ANALYSIS OF EXON3-EXON6 WAXY GENE POLYMORPHISM ON *JAPONICA* RICE

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ABSTRACT

Amylose content is one of the most important characteristics of japonica rice grain quality. The study used 20 japonica rice varieties (Oryza sativa L.) to evaluate chemical quality characteristics to find out the correlation between traits and identify varieties with different amylose contents to investigate waxy gene region polymorphisms. The results determined that there was a strong correlation between amylose content and gel consistency (r = 0.75), and the analysis found 4 groups with different amylose content: glutinous, low, medium, and high. The results of gene sequencing from exon 3 to exon 6 of the waxy genes in 6 rice varieties in 4 amylose groups can distinguish glutinous rice varieties (IR4625), low amylose groups (DS01 and J01), intermediate amylose group (grassy), and high amylose group (Padi Pohon Batu).

Keywords: Amylose content, SNP marker, waxy gene.

INTRODUCTION

Vietnam is the world's leading rice exporter, but the export value is not high mainly because it is produced from high-yielding varieties of medium-quality rice (FAO 2018). In recent years, Vietnam has turned to rice production in the direction of improving quality to increase the value of exported rice grains on the world market. The group of *japonica* rice varieties is preferred by the markets of Northeast Asian countries and is ready to be imported at very high prices (more than 1000 USD/ton). Some important properties are believed to determine rice quality and commercial value such as amylose content, gel consistency, and gelatinization temperature, in which amylose content plays the most important role. The amylose content in the endosperm is thought to be controlled by the *waxy gene (Wx)* incompletely dominant on rice chromosome 6 (Wang et al. 1995). Lower expression levels of the Wx^b allele are inefficient splicing of the *Wx* intron 1. The G→T mutation reduces the efficiency of the first intron knockdown and subsequently reduces the expression of Granule-bound starch synthase (product of the waxy gene) (Bligh et al. 1998). A single nucleotide polymorphism (SNP) at exon 6 (A→C), Wx^{in}, was associated with the medium amylose trait (Chen et al. 2008; Larkin et al. 2008; Park 2003; Mikami et al. 2008). Another variant is the Wx^{op} (or Wx^{hp}), which carries the same SNP in the intron 1 cleavage site with Wx^a and one SNP (A→G) on exon 4 for the low amylose content trait (Liu et al. 2009). Furthermore, premature termination of translation due to duplication 23 bp in exon 2 of *Wx* induces a shift in the reading frame, which leads to the formation of a terminator triptych and produces a *waxy* phenotype (Mikami et al. 2008; Wanchana et al. 2003). Evaluation of these properties by traditional methods requires time-consuming and labor and is only possible after harvesting. The target gene region sequencing technique to find out the differences between genotypes is one of the effective tools to help breeders

evaluate the genetic variation of amylose content between individuals effectively right away at the seedling stage. Because of the aforementioned reasons, the study "Analysis of waxy gene region polymorphism in *japonica* rice" was carried out.

MATERIALS AND METHODS

Materials

Twenty *japonica* rice varieties provided by the Cuu Long Delta Rice Research Institute (CLRRI) were used in this study, whose list as shown in **Table 1**.

Table 1. List of rice varieties investigated for quality characteristics.

No.	Variety name	No.	Variety name
1	Quimipol	11	ĐS1
2	WC2811	12	J01
3	Secano Do Brazil	13	J03
4	C8429	14	J13
5	Padi Pohon Batu	15	J16
6	WC3532	16	J19
7	GPNO1106	17	Shinmei 01
8	Grassy	18	OM38
9	Tia Bura	19	IR 4625
10	Coppocina	20	KG japonica

Methods

Phenotypic analysis of quality characteristics

Rice varieties were analyzed for rice quality according to the method of Nese Sreenivasulu (2019) with improvements according to Vietnamese national standards including Amylose content according to TCVN 5716-2:2017; Gelatinization temperature according to TCVN5715:1993, and Gel consistency according to TCVN 8369:2010.

The content of amylose was determined based on the principle of measuring the reaction to form amylose - Iodine complex in blue-violet color in acetate buffer pH 4.5-4.8. A spectrophotometer was used to measure the absorbance of the complex at 620 nm. Accordingly, the rice was ground into a fine powder to break down the endosperm structure to support complete dispersion and gelatinization. The test portion was dissolved in a sodium hydroxide solution, then a portion of this solution is mixed with the iodine solution (usually KI). The test sample was measured and calculated inferred from a calibration curve built on known amylose concentrations (standard amylose in different proportions) and evaluated according to the IRRI (2013) standard evaluation system for rice as follows: the amylose content was measured and divided into 5 groups, including glutinous (0 - 5.5%), very low (5.6-12%), low (12.1-20%), medium (20.1-25%), and high (> 25%).

