

REVIEW

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# Effects of soluble starch synthase genes on eating and cooking quality in semi waxy japonica rice with $Wx^{mp}$

Shu Yao<sup>1,2</sup>, Yadong Zhang<sup>1</sup>, Yanqing Liu<sup>1</sup>, Chunfang Zhao<sup>1</sup>, Lihui Zhou<sup>1</sup>, Tao Chen<sup>1</sup>, Qing Yong Zhao<sup>1</sup>, Balakrishna Pillay<sup>2\*</sup> and Cailin Wang<sup>1\*</sup>

## Abstract

The purpose of this study is to reveal the genetic mechanism of the variation of amylose content among different semi waxy or glutinous japonica rice in the background of  $Wx^{mp}$  gene. Sixty-four semi waxy lines derived from the hybrid progenies of Wujing 13 and Milky Princess (Kantou 194) with polymorphism in soluble starch synthase gene  $SSIIa$  ( $SSII-3$ ) and  $SSIIIa$  ( $SSIII-2$ ) but no polymorphism in other starch synthase related genes were used as test materials. The genotypes of  $SSIIa$  and  $SSIIIa$  allele were identified by molecular markers, and the allelic effects of  $SSIIa$  and  $SSIIIa$  gene on amylose content (AC), gel consistency (GC), gelatinization temperature (GT) and rapid visco analyzer (RVA) profile characteristics were analyzed. The significant effects of  $SSIIa$  and  $SSIIIa$  alleles and the interactive effects between two genes on AC, GT, GC and RVA profile characteristics were found. The  $SSIIa$  and  $SSIIIa$  alleles from Wujing13 shown positive effects on AC with an average increase of 1.87 and 1.23% in 2 years respectively. There was no significant effect on GT for  $SSIIa$  or  $SSIIIa$  allele but remarkable influence on GT when the co-existence of the two genes. The genotype  $SSIIa^{mp}SSIIIa^{mp}$  shown 1.34 °C higher GT than genotype  $SSIIa^{wj}SSIIIa^{wj}$  ( $mp$  and  $wj$  indicated that the gene was derived from Milky Princess and Wujing 13 respectively, the same as in the below). Different genes and alleles resulted in significant different GC. The genetic effect of  $SSIIa^{wj}$  and  $SSIIIa^{mp}$  on GC was 8.74 and 9.62 mm respectively. The GC of  $SSIIa^{wj}SSIIIa^{mp}$  was 10.64 and 16.95 mm higher than that of  $SSIIa^{mp}SSIIIa^{wj}$  and  $SSIIa^{wj}SSIIIa^{wj}$ , respectively. The allele  $SSIIa^{wj}$  could increase the peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV) and breakdown viscosity (BDV), while decrease the consistency viscosity (CSV) and setback viscosity (SBV). However for the allele  $SSIIIa^{wj}$  the opposite was true. The genotype  $SSIIa^{wj}SSIIIa^{mp}$  had the largest PKV, HPV and CPV, the genotype  $SSIIa^{wj}SSIIIa^{wj}$  had the largest BDV and CSV, but the genotype  $SSIIa^{wj}SSIIIa^{mp}$  had the least SBV. According to the comprehensive effect of each trait, the genotype  $SSIIa^{wj}SSIIIa^{mp}$  was the best. The allelic variation and interaction effect of  $SSIIa$  and  $SSIIIa$  genes have important reference value for improving cooking and eating quality of semi waxy japonica rice.

**Keywords:** Soluble starch synthase genes, Amylose content, Gel consistency, Gelatinization temperature, RVA profiles characteristics, Genetic effects

\* Correspondence: pillayb1@ukzn.ac.za; cailin\_wang@163.com

<sup>2</sup>Discipline of Microbiology, School of Life Science, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Durban 4000, South Africa

<sup>1</sup>Institute of Food Crops, Jiangsu Academy of Agricultural Sciences/Jiangsu High Quality Rice Research and Development Center/Nanjing Branch of China National Center for Rice Improvement, Nanjing 210014, China



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## Introduction

Rice is one of the three most important food crops in the world. More than half of the world's population take rice as a source of daily diet carbon intake. The good quality of rice is the major objective for breeding researchers and consumers (Wang et al. 2012b). The rice quality is usually divided into four aspects: appearance quality, milling quality, eating and cooking quality and nutritional quality. Eating and cooking quality (ECQ) is the most important rice quality trait that has attracted much research efforts. The amylose content (AC), gelatinization temperature (GT), gel consistency (GC) and rapid visco analyzer (RVA) profile characteristics are the most important determining traits for ECQs, and all the traits are related to each other closely (Jin et al. 2001; Sui et al. 2005). Starch synthesis is controlled by multiple starch synthases, each starch synthase is encoded by a corresponding gene (Hannah and James 2008). Therefore, it is of great significance to study the influence of genes related to rice starch synthesis on rice quality and its inheritance, and it is also the basis for further research on rice quality improvement.

The starch content in the milled rice accounts for 90 % (Vandeputte and Delcour 2004). A large number of studies have verified that the AC is one of the main factors that determine the ECQs of rice. The rice with high AC is usually expansive, loose and of pale color, and it is stiff after cooled and has poor palatability, while the rice with low AC is less expansive, fluffy and soft, which is popular among consumers (Huang et al. 2003; Sato et al. 1996; Zhao et al. 2019).

