



UNIVERSITY OF
KWAZULU-NATAL

INYUVESI
YAKWAZULU-NATALI

**A genetic study of the *SSIIa* and *SSIIIa* genes of rice under
the control of *Wx-mq* gene**

Submitted in fulfilment of the academic requirements for the degree of Doctor of
Philosophy (PhD) in the Discipline of Microbiology; School of Life Sciences; College
of Agriculture, Engineering and Science at the University of KwaZulu-Natal (Westville
Campus), Durban.

Shu Yao (Reg. No. 216075167)

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2018

As the candidate's supervisors, we have approved this thesis for submission.

Supervisor: Prof. Balakrishna Pillay _____

Co-Supervisors: Prof. Cailin Wang _____

A handwritten signature in black ink, enclosed in a light green rectangular box. The signature appears to read "Cailin Wang".

PREFACE

The experimental work described in this thesis was carried out in Jiangsu Academy of Agricultural Sciences, Nanjing, China and School of Life Sciences, University of KwaZulu-Natal (Westville Campus), Durban, South Africa from May 2013 to September 2018, under the supervision of Prof. Balakrishna Pillay and Prof. Cailin Wang.

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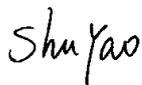
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DECLARATION 2 – PUBLICATIONS

Details of contributions to publications that form part and/or include research presented in this thesis (include publications in preparation, submitted, in press and published and give details of the contributions of each author to the experimental work and writing of each publication).

Publication

Title: The Amylose Content in Good Eating Quality Rice Under different Nitrogen Rates and Sowing Date

Journal: *Chinese Journal of Rice Science*

Authors: Shu Yao – Conceptualisation, Experimental design, Data analysis, Drafting and editing of manuscript

Yanqing Liu – Data collection

Cailin Wang – Conceptualisation, Experimental design, and editing of manuscript

Presentations

Title: The Amylose Content in Good Eating Quality Rice Under different Nitrogen Rates and Sowing Date

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Symposium: The ninth session of Japanese Society for rice quality and Palatability.

Title: Variation of amylose content in rice under the background of *Wx-mq* Gene

Time: March 14-20, 2016. East Hiroshima, Japan

Symposium: China and Japan good quality *japonica* rice comparison seminar.

Title: Distribution of blast resistance genes and resistance to Panicle Blast in Japonica Rice Varieties in Jiangsu

Time: October 19-24, 2016. International Rice Research Institute, Philippines

Symposium: The Seventh World Rice Blast Conference.

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ABSTRACT

Rice is one of the main cereal crops in the world. For a long time the main goal of breeding is to get high yield. With the development of market-oriented economy and the change of consumption concept, people have more demands on rice quality, especially the good appearance and good taste rice is well liked by the people. Amylose content in rice is the main factor affecting the quality of rice taste. Amylose content is recognized as a main index to identify the quality of rice taste. Rice with low amylose content is more and more popular because of its soft and elastic rice texture. Cailin Wang team of the Institute of Food Crops, Jiangsu Academy Agricultural Science developed a series of good eating quality *japonica* rice varieties, such as Nanjing 46, Nanjing 5055, Nanjing 9108, Nanjing 505 and Nanjing 2728, with the semi-glutinous gene *Wx-mq* with low amylose content, which had been popularized in Jiangsu Province and the surrounding provinces in China, and achieved great success and development. High quality brand rice is well received by consumers. However, in the breeding practice we found that the cooking and eating quality between different low amylose content varieties with the same gene background of *Wx-mq* was very different even if they were derived from the same cross combination. In order to clarify the difference and the reason of cooking and eating quality of the semi-glutinous *japonica* rice varieties (lines) with *Wx-mq* gene, the genetic and environmental aspects were discussed in this study, which will provide a theoretical basis for the breeding of new varieties of good eating quality rice and the production of high quality rice.

A total of 124 new semi-glutinous *japonica* rice varieties (lines) with *Wx-mq* gene were selected as materials to determine the amylose content (AC) of each variety (line), and to analyze the AC value differences in the *Wx-mq* gene of semi-glutinous rice from different backgrounds and different ecological types. The results revealed that the AC value here had a great variation in the semi-glutinous lines with the same *Wx-mq* gene, even the same hybrid combination has a variation of 6.34% to 11.70%. The variation range of the semi-glutinous lines changes with the genetic background and the growth period. The hybrids of Wuxiangjing 14 and Kanto 194 were relatively higher in the AC values but comparatively smaller in the variation range. Different

maturity varieties have different amylose content: middle maturing medium *japonica* > late maturing medium *japonica* > late maturing medium *japonica*, and the medium *japonica* rice has a large variation range than the late *japonica* rice.

The experimental material is 64 semi-glutinous lines derived from the hybrids of Wujing 13 and Kanto194 with manifestation polymorphism in starch synthase gene *SSIIa* and *SSIIIa*. The *SSIIa* and *SSIIIa* genotypes were identified by molecular markers on the semi-germinated lines and the genetic effects of *SSIIa* and *SSIIIa* on amylose content, gel consistency, gelatinization temperature, and RVA profiles characteristics were analyzed under the same background of *Wx-mq* gene. The results showed that *SSIIa* and *SSIIIa* had significant effects on the characteristic values of AC, gelatinization temperature (GT), gel consistency (GC) and rice starch viscosity profiles (tested using a Rapid Visco-analyzer, RVA), and the two genes of which have interactive effects. The *SSIIa* and *SSIIIa* genes from Wujing 13, a non-waxy parent, had a tendency to increase AC value with a respective increment of 1.87 and 1.23 percentage and the results were similar in two years. There was no significant difference in gelatinization temperature between *SSIIa* and *SSIIIa* genotypes of different parents, while the gelatinization temperature of genotype *SSIIa^{-mq}SSIIIa^{-mq}* was significantly higher than that of *SSIIa^{-b}SSIIIa^{-b}*. The result indicates that the gelatinization temperature was not significantly affected by single *SSIIa* and *SSIIIa* gene but remarkably influenced by the combination of the two genes. The gelatinization temperature of genotype *SSIIa^{-mq}SSIIIa^{-mq}* was 1.34 °C higher than that of *SSIIa^{-b}SSIIIa^{-b}*. The gel consistency among different genotypes was significantly different. For example, the *SSIIa* gene from Wujing 13 (*SSIIa^{-b}*) and *SSIIIa* gene from Kanto 194 (*SSIIIa^{-mq}*) could increase the gel consistency of 8.74mm and 9.62mm respectively. From the joint effect of the two genes, the gel consistency of genotype *SSIIa^{-b}SSIIIa^{-mq}* was 10.64 mm higher than that of genotype *SSIIa^{-mq}SSIIIa^{-b}* and was 16.95 mm higher than that of *SSIIa^{-b}SSIIIa^{-b}*. The *SSIIa* gene (*SSIIa^{-b}*) from Wujing 13, a non-waxy parent, increased the peak viscosity, hot paste viscosity, cold paste viscosity and breakdown value, while it decreased the consistence value and setback value in the meanwhile. However, the effect of *SSIIIa* gene *SSIIIa^{-b}* from Wujing 13 was just the opposite, which decreased the peak viscosity, hot paste viscosity, cold paste viscosity, breakdown Value and increased the consistence value and consistence value. In terms

of the joint effects of the two genes, the peak viscosity, hot paste viscosity and cold paste viscosity were the largest in $SSIIa^{-b}SSIIIa^{-mq}$ genotype, the breakdown value and consistence value were the largest in $SSIIa^{-b}SSIIIa^{-b}$ genotype, and the setback value in $SSIIa^{-b}SSIIIa^{-mq}$ genotype was the least. According to the manifestations of all characters, the 4 genotype eating quality of $SSIIa$ and $SSIIIa$ site were the best in $SSIIa^{-b}SSIIIa^{-mq}$ genotype, 11.54% of AC value, comparably lower GT, gel consistency, peak viscosity, hot paste viscosity, cold paste viscosity and breakdown value were the largest, and the consistence value was the lowest.

Through the process of four levels, high (450 kg/hm²), medium (300 kg/hm²), low (150 kg/hm²) and no nitrogen application (control) and sowing by stages as well as short sunshine control, the influence on the AC values of the semi-glutinous *japonica* rice of conditions such as nitrogen dose and the seeding dates were studied. The results showed that the amount of nitrogen fertilizer had a significant effect on AC value of semi-glutinous *japonica* rice. With the increase of nitrogen application rate, the AC value decreased, and the AC value of high nitrogen treatment was the lowest in two years. The AC value of *japonica* rice with good eating quality was also significantly affected by sowing date, which will decrease with the delay of the sowing date. And it mainly relates to the temperature of the days between 6-15 after heading. The result was that higher temperature could improve the amylose content. This has also been confirmed by the results of short sunshine treatments. The results of this study have a guiding significance for the preservation and cultivation of the semi-glutinous fine *japonica* rice.

This thesis is dedicated to:

My husband, Binqian Luo & my daughter, Jiarui Luo

for their love, encouragement and support during the course of my academic career

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ABBREVIATIONS

English abbreviations	English full name
ECQ	Eating and cooking quality
AAC	Apparent amylose content
AC	Amylose content
GC	Gel consistency
GT	Gelatinization temperature
RVA	Rapid visco-analyser
AGPP	ADP glucose pyrophosphorylase
GBSS	Granule bound starch synthase
SS	Souble starch synthase
<i>W_x</i>	Waxy gene
SSS	Souble starch synthase
SBE	Starch branching enzyme
DBE	Starch debranching enzyme
PKV	Peak viscosity
HPV	Hot viscosity
CPV	Cool viscosity
BDV	Breakdown
SBV	Setback
CSV	Consistence
PeT	Peak time
PaT	Pasting temperture
<i>Isa</i>	Gene encoding isoamylase
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
SSR	Simple Sequence Repeat
STS	Sequence-tagged site

CAPS	Cleaved-amplified polymorphism sequence
PCR	Polymerase chain reaction
CTAB	Cyltrimethylammonium Bromide
EDTA	Ethylene Diamine Tetraacetic Acid
Tris	Tris(hydroxymethyl)aminomethane
AP	Ammonium persulphate
TEMED	N,N,N',N'-Tetramethylethylenediamine
DuRed	DuRed nucleic acid gel stain
bp	base pair
cM	centimorgan

CHAPTER ONE

INTRODUCTION

Introduction

1.1 Background

Rice is one of the most important major food crops, as the living standards of society and the economy have significantly improved during the past decades, studies have focused on rice (*Oryza sativa* L.) quality traits, such as cooking and eating quality, more and more people are high to the requirement of rice quality. Previous studies showed that amylose content is an important factor affecting eating quality of rice (Matsuo *et al.*, 1990; Hushibuchi, 1992). The heredity of rice amylose content is very complex (Yi *et al.*, 2013). *Indica* rice was controlled by the Wx^a gene, the amylose content is generally between 21%-30%; *japonica* rice was controlled by the Wx^b gene, the amylose content is generally between 15%-20%; glutinous rice was controlled by the recessive wx gene, the amylose content is lower than 2% (Zhou *et al.*, 2015). There is a class of mutants in the gene locus, such as $Wx-mq$, the amylose content is between those of *japonica* rice and glutinous rice, so it is called semi-glutinous rice or low amylose content rice. Low amylose content rice shows cloudy and milky white endosperm appearance and slightly worse transparent. However, the surface of the steamed rice is gloss translucent, soft and flexible. Combining the softness of glutinous rice and the flexibility of *japonica* rice, low amylose content rice has better eating quality, not retro-gradation after cold and puffing good with high commodity value (Zhu *et al.*, 2004). Therefore, development of good eating quality rice varieties with low amylose content to meet the increasing market demand has become a new research direction of rice breeding in current. In recent years, low amylose content of rice to texture characteristics of the soft and elastic, more and more get the favour of people (Zhu *et al.*, 2004).

Rice amylose catalyzed synthesis of granule combined with starch synthase (*GBSS*) is encoding by rice waxy gene (Wx). And control of low amylose gene $Wx-mq$ is through chemical mutagen N - methyl - N - nitroso urea deal with *Japanese* rice varieties of the light, the waxy gene (Wx) coding area happened two base replacement, in turn affects the *GBSS* activity of enzymes, causing low amylose. $Wx-mq$ has been applied in breeding, Japanese breeders use rice

containing the gene resources Milky Queen, created low amylose content rice varieties "New-hikari". In China, our laboratory development designed to distinguish containing or not containing *Wx-mq* gene homozygous genotype and heterozygous genotype of CAPS (cleaved amplified polymorphic sequence), and successfully used the auxiliary out good eating quality rice breeding Nanjing 46, Nanjing 5055, Nanjing 9108 (Zhu *et al.*, 2013).

There had significant differences in grain amylose content among different rice varieties. The genetic expression of amylose content was significant different in the offspring of different parents with different amylose content (Gonzalez *et al.*, 2013). The amylose content is also affected by the external environment condition. There were many results related to rice amylose and low amylose content has been published, mainly about the genetic characteristics of amylose content and the rice appearance quality, cooking quality and their correlation with agronomic traits. However, the genetic characteristics and molecular mechanism on the difference of low amylose content in rice is not so clear. In breeding practice, we found that there existed significant differences in amylose content, palatability and eating quality among low amylose rice lines even if originated from the same cross combination with the same background of *Wx-mq* gene. It implied that starch synthesis related genes other than the *Wx* locus have a large influence on amylose content and eating quality.

Cooking and eating quality is an important aspect of rice quality. It was closely related to amylose content (AC), gelatinization temperature (GT), gel consistency (GC) and rice starch viscosity (tested using a Rapid Visco-analyzer, RVA). In order to clarify the difference and the reason of cooking and eating quality of the semi-glutinous *japonica* rice varieties (lines) with *Wx-mq* gene, the genetic and environmental aspects were discussed in this study, which will provide a theoretical basis for the breeding of new varieties of good eating quality rice and the production of high quality rice.

In this study, the genotypes of starch synthesis genes were systematically screened among different rice varieties using molecular markers. Sixty-four breeding lines derived from the cross of "Kanto 194" and "Wujing 13" were used to evaluate the genetic effects of *SSIIa* and *SSIIIa* genes by detecting genotypes and measuring AC, GT, GC and RVA. We also studied the

effects on amylose content of different nitrogen rates and sowing dates of the good eating quality *japonica* rice.

1.2 Scope of this study

Rice is one of the important food crops in the world, more than half the world's people are regarding rice as the staple food. Amylose content was usually used as a main index for the identification of rice eating quality. There existed significant differences in grain amylose content among different rice varieties. The genetic expression of amylose content was significant different in the offspring of different parents with different amylose content.

In this study, it is the first time to explore the reasons for the difference in the eating quality of semi-glutinous good eating quality *japonica* rice were discussed from two aspects, namely heredity and environment. It clarifies the genetic effect of *SSIIa* and *SSIIIa* on AC, GT, GC and RVA profiles and interaction effects under the background of *Wx-mq* gene, identifies the best eating quality of *SSIIa^bSSIIIa^{-mq}* genotype, and provides a theoretical basis for the molecular design breeding semi-glutinous type *japonica* rice with good eating quality. It makes sure that the excessive amount of nitrogen fertilizer and the high temperature of 6-15 d after heading could descend AC, the appearance and the eating quality of semi-glutinous *japonica* rice and increase PC, which provides a theoretical basis for the cultivation of good eating semi-glutinous *japonica* rice.

1.3 Hypothesis

It is hypothesized that under the same genetic background, rice varieties with *SSIIa^bSSIIIa^{-mq}* genotype have the best eating quality and appearance. It is further hypothesized that molecular design breeding will help to develop the semi-glutinous rice varieties with optimum amylose content.

1.4 Aim

In order to clarify the difference and the reason of cooking and eating quality of the semi-glutinous *japonica* rice varieties (lines) with *Wx-mq* gene, and to provide a theoretical basis for the breeding of new varieties of good eating quality rice and the production of high quality rice.

1.5 Specific objectives

The objectives of this study were:

1.5.1 To analyze the difference of amylose content between different varieties of the same combination or different combination or different ecological types.

1.5.2 To analyze effect on amylose content of nitrogen application, sowing date and short daylight treatment, it is clear that the variety itself is the most important factor affecting amylose content.

1.5.3 To identify the *SSIIa* and *SSIIIa* genotypes of semi-glutinous lines with *Wx-mq* allele.

1.5.4 To analyze the genetic effects of *SSIIa* and *SSIIIa* genes on amylose content, gel consistency, gelatinization temperature and RVA values under the same *Wx-mq* gene background.

1.5.5 To establish a set of method to breed the semi-glutinous rice variety with the most suitable amylose content by molecular marker-assisted.

1.6 Key questions to be answered

1.6.1 How to determine the cooking and eating quality of rice?

1.6.2 Under the same genetic background, why has big difference of amylose content between varieties which from the same combination?

1.6.3 Under the *Wx-mq* gene background, which genotype has the best amylose content?

1.6.4 How to control the influence of environmental conditions on amylose content of rice?

1.6.5 How to grow good eating quality rice varieties better?

1.6.6 Under the *Wx-mq* gene background, How to cultivate different amylose content rice varieties by molecular design breeding?

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CHAPTER TWO

LITERATURE REVIEW

Literature Review

1.1 Introduction

Rice is one of the three most important food crops in the world. More than half of the world's population uses rice as a source of daily carbon intake. China's population is in the forefront of the world, the production and consumption of rice play a pivotal role in global food production (Kotaro *et al.*, 2011; Lu *et al.*, 2005; Jiang *et al.*, 2007). In recent years, the improvement of rice quality has become a new and important goal in rice breeding. In the past few decades, breeders have made use of dwarf breeding and heterosis technology to improve the yield of rice and increase the area of rice production so as to meet the demand of consumption (Xue *et al.*, 2008; Jeon *et al.*, 2011). In recent years, rice breeders in China have tried to make use of the heterosis between *indica* and *japonica* subspecies for super rice breeding which makes it possible to further improve the yield level of rice varieties (Song *et al.*, 2016). At present, with the increase of output, the market competition is becoming more and more fierce. It is difficult to bring about the corresponding economic benefits by simply pursuing the output. Furthermore, with the continuous increase of new varieties, the market competition is more fierce. People's choice of range of rice is relatively large. In order to meet the demands of the market and people's lives, the rice cooking and eating quality of and appearance quality are most important and most worthy of attention. Therefore, the main direction of rice breeding improves from high yield to rice eating quality.

1.2 Determinants of rice cooking and eating quality

Cooking and eating quality of rice refers to the physical, chemical and sensory characteristics of rice during cooking and eating. Due to the high correlation between cooking quality and eating quality, it is commonly referred to as cooking and eating quality (Wang, 2015). Eating quality is a complex trait; it mainly refers to the sensory impression left by rice when it is observed, chewed and eaten. The cooking quality mainly includes the water absorption, extensibility, expansibility and cooking time of rice. Different regions of the consumer groups

for tasting quality requirements are different. The quality identification of rice cooking and eating have strict standards. Rice cooking should grasp the appropriate ratio of rice water and cooking time. Experienced people form a taste group to identify in turn the taste of each index. However, the taste of people will be different with age, gender and region, so there is subjective deviation in this method. In order to determine accurately the cooking and eating quality of rice, the physicochemical index of rice starch, i. e. amylose content (AC), is generally determined. Gelatinization temperature (GT), Gel consistency (GC) Rice starch viscosity (RVA) Equal index to comprehensive evaluation.

1.2.1 Amylose content (AC)

The rice starch is divided into amylose and amylopectin according to their different structures, while the quality of rice starch depends on the structure and proportion of amylose and amylopectin. Amylose content (AC) generally refers to the percentage of amylose in dry weight of rice flour, which is one of the main indexes to identify rice quality. AC mainly relates to the stickiness, swelling, glossiness and softness of rice (Mo *et al.*, 1993). The content of amylose in rice is divided into four types according to International Rice Research Institute (IRRI), which are waxy (AC is 0-2%), low (AC is 8-20%), middle (AC is 20-25%) and high (AC>25%) (Shaokai *et al.*, 1984). The rice with high amylose content usually has a large expansibility, the rice is loose and of pale color, and it is stiff after cooled and has poor palatability, while the rice with low amylose content is less expansive, the rice is fluffy and soft, it is favored by people. Low amylose content rice is commonly known as soft rice, and its amylose content is in 5-15%, which is an intermediate type between the general rice and glutinous rice, it is also commonly known as semi-glutinous rice. Its endosperm appearance is cloud shaped, milky white, with slightly poor transparency. The surface of rice is lustrous, soft and elastic. It combines the softness of glutinous rice and the elasticity of *japonica* rice; it is not stiff after cold, and has better quality of taste.

So far, 14 mutant genes with low amylose content in rice have been reported. According to their allelic relationship with *Wx* gene, they can be divided into two groups: *Wx* allele and non-allele. The translucent endosperm mutant gene *Wx-mq* belongs to the low amylose content gene with

Wx allele. Japanese rice breeders have used translucent endosperm mutant genes to produce rice varieties with good eating quality and low amylose, such as Milky Queen and Kanto 194 (Sato *et al.*, 2000). In order to improve the eating quality of *japonica* rice in Jiangsu Province, our team used Kanto 194, a germplasm derived from Japan with low amylose content, to improve the quality of *japonica* rice. Nanjing 46, Nanjing 9108, Nanjing 5055 and other good varieties of rice with low amylose content, good disease resistance and high yield were cultivated, which promoted the breeding of high-quality *japonica* rice in Jiangsu to a new level.

1.2.2 Gelatinization temperature (GT)

Gelatinization temperature (GT) refers to the critical temperature at which expansion becomes dissolved after the loss of birefringence and crystallization during water absorption heating (Khush *et al.*, 1979). This depends on the temperature and time of the cooking of the rice. Because of the difference of rice varieties, the gelatinization temperature of rice starch varies from 55 °C to 79 °C, which is usually divided into three types of low (GT < 70°C) middle (70 °C < GT < 74°C) and high (GT > 74°C). Because gelatinization temperature is highly correlated with setback value, and setback value is easy to measure. So, gelatinization temperature is usually replaced by setback value (Yan *et al.*, 2001). Setback value are usually divided into seven levels: 1-3 Grade belongs to high gelatinization temperature ($\cong 75^{\circ}\text{C}$), Grade 4-5 belongs to medium gelatinization temperature (70-74°C) and Grade 6-7 belongs to low gelatinization temperature ($\cong 69^{\circ}\text{C}$) (Gao *et al.*, 2003). The level of GT is closely related to the number of AC, and it also may be related to the spatial structure and synthesis of amylopectin (Yan *et al.*, 2001). The gelatinization temperature of *indica* rice varieties is either medium or low. The gelatinization temperature of *japonica* rice varieties is low, while that of good *indica* rice and *japonica* rice varieties is usually medium and low.