Gelatinization temperature was determined by using 1.7% potassium hydroxide solution to decompose 6 whole grains of milled rice at 30^0C for 23 hours. The shape and degree of decomposition of rice grains after treatment were evaluated to determine the alkaline decomposition according to the standard evaluation system (TCVN 5715-1993).

Gel consistency was determined by measuring the elongation of the gel after gelatinizing white rice flour in a dilute alkaline solution, water

bath, and cooling. Accordingly, the rice was ground to a fine powder as analyzed for amylose content.

Ground rice flour (100 mg) weighed into test tubes (13 mm in diameter and 100 mm in length) was added 0.2 mL of 0.03% methyl blue solution in 95% ethyl alcohol. Subsequently, the reaction mixture was introduced into a solution containing 2.0 mL of 0.2N KOH, and subjected to gentle agitation using a vortex machine set at a speed of 6. The reaction solution was incubated in a water bath for 8 min, left to cool at room temperature for 5 min, and chilled in an ice bath for 20 min. Afterward, the tubes were horizontally placed on a flat table that was covered with parchment paper to allow the gel to flow out slowly for 1 h until the gel thickness. Gel length (mm) from the bottom of the tube to the top of the gel was measured and calculated according to the protocol previously described by the IRRI (2013).

Analysis of exon 3-6 region of Waxy gene in some rice varieties

DNA extraction: After phenotypic evaluation, 6 rice varieties with different amylose contents were selected, and DNA was extracted according to the CTAB procedure described by Rogers and Bendich (1988) with suitable modifications to the laboratory. DNA quality was checked by electrophoresis on 1% agarose gel. Quality DNA samples were stored at -20°C in the refrigerator prior to being used in further experiments.

Primer design: Primer Wx3_6 was designed using Primer3 software (https://bioinfo.ut.ee/primer3-0.4.0/). The *waxy (Wx)* gene sequence information of *japonica* rice was downloaded from the gene bank of rice (https://rapdb.dna.affrc.go.jp). The primer was designed to clone a 756 bp DNA fragment of the *Wx* gene region from exon 3 to exon 6. Primer information is described as follows: F-forward primer sequence: 5' GCAGATCAAGGTTGCAGACA3' and reverse R: 5'GTTGTGGATGCAGAAAGCAA3'. The primer length is 20 nucleotides, GC content is 50%, the primer binding temperature is 60°C, and the PCR product is 756 bp.

***Waxy* gene PCR reaction**

The PCR reaction was conducted in 50 µL total volume, containing 33.5 µL of sterile double distilled water, 10 µL of PCR solution (5X buffer), 2.0 µL of forward and reverse primer (10 pmol), 0.5 µL of Taq DNA polymerase solution (5unit/µL), and 2.0 µL of total DNA (~50 ng). The heating process was carried out using a DNA thermal cycler-model: GeneAmp PCR System 9700 (USA) according to an automatic program including the denaturation initiation phase at 94°C for 5 minutes and followed by 30 cycles with steps: denaturation, double-stranded DNA cleavage at 94°C for 30 seconds, primer binding at 60°C for 60 seconds, elongation at 72°C for 60 seconds, and stabilization product at 72°C for 7 minutes, samples were stored at 4°C. PCR products were electrophoresed on 2% agarose gel supplemented with safe view dye to check the quality. Qualified samples were subjected to GENLAB for sequencing.

Data analysis

The phenotypic data were collected and graphed on Microsoft Excel 2019. Correlation analysis was processed by Statgraphics 19 software. Exon 3 to exon 6 Waxy gene region sequence was aligned with the reference gene sequence of the Nipponbare rice variety on the NCBI gene bank using BioEdit V7.2.6.1 software to detect SNPs.