The *Wx* gene is the main determining factor of AC; it plays a decisive role in the ECQs. *Wx<sup>mp</sup>* is the gene that has low AC and is allelic with the *Wx* gene. Japanese breeders have produced a series of rice varieties with good eating quality and low AC using this gene, such as Milky Princess (Kantou 194). Semi waxy rice is a milky white and cloudy variety whose AC is located between round-grained non-glutinous and glutinous *japonica* rice (Sato et al. 2001; Tomita et al. 2007). In order to improve the eating quality of *japonica* rice in Jiangsu Province, our research team developed Nanjing 46, Nanjing 9108, Nanjing 5055, Nanjing 2728, Nanjing 3908 and other good rice varieties with low AC, good disease resistance and high yield by using Kantou 194 (Wang et al. 2009, 2012a, 2013; Cai et al. 2019; Chen et al. 2019).

It has been shown that under the background of the same major gene *Wx<sup>mp</sup>*, the AC of different semi waxy rice varieties (lines) is quite different (Yu et al. 2012). This is mainly due to the polymorphism of genes related to starch synthesis carried by parents, in addition to environmental factors such as the amount of nitrogen fertilizer used in the later stage of rice growth and the temperature during the filling stage (Yao et al. 2016). A

large number of studies have shown that the ECQ of rice is controlled by other genes related to starch synthesis besides *Wx*. So far, more than 20 genes related to starch synthesis have been reported, including *AGPlar*, *AGPis*, *AGP<sub>sma</sub>* 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*, *SSIIId*, *SSIIe*, *SSIIIf*, *SSIIg* and genes encoding starch branching enzymes *SBE1*, *SBE3*, *SBE4* and genes encoding deb-branching enzymes *ISA*, *PUL* (Hannah and James 2008; Nakamura 2002; James et al. 2003). Among them, starch synthase gene *SSIIa* is the main gene controlling rice GT, Umemoto et al. (2002) located *SSIIa* gene at the *ALK* site of the short arm of chromosome 6 of rice. The *SSIIId* gene is located on chromosome 8 and consists of 14 exons and 13 introns (Crofts et al. 2012). The deletion or mutation of *SSIIId* gene will lead to the change of starch-related traits in grain.

There are many reports on the genetic effects of the starch synthase genes, the results are different due to different genetic background of *Wx* gene used in the experiments. However, there are few studies on the effects of starch synthase genes on the ECQs under the same major gene background of *Wx* gene. In order to clarify the genetic mechanism of the variation of AC among different semi waxy *japonica* rice with same *Wx<sup>mp</sup>* gene, semi waxy lines derived from Wujing13 and Milky Princess, which show polymorphisms in starch synthase gene *SSIIa* and *SSIIId*, were selected as test materials. The *SSIIa* and *SSIIId* genotypes were identified by molecular markers in semi waxy lines with *Wx<sup>mp</sup>* alleles. The genetic effect of *SSIIa* and *SSIIId* on AC, GC, GT and RVA profiles characteristics were explored in the same *Wx<sup>mp</sup>* gene background to provide the theoretical basis for the breeding of new varieties with excellent eating quality.

## Materials and methods

### Test materials

In order to illuminate the effect of *SSIIa* and *SSIIId* genes related to starch synthesis on ECQ in the background of *Wx<sup>mp</sup>* gene, semi waxy lines with different alleles of *SSIIa* and *SSIIId* genes were developed by crossing semi-waxy rice lines Milky Princess, which contain *Wx<sup>mp</sup>* gene, with *japonica* rice variety Wujing 13, which contain *Wx<sup>b</sup>* gene. Milky Princess and Wujing 13 have different alleles of *SSIIa* and *SSIIId* genes and same alleles of other starch synthesis genes.

In 1999, Wujing 13, a *japonica* rice cultivar bred by Wujin Rice Research Institute of Jiangsu Province, was used as female parent, and Milky Princess, a semi waxy rice variety introduced from Japan, was used as male parent. Sixty-four stable lines obtained by multiple

generations of inbreeding were used as experimental materials (Fig. 1).

#### Planting and design of test materials

In 2013 and 2014, the experimental materials were planted in the Nanjing experimental field of the Institute of food crops, Jiangsu Academy of Agricultural Sciences. Each line was planted as a plot, with 4 rows and 10 plants in each row. The row and plant spacing were 27 and 17 cm respectively. The test materials are arranged in order and repeated 3 times. The experimental materials were sowed on May 10 and transplanted on June 10. Field management was the same as field production. The young leaves at tillering stage were sampled for DNA extraction, and the seeds were harvested after ripening for the determination of physicochemical indicators and rapid visco analyzer (RVA) profile characteristics.

#### Genotypic detection

##### Extraction of DNA from leaves

At the peak of tillering stage, the fresh young leaves of 5 individual plants were taken continuously from the second plant in the middle of each plot, and DNA (Murray and Thompson 1980) was extracted by CTAB method.

##### 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 (Cai et al. 2002; Yan et al. 2005; Wang 2006). 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 (Chen et al. 2013). The primers were synthesized by Shanghai Sangon Biotechnology Co., Ltd.

20  $\mu\text{L}$ , 10 x Buffer 1  $\mu\text{L}$ , dNTP 0.20  $\mu\text{L}$ , *Taq* DNA polymerase 0.50  $\mu\text{L}$ , upstream and downstream primers 1  $\mu\text{L}$ , template DNA 1.00  $\mu\text{L}$ .