1.2.3 Gel consistency (GC)

Gel consistency (GC) is generally determined by measuring the length of rice gel after cooling. It can reflect the elongation of rice glue after cooling (Yu, 2001). Gel consistency can reflect the comprehensive effect of amylose and amylopectin molecules on amylose content in

endosperm (Li *et al.*, 2007) which is closely related to the softness and viscosity of rice. Gel consistency can usually be divided into three levels: soft ($\cong 61$ mm), middle (41-60 mm) and hard (≤ 40 mm). *Indica* rice is usually of hard gel consistency variety, *japonica* rice mostly belongs to soft gel consistency variety, while glutinous rice gel consistency is generally more than 90 mm. The rice with low GC content is loose and dry, hard after cooling with rough surface and poor taste. The rice with high GC content has a smooth and elastic surface and remains soft after cooling. Therefore, in the breeding utilization, the rice with good eating quality is the best combination of soft gel consistency and middle amylose content.

1.2.4 Rice starch viscosity (RVA profiles characteristics)

Rice starch viscosity refers to the viscosity curve formed by a series of changes in the viscosity of starch paste as determined by the rapid viscosity analyzer (RVA) during heating, continuous high temperature, and cooling. Its characteristic values mainly include the peak viscosity, (Peak viscosity, PKV) Heat slurry viscosity (Hot paste viscosity, HPV), Cold paste viscosity (Cool viscosity, CPV) Breakdown Value (Breakdown value, BDV, = peak viscosity-viscosity of hot pulp) Setback value (Setback value, SBV, = cold paste viscosity peak viscosity) And response value (Consistency value, CSV, = cold paste viscosity-Hot slurry viscosity) (Jia *et al.*, 2008).

Because of the rapid, convenient and accurate determination of RVA, and its reproducibility, RVA has been applied to the determination of rice quality (Bason *et al.*, 1994). Jia *et al.* (2008) results showed that the difference of cooking and eating quality among rice varieties could be reflected by the characteristic value of RVA profile, especially BDV, SBV and CSV which could effectively reflect the quality of rice taste. Sui *et al.* (2005) concluded that the characteristics of RVA profile are closely related to the taste quality. The results show that the breakdown Value, the setback value and the consistence value can accurately evaluate the quality of the taste, so it can be used as an effective index for selecting good varieties of taste quality. Some studies have shown that the characteristic value of RVA profile is closely related to AC and GC of rice, that is, the higher the AC of rice varieties, the smaller the breakdown Value, but the greater the consistence value, the setback value and the viscosity of cold glue (Hu *et al.*, 2004). Therefore,

the characteristic value of RVA profile can be used as a reference when evaluating rice eating quality.

1.3 Starch synthesis pathway and key enzymes

1.3.1 Composition of starch

Starch as the main component of rice endosperm, can be divided into amylose and amylopectin according to their molecular structure and chemical properties. Amylose belongs to linear branching, and its basic unit is α -D-glucose. Each basic unit is connected by α -1, 4 glycoside bond, and its branches are few or none. Amylopectin is a kind of high branched glucose polymer, which is connected by α -1, 4 glycoside bond and then connected by α -1, 6 glycoside bond (Xu *et al.*, 2008). The content and proportion of amylose and amylopectin are important factors affecting the cooking quality of rice.

1.3.2 Starch synthesis pathway

The regulation system of starch synthesis in rice is complex. Starch is the main component of milled rice, and the process of starch synthesis in grain is the formation process of rice quality traits (Cai *et al.*, 2004). The process is not only related to the yield of rice but also its quality. A large number of studies showed that photosynthetic products were transported from leaves to grains through phloem in the form of sucrose, and starch was formed after a series of enzymatic reactions. Sucrose in rice grain was degraded into glucose, fructose and uridine diphosphate glucose, which was mainly caused by sucrose invertase and sucrose synthase, and then by hexokinase, fructose kinase and glucose phosphate translocation enzyme. After conversion to glucose 1-phosphate, it enters the amyloplast and then forms ADPG under the catalysis of AGPase and other enzymes. Finally, the starch synthase is synthesized by starch synthetase (*SS*), starch branching enzyme (*SBE*), synthesis of amylose and amylopectin by enzymes such as starch debranching enzyme (Ball *et al.*, 1998) (Figure 2.1).

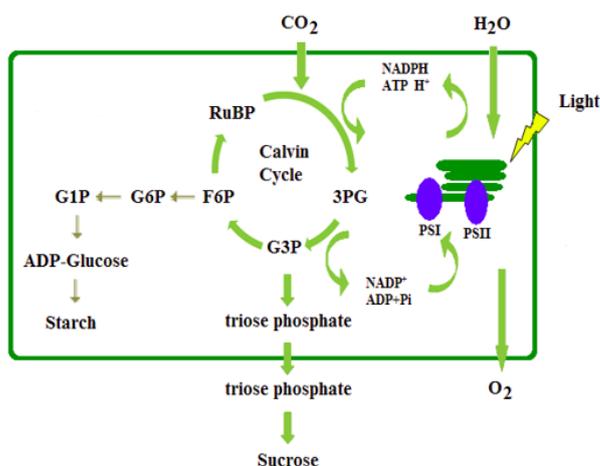


Fig. A

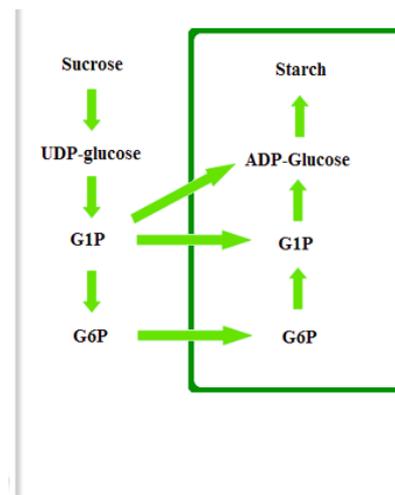


Fig. B

A. Starch synthesis process in chloroplast. B. Starch synthesis process in starchy body.

Fig 2.1 the synthesis pathway of starch in a cell (Ball *et al.*, 1998)

1.3.3 Key enzymes and genes for starch synthesis

At present, more than 20 genes related to starch synthesis have been reported, including *AGPlar*, *AGPis*, *AGP_{sma}* genes encoding ADP-glucose pyrophosphorylase subunits and multiple genes produced by different splicing modes, genes encoding starch synthase, such as *Wx*, *GBSSII*, *SSI*, *SSIIa*, *SSIIb*, *SSIIc*, *SSIIIa*, *SSIIIb*, *SSIVa*, *SSIVb* and genes encoding starch branching enzymes *SBE1*, *SBE3*, *SBE4* and genes encoding debranching enzymes *ISA*, *PUL* (Hannah *et al.*, 2008; Nakamura *et al.*, 2002; James *et al.*, 2003) (Figure 2.2). In recent years, some scholars believe that starch phosphorylase and dismutase are also involved in starch synthesis, and the coding gene of starch phosphorylase and dismutase is *PHO1*, *PHO2*, *DPE*.

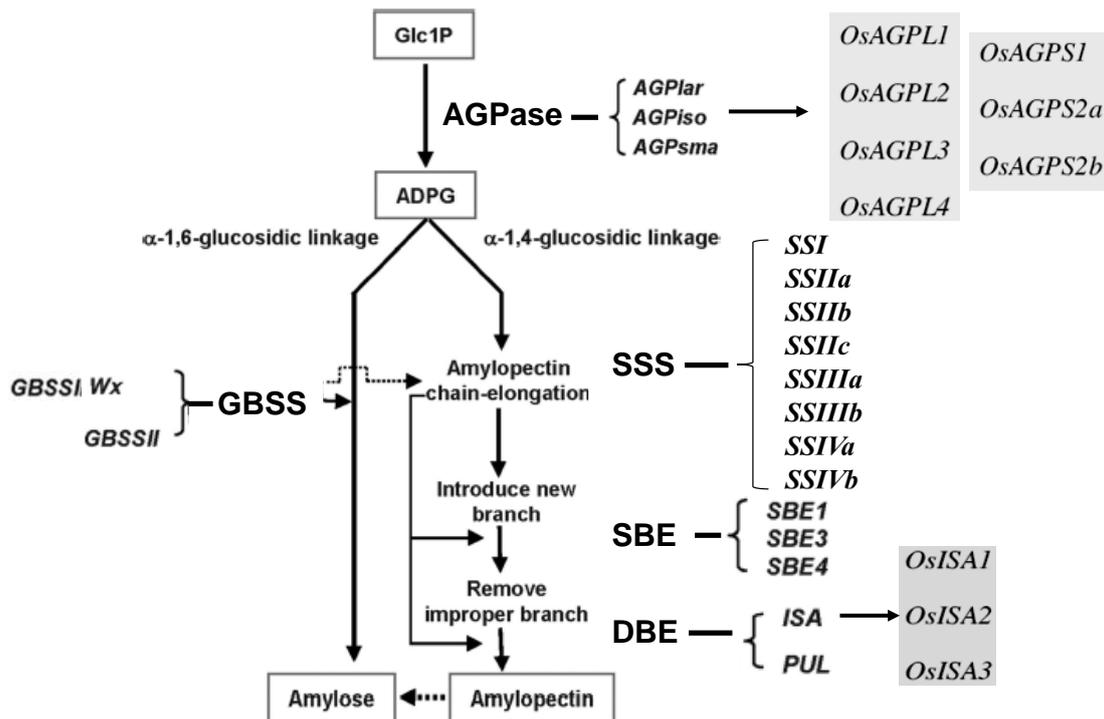


Fig 2.2 Enzymes and regulatory genes involved in rice starch-synthesis pathway (Hannah *et al.*, 2008)

1.3.3.1 ADP glucose pyrophosphorylase (AGP)

AGP is the rate-limiting enzyme of starch synthesis and the first enzyme involved in starch synthesis, catalyzing G-1-P (1- Phosphoric acid glucose) pyrophosphoric acid and ADPG were synthesized with ATP. ADPG as the substrate of starch synthase, and also the initial glucose donor for biosynthesis, is used to synthesize amylose and amylopectin. In rice, encoding AGPase has six genes, four large subunits and two small subunits. AGPP belongs to the allosteric regulatory enzyme, which is positively activated by glycerol 3-phosphate, but negatively inhibited by inorganic phosphoric acid (Beckles *et al.*, 2001). In the endosperm of rice, the enzyme activity is consistent with the starch accumulation process and is also related to the grain filling rate, but there is no direct relationship with the starch composition (Yasunori N, *et al.*, 2010).

1.3.3.2 Starch synthase (SS)

Starch synthase can generally be classified into two categories according to the state of existence in the amyloplast: Granular bound starch synthase (GBSS) which is responsible for

amylose synthesis; and soluble starch synthase (SSS) which is responsible for amylopectin synthesis.

(1) Granular binding starch synthase (GBSS)

Granular binding starch synthase (GBSS), a starch synthase that is mostly studied, plays a major role in amylose synthesis. It is mainly controlled by the waxy gene *Wx* site (Wang *et al.*, 2008). There are two GBSS genes in rice which are respectively *GBSSI* and *GBSSII*. *GBSSI* is mainly responsible for the synthesis of amylose in endosperm, while *GBSSII* is mainly responsible for the expression of nutrients such as leaves and stems. The previous research showed that there was no amylose in endosperm if GBSS was absent in rice endosperm.

Since amylose content is the main influencing factor of rice cooking and eating quality, it is very important to study *Wx* locus. Studies have shown that there are three alleles in the locus: *Wx*, *Wx^a* and *Wx^b* are found in waxy rice, *indica* rice and *japonica* rice respectively (Hirano *et al.*, 1991). *Wx* recessive genes encoding GBSS <2% content of amylose containing the genotype of rice is glutinous rice. In *indica* rice, *Wx^a* is the main content and amylose content is generally high. In *japonica* rice, *Wx^b* is the main content and amylose content is low. (Wu *et al.*, 2006). In recent years, with the further study of the molecular level of *Wx* gene expression regulation mechanism, people have a further understanding of the difference of allele expression. Some studies have shown that the main reason for the difference of amylose content among rice varieties is the post-transcriptional regulation of *Wx* gene. High amylose content (>20%) *Wx* gene is a mature transcription material with a molecular weight of 2.3 kb, while amylose content is low or low in rice varieties. (6-16%) In addition to transcribing mature transcripts, the *Wx* gene also has the precursor mRNA of the first intron and molecular weight of 3.3 kb (Sun *et al.*, 2005). Glutinous rice varieties have no mature transcripts of *Wx* gene and therefore have little or no amylose. Further studies have shown that this post-transcriptional regulation is closely related to the splicing efficiency of intron 1 of *Wx* gene. If the base of the first intron 5' end shear site is G, the splicing efficiency of the transcription product of the *Wx* gene is high, and it can produce more mature mRNA and corresponding GBSS enzymes, and

finally show a high amylose content in the endosperm. When the base of the shear site changed from G to T, the splicing efficiency decreased, the amount of mature mRNA and GBSS decreased, and the amylose content in endosperm decreased (Lv *et al.*, 2005). A large number of studies have shown that in *indica* rice, the first intron 5' site of *Wx* gene is usually GGN, while in *japonica* rice it is TX, which is consistent with the higher amylose content in *indica* than in *japonica*. The G and T single nucleotide polymorphism of *Wx* gene in rice varieties could explain more than 80% of the variance of amylose content variation.

At present, there are 14 mutant genes with low amylose content that can be used in rice breeding. Most of them are controlled by a pair of recessive genes and mainly distributed in rice on chromosomes 6, 9, 10 and 12 (Wang *et al.*, 2012). *Wx-mq* is a low amylose content mutation gene obtained by treating Japanese rice varieties Koshihikari with chemical mutagens *N*-methyl-*N*-nitrosourea (MNU). In the mutation gene, two base substitutions occurred in the coding region of *Wx*, which affected the activity of *Wx* protein and reduced the amylose content of *Wx* protein (Sato *et al.*, 2002). *Wx-mq* gene is widely used in breeding while the genetic mechanism is deeply studied. By using the rice resource Milky Queen containing this gene Japanese breeders developed rice varieties "New-hikari" and "Kanto 194" with good eating quality and low amylose content (Tomit *et al.*, 2007; Sato *et al.*, 2008; Wang *et al.*, 2008). Wang *et al.* (2008) developed new varieties of *japonica* rice with good eating quality, such as "Nanjing 46", "Nanjing 5055" and "Nanjing 9108" by using "Kanto 194".

(2) Soluble starch synthase (SSS)

Soluble starch synthase (SSS) mainly exists in plastid matrix, it includes all starch synthase except GBSS. There are many isozymes or isoforms in SSS. So far, eight different isoforms have been reported in rice: *SSI*, *SSIIa*, *SSIIb*, *SSIIc*, *SSIIIa*, *SSIIIb*, *SSIVa*, and *SSIVb*. Other different isoforms have also been reported in other species (Craig *et al.*, 2000). The activity of each isotype in different plant tissues is different, so it plays a different role in amylopectin synthesis. *SSSI* was more conserved in evolution and had little difference among different plants. There are three types of *SSII*: *SSII-1* (*SSIIc*), *SSII-2* (*SSIIb*) and *SSII-3* (*SSIIa*). Different *SSII* between the same types of expression specificity in the organization has some differences, For

example, *SSIIb* in rice is mainly expressed in leaves of rice, while *SSIIa* is mainly expressed in endosperm cells. *SSII* plays an important role in the synthesis of amylopectin. When the three isoforms of amylopectin are mutated, the proportion of each branch of amylopectin changes greatly, and the content of dextran chain in medium length obviously decreases. This indicates that it plays an important role in the synthesis of amylopectin with medium length. (Craig *et al.*, 1998). The results show that *SSIIa* is the main gene of gelatinization temperature in rice. It specifically acts on the extension of short A and B1 chains in branch clusters and the synthesis of long B1 chains, which determine the structural characteristics of amylopectin (Zhang *et al.*, 2001).

1.3.3.3 Starch branching enzyme (SBE)

Starch branching enzyme (*SBE*) plays an important role in the formation of starch amylopectin. There are at least three SBE isoforms in rice. *SBE1*, *SBE3* and *SBE4/SBE1* genes are composed of 14 exons and 13 introns. They play different roles in the synthesis of amylopectin. In rice, *SBE1* mutants show moderate length and slight decrease of long chain branching. The results indicate that *SBE1* plays an important role in synthesizing these branched strands, and the functional deficiency of *SBE1* can't be compensated by *SBE3* or *SBE4*. (Nakamura *et al.*, 2002; Blauth *et al.*, 2002). Some researchers used mutants to find that *SBE3* may play a role in the branching synthesis of medium length such as A chain and B1 chain, while the activity of *SSSI* in mutants is also significantly decreased, so there may be interaction between *SBE3* and *SSSI* protein and it participates in the synthesis of amylopectin. No changes in the structure of amylopectin were found in the mutant that expressed in the early time of *SBE4* in rice, so *SBE4* did not play a key role in amylopectin synthesis.

1.3.3.4 Starch debranching enzyme (DBE)

The function of *DBE* is contrary to the function of *SBE*, which is the α -1, 6 glycoside bond of amylopectin, and there are two types of *ISO* amylase (*ISA*) and branched amylase (*PUL*). The α -1, 6 branch of glycogen can be hydrolyzed by *ISA*, but the α -1, 6 branch of amylopectin can't be hydrolyzed. Although *PUL* can't use glycogen as a substrate, it can effectively decompose

the α -1, 6 branch of amylopectin (Smith *et al.*, 1997).

1.4 Effects of environmental conditions on the cooking and eating quality of rice

1.4.1 Sowing date

The cooking and eating quality of rice is not only controlled by the genetic genes of rice varieties, but also influenced by environmental conditions. It is an important technical measure for rice to realize high yield and good quality by reasonably regulating the sowing time of rice, and making full use of the local climate resources to improve the rice quality. The essential reason that sowing date affects the cooking and eating quality of rice is temperature. Research shows that high temperature increases gelatinization temperature and hardens the gel consistency, and also affects the amylose content. However the effect varies with varieties (Qin, 2006). The changes of amylose content and protein content in rice were studied at different latitudes by Shen *et al.*, (2007) with four different types of rice varieties in 5 sowing dates at 4 latitudes in Jiangsu Province.. The amylose content and protein contents were varied with different varieties. Because of the synergistic effect of latitude and sowing date, each type of varieties had its own variation characteristics. In the range of 0-300 kg/hm², the gel consistency and amylose content of rice varieties decreased with the increase of nitrogen level. The sensitivity of rice taste value to nitrogen fertilizer varies with the varieties of rice (Gao, 2010).

1.4.2 Cultivation density

The formation of rice quality is mainly controlled by genetic and environmental factors. Among many environmental factors, reasonable cultivation density is an important prerequisite for establishing ideal rice population structure and obtaining high quality and high yield. The planting density includes row spacing, plant spacing and transplanting density. With the increasing shortage of agricultural land and the intensification of non-point source pollution caused by unreasonable fertilization, the research on high yield and high quality techniques for optimizing the cultivation density of rice has been paid more and more attention. At present,

the effect of planting density on rice quality has not been decided. Xie *et al.* (2007) and other studies believe that increasing planting density can increase the whole head rice rate, reduce the reduction RVA profile characteristics, consistence value and the hardness of rice texture. Wu *et al.* (2005) and others thought that planting density had significant linear negative correlation with grain percentage, chalkiness, amylose content, inverted conic distribution relationship with head rice percentage, and close linear positive correlation with protein content. Xu *et al.* (2008) and other studies also showed that the protein content of rice increased with the increase of planting density, but Zhou *et al.* (2001) and Han *et al.* (1997) thought that sparse planting could increase the protein content of rice. Qian (2006) and so on thought that with the increase of the number of seedlings planted in each hole, the yield and the characteristic value of RVA profile characteristics showed parabola change, while the main quality characters showed a tendency of deterioration, but each index varied with the number of planted seedlings. Zhang *et al.* (2012) believe that row spacing plays an important role in cooking and eating quality and RVA characteristics.(except for the regenerative consistency value)There were significant or extremely significant effects on cooking and eating quality, processing quality and RVA profile characteristics of transplanting seedling density in hole. (Except for minimum viscosity and final viscosity) There were significant or extremely significant effects on cooking quality traits and RVA characteristics. The results showed that the effect of planting density on the main rice quality index was as follows: the chalkiness rate and chalkiness were the biggest, followed by amylose, protein and gel consistency. Up to now, most of the effects of planting density on rice quality have remained at the technical level or statistical analysis level, so the molecular mechanism of how these cultivation factors affect the rice quality is still relatively weak. It needs to be fully excavated in subsequent work.

1.4.3 Fertilizer

Among the three elements of nitrogen, phosphorus and potassium, nitrogen has the greatest influence on rice quality (Lin *et al.*, 2012). Nitrogen fertilizer is one of the important factors that influence the rice cooking and eating quality. Applying appropriate nitrogen fertilizer can not only improve the yield, but also improve the appearance and cooking and eating quality of

rice. Rice cooking and eating quality depends largely on the grain of the amylose content and protein, nitrogen is important measures for high-yield cultivation of rice production, nitrogen fertilizer on rice plant carbon and nitrogen metabolism in the body, to determine in rice amylose and protein synthesis and accumulation. There have been many reports about the relationship between nitrogen nutrition and rice quality. The results showed that with the increase of nitrogen nutrition, the protein content of rice increased, the glue consistency became shorter, the maximum viscosity decreased, and the cooking food quality became worse (Chen, 2012). Studies have also shown that nitrogen fertilization has no significant effect on the gelatinous consistency of hybrid early rice. Applied amount of nitrogen fertilizer on rice quality formation, the influence of many research point of view more consistent, increase nitrogen can improve the head rice rate and protein content, gelatinization temperature, reduce the rate of chalk white and white chalk degree, shorten the gel consistency. But there were also scholars study shows that with the increase of nitrogen, gel consistency get shorter, lower amylose content (Lin Qing *et al.*, 2012).

1.4.4 Harvest time

The harvest time after rice ripening had little effect on the economic yield, but it has a great influence on rice quality. Suitable harvest period can provide the best rice quality, early harvest or harvest that is too late will reduce rice quality in varying degrees, and postpone the harvest time which can reduce the ration of green rice and improve the palatability of rice. From the view of the cooking and eating quality, the harvest period had little effect on protein content, but had great effect on amylose and fatty acid, but had no obvious effect on the taste value. Jiang *et al.* (2006) and Wu *et al.* (2007) showed that the protein content of rice increased slightly with the delay of harvest date, and was higher at the yellow ripening stage. Huang *et al.* (2008) and other researchers thought that with the delay of harvest date, the alkali elimination value of rice increased, the gelatinization temperature decreased, the gel consistency gradually became longer and the gel consistency became soft. At the same time, the amylose content increased, the crude protein content increased first, followed by the heading of the early rice and the reduction of the heading of both middle and late rice. Li *et al.* (2008) found that with the harvest,

the protein content increased first, then decreased, and then increased again. Li (2014) and others thought that stage harvest had a great influence on the palatability of rice, and there were significant differences in breakdown Value and alkali elimination value in different harvest periods, which were the main factors influencing the difference of taste value in different harvest periods.