RESULTS AND DISCUSSION

Evaluation of the phenotypic and quality traits of rice varieties

The cooking quality of rice grains is mostly influenced by amylose content, which is a significant chemical property. Consequently, rice varieties that possess a high amylose concentration will exhibit elevated levels of swelling and disintegration throughout the cooking process. On the other hand, rice that contains a low amount of amylose exhibits a soft and malleable texture after being cooked. In contrast, rice with an extremely low amylose concentration, such as glutinous rice, takes less water for cooking and results in rice that is less

swollen, malleable, and sticky (Nguyen Ngoc De 2008). The findings of the assessment conducted on rice types in relation to their amylose content were shown in **Figure 1.**

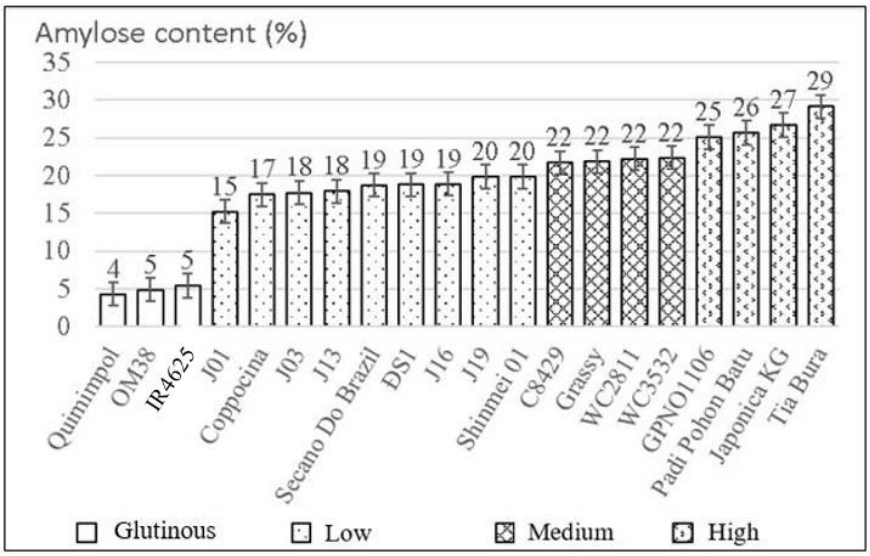

Figure 1. Amylose content of rice varieties (F=203.3**, CV=4.43%).

The results of **Figure 1** showed that these rice varieties with amylose content differed from each other at the 1% statistical significance level, belonging to 4 groups according to the standard evaluation system for rice (IRRI 2013): glutinous, low, medium, and high amylose content, variable from 4.3% to 29.2%. Specifically, three glutinous rice varieties (Quimipol, OM38, and IR4625) with amylose content of 4.3%, 4.9%, and 5.5% variation accounted for 15% variation. Nine varieties had low amylose content, ranging from 15.2 to 19.9% (accounting for 45%), including J01, Coppocina, J03, J13, DS1, Secano Do Brazil, J16, J19 and Shinmei 01. Four varieties C8429, Grassy, WC 2811, and WC 3532, (20%) had medium amylose content (21.7-22.4%). Varieties with high amylose content (25.1-29.2%) as GPNO 1106, Padi Pohon Batu, KG *japonica*, and Tia Bura accounted for a 20% variation in total.

Gelatinization temperature is the temperature at which denatured starch granules gelatinize without being reconstituted, they indicate the time required to cook the rice, so it is considered one of the important factors in determining the quality of cooked rice (Nguyen Ngoc De 2008). Based on gelatinization temperature, TCVN 5715-1993 divides rice varieties into the following categories: rice varieties with high gelatinization temperature (scales 1, 2, 3): >74^{o}C, rice varieties with intermediate gelatinization temperature (scale 4, 5): 70–74^{o}C, rice varieties with low gelatinization temperature (scale 6, 7): <70^{o}C.

The gelatinization temperature analysis of 20 rice varieties showed that the rice varieties with alkali digestion reaction according to the IRRI (2013) were divided into 4 groups, but only when converting to gelatinization temperature according to TCVN 5715-1993, there were only 2 groups of high and low gelatinization temperature (**Table 2**). There was one rice variety with an alkali digestion scale of 1 (accounting for 5%) was Quimipol; 8 rice varieties with an alkali digestion scale of 2 (accounting for 40%) were WC2811, Secano Do Brazil, C8429, WC3532, GPNO1106, Coppocina, OM38, IR4625; three rice varieties with alkali digestion scale 3 (15%) were Padi Pohon Batu, Grassy, Tia Bura; 8 rice

varieties with alkali digestion scale 6 (accounting for 40%) were DS1, J01, J03, J13, J16, J19, Shinmei01, KG *japonica*, thus, there were 12 varieties with high-gelatinization temperature (>74^{o}C) and 8 varieties with low-gelatinization temperature (<70^{o}C), none of which had an intermediate gelatinization temperature (70-74^{o}C).