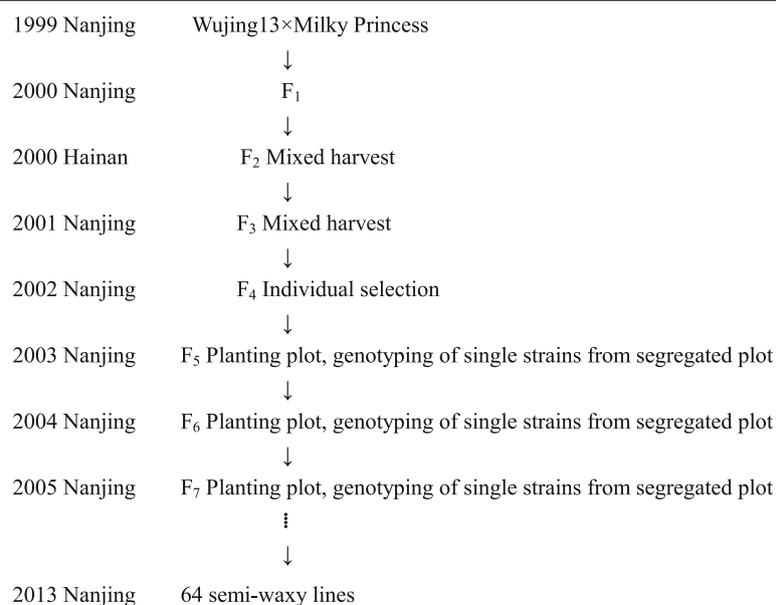
##### PCR amplification program

The PCR reaction was amplified on an Eppendorf-5330 PCR machine. Total volume 10  $\mu\text{L}$ , ddH<sub>2</sub>O 6.55  $\mu\text{L}$ , 10 x Buffer 1.00  $\mu\text{L}$ , dNTP 0.20  $\mu\text{L}$ , *Taq* DNA polymerase 0.50  $\mu\text{L}$ , upstream and downstream primers 1  $\mu\text{L}$ , template DNA 1  $\mu\text{L}$ .

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 (Dian et al. 2005; Jiang et al. 2004).

The four-primer PCR amplification program was pre-denatured at 94 °C for 5 min and denaturation at 94 °C for 30 s, renaturation at 65 °C for 30 s, extension at 72 °C for 1 min, in which repeated steps 2–4 for 30 times.

Agarose gel (1.5%) or polyacrylamide gel (9%) was selected according to the size of the PCR amplification products and the resolution of the gel.



**Fig. 1** Development of 64 semi-waxy lines derived from Wujing 13/Milky Princess

**Table 1** Molecular markers of *SSIIa*, *SSIIIa* and *Wx<sup>mp</sup>* genes used in this study

Genes	Primer sequences	Product size (bp)	Annealing temperature (°C)
<i>SSIIa</i> (L22)	F: CTTTGATAGTTCGAATGGTT	251/248	55
	R: CAATGTTTCTCCGTGATGAT		
<i>SSIIIa</i> (L25)	F: GAACTTGTGCCTTAAGCTGACTG	197/177	55
	R: GGAATAGTAAGCCGAAGGACTT		
<i>Wx<sup>mp</sup></i>	O-F: 5'-ATGTTGTGTTCTTGTGTTCTTTGCAGGC3'	439/200	65
	O-R: 5'-GTAGATCTTCTACCCGGTCTTTCCCAAA3'		
	I-F: 5'-GGGTGAGGTTTTTCCATTGCTACAATCG3'	439/292	65
	I-R: 5'-GTCGATGAACACACGGTCGACTCAAT3'		

### Determination of rice quality traits

#### Pretreatment of rice samples

The rice seeds were harvested from every single plant after full maturation, the rice flour was sifted through mesh after rough milling of rice to determine AC, GC and starch viscosity.

#### Determination of AC

The determination of AC followed the standard issued by the Ministry of Agriculture, NY147–88 (1988). The absorbance value and the standard curve of AC measured with four standard samples (1.5, 10.4, 16.2 and 26.5%) of known AC were made, and the AC of the tested samples was calculated according to this standard curve.

#### Determination of GT

The GT were determined by DSC (200-f3, Netzsch Germany) as described by Yan et al. (2010).

#### Determination of GC

The GC was measured according to Chinese national standard GB/T 22294–2008 (2009).

#### Determination of the RVA parameters

The 3-D viscosity rapid-measuring instrument made by Newport Scientific in Australia and the matching software TCW were used to measure the results according to the operating rules of the American Association of Cereal Chemists. The viscosity unit was expressed in cP. The viscosity parameters of starch have five first-order data, including the peak viscosity (PKV), the hot paste

viscosity (HPV), the cool paste viscosity (CPV), the initial temperature (PaT) and the peak time (Pet) and 3 s-order data, including the breakdown viscosity (BDV=PKV-HPV), the setback viscosity (SBV=CPV-PKV), the consistence viscosity (CSV=CPV-HPV).

For all the quality traits, each sample was tested three times. The average was taken as the phenotypic value of the traits.

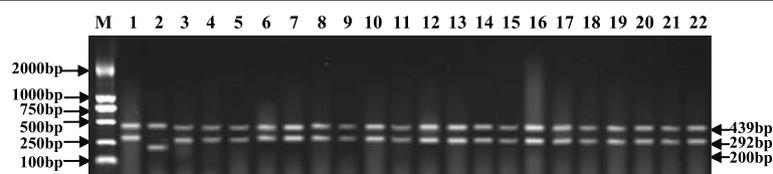
#### Statistical analysis

Variance analysis and difference significance test were conducted according to the method by Mo (1992). Multiple comparisons of the Duncan method were performed using the Statistical Product and Service Solutions (SPSS) software version 20.