1.4 Effects of starch synthesis-related genes on cooking and eating quality in rice

1.4.1 Effect of *Wx* on cooking and eating quality

Wx is a major gene controlling amylose content. In addition, it plays an important role in amylose synthesis. *Wx* is also the main gene of gel consistency (GC) and also has a certain effect on gelatinization temperature (GT). Some researchers believe that the gene loci that control AC, GC and GT are the same. Tian *et al.* (1999) believe that AC, GC and GT are controlled by the same locus, and that this locus is linked or is closely linked to the *Wx* locus (Tian *et al.*, 1999). As the main effect gene for controlling AC, the contribution rate of *Wx* gene to AC was 77.9% (Wu *et al.*, 2006). Bao (2003) and others have pointed out that the main locus controlling GC is a locus closely linked to the *Wx* locus, and because GC is significantly related to AC, it is inferred that this locus is *Wx* gene locus (Bao *et al.*, 2003). Results from Sun (2005) and Tian *et al.* (1999) showed that GC was closely related to *Wx* gene, controlled by *Wx* gene or closely linked to *Wx* gene, and the correlation coefficient between AC and GC was -0.96. He *et al.* (2008) used the F₂ populations of "Suyunuo" and "Yangwunuo 4". The results showed that *SBEI*, *SBEIII*, *SSSI*, *SSSIIa*, *PUL* and *ISA* had little effect on GC when *Wx* gene did not exist in F₂ population. This suggests that *Wx* gene plays an important role in GC mutation. Li *et al.* (2010) showed that the GC of different *Wx* alleles was very different, and there was a very significant negative correlation with AC; the correlation coefficient was -0.94, which indicated that the different alleles of *Wx* were the regulating genes or major genes of GC. Tian *et al.* (2007) used "Zhenshan 97B" and "Minghui 63" as parents to construct recombinant inbred lines. The QTL of AC, GC and GT were mapped to the same locus on the short arm of low 6 chromosome,

which contained *Wx* locus. So it is assumed that these three markers may be controlled by the *Wx* gene at the same time, or by a gene that is closely linked to it (Tian *et al.*, 2007). He *et al.* (2006), using “Nanjing 11/Balilla” DH population study, found that only *Wx* has effects on AC, GC and GT (Table 2.1), It can explain 58.5% of the apparent variation in AC, which can also explain 38.9% of the GC apparent variability, but only 4.7% of the GT variation can be explained.

Table 2. 1 Contributions of the starch-synthesis genes investigated on AC、GC and GT variations in the DH population (He *et al.*2006)

Gene	R ²		
	AC (%)	GC (%)	GT (%)
<i>Wx</i>	58.5	38.9	4.7
<i>SSSI</i>	1.7	2.8	
<i>PUL</i>	1.3		
<i>SSSIIa</i>			25.5

In addition, *Wx* gene plays a key role in the eigenvalues of RVA profile. Bao *et al.* (2006) used the QTL mapping of DH population with narrow Ye Qing 8 and Jingyin 17. It was found that the main RVA profile characteristics were all related to *Wx* gene, and the RVA profile of rice was mainly controlled by *Wx* gene. There was a correlation between AC and several RVA profile characteristics. In general, with the increase of AC, the peak viscosity, hot paste viscosity, cold paste viscosity, attenuation value and consistence value of rice starch will increase with the increase of AC (Wu *et al.*, 2006). Wu *et al.* (2006) used near-isogenic lines constructed with glutinous rice as the background. The results showed that when the *Wx* gene of Guizhao No. 2 was replaced by *waxy* gene, the peak viscosity, hot paste viscosity, cold paste viscosity and breakdown Value of *Wx* gene decreased correspondingly. A study by Bao *et al.* (2000) on QTL Positioning found that except for the peak viscosity, all the viscosity characters of RVA profile characteristics were controlled by *Wx* gene. Only *Wx* gene effect could be detected in both environments, but other QTL could not be detected, which indicated that RVA profile characteristics was also affected by genotype and environment effect (Bao *et al.*, 2000).

From Figure 2.3 (Tian *et al.*, 2009), it can be intuitively observed that each gene has three quality traits (AC, GC and GT). First, *Wx* is the only major gene that affects both AC and GC, but it has little effect on GT. *SSIIa* is a major gene that controls GT, but it has little effect on AC and GC. Secondly, the genes *ISA* and *SBE3* affect both GC and GT. Thirdly, only one quality trait is affected by several micro effector genes, such as *SSIIIa*, *AGPlar*, *PUL*, *SSI* mainly affected AC, *AGPiso* mainly affected GC, *SSIVb* mainly affected GT. The association among AC, GC and GT are caused by the combination of related genes and unequal haplotype combinations.

Soluble starch synthase, branching enzyme and debranching enzyme genes all affect the structure of amylopectin and make the cooking and eating quality of different varieties different. Some researchers have found that *SSIIa* is sequentially different in different rice varieties, especially in the gene coding region, where base substitution occurs, which changes the amino acid sequence. Finally, the change of *SSSI* enzyme activity affected the synthesis of amylopectin with medium length branching chain, and changed the structure of starch crystal layer, and finally showed the change of gelatinization temperature. Fujita *et al.* (2006) used four rice mutants with different degrees of *SSSI* deficiency. It was found that the change of amylopectin chain length distribution was positively correlated with the degree of decrease in activity of amylopectin. Wu *et al.* (2006) used glutinous rice as the background to construct near-isogenic lines as the research material. The results showed that *SBE1*, *SSI*, *SS IIa* and *SS IIIb* genes all had a significant effect on the RVA profile characteristics, and the most obvious effect was *SS IIa*, which showed that the peak viscosity of the gene was reduced to 176.8 when it was replaced by the corresponding gene of Suyunuo, which was closer to the parent Suyunuo (62.7). When the *Wx* and *SS IIa* gene were simultaneously replaced by the corresponding gene of Suyunuo, the peak viscosity was obtained. (PKV) further down to 89.1, this phenomenon is also reflected in the viscosity of thermal adhesives. (HPV) Cold paste viscosity (CPV) And breakdown Value (BDV) Equal eigenvalues. Kang *et al.* (2015) results showed that the interaction effect of *SSIIIb* gene and *SS I* gene had significant effect on GT, AAC, GC, PKV and SBV, while *SSIIIb* gene had significant effect on GT, PKV, BDV and peak time (PeT). Wu

et al. (2006) found that genes related to amylopectin synthesis, such as *SSSI*, *SSSIIa*, *SSSIIIb* and *SBE1*, all had an effect on RVA profile, but *Wx* gene has apparent epistatic effects on these genes.

Han *et al.* (2004) designed the STS and CAPS markers based on the differences between indica type rice and japonica rice and used 40 japonica rice varieties as materials to study their functional differences. The results showed that the starch RVA profile characteristics of varieties with different *SAE1* and *SBE3* alleles show significant differences in the hot paste viscosity, cool viscosity, peak viscosity, and consistence. Zhang *et al.* (2006) analyzed the *SBE1* and *SBE3* loci and found that the *SBE1* and *SBE3* loci with different sources had a greater impact on AC, GC, SBV, and CSV, and the differences between the two groups reached significant or extremely significant levels. This result also initially implies that branching enzymes that control the formation of starch branching structures have important effects on amylose content, gel consistency, and setback viscosity and consistence.

Using indica rice variety Guizhao 2 and japonica glutinous rice variety Suyunuo as donors and recurrent parents, the near-isogenic lines of *PUL* gene were established. The results showed that the gene had some influence on amylose content and gel consistency of rice. However it does not reach a significant level (Yan *et al.*, 2010). In addition, under the background of glutinous rice, the effect of *PUL* gene on RVA profile characteristics was most significant, including peak viscosity, hot paste viscosity, cold paste viscosity, setback value, pulping temperature and peak time, all of which were affected by *PUL* gene (Kang *et al.*, 2015). The effects of amylopectin synthase related genes on the physicochemical indexes of rice starch were analyzed by stepwise regression method. Two loci, such as *PUL* and *SSS I*, were detected to affect GC, and the effect values were -2.11 and 0.663, respectively (Zhang *et al.* 2006).

1.5 The purpose and significance of this study

According to the literature review, *Wx* gene plays a leading role in cooking and eating quality of rice. Because most genetic studies are based on different rice varieties or populations of indica and japonica hybrid combinations, the test materials contain different *Wx* alleles.

Therefore, the significant effect of *Wx* gene was detected in the results of the study, while the effect of other genes related to starch synthesis was very small and easy to be masked by *Wx* gene. Previous research in our laboratory found that when the major gene *Wx* and its allele are the same, the amylose content of different varieties or lines will also be very different, which is reflected in the sister lines that are cultivated by the same parental combination. The major gene is the same as the semi-glutinous *Wx-mq* and its amylose content is also different. In order to analyze the reasons of the difference of amylose content in *japonica* rice with good eating quality under the same gene background, the genetic and environmental aspects will be studied in this study.

On the one hand, the polymorphism of starch synthase genes *SSIIa*, *SSIIIa* and *PUL* were extensively screened by screening the molecular markers of starch synthase genes, and the polymorphism of starch synthase genes *SSIIa*, *SSIIIa* and *PUL* were used among the populations of “Wujing 13/kanto 194” and “Ning 0145/Wuyunjing 21”. The *SSIIa*, *SSIIIa* and *PUL* genotypes of “Wujing 13/kanto 194” and “Ning 0145/Wuyunjing 21” breeding lines with *Wx-mq* alleles were identified, and the background of the same *Wx* allele was evaluated (*Wx-mq*). The effects of *SSIIa*, *SSIIIa* and *PUL* on amylose content, gel consistency, gelatinization temperature and characteristic value of RVA profile were studied to explore the genetic factors of rice cooking and eating quality variation under the same *Wx-mq* gene background.

On the other hand, the amylose content in good eating quality rice were investigated under different nitrogen rates and sowing dates through four nitrogen application levels of high (450 kg/hm²), medium (300 kg/hm²), low (150 kg/hm²) and zero (control), sowing date and short daylight treatment by using the semi-glutinous new *japonica* rice varieties (lines) with different amylose content in 2015 and 2016.

In this study, the genetic and environmental aspects of rice cooking and eating quality variation under the same *Wx-mq* gene background were comprehensively discussed, and a molecular marker assisted breeding platform was established to screen the most suitable amylose content in semi-glutinous rice varieties. The excellent cultivation technology system for the good eating *japonica* rice provides a solid theoretical basis and strong technical support for the breeding of

new rice varieties with good eating quality.

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CHAPTER THREE

VARIATION OF AMYLOSE CONTENT IN RICE UNDER THE BACKGROUND OF *Wx-mq* GENE

Variation of Amylose Content in Rice under the Background of *Wx-mq* Gene

Shu Yao^{1,2}, Prof. Balakrishna Pillay², and Prof. Cailin Wang^{1*}

1. Institute of Food Crops, Jiangsu Academy of Agricultural Sciences/Jiangsu High Quality Rice R&D Center/Nanjing Branch of China National Center for Rice Improvement, Nanjing 210014, China
2. Discipline of Microbiology, School of Life Science, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa.

*Corresponding author. Tel: +25 84390317; Fax: Tel: +25 84390317

E-mail address: clwang@jaas.ac.cn (Cailin Wang)

Abstract

Rice amylose content (AC) is one of the main factors that affect the taste quality of rice. The semi-glutinous *japonica* rice with AC between 5% and 15% has the characteristics of soft rice, oily luster, cold rejuvenation, and good expansibility, and is increasingly favored by people. In order to clarify the genetic stability of semi-glutinous *japonica* rice AC, we choose a new *japonica* rice variety containing *Wx-mq* gene developed by our team as the material to determine the AC of each variety (strain) and analyze differences in AC between different backgrounds and different ecological types. The results show that there was a great difference in AC between the strains containing the same *Wx-mq* gene, even though the variation of AC between strains from the same cross was between 6.31% and 11.70%. There are differences in the variation of AC between different genetic backgrounds and semi-glutinous strains at different growth stages. The AC of the hybrids between Wuxiangjing14 and Kanto194 was relatively high, and its amplitude was relatively small; the amylose contents of different ripening varieties (lines) were middle-maturing > late mature > late, the variation of *japonica* is

larger than that of late *japonica*. The results of this study can provide reference for the selection of low amylose content varieties with good eating quality.

Keywords: good eating quality *japonica* rice; amylose content; *Wx-mq* gene; different ecological types

1.1 Introduction

Amylose content (AC) in rice is one of the main factors that affects the taste quality of rice (Cheng *et al.*, 2014). Semi-glutinous *japonica* rice with 5%-15% AC has the characteristics of soft rice, appearance of oil sheen, non-return, and good puffiness, which is favored by people. In order to clarify the genetic stability of semi-glutinous *japonica* rice AC, we chose a new *japonica* rice variety containing *Wx-mq* gene which was developed by our team. In this chapter we analyze the difference of AC between different backgrounds and different ecological types of semi-glutinous materials with *Wx-mq* gene. The results show that there was a great difference in AC between the semi-glutinous strains containing the same *Wx-mq* gene, even though the AC of semi-glutinous varieties from the same hybrid combination varied from 6% to 14%. The variation range of AC was different among the semi-glutinous varieties with different genetic backgrounds and different growth period. The AC of hybrid variety from Wuxiangjing14 and Kanto194 is relatively high, and its variation amplitude is relatively small. The amylose content of different mature stage varies: middle maturing medium *japonica* > late maturing medium *japonica* > late *japonica* was higher than that of late *japonica*, and the variation of medium *japonica* was larger than that of late *japonica*. The results of this study can provide reference to the breeding of high quality *japonica* rice varieties with low amylose content.

Rice is one of the main cereal crops in China, and the main goal of breeding is to get high yield for a long time. With the development of market-oriented economy and the change of consumption concept, people have more demands on rice quality, especially good appearance and good taste (Chen, 2003). Amylose content in rice is the main factor affecting the quality of rice taste. Amylose content is recognized as a main index to identify the rice eating quality in

the world. Rice with low amylose content is more and more popular because of its soft and elastic rice texture (Huang *et al.*, 2003; Ming *et al.*, 2003).

It is reported that there are 14 mutant genes with low amylose content available for breeding in rice; most of them are controlled by a pair of recessive genes. *Wx-mq* is a translucent endosperm mutant gene with low amylose content obtained from the treatment of Japanese rice varieties by chemical mutagen N-methyl-N-nitrosourea. *Wx-mq* has been used in breeding. Japanese breeders used the rice resource Milky Queen containing this gene to cultivate rice varieties with low amylose content such as “New-hikari” and “Kanto194”. One of the good eating quality of Kanto194 was known as “milky Princess”. We used the hybrid of Wuxiangjing14 and Wujing13 to select the translucent endosperm mutant gene and resistance gene of stripe disease by screening the appearance and eating quality of several generations and have bred new *Japonica* Rice varieties “Nanjing 46” and “Nanjing 5055” (Wang *et al.*, 2008, Wang *et al.*, 2012). In order to improve the selection efficiency of rice varieties with low amylose content of *Wx-mq* gene, cleaved amplified polymorphic sequence markers were developed and designed by using Tetra-primer amplification refractory mutation system technique (Wang *et al.*, 2009). The allelic mutation amplification was performed to detect the *Wx-mq* gene with low amylose content in rice.

There is a big difference in amylose content of different rice varieties and the inheritance of amylose content in rice is very complicated (Shi *et al.*, 2000). Genetic expression of offspring is different due to their diverse parental amylose content (Kumar *et al.* 1999; Shen *et al.* 1990; Wang, 1992). At the same time, amylose content is also affected by environmental conditions (Huang *et al.*, 2006). There are many researches on amylose and low amylose content in rice at present, which mainly focus on the genetic characteristics of amylose content and the correlation between amylose content and appearance quality, cooking quality and agronomic characters of rice (Li *et al.*, 2008; Wan *et al.*, 2006; Cai *et al.*, 2011). However, there is a lack of research on genetic characteristics of low amylose rice. In this research, amylose content of 124 rice varieties with *Wx-mq* gene were determined. The genetic characteristics of low

amylose content in rice under different genetic background and different ecological types were analyzed to provide reference to the breeding of better eating quality rice.

2.1 Materials and methods

2.1.1 Test materials

The tested materials included 124 new semi-glutinous *japonica* rice varieties (Lines) with *Wx-mq* gene, which were bred by crossing with Japanese high-quality *japonica* rice with Kanto 194 as parent. The main agronomic characters were stable and consistent. There were 84 combinations of Wujing 13/ Kanto 194, 32 combinations of Wujing 15//13/Kanto 194 and 10 combinations of Wuxiangjing 14/Kanto 194.

2.1.2 Test methods

In 2014, 124 materials were planted in the experimental field of food crops Research Institute of Jiangsu Academy of Agricultural Sciences. Sowing was done on May 15 and transplanting was done on June 16. Randomized block design, twice repeated and 8 rows each plot, 40 plants each row. The row and plant spacing were 27cm and 13cm respectively. Field management was consistent with field production. Each strain was charged with mature seeds, threshing and drying, rough and milled rice, and whirlwind milling for 100 purposes. The moisture of each sample stabled at 13.5% (Tang *et al.*, 2013).

2.1.2.1 *Wx-mq* gene detection

Thirty days after transplanting, fresh young leaves were sampled in each plot which were used to extract the DNA with CTAB method (Murray, 1980), and ARMS-PCR was used to detect the low amylose content gene *Wx-mq* (Yao *et al.*, 2010). Its forward outer primer *Wx-mq*-O-F: 5' - ATGTTGTGTTCTTGTGTTCTTTGCAGGC-3' ; reverse outer primer *Wx-mq*-O-R: 5' - GTAGATCTTCTCACCGGTCTTTCCCAA-3' ; forward internal primer *Wx-mq*-I-F: 5' - GGGTGAGGTTTTTCCATTGCTACAATCG-3' ; the reverse internal primer *Wx-mq*-I-R: 5' - GTCGATGAACACACGGTCTGACTCAAT-3' . PCR amplification reaction was modified slightly according to the report by Chen *et al.*, (2013), and 20 μ L PCR reaction system including

template DNA 1 μL , 10 $\text{mmol}\cdot\text{L}^{-1}$ dNTPs 0.4 μL , 4 $\text{pmol}\cdot\mu\text{L}^{-1}$ primer 2.0 μL , 10 \times buffer 2.0 μL , 5 $\text{U}\cdot\mu\text{L}^{-1}$, Taq enzyme 0.2 μL , ddH₂O 14.4 μL was amplified by Biometra PCR. The reaction procedure was as follows: pre-degeneration at 94 °C for 5 min, then denaturation at 95 °C for 30 S, renaturation at 65 °C for 30 s, extension at 72 °C for 1 min, 35 cycles; extension at 72 °C for 10 min, cooling at 4 °C for 10 min. Indication was added to the amplification product, and reserved for later use. The reaction product was electrophoresed on a 1.5% agarose at voltage of 120V for 25 min. Ethidium bromide was stained and observed under ultraviolet lamp, and the result was recorded after observed under a Bio RAD gel imaging system.

2.1.2.2 Determination of amylose content

Pretreatment for determination of amylose content was processed according to the Ministry of Agriculture standard “The NY147-88 method for the determination of quality” (1988), the amylose content of selected samples were 1.50%, 10.40%, 16.20% and 26.50% respectively. This was calculated according to the standard curve to the standard sample, and respected for 3 times with the United States BioTek Epoch ultra-micro total wavelength enzyme mark instrument in 620 nm determination of light absorption value.

2.1.2.3 Definition of maturity type

The experimental materials were divided into three maturing stages, middle maturing medium *japonica*, late maturing medium *japonica* and late *japonica*, and the corresponding control varieties were Xudao 3, Huadao 9 and Zhendao 11, respectively. Compared with the control varieties, the heading date of testing variety (Line) was the same as that of the control. The difference between the heading date and the control group was the same maturity type in the range of 3 days. In addition, varieties whose heading date was later than Zhendao 11 were regarded as the late *japonica* type.

2.1.2.3 Statistical analysis

The data were analyzed with Excel 2016 and SPSS17.0 software.

3.1 Results

3.1.1 Identification of *Wx-mq* gene of experimental varieties (lines)

Kanto 194 containing *Wx-mq* gene and Wujing 13, Wujing 15 and Wuxiangjing 14 parental varieties without the *Wx-mq* gene were used as controls, the DNA of 124 rice varieties (lines) was amplified by four-primers ARMS-PCR, and the amplification products were detected by 1.5% agarose gel electrophoresis. The specific bands of 439 bp were amplified from 124 rice materials and control varieties, and 292 bp bands were amplified from 124 rice materials and Kanto 194, but 292 bp bands could not be amplified from Nanjing 44, a rice material without *Wx-mq*. But it can amplify one 200 bp specific bands (Figure 3.1). The results show that 99 different rice varieties tested (lines) contain the *Wx-mq* gene.

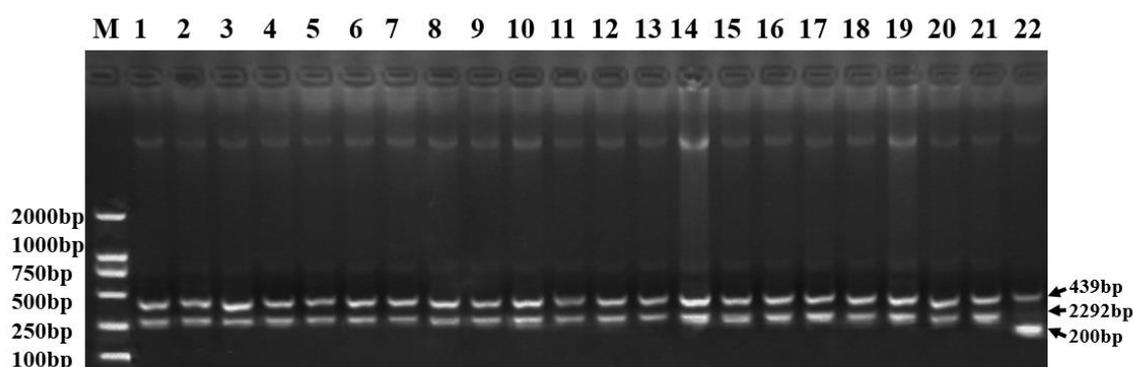


Fig 3.1 Molecular detections with Tetra-primer ARMS-PCR in different rice varieties or lines

M: DNA marker DL2000; 1-8: Varieties or lines of Wujing 13 / Kanto 194; 6-10: Varieties or lines of Wuxiangjing 14 /Kanto 194; 9-16: Varieties or lines of Wujing 13 // Wujing 13 / Kanto 194; 17-20: Varieties or lines of Nanjing 44 // Wujing 13 / Kanto 194; 21: Varieties with *Wx-mq* gene, Kanto 194; 22: Varieties without *Wx-mq* gene, Wujing 13

3.2.1 The difference of amylose content among different varieties (lines)

The results show that the AC of 124 semi-glutinous materials was distributed between 6.31% - 11.70% (Figure 3.2). The average value was 7.88% and the coefficient of variation was 10.4%. The AC of the majority of the materials was between 8%-10%, 116 in total, and accounting for

93.5%. Less than 8% of the materials were only 4, accounting for 3.2%, and more than 10% of the materials were only 3, accounting for 2.4%.