Table 2. Results of gelatinization temperature analysis of 20 *japonica* rice varieties.

Rice varieties	Alkaline decay (grade)	Gelatinization temperature
Quiminpol.	1	
WC2811, Secano Do Brazil, C8429, WC3532, GPNO1106, Coppocina, OM38, IR4625.	2	High (> 74 ^{o}C)
Padi Pohon Batu, Grassy, Tia Bura.	3	
DS1, J01, J03, J13, J16, J19, Shinmei 01, KG japonica.	6	Low (< 70 ^{o}C)

The gel consistency of *japonica* varieties tends to be related to amylose content, normally, varieties with low amylose content have soft gel consistency (Nguyen Ngoc De 2008). The results presented in **Figure 2** showed that 20 rice varieties were divided into 5 different gel consistency levels, at 1% statistically significant, and the gel consistency of the varieties ranges from 32.7-94.5 mm. There were 11 varieties classified as very soft gel consistency ranging from 83.9-94.5 mm (55%), including J13, RD1, Coppocina, J03, Quimimpol, C8429, J01, Secano Do Brazil, Shinmei 01, IR 4625 and OM 38; five varieties of the soft gel consistency group, ranging from 67.6-76.7 mm (25%), including WC 3532, Grassy, WC 2811, J16 and J19; only 1 variety (accounting for 5%) belongs to the medium category, GPNO 1106 with gel consistency of 42.9 mm. Two hard gel consistency varieties (10%) recorded were KG *japonica* and Padi Pohon Batu with gelatinization lengths of 35.5 mm and 37.8 mm, respectively. The other variety Tia Bura had a very hard gel consistency (31.7 mm).

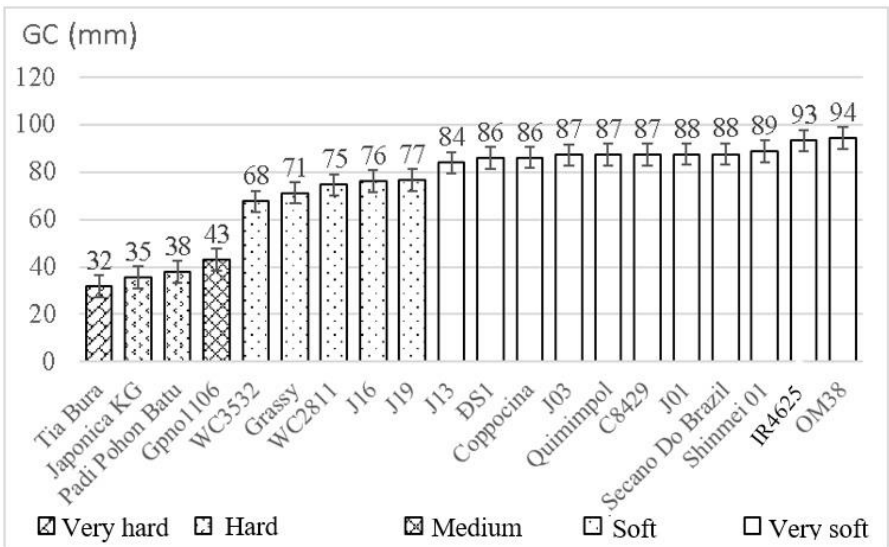

Figure 2. The gel consistency of rice varieties (F=595.1**, CV=2.07%).

Correlation analysis of the chemical characteristics of rice grains (**Table 3**) showed that only gel consistency and amylose content were inversely correlated with each other, that is, varieties with lower amylose content gave longer gel length, or soft gel consistency, and vice versa. In terms of the degree of correlation, this pair of traits have a strong correlation (correlation coefficient is 0.75), that is, based on the results of one trait, it is possible to calculate the results of the other trait. The correlation equation $Y = –0.25X + 37.3$ determines the amylose content when the gel consistency is known with an accuracy of 75%, where X is the gel consistency and Y is the amylose content.

Table 3. Correlation coefficients on chemical characteristics of 20 rice varieties.