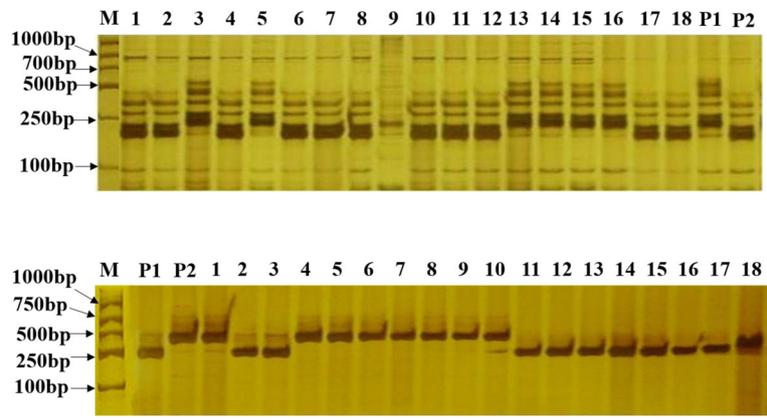
### Results

#### Detection of *Wx<sup>mp</sup>* genotypes

According to the principle of Mendelian inheritance, there are three genotypes of *Wx<sup>b</sup> Wx<sup>b</sup>*, *Wx<sup>mp</sup> Wx<sup>mp</sup>* and *Wx<sup>b</sup> Wx<sup>mp</sup>* in the segregation generation of *japonica* rice with *Wx<sup>b</sup>* gene and semi waxy *japonica* rice with *Wx<sup>mp</sup>* gene. Three genotypes could be detected simultaneously by using the PCR system of four primer blocked amplification mutation system (Chen et al. 2013). The results showed that the specific bands of 439 bp could be amplified from 64 semi waxy lines and two parents. In addition, 292 bp specific bands could be amplified from 64 semi waxy lines and Milky Princess, but 292 bp bands could not be amplified from Wujing13 without *Wx<sup>mp</sup>*, but a specific band of 200 bp could be amplified from



**Fig. 2** Detection of the *Wx<sup>mp</sup>* gene in 64 semi waxy lines



**Fig. 3** Detection of *SSIIa* and *SSIIIa* gene in 64 semi waxy lines

Wujing13 without  $Wx^{mp}$  (Fig. 2), which indicated that all of 64 samples contained  $Wx^{mp}$  gene.

**Detection of genotypes *SSIIa* and *SSIIIa***

The STS markers L22 and L25 designed by *SSIIa* and *SSIIIa* loci were used to detect 64 semi waxy lines (Fig. 3). The results showed that the lines of the gene *SSIIa<sup>mp</sup>* (*mp* indicate that the gene was derived from the semi-waxy parent Milky Princess) and *SSIIa<sup>wj</sup>* (*wj* indicate that the gene originated from non-waxy parent Wujing13) were 23 and 41, the lines of the gene *SSIIIa<sup>mp</sup>* and *SSIIIa<sup>wj</sup>* were 49 and 15, respectively. There were 10, 13, 39 and 2 lines of *SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup>*, *SSIIa<sup>mp</sup>SSIIa<sup>wj</sup>*, *SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup>* and *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>* genotypes of the two genes, respectively (Table 2).

**Variation of AC, GC and GT**

The AC, GT and GC of 64 semi-waxy lines were measured. The results showed that (Fig. 4) the AC ranged from 7.6 to 14.0%, among which 49 were distributed between 9 and 11%, accounting for 76.6%. The GT of 64 materials were distributed between 68 and 72 °C, mainly concentrated at 69 to 71 °C, but the variation is not large and the coefficient of variation is 1.56%. The GC of 64 materials were mainly between 50 and 102 mm, the average is 79.6 mm, and the coefficient of variation is 16.05%.

**Variation of RVA parameters**

The distribution of the RVA profile characteristics of 64 semi waxy lines was shown in Fig. 5. It can be seen from Fig. 5 that the variation range of the PKV was between 1746 and 3511 cP, and there were 57 lines distributed between 2000 and 2900 cP, accounting for 89.1%. The variation range of the HPV was between 925 and 2160 cP, and 60 of them were distributed between 1100 and 1900 cP, accounting for 93.8%. The variation range of CPV was between 1533 and 2769 cP, and 54 of them were distributed between 1800 and 2400 cP, accounting for 84.4%. The variation range of BDV was between 617 and 1646 cP, among which 59 were distributed between 800 and 1600 cP, accounting for 92.2%. The variation range of SBV was between -1018 and -1 cP, among which 50 were distributed between -900 and -500 cP, accounting for 78.1%. The variation range of CSV was between 480 and 752 cP, among which 60 were distributed between 550 and 700 cP, accounting for 93.8%. The variation of SBV was the largest, the coefficient of variation reached 42.4%, followed by the BDV, HPV, PKV, CPV, and CSV.