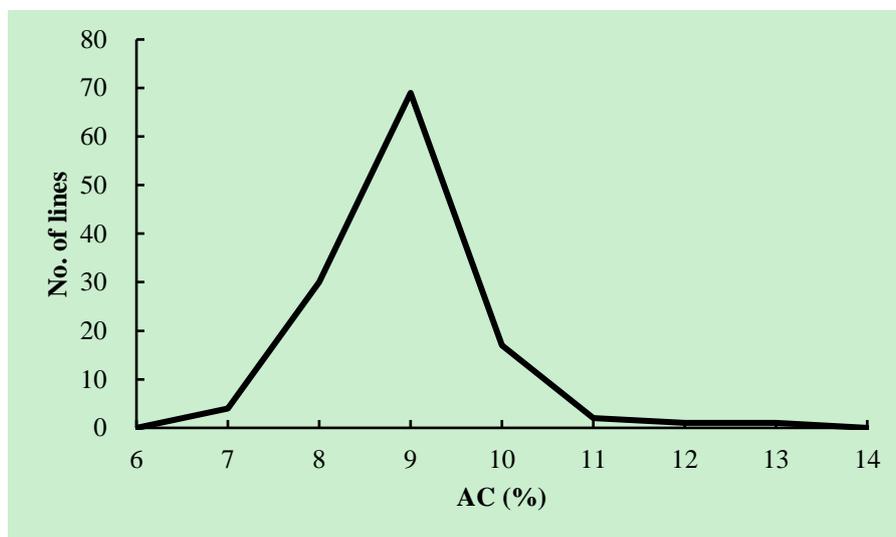


Fig 3.2 Amylose content distribution of 124 semi-glutinous lines

3.3.1 The difference of AC of varieties from different combinations (lines)

The results of comparison and analysis of AC of semi-glutinous materials from different combinations are shown in Table 3.1. The AC of semi-glutinous materials derived from different combinations was to some extent different. The AC of semi-glutinous materials from the Kanto 194 and Wujing 13, was the highest, with an average of 8.01%. The second was the semi-glutinous materials from Wujing 15// Wujing 13/ Kanto 194, and the average value of AC was 7.71%. The AC coefficient of variation of semi-waxy materials from these two combinations was large as well. The AC of semi-glutinous materials from Wuxiangjing 14 /Kanto 194 was the lowest and the coefficient of variation was the lowest. But the amylose content in different combinations was not significant.

3.4.1 Difference of amylose content of varieties among different ecological types (Line)

One hundred and twenty four tested materials were divided into three types according to their maturity: medium maturing medium *japonica* late maturing medium *japonica* and late *japonica*. The analysis results of amylose content in different ripening periods are shown in Table 3.2.

The results showed amylose content: medium maturing medium *japonica* > late maturing medium *japonica* > late *japonica*, but there was no significant difference in amylose content among different ecological types. The variation range of late maturing medium *japonica* was 5.40%-11.85%, and its coefficient of variation was 15.27%.

Table 3.1 Statistical parameters of the amylose content of different genetic backgrounds

Cross	No	Min (%)	Max (%)	Mean (%)	Sd	CV (%)
Wujing13/Kanto 194	87	6.34	11.70	8.01 a	0.87	10.86
Wuxiangjing14/ Kanto194	22	6.64	8.29	7.48 a	0.39	5.21
Kanto 194/89-94	7	6.51	8.41	7.64 a	0.68	8.89
Wujing 15 //Wujing 13/ Kanto 194	8	6.31	8.99	7.71 a	0.99	12.79
Total	124	6.31	11.70	7.88 a	0.82	10.40

Note: Data with same letters in the same column indicated no difference at 0.05 level

Table 3.2 Statistical parameters of the amylose content of different maturity materials

Type	No	Mean (%)	Sd	Min (%)	Max(%)	CV(%)
Medium maturing <i>japonica</i>	54	9.11 a	1.03	6.65	10.86	11.25
Late maturing medium <i>japonica</i>	40	9.04 a	1.38	5.40	11.70	15.27
<i>Late japonica</i>	30	8.81 a	1.16	6.71	10.64	13.13

Note: Data with same letters in the same column indicated no difference at 0.05 level

Taking 87 combinations of Wujing 13/Kanto 194 as an example, the difference of amylose content of different varieties in maturing periods in the same combination was analyzed (Table 3.3). In combinations of Wujing 13/Kanto 194, amylose content: medium maturing medium *japonica* > late maturing medium *japonica* > late *japonica*. According to the variation range and coefficient of variation, the variation range of late maturing medium *japonica* was 5.40%, the maximum was 11.85%, and the coefficient of variation was 15.70%. The variation of medium maturing medium *japonica* rice was less, with the range of 8.29%-10.42%, and the coefficient of variation was 7.01%.

Table 3.3 Statistical parameters of the amylose content of the same cross

Type	No	Mean (%)	Sd	Min (%)	Max (%)	CV (%)
Medium maturing <i>japonica</i>	37	9.39 a	0.66	8.29	10.42	7.01
Late maturing medium <i>japonica</i>	27	9.25 a	1.45	5.40	11.85	15.70
Late <i>japonica</i>	23	8.64 a	1.18	6.71	10.29	13.69

Note: Data with same letters in the same column indicated no difference at 0.05 level.

4.1 Discussion

The rice eating quality is affected by many factors such as varieties, habitat conditions, cultivation conditions, harvesting time, drying methods, storage conditions, processing quality, and cooking methods. The difference in eating quality between varieties was mainly related to amylose content, protein content, gel consistency, gelatinization temperature, and RVA profile characteristics. Amylose content was the main factor affecting the eating quality, and was mainly related to the genetic characteristics of the variety. The gel consistency, gelatinization temperature, and RVA profile characteristics are mainly related to amylose content.

The amylose content is mainly controlled by the major gene *Wx* located on chromosome 6. *Wx* is a complex allele, which is *Wx^a* in *indica* and the amylose content is generally above 20%; *Wx^b* in *japonica* rice and the amylose content is generally between 15% and 20%;, *wx* in glutinous rice and the amylose content is generally below 2%; in semi-glutinous rice, the *Wx* is mutant gene such as *Wx-oq*, *Wx-in*, *Wx-mq*, and so on. With content ranging from 5% to 15%, the low-amylose rice is characterized by fluffy texture, glossy appearance of the cooked rice, soft texture of cooled rice, and excellent expansibility for food processing, which can be used as not only cooked rice directly, but also as good material for instant, mixed rice and expanded foods. It is deeply favored by consumers.

Japan, South Korea and other Southeast Asian countries have paid attention to rice breeding with low amylose content for a long time. Since the mid-1980s in Japan, screening and breeding programs for low amylose content mutants has been carried out, and a series of high-quality and low amylose content varieties such as Milky Queen and Sari have been bred. In Yunnan and other places of China, the development of low-amylose rice varieties was also started earlier. The “Yunguang” series of soft hybrid *japonica* rice cultivated by Lu Yixuan and other people of the Yunnan Academy of Agricultural Sciences was called “natural formula rice” and welcomed by consumers. Cailin Wang and other people used the *Wx-mq* gene to breed rice varieties with good eating quality in Nanjing 46, Nanjing 5055, and Nanjing 9108, and they were widely promoted in Jiangsu Province and neighboring provinces in China. They have achieved great success and developed high-quality rice variety brands which are welcomed in the consumer market.

Studies have shown that the genetic content of rice amylose content is complex, the amylose content of the parent is different, and the genetic performance of its offspring is also different. The influence of genetic background on the amylose content of different *indica* and *japonica* rice has been reported but relatively few studies have been done on semi-glutinous rice. In order to clarify the genetic traits of the eating quality of *indica* rice varieties containing the *Wx-mq* gene, this chapter analyzed the variation of amylose content in different genetic backgrounds, ripening periods, and varieties (lines). The results showed that although the different varieties

(lines) of semi-glutinous rice contained the same major gene *Wx-mq*, their amylose content had a large variation. Even among the different semi-glutinous varieties (lines) from the same combination, the amylose content still fluctuates greatly. The semi-glutinous rice varieties from Wujing 13/Kanto 194 and Wujing15// Wujing 13/ Kanto 194 have higher amylose content and larger coefficient of variation; while the semi-glutinous rice varieties from Xiangjing 14/Kanto 194 has lower amylose content and smaller coefficient of variation. It was shown that the genetic background had an effect on the amylose content of semi-glutinous rice varieties.

Starch is the most important component in rice endosperm, which accounts for 90% of polished rice. It is composed of amylose and amylopectin. Amylose is catalyzed by the granule-bound starch synthase (*GBSS*) encoded by the waxy gene (*Wx*); amylopectin consists of soluble starch synthase (*SSS*), starch branching enzyme (*SBE*) and starch debranching enzyme (*DBE*) by synergistic synthesis; ADP-glucose pyrophosphorylase (*AGPase*) is responsible for the synthesis of the precursor-ADP glucose (*ADPG*), which is the rate-limiting enzyme in the starch synthesis pathway.

The genes involved in coding these enzymes are called Starch Synthesis-Related Genes (*SSRG*). There are more than 20 including 7 genes such as *AGPL1-4* and *AGPS1-2* encoding *AGPase* enzyme size subunit; encoding Starch Synthetic Enzymes *GBSSI (Wx)*, *GBSSII*, *SSI*, *SSIIa*, *SSIIb*, *SSIIc*, *SSIIIa*, *SSIIIb*, *SSIVa*, *SSIVb*, and other 10 genes; *SBE1*, *SBE3*, *SBE4*, *ISA1*, *ISA2*, *PUL* encoding starch branching enzymes and debranching enzymes six genes were included (Nakamura *et al.*, 2002; Wu *et al.*, 2006; Chen *et al.*, 2008; Hannah *et al.*, 2008; Zhou *et al.*, 2013).

The starch-synthesis related genes contained in different parents are different. Although they all contain the same major gene *Wx-mq*, they are necessarily related to starch synthesis due to different genes involved in starch synthesis. According to Liu *et al.* (2015), the alleles of Wujing 13, Wujing 15 and Wuxiangjing 14 are different at *SSIIa*, *SSIIIa*, and *PUL*, and there are different alleles of Wujing 13 and Kanto 194 in *SSIIa* and *SSIIIa*. There are different alleles of Wujing 15 and Kanto 194 in the genetic locus of *SSIIa* and *PUL*, while Wuxiangjing 14 and Kanto 194 have the same allele in these three genetic locus. This explains why the semi-

glutinous rice varieties from Wujing 13/ Kanto 194 and Wujing 15//Wujing 13/ Kanto 194 have higher amylose content and higher coefficient of variation; The semi-glutinous rice varieties from Wuxiangjing 14/ Kanto 194 have low amylose content and low coefficient of variation. Whether this deduction is correct will be confirmed by research in Chapter 5.

In addition, under the same genetic background, rice amylose content is also affected by temperature, light, fertilizer and other environmental and cultivation factors. Gomez measured the changes of amylose content in 13 varieties at 22°C - 31°C, and the results showed that with the increase of average temperature, the amylose content of low amylose varieties always decreased. However, in medium or high amylose varieties, the amylose content did not change or slightly increase when the temperature was increased. In this chapter, the results of analysis of amylose content of semi-glutinous Lines of different ecotypes were expressed as medium-ripe medium *japonica* rice>late-maturing medium *japonica* rice>late *japonica* rice, and the trend of semi-glutinous Lines of different combinations was consistent. The amylose content of late-maturing medium *japonica* rice and late *japonica* rice were greater than that of medium-ripe medium *japonica* rice, which may be related to the temperature conditions during the heading period of the different types of semi-glutinous Lines. We will study this issue in Chapter 4.

Selection of suitable low amylose rice type based on genetic background and heading date is undoubtedly a feasible method for pedigree breeding (Zhang *et al.*, 2002). The results show that amylose content of varieties (Lines) crossed by Wuxiangjing 14 and Kanto 194 is relatively high, and its range is relatively small. In addition, the amylose content of medium *japonica* was higher than the data of late *japonica*, and the variation of amylose was relatively large. As a result, in rice quality breeding, on the basis of selecting varieties with low amylose content, we can consider both genetic background and heading date to do better orientate selection, and cultivate good eating quality *japonica* rice varieties to the most suitable for the local climate conditions and residents' taste requirements.

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CHAPTER FOUR

THE AMYLOSE CONTENT IN GOOD EATING QUALITY RICE UNDER DIFFERENT NITROGEN RATES AND SOWING DATES

The Amylose Content in Good Eating Quality Rice Under Different Nitrogen Rates and Sowing Dates

Shu Yao^{1,2}, Prof. Balakrishna Pillay², and Prof. Cailin Wang^{1*}

- 1 Institute of Food Crops, Jiangsu Academy of Agricultural Sciences/Jiangsu High Quality Rice R&D Center/Nanjing Branch of China National Center for Rice Improvement, Nanjing 210014, China
- 2 Discipline of Microbiology, School of Life Science, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa.

*Corresponding author. Tel: +25 84390317; Fax: Tel: +25 84390317

E-mail address: clwang@jaas.ac.cn (Cailin Wang)

Abstract

The amylose content in good eating quality rice were investigated under different nitrogen rates and sowing dates through four nitrogen application levels of high (450 kg/hm²), medium (300 kg/hm²), low (150 kg/hm²) and zero (control), sowing date and short daylight treatment by using the semi glutinous new *japonica* rice varieties (lines) with different amylose content in 2015 and 2016. The results showed that the amount of nitrogen fertilizer has significant effect on amylose content of good eating quality *japonica* rice. Amylose content was decreased with the increase of amount of nitrogen fertilizer. Amylose content of the high nitrogen treatment was the lowest in both of the two years. Sowing date has significant influence on amylose content of good eating quality *japonica* rice. The amylose content was decreased with the sowing date postponed. The effect of sowing date on amylose content was related to the temperature during 6-15 days after heading, and the high temperature resulted in the increase of amylose content. This was also confirmed by the short daylight treatment experiment. The results of the study have guiding significance for the high quality cultivation of the good eating quality *japonica* rice.

Keywords: nitrogen application level; sowing date; good eating quality *japonica* rice; amylose content

1.1 Introduction

The content of amylose in rice is mainly controlled by the dominant gene locus *Wx* (Kumar *et al.*, 1986), and also affected by other starch synthesis genes (Kumar *et al.*, 1986; Shen *et al.*, 1990; Wang, 1990). *Indica* rice is under the control of the gene *Wx^a* with an amylose content of 21%-30%; *japonica* rice is under the control of the gene *Wx^b* with an amylose content of 15%-20% in general; glutinous rice is under the control of the recessive gene *wx* with an amylose content lower than 2% (Sano, 1984). There is another type of mutation in the gene loci, such as *Wx-mq* with amylose content between those of *japonica* rice and glutinous rice, called semi glutinous *japonica* rice, also known as low amylose content *japonica* rice (Mikami *et al.*, 1990; Sato *et al.*, 2002). Semi glutinous *japonica* rice has clear and lustrous surface, characterized by both softness of glutinous rice and elasticity of *japonica* rice with high palatability and eating quality, so in this study it is called good eating quality rice (Wang *et al.*, 2009). By interbreeding low amylose content mutant Milky Princess Kanto194 obtained by EMS mutation of Japanese variety Koshihikari with Jiangsu high yield *japonica* rice variety. Wang *et al.* developed good eating quality rice varieties Nanjing 46, Nanjing 5055 and Nanjing 9108, which have been registered and approved in Jiangsu Province for good eating quality, high yield and excellent resistance to diseases (Wang *et al.*, 2008; 2010; 2012). In recent years, they have been used by more than 100 rice processing enterprises in Jiangsu, Zhejiang, Anhui, Shanghai as well other provinces and cities as unprocessed grain for development of high-quality rice, which enjoys a price over 0.2 yuan/kg higher than other varieties. The developed high-quality rice is increasingly well received by consumers and has been repeatedly awarded first-class prize, gold-award rice, good eating quality *japonica* rice and other honors 18 times in Jiangsu Province and the State. It is known as “the most delicious rice” in Jiangsu Province. In 2016, Nanjing 46 won “the outstanding prize” in the competition with Koshihikari in Japan. But we found in the breeding practice that as for *Wx-mq* with the same gene background of a low amylose content, the range of amylose content between different varieties in the same cross

combination can be up to 5.40%-11.85% (Yu *et al.*, 2012). In order to discover the reason for the difference in amylose content between different varieties with low amylose content gene *Wx-mq*, a discussion is required in both heredity and environment.

The study on the effect of environmental factors on amylose content of rice has been reported in many fields such as nitrogen application level and sowing date, but the results are unsatisfactory. Studies by Ji *et al.* (2001), Xu *et al.* (2004), Chen *et al.* (2004), Gao *et al.* (2010) and Li *et al.* (2003) showed that the amylose content decreased gradually with the rise of nitrogen application level. However researchers such as Liu *et al.* (1989), Chen *et al.* (1997,1999) and Zhang *et al.* (2005) obtained completely opposite results, believing amylose content increased with the rise of nitrogen application level. Liu *et al.* (2010) and Liu *et al.* (2004) believed by study that nitrogen application level was in a curve relationship with amylose content: when amylose content is lower than 128.9 kg/hm², amylose content increases with the rise of nitrogen application level, and the amylose content goes up by 0.007% with the rise of nitrogen application level by 1 kg/hm², when nitrogen application level is higher than 128.9 kg/hm², amylose content decreases with the rise of nitrogen application level. But Jin (2002), Zhao *et al.* (2004) and Jiang *et al.* (2004) reported that amylose content was not retarding to nitrogen application with no significant difference between treatments.

There are also many research reports on effects of sowing date on the content of amylose in rice. The results of study on sowing *indica* hybrid rice Yiyou 673 at different dates performed by Li *et al.* (2012) showed that with the delay of sowing date, the content of amylose in Yiyou 673 significantly increased. Zhao *et al.* (2013) studied five *japonica* varieties with different ecological types and found that amylose content increased with the delay of sowing date. This was also confirmed by the study of Zhang *et al.* (2012) on early-maturing late *japonica* variety Yangjing 4227. The results of study on sowing 5 *japonica* varieties performed by Liu *et al.* (2015), sowing early-maturing late *japonica* variety Nanjing 44 and medium-maturing late *japonica* variety Nanjing 46 performed by Zhu *et al.* (2013) showed that amylose content differed greatly during different sowing dates, but there was no obvious change rule. The results

of study on sowing 6 northern *japonica* varieties performed by Qin *et al.* (2004) showed that amylose content first decreased and then increased, but there was no significant change.

Previous studies on the effect of nitrogen application level and sowing date on amylose content mainly focused on *indica* and *japonica* varieties, and the results were not the same. However no systematic research report on semi-glutinous *japonica* rice has yet been seen. In this paper, the effects of nitrogen application level, sowing date and other environmental conditions on amylose content in semi glutinous *japonica* rice were studied by using semi- glutinous new *japonica* rice varieties (lines) developed by our team with low amylose content gene *Wx-mq*, through different nitrogen application levels, sowing date and short daylight treatment.

2.1 Materials and methods

2.1.1 Test materials

In the semi-glutinous new *japonica* rice varieties (lines) developed by our team with low amylose content gene *Wx-mq*, variety G7020 with high amylose content, variety G7012 with low amylose content and approved variety Nanjing 9108 were selected for the test in 2015. Varieties G7101, G7102, G7103, G7104, G7105, G7012 with different amylose contents and approved varieties Nanjing 9108 and Nanjing 5055 were selected for the test in 2016.

2.1.2 Test settings

The tests were conducted at Nanjing experimental base of Institute of Food Crops, Jiangsu Academy of Agricultural Sciences in 2015 and 2016. Fertilizer test was conducted based on sowing on May 10, transplanting on June 9, with seedling age at 30 days. As for sowing at different dates, seedling ages for transplanting were 20 days and 20 days respectively, for the second and third sowing stage. Fertilizer test and sowing at different dates were repeated 3 times in 2015. Fertilizer test and sowing at different dates were repeated 2 times in 2016 (Wu *et al.*, 2009). Split plot design was used in the fertilizer test with fertilizer treatment at the main plot and the variety at the split plot. A total of 187 lines arranged by 11 rows \times 17 lines with

spacing of 27 cm × 13 cm between rows and lines. The rest of field management was conducted by the conventional way (Liu *et al.*, 2016).

2.1.2.1 Fertilizer test

In 2015 and 2016, the fertilizer test was conducted under 4 different nitrogen treatments of high (450 kg/hm²), medium (300 kg/hm²), low (150 kg/hm²) and zero (Control), and in each treatment the total nitrogen amount was used 4 times, where the base fertilizer accounted for 20% of the total amount of nitrogen fertilizer, the organic fertilizer was 200 kg/hm² with nitrogen-phosphorus-potassium accounting for 1.2%, 0.8% and 0.5% respectively. And compound fertilizer was 375 kg/hm² with nitrogen-phosphorus-potassium equally accounting for 15%; tiller fertilizer accounted for 48% of the total amount of nitrogen, used two times. Tiller fertilizer was applied for the first time 4 days later after transplanting (June 12) based on a dosage accounting for 60% of the tiller fertilizer application level, and for the second time 12 days later after transplanting (June 20) based on a dosage accounting for 40% of the tiller fertilizer application level. Panicle fertilizer accounted for 32% of total nitrogen fertilizer application level, used in a lump sum when the remaining leaf primordium number was 3.0-2.5 (July 18). Tiller fertilizer and panicle fertilizer were urea and the nitrogen content was 46%.

2.1.2.2 Sowing date test

There were three sowing dates in 2015 respectively, May 10, May 30 and June 20. In order to verify the results of 2015, the sowing of early-maturing varieties were delayed and late-maturing varieties were put under short daylight treatment in 2016 to make them heading earlier. Medium-maturing medium *japonica* varieties G7101 and G7102 were sowed on both normal sowing date (May 10) and delayed sowing date (June 10), based on the same fertilizing amount, application of frequency and application as medium level treatment in the fertilizer test; late-maturing medium *japonica* variety G7104, late *japonica* variety G7106 and Nanjing 5055 were sowed on May 10 and transplanted to pots on June 9, with 10 pots for each variety and 5 lines in each pot. Among them, 5 pots were under short daylight treatment transplanted 30 days later after transplantation (nine hours in the sun with shading by black cloth from 5:00 PM to 8:00

AM next day) for 15 days, to make them head earlier; another 5 pots were used for control to observe the effect of different heading dates on amylose content. The relationship between the meteorological factors such as the maximum temperature, the minimum temperature, the mean temperature difference and the diurnal temperature difference at different times after heading and amylose content was analyzed.

2.3 Measuring items and methods

Upon maturation, five single lines were randomly selected from each plot to determine amylose content in accordance with the national quality rice standard (GB/T1791-1999) after threshing and drying.

2.4 Data statistics and analysis

According to the method introduced by Mo *et al.* (1986), the variance analysis was performed on the data based on randomized block or split block design test of two factors and multiple comparisons between averages were performed for the factor with significant difference by means of LSD method using SPSS statistical software.

3.1 Results

3.1.1 Effect of nitrogen application level on amylose content

The results of variance analysis (Table 4.1) suggest that there were significant and extremely significant differences in amylose content between different nitrogen treatments in 2015 and 2016, and the difference in amylose content between varieties was significant in two years, but the interaction between nitrogen application level and variety was not significant in 2015, but significant in 2016. It indicates that the content of amylose was significantly affected by nitrogen treatment, but the difference in amylose content between varieties was more obvious and there was interaction between nitrogen application level and variety.