	Gelatinization temperature	Amylose content	Gel consistency
Gelatinization temperature	1		
Amylose content	0.26645	1	
Gel consistency	-0.06637	-0.75153**	1

** is correlated at 1% significance level.

Analysis of exon 3 to exon 6 regions of the *Waxy gene* in some rice varieties

Varieties selected for sequencing were performed PCR with primer pair Wx3_6 to amplify the exon 3 to exon 6 region of the *Waxy gene*. Then, electrophoresis was conducted to test the quality of PCR products. The results of electrophoresis showed that the DNA bands were bright, clear, and unbroken, indicating that the product was of sufficient quality, with low impurity content, and qualified to be subjected to sequencing.

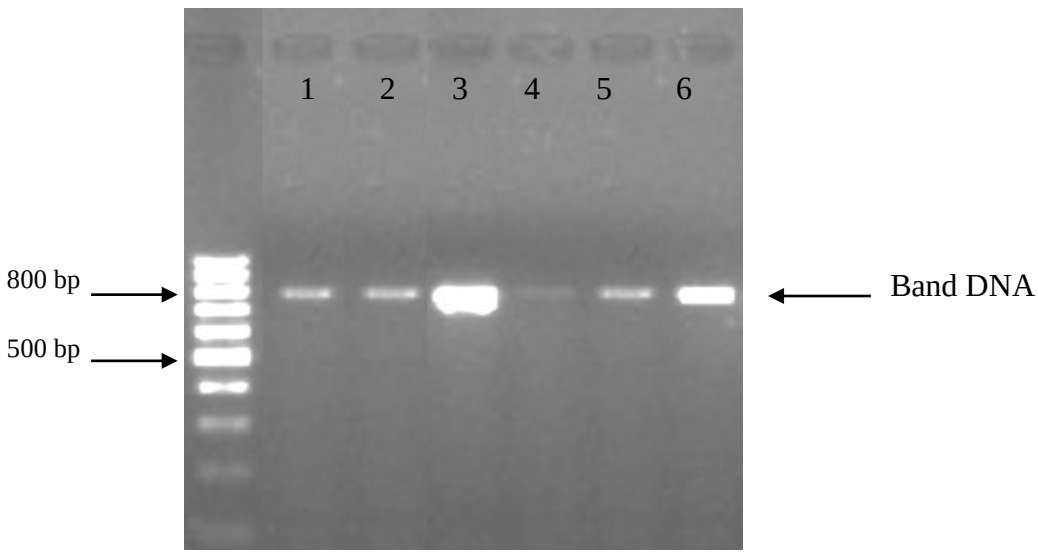

Figure 3. PCR products of exon 3 to exon 6 Waxy genes of rice varieties. 1. IR 4625, 2. J01, 3. DS1, 4. Grassy, 5. C8429, 6. Padi Pohon Batu.

Sequencing the exon 3 to exon 6 regions of the Waxy gene in 6 rice varieties showed a stable signal region of 650 bp long, extending from nucleotides position 137 to nucleotides position 787, and was amplified and sequenced, resulting in 8 nucleotide substitutions (SNPs) when referenced to the Nipponbare rice sequence on the database at nucleotide positions 86, 351, 362, 472, 588, 599, 624 and 636. Of the total six varieties sequenced, one variety had 4 nucleotide substitutions (IR4625), two varieties had 3 nucleotide substitutions (DS01 and J01), two varieties (C8429, Grassy) recorded 1 SNP, and one variety (Badi Pohon Batu) had 7 nucleotide substitutions (**Table 4**).

Among the 8 markers of SNPs, three SNP markers led to none synonymous mutations, and five SNPs markers were synonymous mutations. Three misplaced substitutions occurred at nucleotide positions 351, 599, and 636. Specifically, at nucleotide position 351, the A to C nucleotide change (A351C), resulted in a change of the amino acid Leucine to Phenylalanine at position 117 (L117F), found in Grassy varieties, C8429, DS01, J01, IR4625. At the nucleotide position T599C, which changes the amino acid Histidine to Proline at position 207 (H207P), this change was found in the Padi Pohon Batu rice variety, IR4625. The G636C nucleotide change, resulting in a change of the amino acid Cysteine to Arginine at position 212 (C212R), was present in the Padi Pohon Batu variety, IR4625. The nucleotide changes at positions 86, 362, 472, 588, and 624 did not change the amino acid information on the exon 3 to exon 6 region (**Table 4**). This can be explained by the fact that the genetic code is degenerate, an amino acid can be encoded by more than one set of genetic codes.

