flavorless
19	Nep vo do	-		flavorless
20	Nang co do 2	-		flavorless

4. CONCLUSIONS

In conclusion, the application of molecular marker genes DRR-GL with primers (EFP, IRSP, ERP, IFLP) and Wx-in1 with primers (GF, TR, GR, TF) was explained 90% length grain genotype and amylose trait, respectively. Besides that, Application of molecular marker genes BADH2 with primers (ESP and IFAP, INSP and EAP) were explained exactly 100% aroma trait of varieties in this research.

In this study, Nep trong vo can be selected as a candidate parental high-quality rice with low amylose and soft gel consistency in breeding programs. In addition, Nep tam sac variety has slender grain shape and waxy amylose content. These two varieties can be used for further breeding to improve low amylose content and long grain breeding program.

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