The result of multiple comparisons indicated that the amylose content was the highest in 2015 under zero nitrogen treatment (control); with the rise of nitrogen application level, amylose

content decreased gradually; the content of amylose under high nitrogen treatment was the lowest, with no significant difference from amylose content under low and medium nitrogen treatments, but its difference with amylose content under control was significant up to 5% (Table 4.2). The amylose content under high nitrogen treatment was the lowest in 2016, but that under medium nitrogen treatment was the highest and the difference between the two was at a significant level up to 1%. Amylose content under control and low nitrogen treatment lay in between and there was no significant difference with amylose content under medium nitrogen treatment and high nitrogen treatment.

Table 4.1 Analysis of variance for amylose content in different nitrogen treatment in 2015-2016

Source of variation	2015				2016			
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>
Repetition	2	2.94	1.47	6.23**	1	0.15	0.15	1.63
Treatment(T)	3	3.11	1.04	4.40*	3	1.68	0.56	6.25**
Variety(V)	2	98.50	49.25	209.02**	7	50.72	7.25	81.04**
T×V	6	0.72	0.12	0.51	21	6.62	0.32	3.52**
Error	22	5.18	0.24		31	2.77	0.09	
Total	35	110.45			63	61.94		

* and ** mean significant at the 0.05 and 0.01 probability level, respectively. Same as in the following Tables

Table 4.2 Differences of amylose content in different nitrogen treatment.

Nitrogen treatment	Amylose content/%	
	2015	2016
Control	10.46 a	9.04 AB
Low level	10.34 ab	8.90 AB
Medium level	9.93 ab	9.17 A
High level	9.74 b	8.73 B

* Values followed by different lowercase and uppercase letters in a column indicate significant difference at 0.05 and 0.01 levels respectively. Same as in the following tables.

Table 4.1 shows that the difference in amylose content between varieties was extremely significant in the two years, which is the main reason of amylose content difference. The results

for multiple comparisons (Table 4.3) show that the difference in amylose content between 3 varieties was extremely significant in 2015. Amylose content of 8 varieties in 2016 were classified in general into high and low types, where G7101, G7102, G7103, G7104 were of a type with high amylose content, up to over 9%, with a significant difference up to 1% with the other 4 varieties (lines). Amylose content of G7101 was the highest with a significant difference up to 1% with G7102, G7103 and G7104; G7105, G7012, Nanjing 9108 and Nanjing 5055 were lower in amylose content, all below 9%, where amylose content of G7012 was the lowest with a significant difference up to 1% with G7105.

Table 4.3 Differences of amylose content among different varieties.

Year	Variety	Amylose content/%
2015	G7020	12.37±0.46A
	Nanjing 9108	9.54±0.28 B
	G7012	8.44±0.36 C
2016	G7101	10.66±0.37A
	G7102	9.63±0.36B
	G7104	9.51±0.35B
	G7103	9.15±0.81B
	G7105	8.53±0.57C
	Nanjing 9108	8.30±0.08CD
	Nanjing 5055	8.07±0.01CD
	G7012	7.83±0.12D

3.1.2 Effect of Sowing Date on Amylose Content

The results of variance analysis (Table 4.4) suggest that the difference in amylose content between 3 different sowing dates in 2015 was significant, but the difference in amylose content between varieties was extremely significant and there is no significant interaction between sowing date and variety. It indicates that the content of amylose was significantly affected by

sowing date, but the difference in amylose content between varieties was more obvious and there was no interaction between sowing date and variety.

The results of multiple comparisons (Table 4.5) show that amylose content of rice sowed on May 10 was the highest and that in rice sowed on May 30 was the lowest. The difference between the two sowing dates was significant. Amylose content of rice sowed at June 20 lay in between, but its difference in amylose content with rice sowed on May 10 and May 30 was not significant.

Table 4.4 Analysis of variance for amylose content in different sowing date (2015)

Source of variation	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>
Repetition	2	0.01	0.00	0.01
Sowing date(SD)	2	3.90	1.95	4.91*
Variety(V)	2	79.36	39.68	99.88**
SD×V	4	1.47	0.37	0.93
Error	16	6.36	0.40	
Total	26	91.09		

Table 4.5 Differences of amylose content in different sowing date and its relationship with the temperature during 6-15 days after heading in 2015.

Sowing date	Amylose content/%	Heading date	6-15 d Temperature during 6-15 days after heading/°C			
			Highest(H)	Lowest(L)	Mean	Temperature difference between H and L
05-10	9.95a A	08-20	30.5 A	23.0 aA	26.8 aA	7.5 aA
05-30	9.08 bA	08-27	28.7 B	21.5 bB	25.1 bB	7.3 bB
06-20	9.22 abA	09-02	28.2 C	21.3 bB	24.7 cB	6.9 cC

In order to explore the reason for the difference in amylose content between different sowing dates, the relationships between the meteorological factors such as the maximum temperature, the minimum temperature, the average temperature difference and the diurnal temperature difference at different times within 30 days after heading and amylose content were statistically analyzed with 5 days as a period and differential. The results showed that the maximum

temperature, the minimum temperature, the mean temperature difference and the diurnal temperature difference within 6-15 days after heading were closely related to amylose content, which indicated that amylose content of semi glutinous new *japonica* rice was higher with grain filled at a higher temperature within 6-15 days after heading (Table 4.5). In view of the close relationship between the 4 meteorological factors, the relationships between mean daily temperature, diurnal temperature difference and amylose content were discussed in this study for easier reference.

In order to further confirm the effect of temperature on amylose content, in 2016, sowing of medium-maturing medium *japonica* varieties G7101 and G7102 with a short growth period was delayed by 1 month (June 10), and late-maturing medium *japonica* variety G7104, late *japonica* variety G7106 and Nanjing 5055 with a long growth period were put under short daylight treatment to make the heading earlier. The results showed that medium-maturing medium *japonica* varieties G7101 and G7102 sowed at a delayed time headed sprouting 10-12 days late and the amylose content decreased by 1.17%-1.33% compared with normal sowing, with a difference up to a significant level of 1% (Table 4.6). Late-maturing medium *japonica* variety G7104 and late *japonica* variety G7106 headed sprouting about 10 days early after short daylight treatment and amylose content increased by 1.07%-1.16% with a difference up to a significant level of 1%; as for Nanjing 5055 after short daylight treatment, its amylose content was not significantly different from that in the control (Table 4.7).

Table 4.6 Amylose content of two medium *japonica* strains with medium maturation in different sowing date and its relationship with the temperature during 6-15 days after heading in 2016

Variety	Sowed at May 10				Sowed at June 10				DAC
	AC/%	HD	MT/°C	TD/°C	AC/%	HD	MT/°C	TD/°C	
G7101	11.70	08-12	25.2	6.3	10.37	08-22	23.7	5.0	1.33**
G7102	11.00	08-09	24.4	6.2	9.83	08-21	23.8	5.0	1.17**

* and ** mean significant at the 0.05 and 0.01 probability level, respectively

Table 4.7 Amylose content of three *japonica* strains with late maturation under short daylight treatment and its relationship with the temperature during 6-15 days after heading in 2016

Variety	Short daylight treatment				Control				Difference in amylose content/%
	Amylose content/%	Heading date	Mean temperature during 6-15 days after heading/°C	Temperature difference between day and night during 6-15 days after heading/°C	Amylose content/%	Heading date	Mean temperature during 6-15 days after heading/°C	Temperature difference between day and night during 6-15 days after heading/°C	
G7104	8.61	08-10	25.1	6.4	7.45	08-20	24.1	5.1	1.16**
G7012	6.93	08-13	25.1	6.3	5.86	08-24	24.4	5.5	1.07**
Nanjing 5055	7.59	08-14	25.0	6.2	7.65	08-25	24.8	5.9	-0.06

The statistical results showed that the mean daily temperature and diurnal temperature difference within 6-15 days after heading for medium-maturing medium *japonica* varieties G7101 and G7102 were both lower than those for sowing on May 10 (Table 4.7). The mean daily temperature and diurnal temperature difference within 6-15 days after heading for late-maturing medium *japonica* variety G7104 and late *japonica* variety G7106 after short daylight treatment were both higher than those for the control; the difference in mean daily temperature and diurnal temperature difference between Nanjing 5055 after short daylight treatment and the control was smaller (Table 4.7). The results also showed that amylose content of semi glutinous new *japonica* rice increased with grain filled at a higher temperature within 6-15 days after heading.

4. 1 Discussion

4.1.1 Effect of nitrogen application level on amylose content

Studies have shown that the main factors affecting rice eating quality include amylose content, protein content, gelatinization temperature, gel consistency and the like (Matsuo *et al.*, 1990). The eating quality is negatively correlated with amylose content and protein content, and amylose content has the greatest influence on the eating quality with a determining coefficient as high as 92% (Zhang *et al.*, 2007). Our breeding practice shows that semi-glutinous new *japonica* rice with low amylose content gene *Wx-mp* has good eating quality and the semi glutinous new *japonica* rice with 10%-14% has both excellent eating quality and good appearance. Nitrogen fertilizer affects the eating quality of rice by two ways, the first is to directly affect the eating quality by influencing protein content; the second is to indirectly affect the eating quality by influencing amylose content. Therefore, it is important to study the effect of nitrogen fertilizer on amylose content of semi-glutinous *japonica* rice for breeding and cultivation of good eating quality *japonica* rice.

The results of previous studies on the effect of nitrogen application level on amylose content of rice can be grouped into four categories. The first is that the increase of nitrogen application level leads to the decrease of amylose content (Jin *et al.*, 2001; Xu *et al.*, 2004; Chen *et al.*,

2004; Gao *et al.*, 2010; Li *et al.*, 2003), the second is that the increase of nitrogen application level results in amylose content rising (Liu *et al.*, 1989; Chen *et al.*, 1997; Chen *et al.*, 1999; Liu *et al.*, 2005), the third is that within certain nitrogen application level, nitrogen application level is in a curve relationship of an initial rise and then a fall with amylose content (Zhang *et al.*, 2010; Jin *et al.*, 2004), and the fourth is that nitrogen application level has no effect on amylose content (Jin *et al.*, 2002; Zhao *et al.*, 2004; Jiang *et al.*, 2004). The results obtained by different researchers are not the same, which may be related to different genotypes of amylose content of tested varieties and different test conditions. This significant interaction between nitrogen treatment and variety in 2016 in this study also confirms that the effects of nitrogen application level on amylose content between different varieties are at a different response level.

The results of the test using good eating quality *japonica* rice with a low amylose content show that the amylose content decreases with the increase of nitrogen application level when the nitrogen level is below 450 kg/hm². It agrees with the results of the study performed by Jiang *et al.* (2015) using semi-glutinous *japonica* Nanjing 9108 and Nanjing 5055, and the study performed by Chen *et al.* (2012) using 50 early-maturing late *japonica* varieties developed in the near 10 years in Jiangsu Province.

4.1.2 Effect of temperature on amylose content

Previous studies showed that the effect of temperature on amylose content of rice during grain filling period was related to amylose content of the variety, but different researchers obtained different results. Studies by Sun *et al.* (1993) showed that the effect of temperature on amylose content varied with variety type, and high temperature made amylose content of low amylose content varieties decrease and that of high amylose content varieties rise. Studies by Mong *et al.* (1994) showed that low temperature in the productive phase would make amylose content of low and medium amylose content varieties rise and that of glutinous varieties decrease. The temperature 5-10 days later after flowering significantly affected amylose content. Studies by Cheng *et al.* (2000) suggested that amylose content of many varieties was in a quadratic curve relationship with the temperature in the productive phase and amylose content of high amylose content varieties in general reached the maximum at a higher temperature, but low amylose

content varieties were the opposite. For a particular variety, temperature in the productive phase within a certain temperature range was positively correlated with amylose content of rice, but negatively correlated in another temperature range. Zhou *et al.* (1994) and Chen *et al.* (1996) also held a similar view. Jia *et al.* (1991) believed the effect of temperature on amylose content of rice was different in different periods during the grain-filling period: in the first 18 days of the grain-filling period the high temperature was not conducive to accumulation of amylose content but conducive to that 18 days later, showing a positive effect. Our tests in the past two years show that high temperature makes amylose content of good eating quality *japonica* rice increase and the effect of temperature is mainly produced 6-15 days later after heading. The test results of sowing at different stages in 2015 showed that amylose content of rice sowed at the first stage was the highest; the maximum temperature, the minimum temperature, the mean temperature difference and the diurnal temperature difference 6-15 days later after heading were 30.5 °C, 23 °C, 26.8 °C and 7.5 °C respectively, the highest in the 3 sowing stages. Amylose content of the rice sowed at the 2nd and 3rd stages were 9.08% and 9.22% respectively, obviously lower than that sowed at the first stage. The maximum temperature, the minimum temperature, the mean temperature difference and the diurnal temperature difference 6-15 days later after heading were also obviously lower than those of the first stage, with no significant difference between the 2nd and 3rd stages, which agreed with the change trend of amylose content. This was also confirmed in the 2016 study either by short daylight treatment or by the test of delayed sowing. In addition, Nanjing 9108 and G7012 were put in the test in 2015 and 2016 respectively, and amylose content of Nanjing 9108 and G7012 were 9.55% and 8.3% respectively in 2015, 1.25% and 0.47% higher than 8.3% and 7.83% in 2016, which completely agreed with a change in the maximum temperature 6-15 later after heading in 2015 (33 °C and 29 °C) and the highest temperature in 2016 (27 °C and 28 °C).

Nitrogen application level also has certain effect on heading date. In order to determine whether the effect of different nitrogen treatments on amylose content covers the effect of temperature, the heading date under different nitrogen treatments and the temperature 6-15 days later after heading were analyzed in this study. The results showed that the number of days from sowing

to heading in 2015 under 4 different nitrogen treatments was 98.4-102.9, and the number in 2016 was 99.3-102.1. Although nitrogen treatment had a tendency to postpone heading date, the effect was little, and the temperature change 6-15 days later after heading was also not greatly. Therefore, the effect of nitrogen treatment on amylose content is mainly caused by different nitrogen application levels.

4.1.3 Enlightenment of the results on high quality cultivation of the good eating quality *japonica* rice

Good eating quality *japonica* (semi-glutinous *japonica* rice) is a special variety with amylose content lying between glutinous and *indica*, characterized by low amylose content, softness and shiny appearance, good elasticity and good eating quality. But the milled rice is translucent in milky white, close to glutinous rice's white when dried with amylose content below 8%. In order to maintain good eating quality characteristics of semi-glutinous rice and transparency of rice grain, amylose content is better in a range of 10%-14%. The results of the study show that amylose content should be increased and protein content should be decreased as far as possible in the cultivation strategy in order to maintain the eating quality of the good eating japonica rice. The rice should be sowed at suitable time to ensure grain filling at a suitable temperature 6-15 days later after heading. In the application of nitrogen, organic manure should be used as far as possible with less use of chemical nitrogen, especially in the late stage it is required to avoid the use of nitrogen fertilizer, preventing excessive nitrogen application from lowering the eating quality as a result of lowering amylose content and raising protein content.

It must be pointed out that although nitrogen application level and sowing date have a significant effect on amylose content, variety is the main factor that leads to the difference in amylose content in either nitrogen fertilizer test or sowing date test. The variance of amylose content between varieties in the fertilizer test accounted for 89.2% and 76.2% of the total respectively in 2013 and 2014, and the variance of amylose content between varieties in the sowing date test accounted for 87.1% of the total in 2015. Therefore, we will focus on genetic research on the molecular basis causing the difference in amylose content of the good eating quality *japonica* rice.

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CHAPTER FIVE

EFFECTS OF *SS IIa* AND *SS IIIa* GENES ON COOKING AND EATING QUALITY TRAITS

Effects of *SSIIa* and *SSIIIa* Genes on Cooking and Eating Quality Traits

Shu Yao^{1,2}, Prof. Balakrishna Pillay², and Prof. Cailin Wang^{1*}

1. Institute of Food Crops, Jiangsu Academy of Agricultural Sciences/Jiangsu High Quality Rice R&D Center/Nanjing Branch of China National Center for Rice Improvement, Nanjing 210014, China

2. Discipline of Microbiology, School of Life Science, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Private Bag X54001, Durban, 4000, South Africa.

*Corresponding author. Tel: +25 84390317; Fax: Tel: +25 84390317

E-mail address: clwang@jaas.ac.cn (Cailin Wang)

Abstract

Sixty-four semi-glutinous lines derived from hybrid progenies of Wujing 13 and Kanto 194 with manifestation polymorphism in starch synthase gene *SSIIa* and *SSIIIa*, were selected as materials. *SSIIa* and *SSIIIa* genotypes were identified by molecular markers. Genetic effects of *SSIIa* and *SSIIIa* on the amylose content, gel consistency, gelatinization temperature and RVA profile characteristics in the same *Wx-mq* gene background were studied. The results showed that *SSIIa* and *SSIIIa* had significant effects on the characteristic values of AC, GT, GC and RVA profile, and the two genes of which have interactive effects. The *SSIIa* and *SSIIIa* genes from Wujing13, a non-waxy parent, had a tendency to increase AC value with a respective increment of 1.87 and 1.23 percentage and the results were similar in two years. There was no significant difference in gelatinization temperature between *SSIIa* and *SSIIIa* genotypes of different parents, while the gelatinization temperature of genotype *SSIIa^{-mq}SSIIIa^{-mq}* was significantly higher than that of *SSIIa^{-b}SSIIIa^{-b}*. The result indicates that the gelatinization temperature was not significantly affected by single *SSIIa* and *SSIIIa* genes but remarkably influenced by the combination of the two genes. The gelatinization temperature of genotype *SSIIa^{-mq}SSIIIa^{-mq}* was 1.34 °C higher than that of *SSIIa^{-b}SSIIIa^{-b}*. The gel consistency among

different genotypes was significantly different. For example, the *SSIIa* gene from Wujing 13 (*SSIIa^{-b}*) and *SSIIIa* gene from Kanto 194 (*SSIIIa^{-mq}*) could increase the gel consistency of 8.74mm and 9.62mm respectively. From the joint effect of the two genes, the gel consistency of genotype *SSIIa^{-b}SSIIIa^{-mq}* was 10.64mm higher than that of genotype *SSIIa^{-mq}SSIIIa^{-b}* and was 16.95mm higher than that of *SSIIa^{-b}SSIIIa^{-b}*. The *SSIIa* gene (*SSIIa^{-b}*) from Wujing 13, a non-waxy parent, increased the peak viscosity, hot paste viscosity, cold paste viscosity and breakdown Value, while it decreased the consistence value and setback value in the meanwhile. However, the effect of *SSIIIa* gene *SSIIIa^{-b}* from Wujing 13 was just the opposite, which decreased the peak viscosity, hot paste viscosity, cold paste viscosity, breakdown Value and increased the consistence value and consistence value. In terms of the joint effects of the two genes, the peak viscosity, hot paste viscosity and cold paste viscosity were the largest in *SSIIa^{-b}SSIIIa^{-mq}* genotype, the breakdown Value and consistence value were the largest in *SSIIa^{-b}SSIIIa^{-b}* genotype, and the setback value in *SSIIa^{-b}SSIIIa^{-mq}* genotype was the least. According to the manifestations of all characters, the 4 genotype food quality of *SSIIa* and *SSIIIa* site were the best in *SSIIa^{-b}SSIIIa^{-mq}* genotype, 11.54% of AC value, comparably lower GT, gel consistency, peak viscosity, hot paste viscosity, cold paste viscosity and breakdown Value were the largest, and the consistence value was the lowest.

Keywords: starch-synthesizing genes; amylose content; gel consistency; gelatinization temperature; RVA profiles characteristics; genetic effects

1.1 Introduction

In order to clarify the reason for the difference of amylose content in different semi-waxy lines with the same *Wx-mq* gene, the previous chapter analyzed the effect of nitrogen application and sowing date (temperature) on amylose content of semi-glutinous *japonica* rice. The results show that the amount of nitrogen fertilizer and sowing date had a significant effect on amylose content of semi-glutinous *japonica* rice. Both increasing nitrogen application and delaying sowing date could be able to reduce amylose content. However, the reduction was modest

within 1% at most. The results show that the difference of amylose content in different semi-waxy lines with the same *Wx-mq* gene was mainly caused by genetic factors.

In this chapter, the semi-glutinous lines derived from Wujing13 and Kanto194, which show polymorphisms in starch synthase gene *SSIIa* and *SSIIIa*, were selected as test materials. The *SSIIa* and *SSIIIa* genotypes were identified by molecular markers in semi-glutinous lines with *Wx-mq* alleles. The genetic effect of *SSIIa* and *SSIIIa* on amylose content, gel consistency, gelatinization temperature and RVA profiles characteristics were explored in the same *Wx-mq* gene background to provide the theoretical basis for the breeding of new varieties with excellent eating quality.

1.2 Materials and methods

1.2.1 Test materials

In order to clarify the effect of *SS IIa* and *SS IIIa* genes related to starch synthesis on cooking and eating quality in the background of *Wx-mq* gene. We used different genotypes of *Wx*, *SS IIa* and *SS IIIa* genes were constructed by crossing semi-waxy rice lines, which contain *Wx-mq* gene, with *japonica* rice varieties (*Wx-b*), which have different loci of *SS IIa* and *SS IIIa* genes.

In 1999, Wujing 13, a *japonica* rice cultivar bred by Wujin Rice Research Institute of Jiangsu Province, was used as female parent, and Kanto194, a semi-glutinous rice variety imported from Japan, was used as male parent. Sixty-four stable lines obtained from multi-generation inbred rice were used as experimental materials (Fig 5.1). Wujing13 and Kanto194 have different alleles at *SSIIa* and *SSIIIa* loci.

1.2.2 Planting and Design of Test Materials

Every line was divided into a small area with 4 rows and 10 plants per row in each plot. The row and plant spacing were 27cm and 17cm respectively. The test materials were arranged in sequence and repeated twice. In 2013 and 2014, sowing on done May 10 and transplanting on June 10. Field management is consistent with field production (Pai *et al.* 2011). The young

leaves at full tillering stage were used to extract DNA, and the seeds were harvested after ripening for the determination of physical and chemical indexes and quality traits of rice.