This result is similar to the study of Biselli et al. (2014) investigating the polymorphism of the exon 6 regions of the *waxy gene* on 127 rice varieties with amylose content from glutinous to high amylose content, reported 8 SNP, which could explain 79.5% variation in amylose content. However, the results of this study were lower than the study of Jayamani et al. (2007) on 39 rice varieties with low to high amylose content, 11 SNPs were found on the *waxy* gene segment.

The results of sequencing exon 3 to exon 6 regions of the *waxy* gene region were consistent with the results of a phenotypic evaluation of the amylose content trait of rice varieties. Specifically, the variety IR4625 belongs to the glutinous rice group with 5 SNPs, and the DS1 and J01 varieties (low amylose) have 3 SNPs. There was 1 SNP for varieties with intermediate amylose content (Grassy and C8429). Padi Pohon Batu belongs to the group of high amylose content, with 7 SNPs found on the exon 3 to exon 6 region of the *waxy* gene segment (**Table 4**). This result contributes to strengthening the hypothesis of single nucleotide changes in genotype (SNP) compared to the original gene sequence, helping to select materials with different amylose content for the selection of good quality *japonica* rice varieties in the Mekong Delta.

In short, the glutinous rice variety IR4625 has 5 SNPs that distort 3 amino acids (L117F, H207P, C212R). Varieties DS01 and J01 (low amylose) have 3 SNPs that distort 1 amino acid (L117F). Grassy and C8429 were two varieties belonging to the intermediate amylose group with 1 SNP distorting 1 amino acid (L117F). Padi Pohon Batu (high amylose) has up to 7 SNPs that distort 2 amino acids H207P and C212R. This result showed that the SNP at the A351C site that changed the L117F amino acid appeared in *japonica* rice varieties with low to intermediate amylose content, while the SNP at the T599C site changed the amino acids H207P and G636C, changed C212R amino acid appeared in *japonica* rice varieties with high amylose content or glutinous rice when the addition of SNP changed amino acid L117F. Thus, the combination of these SNPs may be related to the different amylose content of rice varieties.

Table 4. Exon 3 to exon 6 region polymorphisms of *waxy gene*.

Location of SNP	**C86A**	**A351C**	**C362T**	**G472A**	**A588G**	**T599C**	**T624C**	**G636C**	**Rice varieties (amylose group)**
Amino acids change	Synonymous mutation	L117F	Synonymous mutation	Synonymous mutation	Synonymous mutation	H207P	Synonymous mutation	C212R	
	C	**A**	**C**	**G**	**A**	**T**	**T**	**G**	**Nipponbare**
		C			G	C		C	IR4625 (Glutinous)
	A	C			G				DS01(low)
	A	C			G				J01 (low)
		C							Grassy, (medium)
		C							C8429 (medium)
	A		T	A	G	C	C	C	Padi PB (high)

CONCLUSIONS

Phenotypic survey of grain quality traits of 20 *japonica* rice varieties revealed a significant inverse relationship between amylose content and gel consistency. Additionally, the survey identified three glutinous rice varieties, nine varieties with low amylose content, four varieties with intermediate amylose content, and four varieties with high amylose content. The SNPs in the exon 3 to exon 6 regions were able to distinguish the glutinous rice group (IR4625), the low amylose group (DS01 and J01), the intermediate amylose group (Grassy), and the high amylose group (Padi Pohon Batu). Further investigation is warranted to examine the impact of amino acid substitutions, namely L117F, H207P, and C212R, on the three-dimensional conformation of the Granule bound starch synthase enzyme, which is the resultant product of the waxy gene. Additionally, it is crucial to elucidate the biological roles associated with these substitutions and their role in modulating alterations in amylose content.

COMPETING INTERESTS

The authors declare they have no conflict of interest, financial or otherwise.

AUTHOR'S INFORMATION AND CONTRIBUTIONS

Tran Dinh Gioi conceived, designed the experiments, and wrote the first draft manuscript. Le Ngoc Lel and Le My Linh conducted the experiments. Nguyen Khac Thang analyzed and interpreted the data, and Nguyen Thi Pha is the supervisor. All authors revised and approved the final submitted version.

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REFERENCES

Biselli C, Cavalluzzo D, Perrini R, Gianinetti A, Bagnaresi P, Urso S,... & Valè G (2014) Improvement of marker-based predictability of Appearance Amylose Content in japonica rice through GBSSI allele mining. Rice 7(1):1-18.