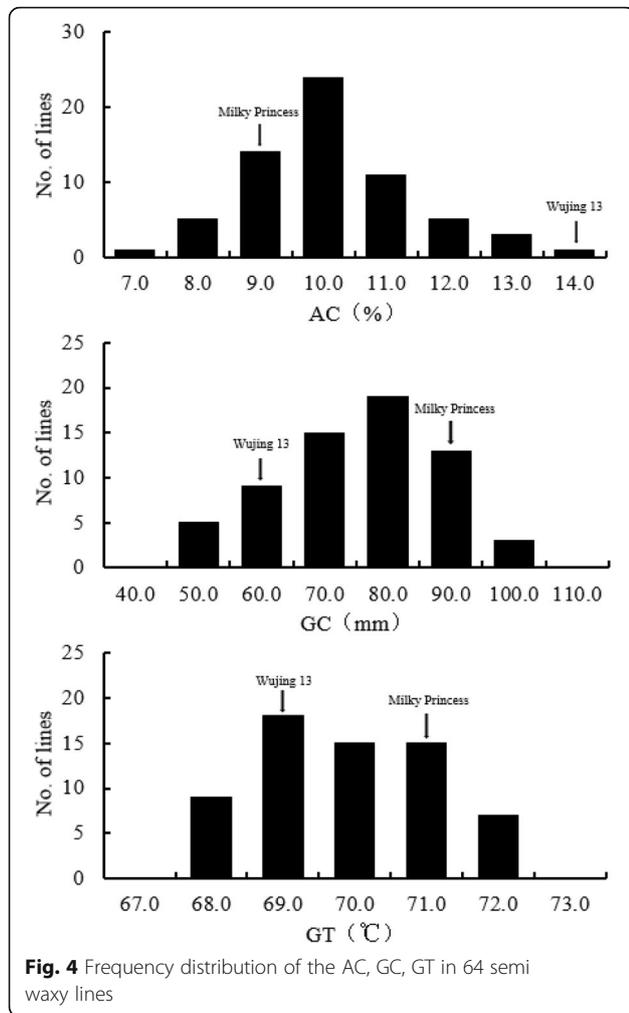
**Genetic effects of *SSIIa* and *SSIIIa* alleles on AC**

The results of ANOVA for AC of 64 semi waxy lines planted in 2013 and 2014 are shown in Table 3. Table 3 showed that there were significant differences in AC between lines, years and the interaction between years and lines. Further analysis showed that although the

**Table 2** The genotypes of *SSIIa* and *SSIIIa* locus for 64 semi waxy lines derived from Wujing 13/ Milky Princess with  $Wx^{mp}$  gene

Single genotype	Number of strain	Double genotype	Number of strain
<i>SSIIa<sup>mp</sup></i>	23	<i>SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup></i>	10
<i>SSIIa<sup>wj</sup></i>	41	<i>SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup></i>	13
<i>SSIIIa<sup>mp</sup></i>	49	<i>SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup></i>	39
<i>SSIIIa<sup>wj</sup></i>	15	<i>SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup></i>	2

*mp* Indicated that the gene was derived from the semi waxy parent of Milky Princess, and *wj* Indicated that the gene was derived from the parent of Wujing13



difference of AC among the four genotypes of *SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup>*, *SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup>*, *SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup>* and *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>* was significant at 1% level, the differences among the four genotypes were even greater. Except for the insignificant difference of AC between genotype *SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup>* and *SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup>*, the difference of AC among other genotypes was significant at 1% level.

Table 4 lists the average AC of different genotypes of *SSIIa* and *SSIIIa* loci in 64 semi waxy lines in 2013 and 2014. It can be seen from the table that the AC of the genotype of non-waxy parents was higher than that of semi waxy parents, regardless of the *SSIIa* or *SSIIIa* loci. On average, the AC of *SSIIa<sup>wj</sup>* genotype was 1.87% higher than the data of *SSIIa<sup>mp</sup>* genotype. The AC of *SSIIIa<sup>wj</sup>* genotype was 1.23% higher than the figure of *SSIIIa<sup>mp</sup>* genotype, which indicated that the effect of *SSIIa* was higher than that of *SSIIIa*.

Table 5 lists the average, maximum, minimum values 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<sup>mp</sup>SSIIIa<sup>mp</sup>* and *SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup>* was the lowest and there was no significant difference between them, the AC of genotype *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>* was the highest, the AC of genotype *SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup>* was in the middle, and the difference of the three genotypes was significant at 1% level.