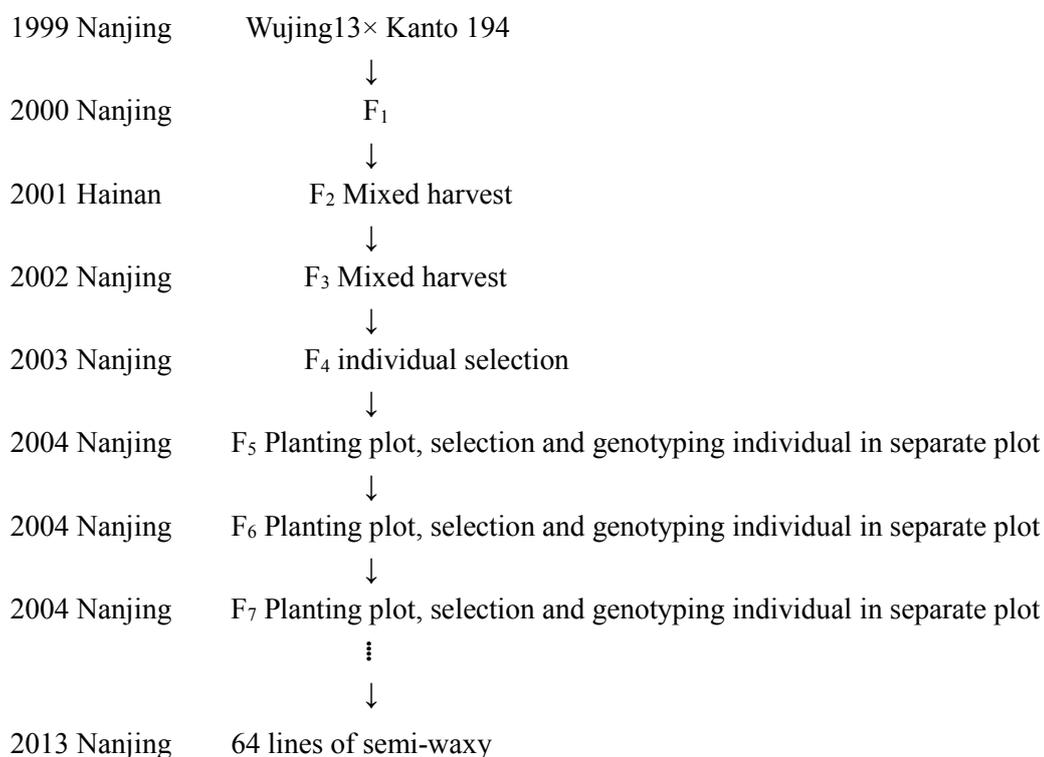


Fig 5.1 Planting and experimental design of test materials

1.2.3 Genotypic detection

1.2.3.1 Extraction of DNA from leaves

At the peak of tillering stage, the fresh young leaves of 6 individual plants were taken continuously from the second plant in the middle of each plot, and DNA (Murray, 1980) was extracted by CTAB method. The actions are as follows:

(1) The blade was added into 2 centrifuge tubes with diameter of 4 mm steel beads and freeze-dried for 8 hours.

(2) The tissue was crushed by grinder and pour the steel beads out.

(3) 1mL 2% CTAB extract (65 °C preheating) was added and mixed with whirlpool concussion, and then put it into the 65°C oven for metal bath for 30 min.

(4) 400 μL chloroform was added in the ventilated kitchen and fully shaken for 10min.

(5) 12000 rpm centrifuge for 10min, took up 400 μ L and added 1.5 mL to the centrifuge tube, added 400 μ L isopropanol (-20 preheating), and mixed it into -20 $^{\circ}$ C refrigerator for 1h.

(6) The mixture was centrifuged for 10 min at 12000 rpm. The supernatant was discarded and the rest should be rinsed with ethanol for 2 times. Dry naturally (or put in oven 50 $^{\circ}$ C for 30 min), add double steamed water dissolved, to be used.

1.2.3.2 Design of molecular marker primers

In this study, we used the molecular marker information of regulatory genes in the starch synthesis pathway reported in the literature to synthesize primers (Wan *et al.*, 2006; Cai *et al.*, 2002; Yan *et al.*, 2005). The primers for detection of molecular markers were synthesized by Shanghai Sangon Biotechnology Co., Ltd. Table 1 is the molecular marker and primer sequence of starch biosynthesis related genes for semi-waxy lines.

PRIMER1: primer design for tetra-primer ARMS-PCR was used to design four primers to amplify the obstructed mutation system. The primers were synthesized by Shanghai Sangon Biotechnology Co., Ltd.

20 μ L, 10 x Buffer 1 μ L, dNTP 0.20 μ L, *Taq* DNA polymerase 0.50 μ L, upstream and downstream primers 1 μ L, template DNA 1.00 μ L.

Table 5.1 Molecular markers of *SSIIa*, *SSIIIa* and *Wx-mq* genes used in this study

Genes	Primer sequences	Product size (bp)	A.T.($^{\circ}$ C)
<i>SSIIa</i> (L22)	F: CTTTGATAGTTCGAATGGTT R: CAATGTTTCTCCGTGATGAT	248/344	55
<i>SSIIIa</i> (L25)	F: GAACTTGTGCCTTAAGCTGACTG R: GGAATAGTAAGCCGAAGGACTT	197/177	55
<i>Wx-mq</i>	O-F: 5' -ATGTTGTGTTCTTGTGTTCTTTGCAGGC3' O-R: 5' -GTAGATCTTCTCACCGGTCTTTCCCCAA3'	439/200	65
	I-F: 5' -GGGTGAGGTTTTTCCATTGCTACAATCG3' I-R: 5' -GTCGATGAACACACGGTTCGACTCAAT3'	439/292	65

1.2.3.3 PCR amplification program

The PCR reaction was amplified on an Eppendorf-5330 PCR machine. Total volume 10 μL , ddH₂O 6.55 μL , 10 \times Buffer 1.00 μL , dNTP 0.20 μL , *Taq*DNA polymerase 0.50 μL , upstream and downstream primers 1 μL , template DNA 1 μL .

The reaction procedure was as follows: pre-degeneration at 94 $^{\circ}\text{C}$ for 5 min, then denaturation at 95 $^{\circ}\text{C}$ for 30 s, renaturation at 65 $^{\circ}\text{C}$ for 30 s, extension at 72 $^{\circ}\text{C}$ for 1 min, 35 cycles; extension at 72 $^{\circ}\text{C}$ for 10 min, cooling at 4 $^{\circ}\text{C}$ for 10 min. Indication was added to the amplification product, and reserved for later use. (Dian *et al.* 2005; Jiang *et al.* 2004).

The four-primer PCR amplification program was pre-denatured at 94 $^{\circ}\text{C}$ for 5 min and denaturation at 94 $^{\circ}\text{C}$ for 30 s, renaturation at 65 $^{\circ}\text{C}$ for 30 s, extension at 72 $^{\circ}\text{C}$ for 1 min, in which repeated steps 2-4 for 30 times.

1.2.3.4 Enzyme digestion reaction system

Enzyme cutting system: PCR amplification product 10 μL , endonuclease 1 μL , 10 x Buffer 2 μL , 0.1%BSA 2 μL , ddH₂O 5 μL . The digestion time was 1h at 37 $^{\circ}\text{C}$. The products were detected by 1.5% agarose gel or 9% polyacrylamide gel electrophoresis. The endonucleases used in the experiment were purchased from TaKaRa Company.

1.2.3.5 Detection of PCR products

One point five percent Agarose gel or 9% polyacrylamide gel was selected according to the size of the PCR amplification products and the resolution of the gel.

(1) 1.5% Agarose gel

A 2 μL indicator was added to the PCR amplification products, and 1.5% agarose gel was prepared (1 μL Du Red staining agent was added to the gel), the sample volume of the PCR product was 8 μL , and ran for 30 minutes at 90 V voltage, and the result was recorded by gel imaging system.

(2) 9% Polyacrylamide gel

Nine percent, polyacrylamide gel formulation; distilled water 27 μL , 10 x TBE Buffer 4 μL , 40% acrylamide 9 μL , TEMED 20 μL , 10% AP (summer 400 μL).

Electrophoresis: the 2 μL bromine phenol blue indicator was added into the amplified products of the PCR, the sample volume was 1.5 μL , and the electrophoresis time was 1.5 hours (according to the size of the product fragment).

Washing: glue was stripped from the glass plate to 0.1% AgNO_3 solution slowly and was put on the table to silver stain for 15 min. Silver staining solution should be poured into the waste recycling bins, and glue washed with distilled water 2 times, and then color-substrate solution was poured (200 ml water, 3 g NaOH, 2 ml formaldehyde) to show clear bands on rotary shaker color. Drained of Color-substrate solution, rinsed with distilled water once and glue was taken to observe and photograph.

1.2.4 Determination of rice quality traits

1.2.4.1 Pretreatment of rice samples

The rice seeds were harvested from every single plant after full maturation, then the rice flour was sifted through mesh after rough and milled rice which was used to determine AC, gel consistency and starch viscosity.

1.2.4.2 Determination of AC

The determination of AC was carried out in accordance with the standard issued by the Ministry of Agriculture, NY147-88(1988).

(1) 50 mg dry rice flour was put in a 50 mL test tube (which should be accurate enough at 1/10000),

(2) A 0.5 ml anhydrous ethanol was added to the test tube, the test tube gently shaken to disperse the sample, and then added 4.5 ml 1.0 mol/L NaOH solution and mixed well,

(3) The test tube was put in a boiling water bath for 20 minutes (simmering and vibrating to avoid caking), cooled to the room temperature and mixed with distilled water,

(4) Five ml solution was added to a 100 ml volumetric flask with approximately 50 ml of distilled water (blank control solution of NaOH uptake 5 ml 0.09 mol/L); the addition of 1 ml 1 mol/L acetic acid solution was to acidify the sample and then added 1.5 ml 0.02% iodine solution to the sample, before set the volume with distilled water and 20 min standing.

(5) The absorptivity was determined by spectrophotometer at 620 nm with a 1 cm colorimetric dish, and each sample was repeated for 3 times.

(6) The absorbance value and the standard curve of AC measured with four standard samples (1.5%, 10.4%, 16.2%, 26.5%) of known AC were made, and the AC of the tested samples was calculated according to this standard curve.

1.2.4.3 Determination of gelatinization temperature

The data of gelatinization temperature was replaced by the initial pasting temperature of RVA eigenvalue.

1.2.4.4 Determination of gel consistency

(1) 100 mg of white rice flour was weighed and placed it at the bottom of 10 mL test tube, we tried not to dip the rice flour into the wall of the test tube.

(2) 0.2 mL, 0.025% thymoblue indicator indicator was added, and oscillate with the oscillator to make the bottom rice noodles moist and disperse, and then 2 mL 0.2 mol/L KOH solution was added and oscillated with the oscillator to avoid its sedimentation and agglomeration.

(3) When the rice flour was completely dispersed, that is to say, without any caking, the test tube was put into a boiling water bath pot immediately, covered the test tube with a glass ball, adjust the water surface height was adjusted so that the water level was higher than the test tube liquid level. The height of rice glue was kept at about 2/3 of the test tube (it could be adjusted with a hair dryer), gelatinized for 8 minutes.

(4) After gelatinization, take out the test tube was taken out, remove the glass ball was removed and cooled at room temperature for 5 min, and then cooled in ice water mixture for 20 min.

(5) At room temperature of 25 ± 5 °C, the test tube was placed on the horizontal table. The length of the flow of glue (the length of the bottom of the tube to the front of the gel glue) was measured in mm.

(6) Each sample was determined twice. If the absolute difference between the two results is less than 7 mm, the average value is used as the determination result, and the final result is taken as an integer. If the absolute values of the two results exceed 7 mm, the determination will be redetermined.

1.2.4.5 Determination of the characteristic value of Rice RVA profile

The 3-D viscosity rapid-measuring instrument made by Newport Scientific in Australia and the matching software TCW was used to measure the results according to the operating rules of the American Association of Grain Chemistry (AACL).

The white rice flour of 3 g was weighed and 25 g distilled water with RVA aluminum cup was accurately weighed. The rice flour was mixed with a mixing rod and put into the instrument for determination. The agitator firstly rotated at a speed of 960 rpm for 10 seconds to disperse the rice flour, and then rotated at a speed of 160 rpm to determine the viscosity. The total measuring time was 12.5 min. During this period the temperature variation of the irrigation was as follows: at 50 °C for 1 min and rise to 95 °C, keep 2.5 min in the highest temperature of 95 °C, and cool down to 50 °C for 1.4 min. The viscosity unit was expressed in CP. The viscosity parameters of starch have five first-order data, including the peak viscosity PKV (PKV), the hot paste viscosity (HPV), the cool paste viscosity (CPV), the initial gelatinization temperature (PaT) and the peak time (Pet) and three second-order data, including the breakdown viscosity (BDV), the setback value (SBV), the consistence value (CSV).

1.2.5 Statistical analysis

Excel 2016 and SPSS 20 were used to analyze the data.

1.3 Results

1.3.1 Genotypic test results of tested materials

1.3.1.1 Detection of *Wx-mq* genotypes

According to the principle of Mendelian inheritance, there are three genotypes of *Wx-b Wx-b*, *Wx-mqWx-mq* and *Wx-bWx-mq* in the segregation generation of *japonica* rice with *Wx-b* gene and semi-glutinous *japonica* rice with *Wx-mq* gene. Three genotypes could be detected simultaneously by using the PCR system of four primer blocked amplification mutation system (Chen Tao, *et al.* 2013). The results show that the specific bands of 439 bp could be amplified from 64 semi-glutinous lines and two parents. In addition, 292 bp specific bands could be amplified from 64 semi-glutinous lines and Kanto 194, but 292 bp bands could not be amplified from Wujing13 without *Wx-mq*, but a specific band of 200 bp could be amplified from Wujing13 without *Wx-mq* (Fig 5.2), which indicate that all of 64 samples contained *Wx-mq* gene.

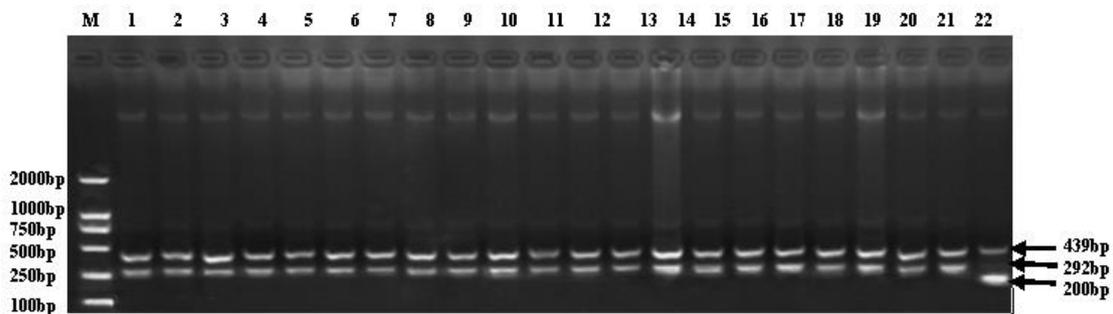


Fig 5.2 Detection of the *Wx-mq* gene in 64 semi-glutinous lines

Note: M: DL 2000bp Marker; 1-20: Part of the semi-glutinous lines; 21: Kanto194; 22: Wujing13

1.3.1.2 Detection of genotypes *SSIIa* and *SSIIIa*

The STS markers L22 and L25 designed by *SSIIa* and *SSIIIa* gene loci were used to detect 64 semi-glutinous lines (Fig 5.3). The results show that the lines of the gene *SSIIa^{-mq}* (mq indicate that the gene is derived from the semi-waxy parent Kanto194) and *SSIIa^{-b}* (b indicate that the gene originated from non-waxy parent Wujing13) were 23 and 41, the lines of the gene *SSIIIa⁻*

mq and $SSIIIa$ 20 were 49 and 15, respectively. There were 10, 13, 39 and 2 lines of $SSIIa$ $^{mq}SSIIIa$ $^{-mq}$, $SSIIa$ $^{-mq}SSIIIa$ $^{-b}$, $SSIIa$ $^{-b}SSIIIa$ $^{-mq}$ and $SSIIa$ $^{-b}SSIIIa$ $^{-b}$ of the two genes (Table 5.2).

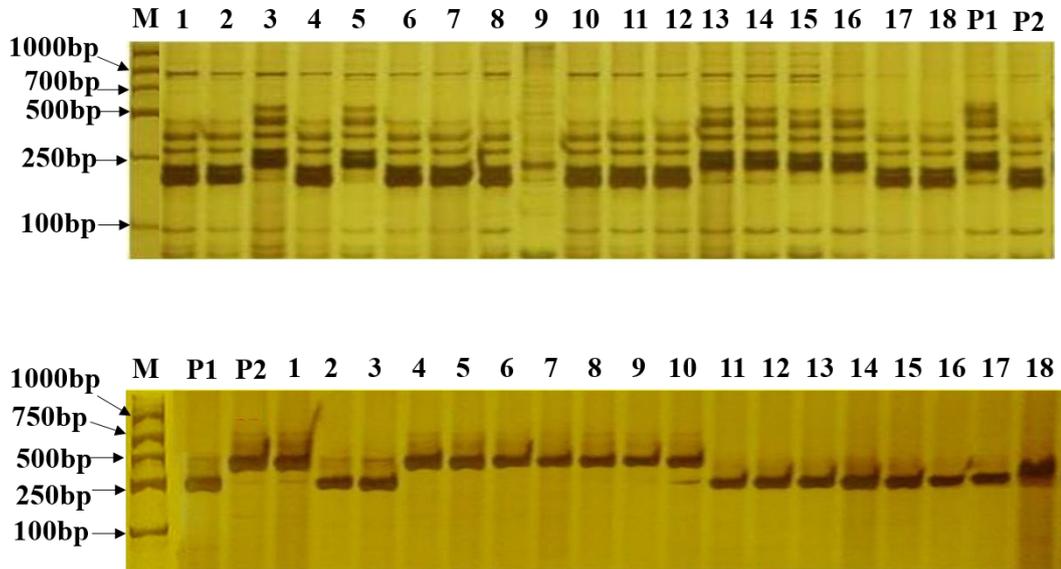


Fig 5.3 Detection of $SSIIa$ and $SSIIIa$ gene in 64 semi-glutinous lines

Note: M: DL 2000bp Marker; 1-18: Part of the semi-glutinous lines; P1: Kanto194; P2: Wujing13

Table 5.2 the genotypes of $SSIIa$ and $SSIIIa$ locus for 63 semi-glutinous lines derived from Wujing 13/Kanto194 with Wx $^{-mq}$ gene

Single genotype	Number of strain	Double genotype	Number of strain
$SSIIa$ $^{-mq}$	23	$SSIIa$ $^{-mq} SSIIIa$ $^{-mq}$	10
$SSIIa$ $^{-b}$	41	$SSIIa$ $^{-mq} SSIIIa$ $^{-b}$	13
$SSIIIa$ $^{-mq}$	49	$SSIIa$ $^{-b} SSIIIa$ $^{-mq}$	39
$SSIIIa$ $^{-b}$	15	$SSIIa$ $^{-b} SSIIIa$ $^{-b}$	2

Note: mq indicated that the gene was derived from the semi-glutinous parent of Kanto194, and b indicated that the gene was derived from the parent of Wujing13.

1.3.2 Variation of cooking and eating quality of different genotypes in the background of *Wx-mq* gene

1.3.2.1 Variation of amylose content, gel consistency and gelatinization temperature

The amylose content (AC), gelatinization temperature (GT) and gel consistency (GC) of 64 semi-waxy lines were measured. The results showed that (Figure 5.4) the AC ranged from 7% to 11%, among which 54 were distributed between 8% and 10%, accounting for 85.71%. The GT of 64 materials are mainly distributed between 68.2°C and 73°C, mainly concentrated at 69-71°C, but the variation is not large and the coefficient of variation is 1.56%. The gel consistency of 63 materials are mainly between 50.0 mm and 102.0 mm, the average value is 79.6 mm, and the coefficient of variation is 16.05%.

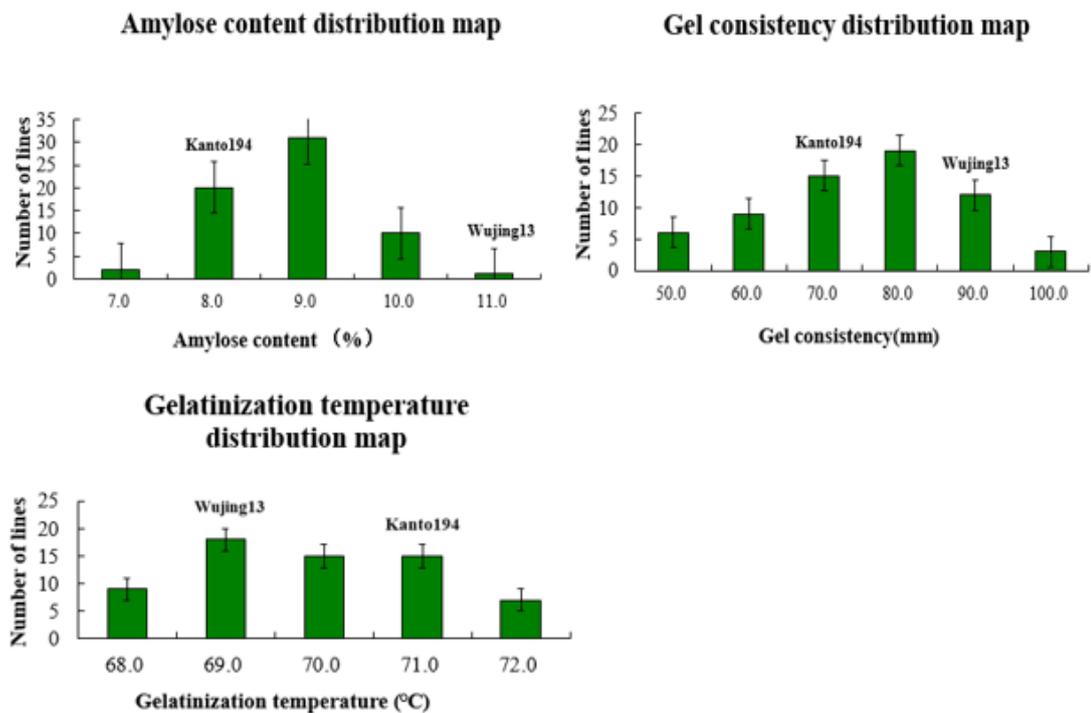


Fig 5.4 Frequency distribution of the AC, GC, GT in 64 semi-glutinous lines

1.3.2.2 Variation of eigenvalues of RVA spectra

The distribution of the eigenvalues of RVA profile characteristics of 64 semi-glutinous lines was shown in Figure 5.5. It can be seen from the distribution map that the variation range of

the peak viscosity was between 1746 cP and 3511cP, and there were 52 parts distributed between 2099cP and 3158cP, accounting for 82.5%. The variation range of the viscosity of the hot pulp was between 925cP and 2160cP, and 51 of them were distributed between 1172cP and 1913cP, accounting for 81%. The variation range of cold viscosity was between 1533cP and 2769cP, and 50 of them were distributed between 1780 cP and 2521cP, accounting for 79.4%. The variation range of breakdown viscosity was between 617 cP and 1646 cP, among which 60 were distributed between 822 cP and 1646 cP, accounting for 95.2%. The variation range of setback values was between -1018cP and -1cP, and the distribution was relatively uniform across the regions. The variation range of **consistency value** was between 480 cP and 752 cP, among which 56 were distributed between 588 cP and 752 cP, accounting for 88.9%. The variation of setback viscosity was the largest, the coefficient of variation reached 42.4%, followed by the breakdown viscosity, the hot paste viscosity, the peak viscosity, the cool paste viscosity, and the **consistency value**.