Bligh HFJ, Larkin PD, Roach PS, Jones CA, Fu H, & Park WD (1998) Use of alternate splice sites in granule-bound starch synthase mRNA from low-amylose rice varieties. Plant Mol Biol 38(3):407-415.

Chen MH, Bergman C, Pinson S, & Fjellstrom R (2008) Waxy gene haplotypes: Associations with apparent amylose content and the effect by the environment in an international rice germplasm collection. J Cereal Sci 47(3):536-545.

FAO (2018) Rice Market Monitor. Volume Xxi Issue No. 1. April 2018.

IRRI (2013) Standard Evaluation System for Rice (SES). Rice Science for Better World. 31pp.

Jayamani P, Negrao S, Brites C, & Oliveira MM (2007) Potential of Waxy gene microsatellite and single-nucleotide polymorphisms to develop japonica varieties with desired amylose levels in rice (*Oryza sativa* L.). J Cereal Sci 46(2):178-186.

Larkin PD, Park WD (2003) Association of waxy gene single nucleotide polymorphisms with starch characteristics in rice (*Oryza sativa* L.). Mol Breed 12(4):335-339.

Liu X, Yu L, Liu H, Chen L, & Li L (2009) Thermal decomposition of corn starch with different amylose/amylopectin ratios in open and sealed systems. Cereal Chem 86(4):383-385.

Mikami I, Uwatoko N, Ikeda Y, Yamaguchi J, Hirano HY, Suzuki Y, & Sano Y (2008) Allelic diversification at the wx locus in landraces of Asian rice. Theor Appl Genet 116 (7):979-989.

Nguyen Ngoc De (2008) Textbook of rice plants. Publishing House of the National University of Ho Chi Minh City. Ho Chi Minh.

Roger SO, and Bendich AJ (1988) Extraction of DNA from plant tissues. Print: Geluin, 5.8., RA Schilperoort and DPS Vetma *(*Eds.*)*. Plant Molecular Biology Manual, Springer Netherlands, Dordrecht, pp. 73-83.

Sreenivasulu Nese (2019) Rice Grain Quality Methods and Protocols: P: 65. Doi: 10.1007/978-1-4939-8914-0.

TCVN 5715-1993. Rice-method for determination of gelatinization temperature through alkaline decomposition. Ministry of Science, Technology and Environment issued under Decision No. 212/QD dated May 12, 1993.

TCVN 5716-2:2017. White rice–determination of amylose content. Ministry of Science and Technology promulgated under Decision No. 3889/QD-BKHCN, dated December 29, 2017.

TCVN 8369:2010. White rice–determination of gel consistency. Ministry of Science and Technology promulgated under Decision No. 2093/QD-BKHCN, dated October 6, 2010.

Wanchana S, Toojinda T, Tragoonrung S, & Vanavichit A (2003) Duplicated coding sequence in the waxy allele of tropical glutinous rice (Oryza sativa L.). Plant Sci 165(6):1193-1199.

Wang ZY, Zheng FQ, Shen GZ, Gao JP, Snustad DP, Li MG,... & Hong MM (1995) The amylose content in rice endosperm is related to the post-transcriptional regulation of the waxy gene. The Plant J 7(4):613-622.

PHÂN TÍCH ĐA HÌNH VÙNG GEN EXON3-EXON6 WAXY TRÊN CÁC GIỐNG LÚA *JAPONICA*

Nghiên cứu sử dụng 20 giống lúa japonica (Oryza sativa L.) để đánh giá các đặc tính phẩm chất hóa học của gạo để tìm ra mối tương quan giữa các tính trạng và xác định các giống có hàm lượng amylose khác nhau cho khảo sát đa hình vùng gen waxy. Kết quả đã xác định được có mối tương quan chặt giữa hàm lượng amylose và độ bền gel (r=0,75) và tìm được 4 nhóm có hàm lượng amylose khác nhau là nếp, thấp, trung bình và cao. Kết quả giải trình tự gen vùng exon 3 đến vùng exon 6 gen waxy của 6 giống lúa ở 4 nhóm amylose có thể phân biệt được nhóm giống lúa nếp (IR4625), nhóm có hàm lượng amylose thấp (DS01 và J01), nhóm amylose trung bình (Grassy) và nhóm có hàm lượng amylose cao (Padi Pohon Batu).

Từ khóa: *Chỉ thị SNP, gen waxy, hàm lượng amylose.*