UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF AMYLOPECTIN AND SOLUBLE STARCH SYNTHASE ACTIVITIES IN ENDOSPERM OF DIFFERENT RICE CULTIVARS (Oryza sativa L)

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By

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CHARACTERIZATION OF AMYLOPECTIN AND SOLUBLE STARCH SYNTHASE ACTIVITIES IN ENDOSPERM OF DIFFERENT RICE CULTIVARS (*Oryza sativa* L.)

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Chairperson: Professor Maziah Mahmood, PhD

Faculty : Institute of Bioscience

Rice (*Oryza sativa* L.) is consumed by more than half of world's population and is also the main staple food in Asian countries. Modernization of agricultural methods has to consider the expansion of rice production areas, crop yield and nutritional quality of the product. Starch in rice is made up of two types of glucose polymers, amylose and amylopectin. Amylopectin is a branched polymer which composed of α-1,4-linked glucose and α-1,6 linked branch points, whereas amylose has no branch points. Both amylose and amylopectin are major criteria in evaluating rice quality. It has been reported that the SSIIa enzyme in rice cultivars contributes to the eating and processing qualities of rice, since it influences structural differences in amylopectin. South East Asia especially Myanmar and Malaysia, the rice cultivars with superior traits have rare potential productivities since there is a lack of information on preferable breeding traits. The present study is focused on characterization of starch

in different rice cultivars since it is a very important factor in determining rice grain quality. A total of sixteen Myanmar rice cultivars, three Malaysian lines and three control cultivars were used in this study. The use of fluorophore-assisted carbohydrate electrophoresis through capillary electrophoresis enabled the classification of the three different types of amylopectin. The six rice cultivars (Khun Ni Shay, Ta Soke Pwa, Thet Nu Saba Net Pyan, MR219, MR219-4 and MR219-9) were to have long (L-type) amylopectin as rich in intermediate chain length of amylopectin (52.5-55.3%), whereas Ka Gaw Saw, Nga Sein, Yoe Wa, Shwe War Tun Kauk Hnyin, Padan Nga Sein, and Ya Ta Pa Lay were observed to have intermediate (M-type) amylopectin as middle rich in intermediate chain length of amylopectin (48.3-49.9%), while Nga Pya Gyi, Na Pal, Shwe Li-2, Kauk Yar Gyi, Khao Pa Phone, Ye Baw Yoe Sein and Khao Nao were indicated to have short (S-type) amylopectin type as low in intermediate chain length of amylopectin (47.2-48.6%). The result of 1.7% KOH test further supported that the L-type and M-type rice cultivars were relatively resistant to alkali solution while the S-type was highly susceptible to alkali degradation. In the case of starch gelatinization characteristics, the M-type and L-type amylopectin generally showed light violet with iodine staining while S-type endosperm was dark violet. The results of differential scanning calorimeter (DSC) analysis showed that the classification of the amylopectin since gelatinization temperature (GT) affected the amylopectin chain length distribution. Low GT indicates a range of 67.2-69.6°C, intermediate GT indicates a range of 70.2-72.5°C while high GT is in the range of 73.6-78.4°C. Generally S-type amylopectin rice has low GT group while M-type and L-type amylopectin rice have intermediate to high GT. The different rice cultivars also showed a wide range of apparent amylose content from 3.0 to 28.1%. This diverse range in amylose content might offer choices for breeders to select cultivars with appropriate amylose content in breeding program. The data from rapid visco analyzer (RVA) analysis and genotyping with SNPs marker of *SSIIa* confirmed the different rice cultivars were of three groups, S-type, M-type and L-type by confirming functional *SSIIa* present or absent, although zymogram assay did not clearly indicate SSIIa enzyme activity in these cultivars except Ta Soke Pwa and MR219-9 had indicated this enzyme activity. This information is useful in enhancing the development of new rice cultivars with desirable quality characteristics in Asian rice cultivars.

Abstrak tesis yang dikemukakan kepada Senate Universiti Putra Malaysia sebagai memenuhi keperluan untuk ljazah Doktor Falsafah

PENCIRIAN AMILOPEKTIN DAN AKTIVITI SOLUBLE STARCH SYNTHASE DI DALAM KANJI ENDOSPERMA BERBEZA PADI (*Oryza sativa* L.)