**Genetic effects of *SSIIa* and *SSIIIa* alleles on GT and GC**

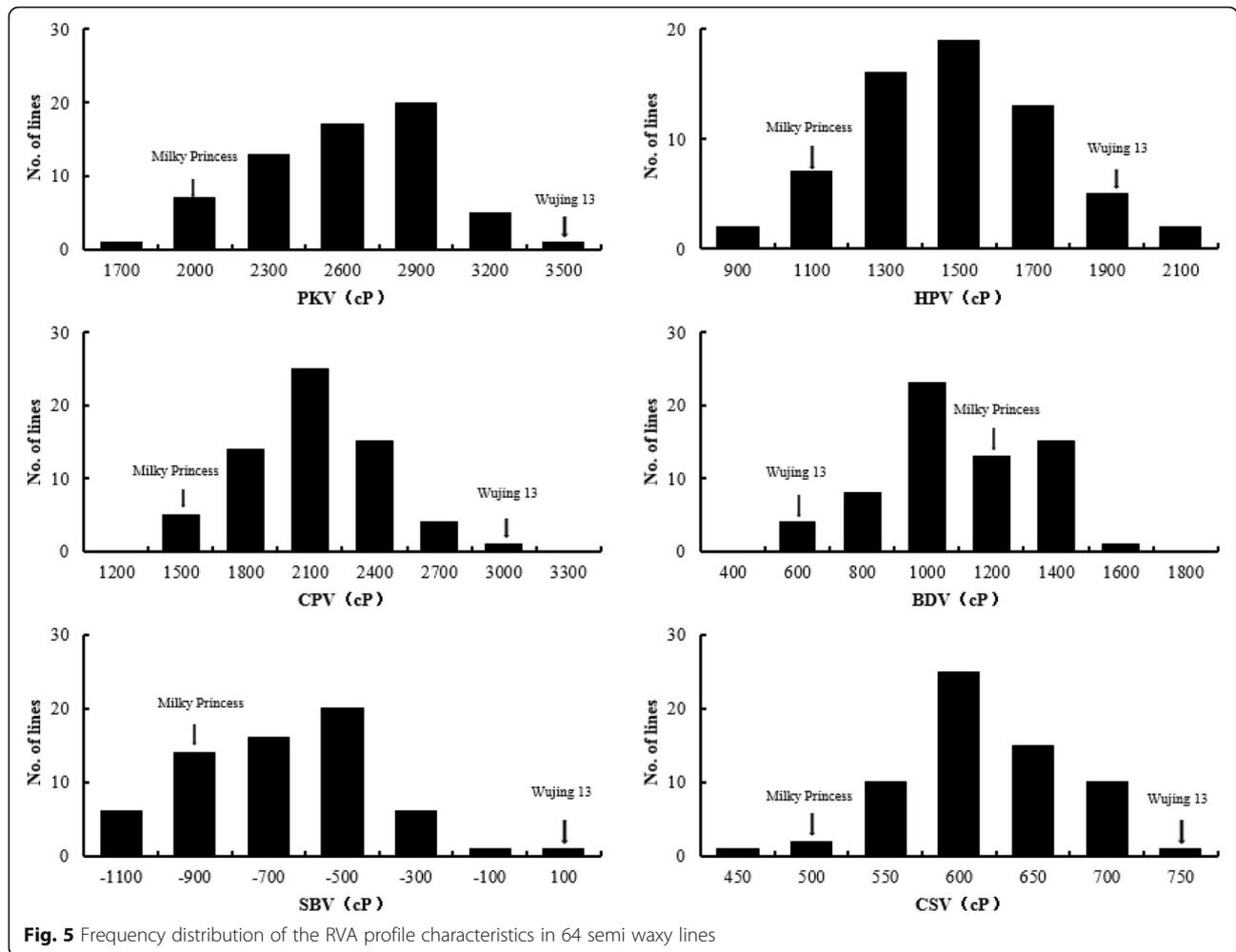
The GT and GC of different genotypes were shown in Table 6. There was no significant difference in GT between *SSIIa* and *SSIIIa* alleles of different parents, but there was significant difference in GC among different genotypes. *SSIIa* gene from Wujing13 and *SSIIIa* gene from Milky Princess increased GC by 8.74 and 9.62 mm respectively.

Multiple comparisons showed that the GT of genotype *SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup>* was the highest, followed by genotype *SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup>*, *SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup>* and *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>*, but the GT of the four genotypes was similar. There was no significant difference between genotype *SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup>* and *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>*, except for genotype *SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup>* and *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>*. The GC of genotype *SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup>* was the highest, followed by genotype *SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup>*, *SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup>* and *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>*. The difference between genotype *SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup>* and *SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup>* was significant, and the difference between genotype *SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup>* and *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>* was significant at 1% level.

**Genetic effects of *SSIIa* and *SSIIIa* alleles on RVA profile characteristics**

The RVA profile characteristics of different genotypes were listed in Table 7. It can be seen from the table that there were significant differences in the RVA parameters among different *SSIIa* alleles; the same was true for *SSIIIa* alleles, except for the HPV and CPV, for which the difference between the alleles was not significant.

The results of multiple comparisons of the RVA parameters among the four genotypes of the two genes showed that there were significant differences in each parameter among the four genotypes. When the *SSIIa* allele was from Milky Princess (*SSIIa<sup>mp</sup>*), the differences of RVA parameters between two *SSIIIa* alleles (*SSIIIa<sup>mp</sup>* and *SSIIIa<sup>wj</sup>*) were insignificant; when the *SSIIa* allele was from Wujing13 (*SSIIa<sup>wj</sup>*), except the SBV was significantly different between two *SSIIIa* alleles (*SSIIIa<sup>mp</sup>* and *SSIIIa<sup>wj</sup>*), other parameters have no significant difference. When the *SSIIIa* allele was from Milky Princess (*SSIIIa<sup>mp</sup>*), except the SBV was significantly different between two *SSIIa* alleles (*SSIIa<sup>mp</sup>* and *SSIIa<sup>wj</sup>*), other parameters have no significant difference. When the *SSIIIa* allele was from Wujing13 (*SSIIIa<sup>wj</sup>*), the differences in PKV, HPV and CPV for two *SSIIa* alleles (*SSIIa<sup>mp</sup>* and *SSIIa<sup>wj</sup>*) were insignificant, the differences in SBV, BDV