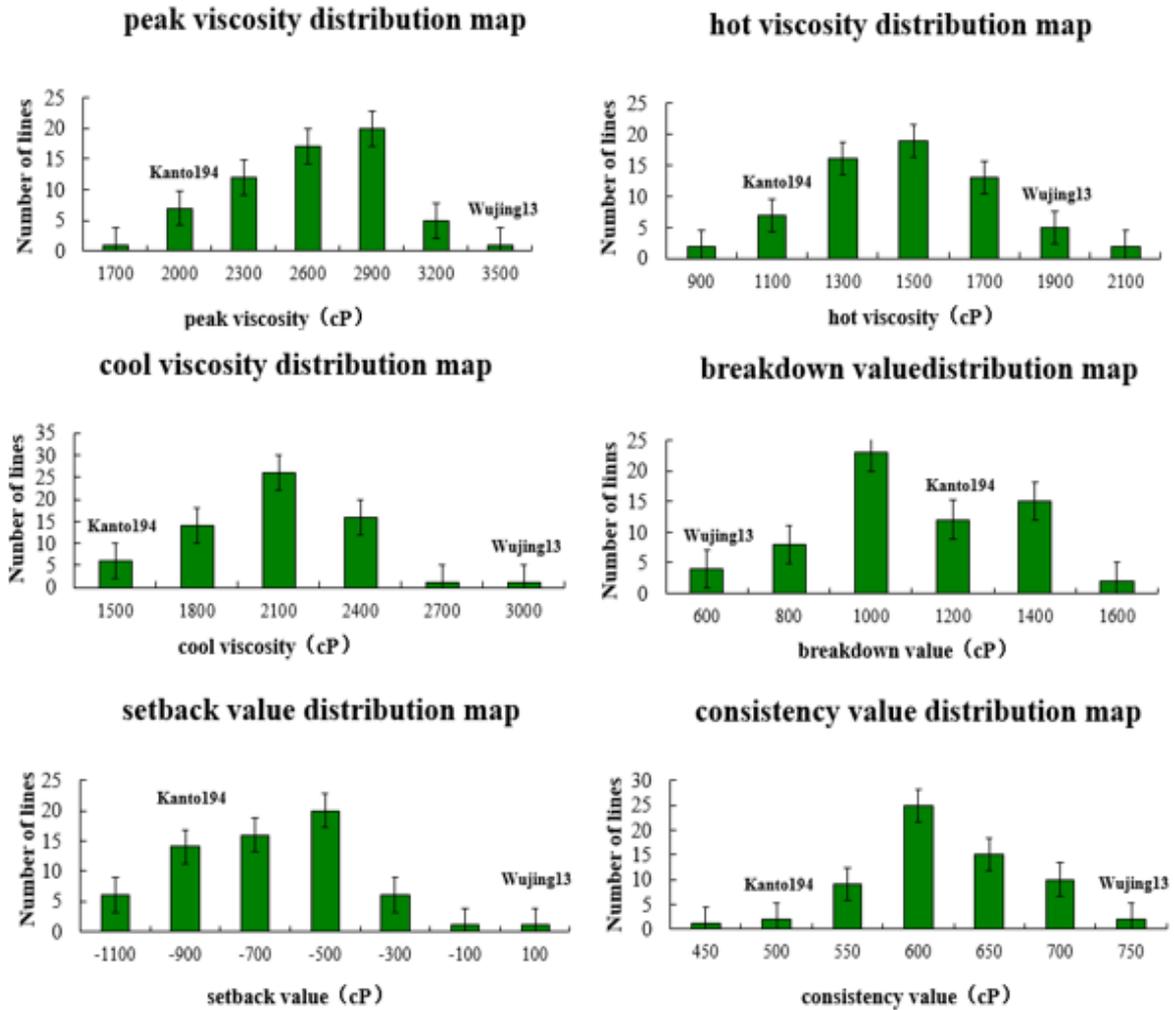


Fig 5.5 Frequency distribution of the RVA profile characteristics in 64 semi-glutinous lines

1.3.3 Difference of cooking and eating quality between *SS IIa* and *SS IIIa* genotypes

1.3.3.1 Analysis of differences between *SS IIa* and *SS IIIa* genotypes in AC

The results of ANOVA for AC of 64 semi-glutinous lines planted in 2013 and 2014 are shown in Table 5.3. Table 5.4 shows that there are significant differences in AC between 64 lines, years as well as years and lines. Further analysis showed that although the difference of AC among the four genotypes of *SSIIa^{-mq}SSIIIa^{-mq}*, *SSIIa^{-mq}SSIIIa^{-b}*, *SSIIa^{-b}SSIIIa^{-mq}* and *SSIIa^{-b}SSIIIa^{-b}* was very significant, the differences among the four genotypes were even greater. Except for the insignificant difference of AC between genotype *SSIIa^{-mq}SSIIIa^{-mq}* and *SSIIa^{-mq}SSIIIa^{-b}*, the difference of AC among other genotypes was extremely significant.

Table 5.3 Analysis of variance on AC in different genotypes of *SSIIa* and *SSIIIa* locus and different years for 64 semi-glutinous lines

Source of variance	df	SS	MS	F value	Significance
Group within the year	4	0.26	0.07	0.72	NS
Years	1	2.42	2.42	26.37	**
Lines	63	268.90	4.27	46.51	**
11 Genotype	9	32.10	3.57	38.87	**
12 Genotype	12	11.91	0.99	10.82	**
21 Genotype	38	66.85	1.76	19.17	**
22 Genotype	1	37.65	37.65	410.30	**
Genotype	3	120.39	40.13	437.32	**
11+12 and 21+22	1	53.24	53.24	580.17	**
11 and 12	1	0.03	0.03	0.30	NS
21 and 22	1	67.12	67.12	731.48	**
Years × Lines	63	60.30	0.96	10.43	**
Experimental error	252	23.12	0.09		
Total variation	383	355.01			

Note: Genotype 11, 12, 21 and 22 represent the four genotypes of *SSIIa^{-mq}SSIIIa^{-mq}*, *SSIIa^{-mq}SSIIIa^{-b}*, *SSIIa^{-b}SSIIIa^{-mq}* and *SSIIa^{-b}SSIIIa^{-b}*, respectively.

2) **: significant at 1% level, NS: not significant.

Table 5.4 lists the average AC of different genotypes of *SSIIa* and *SSIIIa* loci in 64 semi-glutinous lines in 2013 and 2014. It can be seen from the table that the AC of non-waxy parents was higher than that of semi-glutinous parents, regardless of the *SSIIa* or *SSIIIa* loci. On average, the AC of *SSIIa^{-b}* genotype was 1.87% higher than the data of *SSIIa^{-mq}* genotype. The AC of *SSIIIa^{-b}* genotype was 1.23% higher than the figure for *SSIIIa^{-mq}* genotype, which indicate that the effect of *SSIIa* was higher than that of *SSIIIa*.

Table 5.4 AC and genic effect for different genotypes in *SSIIa* and *SSIIIa* locus of 64 semi-glutinous lines in 2013 and 2014

Locus	Genotype	2013	2014	Average	Genic effect
<i>SSIIa</i>	<i>SSIIa^{-mq}</i>	9.05	8.85	8.95	1.87
	<i>SSIIa^{-b}</i>	10.84	10.80	10.82	
<i>SSIIIa</i>	<i>SSIIIa^{-mq}</i>	9.37	9.17	9.27	1.23
	<i>SSIIIa^{-b}</i>	10.51	10.48	10.50	

Table 5.5 lists the average value, maximum value, minimum value and year difference of AC among the four genotypes of *SSIIa* and *SSIIIa* loci of 64 semi-waxy lines in 2013 and 2014. The average AC of the four genotypes was divided into three groups: the AC of genotype *SSIIa^{-mq}SSIIIa^{-mq}* and *SSIIa^{-mq}SSIIIa^{-b}* was the lowest and there was no significant difference between them, the AC of genotype *SSIIa^{-b}SSIIIa^{-b}* was the highest, the AC of genotype *SSIIa^{-b}SSIIIa^{-mq}* was in the middle, and the AC of the three kinds of genotypes was extremely significant. From the genetic effects of single gene in Table 5.4, AC from non-waxy parents were higher than those from semi-glutinous parents. But the AC of *SSIIIa^{-b}* was not higher than the data of *SSIIIa^{-mq}* under the background of *SSIIa^{-mq}* gene, which indicate that there was interaction between *SSIIa* and *SSIIIa* loci.

Table 5.5 Difference of AC for different genotypes in *SSIIa* and *SSIIIa* locus of 64 semi-glutinous lines in 2013 and 2014

Genotype	Mean in 2013	Max.	Min.	Mean in 2014	Max.	Min.	Average	Difference in 2013 and 2014
11	9.06 A	10.70	7.60	8.80 A	10.31	7.67	8.93 A	0.26
12	9.03 A	9.89	8.21	8.89 A	9.66	8.47	8.96 A	0.14
21	9.68 B	11.54	8.08	9.53 B	10.75	7.32	9.61 B	0.15
22	11.99 C	13.96	10.02	12.07 C	13.64	10.49	12.03 C	-0.08

Note: 1) Genotype 11, 12, 21 and 22 represent the four genotypes of *SSIIa^{-mq}SSIIIa^{-mq}*, *SSIIa^{-mq}SSIIIa^{-b}*, *SSIIa^{-b}SSIIIa^{-mq}* and *SSIIa^{-b}SSIIIa^{-b}* respectively.

2) Different capital letters in same column indicates significant at 1% level.

1.3.3.2 Differences in gelatinization temperature and gel consistency among different genotypes

The gelatinization temperature and gel consistency of different genotypes are shown in Table 5.6. There was no significant difference in gelatinization temperature between *SSIIa* and *SSIIIa* genotypes of different parents, but there was significant difference in gel consistency among

different genotypes. *SSIIa* gene from Wujing13 and *SSIIIa* gene from Kanto194 increased gel consistency by 8.74 mm and 9.62 mm respectively.

Table 5.6 Difference of GC and GT for different genotypes in *SSIIa* and *SSIIIa* locus of 64 5.-glutinous lines

Locus	Genotype	Gelatinization Temperature			Gel Consistency		
		Average (°C)	Sd	CV (%)	Average (mm)	Sd	CV (%)
<i>SSIIa</i>	<i>SSIIa^{-mq}</i>	70.59	1.26	1.78	73.78**	13.41	18.18
	<i>SSIIa^{-b}</i>	70.36	1.01	1.44	82.53	10.28	12.46
<i>SSIIIa</i>	<i>SSIIIa^{-mq}</i>	70.49	1.11	1.57	81.47**	11.76	14.43
	<i>SSIIIa^{-b}</i>	70.28	1.11	1.58	71.86	10.89	15.15
<i>SSIIa^{-mq}SSIIIa^{-mq}</i>		70.94a	1.38	1.95	75.71ab	16.29	21.52
<i>SSIIa^{-mq}SSIIIa^{-b}</i>		70.33ab	1.34	1.91	72.31b	11.20	15.49
<i>SSIIa^{-b}SSIIIa^{-mq}</i>		70.37ab	1.02	1.45	82.95a	10.05	12.12
<i>SSIIa^{-b}SSIIIa^{-b}</i>		69.6b	1.21	1.89	66.00b	10.12	15.21

Note: **1% significant level; Different letters after the means in same column indicated significant at 5% level.

Multiple comparisons show that the gelatinization temperature of genotype *SSIIa^{-mq}SSIIIa^{-mq}* was the highest, followed by genotype *SSIIa^{-b}SSIIIa^{-mq}*, *SSIIa^{-mq}SSIIIa^{-b}* and *SSIIa^{-b}SSIIIa^{-b}*, but the gelatinization temperature of the four genotypes was similar. There was no significant difference between genotype *SSIIa^{-mq}SSIIIa^{-mq}* and *SSIIa^{-b}SSIIIa^{-b}*, except for genotype *SSIIa^{-mq}SSIIIa^{-mq}* and *SSIIa^{-b}SSIIIa^{-b}*. But in terms of gel consistency, the gel consistency of genotype *SSIIIa^{-b}SSIIIa^{-mq}* was the highest, followed by genotype *SSIIa^{-mq}SSIIIa^{-mq}*, *SSIIa^{-mq}SSIIIa^{-b}* and *SSIIIa^{-b}SSIIIa^{-b}*. The difference between genotype *SSIIa^{-b}SSIIIa^{-mq}* and *SSIIa^{-mq}SSIIIa^{-b}* was significant, and the difference between genotype *SSIIa^{-b}SSIIIa^{-mq}* and *SSIIa^{-b}SSIIIa^{-b}* was extremely significant.

1.3.3.3 Analysis of characteristic values of Rice RVA profile characteristics among different genotype

The RVA profile characteristics of different genotypes were determined in table 7. It can be seen from the table that as for gene *SSIIa*, there was significant or extremely significant difference in the characteristic values of different parent sources, and in terms of gene *SSIIIa*, there was no significant difference except for hot paste viscosity and cold paste viscosity. The peak viscosity, breakdown viscosity, consistency value and setback viscosity all showed significant or extremely significant difference.

Table 5.7 Difference of RVA profiles for different genotypes in *SSIIa* and *SSIIIa* locus of 64 semi-glutinous lines

Locus	Genotype	PKV	HPV	CPV	BDV	CSV	SBV
<i>SSIIa</i>	<i>SSIIa^{-mq}</i>	2495.0**	1438.0**	2104.83*	1056.91**	666.74*	-390.17**
	<i>SSIIa^{-b}</i>	2939.35	1651.07	2285.4	1288.28	634.33	-653.95
<i>SSIIIa</i>	<i>SSIIIa^{-mq}</i>	2828.43*	1589.65	2227.49	1238.78*	637.84*	-600.94**
	<i>SSIIIa^{-b}</i>	2597.57	1516.14	2191.43	1081.43	675.26	-406.14
<i>SSIIa^{-mq}SSIIIa^{-mq}</i>		2383.5C	1333.2B	1996.5b	1050.3B	663.3B	-387.0a
<i>SSIIa^{-mq}SSIIIa^{-b}</i>		2580.7BC	1518.8AB	2188.2ab	1062.0B	669.4B	-392.6a
<i>SSIIa^{-b}SSIIIa^{-mq}</i>		2942.5A	1655.4A	2286.7a	1287.1A	631.3B	-655.8b
<i>SSIIa^{-b}SSIIIa^{-b}</i>		2816.0AB	1482.0AB	2234.0ab	1334.0A	752.0A	-582.0b

Note: *, ** or different capital and lower case letters after the means in the same column indicated significant at 1% and 5% levels, respectively.

The results of multiple comparisons of the RVA profile characteristics eigenvalues of the four genotypes of the two genes show that there were significant or extremely significant differences in each eigenvalue between the four genotypes. When all the parent sources of *SSIIa* gene are Kanto194 (namely the gene type is *SSIIa^{-mq}*), the differences of characteristic value among different parent sources of *SSIIIa* genes (namely *SSIIIa^{-mq}* and *SSIIIa^{-b}*) are insignificant; when all the parent sources of *SSIIa* gene are Wujing13 (namely the gene type is *SSIIa^{-b}*), except the return value is significantly different in different parent sources of *SSIIIa* genes (namely *SSIIIa⁻*

^{mq} and $SSIIIa^{-b}$), other characteristic values have no significant difference. When all the parent sources of $SSIIIa$ genes are Kanto194 (namely the gene type is $SSIIIa^{-mq}$), except the return value is significantly different in different parent sources of $SSIIa$ genes (namely $SSIIa^{-mq}$ and $SSIIa^{-b}$), other characteristic values have no significant difference. When all the parent sources of $SSIIIa$ genes are Wujing13 (namely the gene type is $SSIIIa^{-b}$), the differences in peak viscosity, hot paste viscosity and cold paste viscosity for different parent sources of $SSIIa$ genes (namely $SSIIIa^{-mq}$ and $SSIIIa^{-b}$) are insignificant, the differences in setback value is significant, and the differences in breakdown value and consistence value are extremely significant. Thus, it is seen the gene affecting RVA profile characteristics is mainly $SSIIa$. The peak viscosity and breakdown value of semi-glutinous series are greater than that of parent Wujing13, and the hot paste viscosity, cold paste viscosity and setback value of semi-glutinous series are smaller than parent Wujing13. In the research of Sui *et al.* (2005), it is deemed that there is an extremely close relationship between the RVA profile characteristics and eating quality index, indicating the feasibility of reflecting eating quality of rice with RVA profile characteristics. The further research indicates that the breakdown value, setback value and consistence value can evaluate the eating quality correctly and can be deemed as effective indicators of selecting material with high eating quality. The varieties with high breakdown value and low setback value and consistence value have better eating quality when they are cooked. The varieties with higher breakdown value in the experiment are $SSIIa^{-b}SSIIIa^{-mq}$ and $SSIIa^{-b}SSIIIa^{-b}$, and there are no significant differences between them. The varieties with low consistence value are $SSIIa^{-b}SSIIIa^{-mq}$ and $SSIIa^{-mq}SSIIIa^{-mq}$, and there are no significant differences between them. The varieties with low setback value are $SSIIa^{-b}SSIIIa^{-mq}$ and $SSIIa^{-b}SSIIIa^{-b}$, and there are no significant differences between them. Thus, it is inferred that the varieties with gene type $SSIIa^{-b}SSIIIa^{-mq}$ have better eating quality when they are cooked.

1.4 Discussion

1.4.1 Genetic effects of *SSIIa* and *SSIIIa*

In this chapter, the semi-glutinous lines derived from Wujing13 and Kanto194, which show polymorphisms in starch synthase gene *SSIIa* and *SSIIIa*, were selected as test materials. The *SSIIa* and *SSIIIa* genotypes were identified by molecular markers in semi-waxy lines with *Wx-mq* alleles. The genetic effect of *SSIIa* and *SSIIIa* on amylose content, gel consistency, gelatinization temperature and RVA profiles characteristics were explored in the same *Wx-mq* gene background to provide the theoretical basis for the breeding of new varieties with excellent eating quality.

The results show that in addition to the control of *Wx*, the cooking and eating quality of rice was regulated by other genes related to starch synthesis. Correlation analysis of Tian *et al.* (2009) shows that *SSII-3* had an effect on AC, GC and GT, besides, *SSI*, *SSIII-2*, *AGPase-L* and *PUL* affect AC as well. *SSII-1*, *SIII-1*, *SSIV-1*, *Sbe3* and *PUL* had an effect on GT. He *et al.* (2006) used DH population of "NJ11/Balilla" and found that in addition to *Wx*, the effect of *SSIIa*, *SssI* and *PUL* genes on AC, GC and GT was detected, and more gene interaction effects were detected.

We did research on the genotypic classification of 64 semi-glutinous lines from Wujing13/Kanto 194 and found that *SSIIa* and *SSIIIa* had an effect on the characteristic values of AC, GT, GC and RVA profiles characteristics. The *SSIIa* and *SSIIIa* gene from Wujing13, a non-waxy parent, had a tendency to increase AC, which increased by 1.87 and 1.23 percent, respectively, and the results were similar in two years. There was no significant difference in gelatinization temperature between *SSIIa* and *SSIIIa* genotypes of different parents, but the gelatinization temperature of genotype *SSIIa^{-mq}SSIIIa^{-mq}* was significantly higher than the figure for *SSIIa^{-b}SSIIIa^{-b}*, which indicates that gelatinization temperature was not significantly affected by single *SSIIa* and *SSIIIa* genes, but the genotype combination of these two genes had a significant effect on the gelatinization temperature. There were significant differences in gel consistency among different genotypes. *SSIIa* gene derived from Wujing 13 and *SSIIIa* gene

from Kanto 194 increased gel consistency by 8.74 mm and 9.62 mm, respectively. According to the combined effect of these two genes, the gel consistency of genotype $SSIIa^{-b}SSIIIa^{-mq}$ was the highest, and the difference with genotype $SSIIa^{-mq}SSIIIa^{-b}$ and $SSIIa^{-b}SSIIIa^{-b}$ was significant. RVA profiles characteristics, especially breakdown viscosity, consistency value and setback viscosity (Bason *et al.*, 2007), can reflect the difference of cooking and eating quality among different rice varieties, and also can be used as supplementary means to evaluate rice eating quality (Su *et al.*, 2014; Chen *et al.*, 2015). Wu *et al.* (2006) indicated that the soluble starch synthase gene $SSIIa$ gene has an important influence on RVA profiles characteristics in glutinous rice (*Oryza sativa L.*). In this study, there were significant or extremely significant differences among the $SSIIa$ genotypes of different parents, but there was no significant difference among the $SSIIIa$ genotypes of different parents. The results show that the effect of gene $SSIIa$ on RVA profile was greater than that of gene $SSIIIa$. Sui *et al.* (2005) considered that rice which has lower hot paste viscosity, cool paste viscosity, setback viscosity, consistence, shorter peak time, lower pulping temperature and higher breakdown viscosity may have better softness, stickiness, cold rice texture and higher comprehensive score. Shu *et al.* (2001) considered that the peak viscosity, breakdown viscosity, setback viscosity and consistency value in RVA profiles characteristics could reflect the eating quality. Both *indica* and *japonica* rice varieties with relatively high peak viscosity and breakdown viscosity, relatively low consistence and setback viscosity have relatively good eating quality. In this study, breakdown viscosity of all semi-glutinous lines was almost higher than the figure for Wujing13, the peak viscosity, hot paste viscosity, cool paste viscosity, consistency value and setback viscosity were lower than the data of Wujing13, while semi-waxy lines have better softness, elasticity and dispersion, which is consistent with previous studies.

There are many enzymes involved in starch synthesis, including granule-bound starch synthase ($GBSS$), soluble starch synthase (SSS), branching enzyme (SBE), debranching enzyme (DBE), and there are more than 20 genes related to the process (Lu *et al.*, 2008; Pandey *et al.*, 2012; Abe *et al.*, 2014). Therefore, starch synthesis is completed by a series of synergistic effects of starch synthase. There are interaction effects among different genes, and the effect between

them is complicated and varied. Changes in a trait are mostly the result of the chain reaction of several biochemical processes controlled by several genes, resulting in complex regulatory networks between genes (Zhang *et al.*, 2011). In this study, the effect of two starch synthase genes on amylose content in rice were analyzed, and the effect of other starch synthase genes on the cooking quality of rice need to be researched in the future.