Oleh

NYO NYO MAR

March 2012

Pengerusi : Profesor Maziah Mahmood, PhD

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Padi (*Oryza sativa* L.) adalah makanan ruji utama di negara-negara Asia dan dimakan oleh lebih dari separuh populasi penduduk dunia. Pemodenan di dalam pengeluaran hasil pertanian perlu mengambil kira pengembangan kawasan pengeluaran pertanian padi, peningkatan hasil tanaman dan kualiti pemakanan untuk memperbaiki tahap kesihatan manusia dan memenuhi citarasa pengguna. Pempolimeran glukosa dari kanji dalam padi boleh dikelaskan berdasarkan kepada dua jenis polimer utama, amilosa dan amilopektin. Amilopektin adalah polimer yang bercabang di mana ia terdiri daripada segmen glukosa α-1,4 yang dihubungkan dengan segmen α-1,6, oleh itu amilopektin lebih besar berbanding amilosa kerana amylose tidak mempunyai cabang. Kualiti padi bukan hanya terletak kepada kandungan amilosa tetapi juga juga bergantung kepada kandungan amilopektin yang mempengaruhi kualiti memasak dan makanan dalam padi. Laporan terdahulu

mendapati bahawa enzim *SSlla* di dalam kultivar padi menyumbang kepada kualiti makanan dan pemprosesan padi kerana ia mempengaruhi perubahan struktur amilopektin. Asia tenggara terutamanya Myanmar dan Malaysia mempunyai kultivar padi dengan ciri-ciri yang unggul dan mempunyai potensi produktiviti yang tinggi tetapi kekurangan maklumat tentang ciri-ciri pembiakan yang sesuai. Kajian ini memfokuskan kepada ciri-ciri kanji di dalam pelbagai kultivar padi kerana ia faktor penting di dalam mengenalpasti kualiti bijian padi. Sejumlah enam belas kultivar padi Myanmar, tiga kultivar Malaysia dan tiga kultivar kawalan telah digunakan di dalam kajian ini. Penggunaan elektroforesis karbohidrat dibantu fluorophore melalui elektroforesis kapilari membolehkan pengkelasan tiga jenis amilopektin. Enam kultivar padi (Khun Ni Shay, Ta Soke Pwa, Thet Nu Saba Net Pyan, MR219, MR219-4 dan MR219-9) dilihat mempunyai amilopektin panjang (jenis-L) yang kaya dengan rantaian perantaraan amilopektin (52.5-55.3%), manakala Ka Gaw Saw, Nga Sein, Yoe Wa, Shwe War Tun Kauk Hnyin, Padan Nga Sein and Ya Ta Pa Lay dilihat mempunyai amilopektin sederhana (jenis-M), iaitu rantaian perantara amilopektin (48.3-49.9%), yang sederhana dan Nga Pya Gyi, Na Pal, Shwe Li-2, Kauk Ya Gyi, Khao Pa Phone, Ye Baw Yoe Sein dan Khao Nao mempunyai amilopektin yang pendek (jenis-S) menandakan rantaian perantara amilopektin (47.2-48.6%) yang rendah. Hasil ujian 1.7% KOH menyokong bahawa kultivar padi jenis-L dan jenis-M adalah tahan terhadap larutan beralkali manakala jenis-S mudah terdedah kepada disintegrasi alkali. Di dalam kes pencirian gelatinisasi kanji, amilopektin jenis-M dan jenis-L biasanya menunjukkan warna ungu cair dengan penunjuk iodin manakala endosperma jenis-S berwarna ungu gelap. Hasil dari perbezaan analisis kalorimeter pengimbas (DSC) mendapati bahawa pengkelasan amilopektin bergantung kepada suhu gelatinisasi (GT) yang mempengaruhi rantaian amilopektin. GT yang rendah berada di antara 67.2-69.6°C, GT pertengahan di antara 70.2-72.5°C dan GT yang tinggi pada 73.6-78.4°C. Hasil daripada kajian ini mendapati variasi GT yang besar di antara kultivar padi yang berlainan. Kultivar padi yang berlainan menunjukkan pelbagai kelas kualiti bijian dengan kandungan amilosa jelas di antara 3.0 hingga 28.1%. Kepelbagaian di dalam kandungan amilosa memberi pilihan kepada kacukan dengan memilih kultivar yang mempunyai kandungan amilosa yang bersesuaian dengan program kacukan. Data daripada penganalisis visco pesat (RVA) dan genotip dengan penunjuk SNP di dalam SSIIa mengesahkan pelbagai kultivar padi boleh dikelaskan kepada tiga kumpulan, jenis-S, jenis-M (atau) jenis-L dengan mengesahkan kehadiran atau ketiadaan *SSIIa*, walaupun analisis zimogram tidak menyatakan aktiviti enzim *SSIIa* di dalam kultivar tersebut kecuali Ta Soke Pwa dan MR219-9 yang mempunyai aktiviti enzim ini. Maklumat in amat berguna dalam meningkatkan pembangunan kultivar padi yang baru dengan ciri-ciri berkualiti yang diingini dalam kultivar padi Asia.