and CSV were significant. Thus, it could be seen that the *SSIIa* locus was the main gene affecting RVA profile characteristics. The PKV and BDV of semi waxy lines are greater than that of parent Wujing13, and the HPV, CPV and SBV of semi waxy lines were smaller than parent Wujing13. Sui et al. (2005) deemed that there was an extreme 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 indicated that the BDV, SBV and CSV can be used to evaluate the eating quality correctly and can be used as effective indicators of selecting material with high eating quality. The varieties with high BDV and low SBV and CSV have better eating quality when they are cooked. The lines with higher BDV in the experiment are *SSIIa*<sup>wj</sup>*SSIIIa*<sup>mp</sup> and *SSIIa*<sup>wj</sup>*SSIIIa*<sup>wj</sup>, and there are no significant differences between them. The varieties with low CSV are *SSIIa*<sup>wj</sup>*SSIIIa*<sup>mp</sup> and *SSIIa*<sup>mp</sup>*SSIIIa*<sup>mp</sup>, and there were no significant differences between them. The varieties with low SBV are *SSIIa*<sup>wj</sup>*SSIIIa*<sup>mp</sup> and *SSIIa*<sup>wj</sup>*SSIIIa*<sup>wj</sup>, and there

are no significant differences between them. Thus, it is inferred that the varieties with gene type *SSIIa*<sup>wj</sup>*SSIIIa*<sup>mp</sup> have better eating quality.

**Interaction effects between *SSIIa* and *SSIIIa* on eating and cooking quality**

It can be seen from Table 4 that the genetic effect of AC for single gene of non-waxy parents in 2013 and 2014, no matter for *SSIIa* locus or *SSIIIa* locus, was higher than that of semi waxy parents. And the average gene effects for *SSIIa* and *SSIIIa* in 2 years were 1.87 and 1.23% respectively. However, in the *SSIIa*<sup>mp</sup> gene background, average gene effect of *SSIIIa* is only 0.03%, while in the *SSIIa*<sup>wj</sup> gene background, average gene effect of *SSIIIa* is up to 2.42%. Similarly, in the *SSIIIa*<sup>mp</sup> gene background, average gene effect of *SSIIa* is only 0.67%; while in the *SSIIIa*<sup>wj</sup> gene background, the average gene effect of *SSIIa* is up to 3.07% (Table 5). It indicates that interaction effect existed between *SSIIa* and *SSIIIa*<sup>wj</sup> genes.

Similarly, the genetic effects of *SSIIa* and *SSIIIa* on GT, GC, PKV, HPV, CPV, BDV, CSV and SBV were not

**Table 3** Analysis of variance on AC in different genotypes of *SSIIa* and *SSIIIa* locus and different years for 64 semi waxy lines

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

Genotype 11, 12, 21 and 22 represent the four genotypes of *SSIIa*<sup>mp</sup>*SSIIIa*<sup>mp</sup>, *SSIIa*<sup>mp</sup>*SSIIIa*<sup>wj</sup>, *SSIIa*<sup>wj</sup>*SSIIIa*<sup>mp</sup> and *SSIIa*<sup>wj</sup>*SSIIIa*<sup>wj</sup>, respectively

NS Not significant

<sup>a</sup> Significance at 1% level

the same under the background of different parent genes (Table 8). Except for the GT, there were significant differences in genetic effect of *SSIIa* and *SSIIIa* under different background of parent for all the other characters, which indicates that there exist interaction effect between *SSIIa* and *SSIIIa* genes for these characters.

## Discussion

### Genetic effects of *SSIIa* and *SSIIIa*

Previous studies have shown that the ECQ of rice was regulated by other genes related to starch synthesis in addition to the *Wx* gene. Tian et al. (2009) revealed that *SSII-3* (*SSIIa*) had an effect on AC, GC and GT, and *SSI*, *SSIII-2* (*SSIIIa*), *AGPase-L* and *PUL* affect AC as well by association analysis. He et al. (2006) detected the effect and interaction of *SSIIa*, *SssI* and *PUL* genes in addition to *Wx* on AC, GC and GT in DH population of “NJ11/Balilla”.

**Table 4** Allelic effect on AC of *SSIIa* and *SSIIIa* genes for 64 semi-waxy lines in 2013 and 2014

Locus	Genotype	2013	2014	Average	Genic effect
<i>SSIIa</i>	<i>SSIIa</i> <sup>mp</sup>	9.05	8.85	8.95	1.87
	<i>SSIIa</i> <sup>wj</sup>	10.84	10.80	10.82	
<i>SSIIIa</i>	<i>SSIIIa</i> <sup>mp</sup>	9.37	9.17	9.27	1.23
	<i>SSIIIa</i> <sup>wj</sup>	10.51	10.48	10.50	

The genotypic classification of 64 semi waxy lines from Wujing13/Milky Princess was carried out in this research. We found that *SSIIa* and *SSIIIa* had an effect on the 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%, respectively, and the results were similar in 2 years. There was no significant difference in GT between *SSIIa* and *SSIIIa* genotypes of different parents, but the GT of genotype *SSIIa*<sup>mp</sup>*SSIIIa*<sup>mp</sup> was significantly higher than that of *SSIIa*<sup>wj</sup>*SSIIIa*<sup>wj</sup>, which indicates that GT was not significantly affected by single *SSIIa* and *SSIIIa* genes, but the interaction of the two genes had a significant effect on GT. There were significant differences in GC among different genotypes. *SSIIa* gene derived from Wujing 13 and *SSIIIa* gene from Milky Princess increased GC by 8.74 and 9.62 mm, respectively. As for the combined effect of the two genes, the GC of genotype *SSIIa*<sup>wj</sup>*SSIIIa*<sup>mp</sup> was the highest, which was 10.64 and 16.95 mm higher than that of genotype *SSIIa*<sup>mp</sup>*SSIIIa*<sup>wj</sup> and *SSIIa*<sup>wj</sup>*SSIIIa*<sup>wj</sup>, respectively. The difference was significant.