1.4.2 Interaction effects between *SSIIa* and *SSIIIa*

Various properties of organism are regulated by a series of genes to form a complicated gene regulation network. Interaction certainly exists among different genes. The biological synthesis approach of rice starchy organism has been very clear, the common substrate ADP glucose of amylose and amylopectin are separately synthesized under catalysis of different enzymes, and competitive relation exists between two synthesis approaches (Asai *et al.*, 2014). Amylose is formed under the catalysis of particles combined with starch synthase, and the amylopectin is synthesized under the cooperation of soluble starch synthetase (SSS), branching enzyme (SBE) and starch debranching enzyme (DBE). There are more than 20 genes participating in the coding of these enzymes, including 10 genes of coding starch synthetase: *GBSSI* (*Wx*), *GBSSII*, *SSI*, *SSIIa*, *SSIIb*, *SSIIc*, *SSIIIa*, *SSIIIb*, *SSIVa* and *SSIVb*; 6 genes of coding branching enzyme (SBE) and starch debranching enzyme (DBE): *SBE1*, *SBE3*, *SBE4*, *ISA1*, *ISA2* and *PUL* (Hannah and James, 2008; Zhou *et al.*, 2013). He *et al.* (2006) has ever detected interaction effect among more starch synthetase genes in the DH population of “NJ11/Balilla”. *SSIIa* and *SSIIIa* starch synthetase genes are involved in the research. From single gene effect, it is seen no matter for *SSIIa* locus or *SSIIIa* locus, the gene of non-semi-glutinous parent Wujing13 is higher than that of Kanto194 for AC. For average two-year gene effect, *SSIIa* and *SSIIIa* account for 1.87% and 1.23% respectively. However, in the *SSIIa*^{-mq} gene background, average gene effect of *SSIIIa* is only 0.03%, while in the *SSIIa*^{-b} gene context, average gene effect of *SSIIIa* is up to 2.42%. Similarly, in the *SSIIIa*^{-mq} gene context, average gene effect of *SSIIa* is only 0.67%; while in the *SSIIIa*^{-b} gene context, the average gene effect of *SSIIa* is up to 3.07% (Table 5.5). It indicates that interaction effect exists between *SSIIa* and *SSIIIa*^{-b} genes (Fig 5.6).

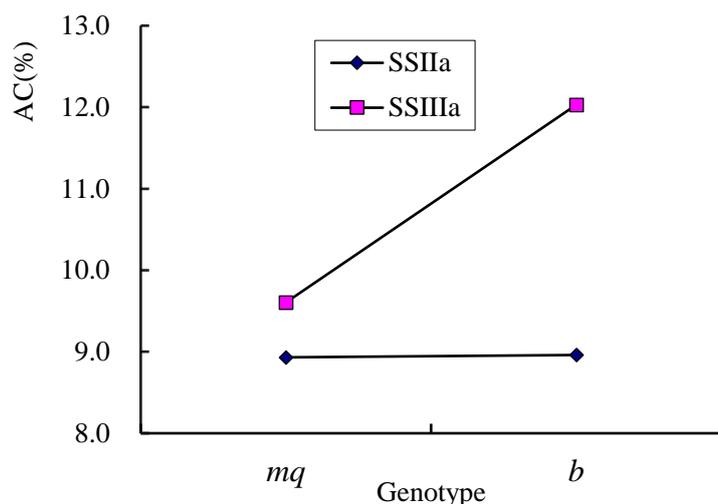


Fig 5.6 Interaction of different genotypes of *SSIIa* and *SSIIIa* locus

Similarly, it is calculated and obtained that the genetic effects of *SSIIa* and *SSIIIa* for GT, GC, peak viscosity, hot paste viscosity, cold paste viscosity, breakdown value, consistence value and setback value in the context of different parent genes are not totally identical (Table 5.8). Except the differences in genetic effect of *SSIIa* and *SSIIIa* for GT in the context of different parent genes are insignificant, the differences in other properties all reach the significant level, indicating interaction effect exists for these properties between *SSIIa* gene and *SSIIIa* gene.

Table 5.8 Genetic effect of *SSIIa* and *SSIIIa* in different background of parent

Parents	AC	GT	GC	PKV	HPV	CPV	BDV	CSV	SBV
The effect of <i>SSIIIa</i> under <i>SSIIa</i> background									
Kanto194	0.03	-0.61	-3.40	197.20	185.60	191.70	11.70	6.10	-5.60
Wujing13	2.42	-0.77	-16.95	-126.50	-173.40	-52.70	46.90	120.70	73.80
Average	1.23	-0.69	-10.18	35.35	6.10	69.50	29.30	63.40	34.10
The effect of <i>SSIIa</i> under <i>SSIIIa</i> background									
Kanto194	0.67	-0.57	7.24	559.00	322.20	290.20	236.80	-32.00	-268.80
Wujing13	3.07	-0.73	-6.31	235.30	-36.80	45.80	272.00	82.60	-189.40
Average	1.87	-0.65	0.47	397.15	142.70	168.00	254.40	25.30	-229.10

1.4.3 Application of the result in improving the eating quality of Japonica Rice

The semi-glutinous *japonica* rice shows milky white and cloud-like opaque phenotype because of its low amylose content, which has an adverse impact on the appearance quality of rice. Therefore, the selection of semi-glutinous genes is of great significance for the cultivation of Japonica rice with good eating quality, because the amylose content of diverse semi-glutinous genes is different (Ni *et al.*, 2011; Caffagni, *et al.*, 2013). Our breeding experience shows that the rice with semi-glutinous gene has better taste quality, but the amylose content of semi-glutinous *japonica* rice is not as low as it can be. The low amylose content (below 8%) may lead to opaque rice grain, bad appearance quality, soft eating quality, and the lack of elasticity. With the increase of amylose content, both the eating quality and the appearance quality is improved. Steamed Rice was crystal (Figure 5.7), elastic, cold but not hard, eating quality and appearance quality are excellent when the amylose content was more than 12%, which satisfies people's eating requirement in the Yangtze River Delta region.



Fig 5.7 the appearance of semi-glutinous rice with different AC

The amylose content of EMS mutant *Wx-mq* derived from *Japonica* Koshihikari is around 9%, but the amylose content of amyloidosis was significantly different among different semi-waxy strains derived from the same combination with *Wx-mq* gene, which indicates that there are other genes affect the synthesis of amylose except for the *Wx* site. This study found that the *SSIIa* and *SSIIIa* alleles of Wujing13 can increase amylose content by 1.87 and 1.23 percent, respectively, under the background of semi-waxy gene *Wx-mq*. Meanwhile the two genes can increase amylose content by 3.1 percent, reaching about 12%. These kind of semi-glutinous lines not only have good eating quality, but also have a bright appearance. Therefore, the

selection of different allelic genotypes of *SSIIa* and *SSIIIa* could make semi-glutinous *japonica* rice both delicious and good-looking.

Of course, there are many genes that affect amylose synthesis. We can provide the theoretical basis for the breeding of ideal semi-glutinous *japonica* rice with more amylose content by researching the genetic effect of these genes.

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CHAPTER SIX

DISCUSSION AND CONCLUSION

Discussion and Conclusion

1 Discussion

Rice is the main food crop of humanity and the staple food for more than 50% of the population in the world. China is a major producer of rice in the world (Khush, 2013; Ghosh *et al.*, 2014). With the great support of the Chinese government and the joint efforts of several generations of rice researchers in China for more than half a century, the problem of food and clothing for a quarter of the human population has been basically solved. After entering the 21st century, people have changed their attention from “eating full” to “eating well”. The goal of rice breeders has also shifted from high yield to good eating quality (Bhattacharya, 2013). Rice quality is divided into appearance quality, processing quality, cooking quality and nutritional quality. Cooking and eating quality is the most important index to evaluate rice quality (Kihupi *et al.*, 2014). Since 1999, the Cailin Wang team of Food Crops Research Institute of Jiangsu Academy of Agricultural Sciences has focused on the improvement of rice eating quality. The gene of low amylose content was used to improve the quality of *japonica* rice in Jiangsu province. The rice varieties “Nanjing 46”, “Nanjing 5055” and “Nanjing 9108” were cultivated in Nanjing rice series. The rice quality meets the taste demand of the residents in the middle and lower reaches of the Yangtze River. The brand rice made from Nanjing series is popular in the southern China rice market and favored by consumers, and has won “Golden Prize” of high-eating quality rice comparison in China and Jiangsu Province more than 20 times. At the same time, Nanjing series varieties with high yield and good comprehensive resistance have become the dominant varieties of rice supply-side structural adjustment in recent years in Jiangsu, Shanghai, Zhejiang, Anhui, Shandong, Henan and other regions.

Starch is the main component of rice, accounting for more than 90%. Therefore, the composition and characteristics of starch are directly related to the rice eating quality (Jin *et al.*, 2005). Rice starch consists of amylose and amylopectin. Amylose is catalyzed by granule bound starch synthase (*GBSS*) encoded by *waxy* gene (*Waxy*, *Wx*). Amylopectin was synthesized by soluble starch synthase (*SSS*), starch branching enzyme (*SBE*) and starch debranching enzyme

(DBE). ADP-glucose was used as substrate to catalyze the synthesis of amylopectin (Jeon *et al.*, 2010). ADP-glucose pyrophosphorylase (adenosine diphosphate glucose pyrophosphorylase AGPase) is responsible for the synthesis of ADP adenosine diphosphate glucose, which is a rate-limiting enzyme in starch synthesis pathway. There are more than 20 genes involved in encoding these enzymes, collectively known as starch synthesis-related genes (SSRG), including AGPL1-4 and AGPS1-2, which encode large subunits of AGPase enzyme, and other 7 genes. There are 10 genes encoding starch synthase *GBSSI (Wx)*, *GBSSII*, *SSI*, *SSIIa*, *SSIIb*, *SSIIc*, *SSIIIa*, *SSIIIb*, *SSIVa* and *SSIVb*, *SBEI*, *SBEIIa*, *SBEIIb*, *ISA1*, *ISA2*, *PUL* and other 6 genes encoding starch branching enzyme and debranching enzyme (Hannah, *et al.*, 2008; Nakamura, 2002; James, *et al.*, 2008). Among them *Wx*, *SSI*, *SSIIa*, *SSIIIa*, *SBEI*, *SBEIIb* and *PUL* are the most important genes for ECQ regulation. Each enzyme has multiple isoforms. Therefore, it is of great significance to identify the genetic effects of the genes encoding these enzymes and their relationship with the eating quality, which is of great significance for the breeding of new *japonica* rice varieties with good eating quality.

1.1 Influence of genetic background on genetic effects of *SS IIa* and *SS IIIa* genes

Rice starch is the result of synergistic reaction of many biochemical processes controlled by multiple genes, and a complex regulatory network is formed between genes. Therefore, the role of any gene will be affected by other genes. The genetic effects of different genotypes at *Wx*, *SBEI* and *SBEI* loci in 53 *indica-japonica* rice varieties were studied with the method of molecular markers by Yan *et al.* (2005). The results showed that there were significant or extremely significant differences in the physicochemical properties of starch among different genotypes, AC, GC, RVA, and the combined effects of the three genes were also significantly different among different genotypes. The genetic effects of *SS IIa*, *SSIIIa* and *PUL* genes were studied with the method of Near-isogenic lines. The results show that the effect of *PUL* gene on RVA profile was the most significant in the background of glutinous rice. Several characteristic values, including peak viscosity, hot paste viscosity, cool paste viscosity, setback

viscosity, sizing temperature and peak time, were all affected by *PUL* gene. Xu *et al.* (2015) research shows that under the same background of main genes (*Wx^b* and *SSIIa*), amylose content in rice is significantly affected by *SSIII-1* and *SBE3 × SSIIIa*, which are involved in amylose content. *PUL × SSIIIa* significantly affected CSV, PeT and PaT; *SSI* and *SSIIIa × SSI × PUL* have great influence on the GC, PKV, HPV, BDV, CPV, CSV and PaT of rice.

The genotypic classification of 64 semi-glutinous lines derived from Wujing13/Kanto194 was studied. The results show that the *SSIIa* and *SSIIIa* genes of Wujing13, a non-waxy parent, increased AC by 1.87 and 1.23 percentage point. The single *SSIIa* and *SSIIIa* gene had no significant influence on the gelatinization temperature, but the genotype combination of these two genes had a significant influence on the gelatinization temperature. The gelatinization temperature of the genotype *SSIIa^{-mq}SSIIIa^{-mq}* was 1.34 °C higher than the figure for *SSIIa^{-b}SSIIIa^{-b}*. In terms of gel consistency, *SSIIa* gene (*SSIIa^{-b}*) from Wujing13 and *SSIIIa* gene (*SSIIIa^{-mq}*) from Kanto194 could increase gel consistency by 8.74 mm and 9.62 mm, respectively. The gel consistency of genotype *SSIIa^{-b}SSIIIa^{-mq}* was higher than the data of genotype *SSIIa^{-mq}SSIIIa^{-b}* and *SSIIa^{-b}SSIIIa^{-b}* respectively. The *SSIIa* gene (*SSIIa^{-b}*) derived from Wujing13, a non-waxy parent, increased peak viscosity, hot paste viscosity, cool paste viscosity and breakdown viscosity, but decreased the consistence and setback viscosity. The effect of *SSIIIa* gene (*SSIIIa^{-b}*) derived from Wujing 13 was opposite, and the peak viscosity, hot paste viscosity, cool paste viscosity and breakdown viscosity were decreased, consistence and setback viscosity increased. The combined effects of these two genes, the peak viscosity, hot paste viscosity, cool paste viscosity, breakdown viscosity of the four traits of *SSIIa^{-b}SSIIIa^{-mq}* genotype is the largest, consistence and setback viscosity of *SSIIa^{-mq}SSIIIa^{-mq}* genotype is the largest. These results suggest that the genetic background has an effect on the genetic effects of the genes associated with starch synthesis.

1.2 Breeding of varieties with different amylose content under the background of *Wx-mq*

The concept of breeding by molecular design was first proposed by Dutch scientists Peleman and van der Voort in 2003, who also registered the trademark “Breeding by design”. Design breeding refers to the use of bioinformatics as a platform and a database of genomics and proteomics to integrate useful information from crop genetics, physiology, biochemistry and biostatistics in crop breeding processes (Dai *et al.*, 2015; Qian *et al.*, 2016). According to the specific crop breeding objectives and growth environment, the best scheme is designed first, and then the molecular breeding method of crop breeding experiment will be carried out. The key of molecular design breeding is to simulate, screen and optimize many factors in the breeding process through the integration of many techniques. In order to improve the foresight and breeding efficiency in crop breeding, the best genotypes, parent selection and progeny selection strategies for achieving target genotypes will be put forward. The transformation from the traditional “breeding according to experience” to directional and efficient “accurate breeding” would be realized. Molecular design breeding consists of the following three steps: (1) Studying genes controlling the target traits and their interrelationships, including the construction of genetic populations, the screening of polymorphic markers, the construction of genetic linkage maps, phenotypic identification of quantitative traits and genetic analysis; (2) Designing target genotypes according to breeding objectives under different ecological conditions. This step uses genetic information for a variety of important breeding traits that have been identified, including the location of genes on chromosomes, genetic effects, biochemical networks and expression pathways from genes to traits, interactions between genes, interaction between genes, genetic background and environment, etc., to simulate and predict phenotypes of possible genotypes and select right genotypes that meet specific breeding objectives; (3) Establishment of breeding program (Wang *et al.*, 2011).

The results showed that under the background of *Wx-mq* gene, amylose content was controlled by *SSIIa* and *SSIIIa* genes, which derived from Wujing13, a non-waxy parent and increased AC

by 1.87 and 1.23 percentage point respectively. Amylose content was closely related to eating quality and appearance quality. People in the middle and lower reaches of the Yangtze River in China prefer soft and waxy semi-glutinous rice, so it is better to have low amylose content. However, amylose content should not be too low. If amylose content is below 8%, rice is too soft and inelastic, and its appearance becomes milky, white and opaque. Our study showed that the semi-glutinous *japonica* rice with amylose content in the range of 10%-14% has not only the best eating quality, but also the good appearance quality. In order to obtain semi-glutinous *japonica* rice varieties suitable for amylose content, the best genotypes could be obtained through the polymerization breeding of different starch synthase related genes, which is the molecular design breeding of eating quality. First of all, we should use the molecular markers of related genes of starch synthesis to detect the alleles of parental materials; the second, select the parent material with different allele to carry on the hybridization; finally, screen out the ideal genotype which accords with the breeding target by the molecular marker-assisted selection of the separation generation.

We have obtained the ideal result through the molecular design breeding of starch-synthesizing genes. A new generation of fresh and semi-glutinous fine edible *japonica* rice varieties with excellent eating quality and good appearance were obtained. This rice variety is participating in the regional experiment of new rice variety in Jiangsu province, which is expected to be applied in production in recent years.

1.3 Cultivation techniques of superior *japonica* rice with good Eating quality and high quality

Because of the semi-glutinous rice with low amylose content is soft and glossy, and its chewing is elastic, and the eating quality is good. However, the rice is milky white, translucent, the dried kernel is close to white glutinous rice when amylose content is lower than 8%. The results showed that the characteristics of semi-glutinous *japonica* rice were mainly controlled by genetic genes, but the environmental conditions have influence as well. In order to keep the good eating quality of semi-glutinous rice and the transparency of rice grain, the amylose

content should be in the range of 10%-14%. Therefore, in order to maintain the good eating characteristics of semi-glutinous *japonica* rice and the transparency of rice grains, in addition to the excellent genes that control starch synthesis in breeding strategy, the amylose content should be raised and protein level should be lower in cultivation strategy. Firstly, sowing at the right stage to enrich the grain filling at the appropriate temperature 6-15 days after heading. Secondly, using organic fertilizer as much as possible and less chemical nitrogen fertilizer, especially to avoid the use of nitrogen fertilizer in the later stage to prevent the loss of amylose content caused by excessive amount of nitrogen fertilizer and the decrease of eating quality caused by the increase of protein content. The third is timely harvest, the best harvest time is when 95% of the grains are ripe and the water content of rice is around 25%.

2 Conclusion

The analysis of AC of 124 semi-glutinous *japonica* rice varieties (lines) bred by our team shows that there was a great difference in AC between the semi-glutinous lines containing the same *Wx-mq* gene, even though the AC of the semi-glutinous strain from the same hybrid combination varied by more than 5 percent. In order to analyze the cause of this difference, 64 semi-glutinous lines with starch synthase gene *SSIIa* and *SSIIIa* polymorphism derived from Wujing 13 and Kanto 194 were chosen as materials, which were analyzed for genetic effects of *SSIIa* and *SSIIIa* on the amylose content, gelatinization temperature, gel consistency and RVA profile characteristics with method of *SSIIa* and *SSIIIa* genotypes by molecular markers on waxy lines in the same genetic background. Meanwhile, through the treatment of high nitrogen (450 kg/hm²), middle nitrogen (300 kg/hm²), low nitrogen (150 kg/hm²) and no nitrogen (control), 4 nitrogen levels, sowing and short day treatment, the effect of nitrogen fertilizer and sowing date on the AC of semi-glutinous *japonica* rice were studied.

The results showed that *SSIIa* and *SSIIIa* had significant effect on AC, GT, GC and RVA profile characteristics, and there was interaction between these two genes. The *SSIIa* and *SSIIIa* genes from non-waxy parents Wujing 13 tended to increase AC by 1.87 and 1.23 percentage point respectively. There were no significant differences between the gelatinization temperature of

SSIIa and *SSIIIa* genotypes from different parents. The gelatinization temperature of genotype *SSIIa^{-mq}SSIIIa^{-mq}* was significantly higher than that of *SSIIa^{-b}SSIIIa^{-b}*, which shows that single *SSIIa* and *SSIIIa* gene had no significant effect on gelatinization temperature, but the two genes combination had significant effect on gelatinization temperature, gelatinization temperature of *SSIIa^{-mq}SSIIIa^{-mq}* was 1.34 °C higher than the data of *SSIIa^{-b}SSIIIa^{-b}*. There was a significant difference in gel consistency between different genotypes. The *SSIIa* gene (*SSIIa^{-b}*) from Wujing13 and *SSIIIa* gene (*SSIIIa^{-mq}*) from Kanto194 could increase the gel consistency by 8.74 mm and 9.62 mm respectively. From the joint effect of two genes, the gel consistency of genotype *SSIIa^{-b}SSIIIa^{-mq}* increased by 10.64 mm and 16.95 mm compared with the figure for genotype *SSIIa^{-mq}SSIIIa^{-b}* and *SSIIa^{-b}SSIIIa^{-b}*. The *SSIIa* gene (*SSIIa^{-b}*) from non-waxy parent Wujing 13 increased the peak viscosity, hot paste viscosity, cool viscosity and breakdown viscosity, and decreased consistence and setback viscosity. The effect of *SSIIIa* gene (*SSIIIa^{-b}*) derived from Wujing13 is opposite, which can reduce the peak viscosity, hot paste viscosity, cool viscosity and breakdown viscosity, and increase consistence and setback viscosity. The associative effect of these two genes: the peak viscosity, hot paste viscosity, cool viscosity were the largest in *SSIIa^{-b}SSIIIa^{-mq}* genotype, and consistency value and setback viscosity were the largest in *SSIIa^{-b}SSIIIa^{-b}* genotype, and the *SSIIa^{-b}SSIIIa^{-mq}* genotype was the smallest in terms of setback viscosity. According to the performance of all characters, *SSIIa^{-b}SSIIIa^{-mq}* genotype had the best eating quality among these 4 genotypes of *SSIIa* and *SSIIIa* loci, the AC was 11.54%, the GT was low, the gel consistency, the peak viscosity, the hot paste viscosity, the cool viscosity and breakdown viscosity were the largest, and the setback viscosity was the lowest.

The result of different nitrogen levels, sowing dates and short day treatments shows that the amount of nitrogen fertilizer had a significant effect on the AC of semi-glutinous *japonica* rice. With the increase of nitrogen fertilizer, AC shows a decreasing trend, and the AC was the lowest in high nitrogen treatment in two years. Sowing time also had a significant effect on the AC of good eating quality *japonica* rice. With the delay of sowing date, AC descended. This effect was mainly related to the temperature of 6-15 d after heading, and higher temperature could

increase amylose content. The result of short daytime treatment confirms it as well. The results of this study have a guiding significance for the optimal cultivation of semi-glutinous *japonica* rice. The good eating quality and appearance quality of semi-glutinous *japonica* rice could be improved by proper seeding, adding organic manure, reducing chemical nitrogen fertilizer and timely harvesting.

3 Innovative points

Rice (*Oryza sativa* L.) is cultivated in a wide range of climatic conditions, and is one of mankind's major staple foods. The interaction of environmental factors with genotype effects major agronomic traits such as yield, quality, and resistance in rice. However, studies on the environmental factors affecting agronomic traits are often difficult to conduct because most environmental factors are dynamic and constantly changing.

1. In this study, it is the first time to explore the reasons for the difference in the eating quality of semi-glutinous good eating quality *japonica* rice were discussed from two aspects, namely heredity and environment. It clarifies the genetic effect of *SSIIa* and *SSIIIa* on AC, GT, GC and RVA profile characteristics and interaction effects under the background of *Wx-mq* gene, identifies the best eating quality of *SSIIa^bSSIIIa^{-mq}* genotype, and provides a theoretical basis for the molecular design breeding semi-glutinous type *japonica* rice with good eating quality.
2. It makes sure that the excessive amount of nitrogen fertilizer and the high temperature of 6-15 d after heading could descend AC, the appearance and the eating quality of semi-glutinous *japonica* rice and increase PC, which provides a theoretical basis for the cultivation of good eating semi-glutinous *japonica* rice.

This study elucidated the effect of *SSIIa* and *SSIIIa* genes on rice cooking and eating quality under the background of *Wx-mq* gene. The genetic effect of other genes related to starch synthesis needs further research. In breeding, the best genotype combination with the best ECQs in the same genetic background should be selected.

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