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I certify that a Thesis Examination Committee has met on 20 March, 2012 to conduct the final examination of Nyo Nyo Mar on her thesis entitled "Characterization of amylopectin and soluble starch synthase activities in endosperm of different rice cultivars" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the degree of Doctor of Philosophy.

Members of the Examination Committee were as follows:

…...…………………………………….
SEOW HENG FONG, PhD
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Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy.

The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institutions.

………………

NYO NYO MAR

Date: 20 March 2012

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LIST OF ABBREVIATIONS

Symbols

°C	Degree centigrade
cP	Centipoises
J/g	Joules per gram
fa	A chain fraction
fb1	B1 chain fraction
fb2	B2 chain fraction
fb3	Long chain fraction
GBSS	Granule Bound Starch Synthase
L-type	Long chain of amylopectin type
M-type	Intermediate chain of amylopectin type
S-type	Short chain of amylopectin type
SSI	Starch Synthase I
SSIIa	Starch Synthase IIa
SSIIb	Starch Synthase IIb
SSIIc	Starch Synthase IIc
SSIII	Starch Synthase III
SSIVa	Starch Synthase IVa
SSIVb	Starch Synthase IVb
ΔH	Gelatinization enthalphy

Abbreviations

AAC	Apparent amylose content
ACR	Amylopectin chain ratio
ADPGlucose	Adenosine 5’ diphosphate glucose
ANOVA	Analysis of variance
APS	Amonium per sulphate
ASV	Alkali Spreading Value
BD	Break down
BPB	BromoPhenol Blue
CPV	Cool paste viscosity
CTAB	Hexadecyltrimethylammonium bromide
DDW	Double distilled water
DNA	Deoxyribonucleic acid
DP	Degree of Polymerization
DSC	Differential Scanning Calorimeter
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
FACE	Fluorophore-assisted carbohydrate electrophoresis
GT	Gelatinization temperature
h	Hour
HPV	Hot paste viscosity
min	Minute
PCR	Polymerase Chain Reaction
PT	Pasting temperature

PV	Peak viscosity
RVA	Rapid Visco Analyzer
s	Second
SAS	Statistically Analysis Software
SB	Setback
SCR	Short chain ratio
SDS	Sodium Dodecyl Sulfate
SGC	Starch Gelatinization Characteristic
SNPs	Single Nucleotide Polymorphisms
SPSS	Statistical Package for the Social Sciences
Tc	Conclusion temperature
TEMED	N,N,N′,N′-Tetramethylethylenediamine
THF	Tetrahydrofuran
To	Onset temperature
Tp	Peak temperature
UPM	Universiti Putra Malaysia

CHAPTER I

INTRODUCTION

Rice is the second most important agricultural crop after maize. There are two cultivated species of rice, *Oryza sativa* L. and *Oryza glaberrima* Steud. They represent the Asian and the African cultivated rice (Kato *et al.*, 1928; cited by Nakamura *et al.,* 2002). Asian cultivated rice (*Oryza sativa* L.) is the most important species cultivated all over the world as it has been providing over 21% of the calories for the world population. *Oryza sativa* L. has two major subspecies, *indica* and *japonica* types which are used for many purposes including production of noodles, snack foods, beer, wine and other industrial applications.

Quality traits including physical appearance, cooking and eating quality, sensory properties and, nutritional values are very important points to consider in rice breeding programs (Fitzgerald *et al.,* 2009). The physicochemical property of cooked rice is one of the most prominent indicators for eating quality which is mainly influenced by rice starch in the endosperm (Takemoto-Kuno, *et al.,* 2006). Apart from eating quality, the grain quality trait is an important determinant for market value and consumer acceptability.

Consumer preferences for rice grain quality differ between countries and locations. Therefore, rice grain quality is very difficult to define since it is determined by specific preferences of the end user and it is highly variable (Singh *et al.,* 2000). Umemoto *et al.* (2008) reported that the breeding of high quality rice generally aims at understanding the genetic factors controlling such as cooked rice texture, grain size and shape, color of pericarp, protein content, and aroma. Production of better quality rice is becoming more important with the expansion of rice cultivation and improvement of consumer living standards. Therefore, improvement of eating quality has become an important goal both in rice breeding programs and in rice production practices (Zhang *et al*., 2003).

Starch is the end product of carbon fixation in photosynthesis and it accumulates in storage organs as energy source for the next generation. Starch contains glucose and its polymer can be divided into two major types, amylose and amylopectin. Amylopectin is one of the polysaccharides present in rice endosperm and that is a highly branched consisting of α-1,4-linked glucose units connected by α-1,6-linked branch points, and are much larger than amylose which has no branch points. The significant differences in starch properties and functionality in rice endosperm are mainly influenced by differentiation between amylose and amylopectin.

Amylopectin chain length distribution is very important role to determine gelatinization temperature (GT) of cooked rice and gelatinization property is one of the most important indicators of the cooking quality and processing characteristics of rice starch (Nakamura *et al.,* 2005; Waters *et al.,* 2006). Gelatinization temperature in rice is mainly controlled by an alkali locus on chromosome six and similar located the soluble synthase IIa (*SSIIa*) gene (Umemoto *et al.,* 2004; Bao *et al.,* 2006a; Waters *et al.,* 2006). The starch granules containing amylopectin with long and intermediate chains can be predicted to be less soluble in alkali solution and to be more resistant to gelatinization in urea solution.

The amylose content is also an important indicator to determine food processing, cooking and eating qualities of rice endosperm since it greatly influences rice quality after being cooked (Bao *et al.*, 2001). Pasting properties using rapid visco analyzer (RVA) serve as predictors for cooked rice textures. RVA analysis alone explains different textural properties in cooked rice and also combination with amylose content of different rice cultivars (Champagne *et al.*, 1999).

The soluble synthase I (SSI) enzyme in the rice endosperm synthesizes very short chain degree of polymerization (DP) ≤ 10 glucose polymers while SSIIa isoform plays a specific role in the elongation of short chains of DP 6-11 to form longer chains of DP 12-24 (Morell *et al.*, 2003; Dian *et al.*, 2005; Fujita *et al.*, 2006). The SSIIa synthesizes chain length distribution of amylopectin of rice starch to be either the *japonica* or *indica* type rice amylopectin by forming short chains (A chain) and elongating long chains (B1 chain) (Umemoto *et al*., 2002; Nakamura *et al.*, 2005).

The breeding target for rice grain quality improvement integrates not only conventional approaches but also molecular genetic analysis. The cultivars with interested genes could yield potential rice cultivars with high quality in breeding program. Hiratsuka *et al.* (2010) revealed that the distribution of different *SSIIa* haplotypes may functionally reflect consumers' preference to rice quality. Additionally, active *SSIIa* synthesizes chain length distribution of amylopectin to be rich intermediate chain length that retrograde faster and become harder texture after being cooked (Umemoto *et al.*, 2008).

Investigations of genetic variation among Myanmar traditional rice cultivars using microsatellite DNA polymorphisms have been carried out to determine grain quality, yield, and agronomic characteristics (Khin *et al.*, 2002; Thein *et al.*, 2002). It was found that Myanmar rice germplasm had wide variation, starch characteristics and this will be useful to improve certain rice cultivars with superior characters for future breeding programs. Myanmar rice germplasm needs to be further investigated for their structural and physicochemical properties of starch containing amylose and amylopectin which can be utilized in grain quality improvement.

There are more than 120,000 different rice cultivars (IRRI, 2008) in the world, a few offers quality acceptable to be grown commercially in Malaysia because limited information for grain quality improvement. The rice germplasm collection at the Rice Gene Bank (Malaysia) stands at 11470 registered accessions (Afizah, 2007). Around 33 modern cultivars with agronomic traits have been developed and released for commercial planting (Abdullah *et al*., 2005). MR219 is newly cultivated rice in Malaysia with superior characters such as minimal input water and disease resistant. The two mutant lines, MR219-4 and MR219-9 were generated by MR219 since it is attracted local market with long grain type and demanded by farmers as high yield cultivar.

In South East Asian countries such as Myanmar and Malaysia, information of rice cultivars with superior traits are limited. Today the breeding programs on rice quality improvement integrate traditional approaches with advanced methods such as biochemical, genetic, and molecular analysis. Moreover a better understanding of functional soluble starch synthase activities especially SSIIa activity in Myanmar and

Malaysian rice cultivars will be useful in rice breeding programs. Apart from that starch physicochemical properties and amylopectin structure are important determinants for eating and cooking quality, but little information has been available for Myanmar and Malaysia rice. Understanding the starch quality of Myanmar and Malaysia rice will facilitate rice breeders to breed rice with good quality. The present study was designed to investigate starch characterization and physicochemical properties of Myanmar rice cultivars and Malaysian lines.

The main objectives of this study were to;

1. determine the apparent amylose content and chain length distribution of amylopectin of different rice cultivars
2. analyze the physicochemical properties of rice flour of different rice cultivars
3. detect soluble starch synthase enzyme activities at milky stage of rice endosperm
4. determine genotypic functional variations of *SSIIa* gene using single nucleotide polymorphism (SNP) markers

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