RVA profiles characteristics, especially BDV, CSV and SBV, can reflect the difference of ECQ among different rice varieties (Bason and Blakeney 2007), 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* has an important influence on RVA profiles characteristics in glutinous rice. In this study, there was almost significant difference between the *SSIIa* alleles from different parents, while there was no significant difference between the *SSIIIa* alleles in several main RVA characteristic values affecting quality, indicating that the effect of *SSIIa* on the RVA characteristic value was greater than that of *SSIIIa*. Sui et al. (2005) considered that the rice which has lower HPV, CPV, SBV, CSV, shorter PeT, lower PaT and higher BDV may have better softness, stickiness, good texture in cold cooking rice and higher comprehensive score. Shu et al. (1998) considered that the PKV, BDV, SBV and CSV in RVA profiles characteristics could reflect the eating quality. Both *indica* and *japonica* rice varieties with relatively high PKV and BDV, relatively low consistence and SBV have relatively good eating quality. In this study, BDV of all semi waxy lines was almost higher than that of Wujing13, the PKV, HPV, CPV, CSV and SBV were lower than that 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

**Table 5** Difference of AC for different genotypes in *SSIIa* and *SSIIIa* locus of 64 semi waxy 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

Genotype 11, 12, 21 and 22 represent the four genotypes of *SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup>*, *SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup>*, *SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup>* and *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>* respectively. Different capital letters in same column indicates significance at 1% level

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 AC 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.

**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). Amylase is formed under the catalysis of particles combined with starch synthase, and the amylopectin is synthesized under the cooperation of SSS, *SBE*

and *DBE*. There are more than 20 genes participating in the coding of these enzymes, including 10 genes of coding starch synthase: *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 2008a, b; Zhou et al. 2013). He et al. (2006) has ever detected interaction effect among more starch synthase genes in the DH population of “NJ11/Balilla”. The results of this study on the genetic effects of *SSIIa* and *SSIIIa* also showed that when the parents of one gene were different, the genetic effects of the other gene were also different. For example, under the background of *SSIIa<sup>mp</sup>* gene, the average gene effect of *SSIIIa* is only 0.03%, while under the background of *SSIIa<sup>wj</sup>* gene, the average gene effect of *SSIIIa* is as high as 2.42%. Similarly, in the background of *SSIIIa<sup>mp</sup>* gene, the average gene effect of *SSIIa* is only 0.67%, while in the background of *SSIIIa<sup>wj</sup>* gene, the average gene effect of *SSIIa* is as high as 3.07% (Table 5). Single gene has no effect on GT, but two genes have significant effect on GT when they exist at the same time. *SSIIa<sup>wj</sup>* and *SSIIa<sup>mp</sup>* increased GC by 8.74 mm and 9.62 mm, respectively, while the GC of genotype *SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup>* was 10.64 mm and 16.95 mm higher than that of genotype *SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup>* and *SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup>*, respectively. For the interaction effects of the two genes on the RVA profile

**Table 6** Difference of GC and GT for different genotypes in *SSIIa* and *SSIIIa* locus of 64 5.-glutinous lines

Locus	Genotype	GT			GC		
		Average (°C)	SD	CV (%)	Average (mm)	SD	CV (%)
<i>SSIIa</i>	<i>SSIIa<sup>mp</sup></i>	70.59	1.26	1.78	73.78 <sup>a</sup>	13.41	18.18
	<i>SSIIa<sup>wj</sup></i>	70.36	1.01	1.44	82.53	10.28	12.46
<i>SSIIIa</i>	<i>SSIIIa<sup>mp</sup></i>	70.49	1.11	1.57	81.47 <sup>a</sup>	11.76	14.43
	<i>SSIIIa<sup>wj</sup></i>	70.28	1.11	1.58	71.86	10.89	15.15
<i>SSIIa<sup>mp</sup>SSIIIa<sup>mp</sup></i>		70.94a	1.38	1.95	75.71ab	16.29	21.52
<i>SSIIa<sup>mp</sup>SSIIIa<sup>wj</sup></i>		70.33ab	1.34	1.91	72.31b	11.20	15.49
<i>SSIIa<sup>wj</sup>SSIIIa<sup>mp</sup></i>		70.37ab	1.02	1.45	82.95a	10.05	12.12
<i>SSIIa<sup>wj</sup>SSIIIa<sup>wj</sup></i>		69.6b	1.21	1.89	66.00b	10.12	15.21

<sup>a</sup> 1% significance level; Different letters after the means in same column indicated significance at 5% level

**Table 7** Difference of RVA profiles for different genotypes in *SSIIa* and *SSIIIa* locus of 64 semi waxy lines

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

<sup>a</sup>, <sup>b</sup> or different capital and lower case letters after the means in the same column indicated significance at 1 and 5% levels, respectively

characteristics, the genotype *SSIIa*<sup>wj</sup>*SSIIIa*<sup>mp</sup> had the largest PKV, HPV and CPV, the genotype *SSIIa*<sup>wj</sup>*SSIIIa*<sup>wj</sup> had the largest BDV and CSV, but the genotype *SSIIa*<sup>wj</sup>*SSIIIa*<sup>mp</sup> had the least SBV. The above results showed that there was interaction effect between *SSIIa* and *SSIIIa* genes on these characters.

#### Application of the result in improving the eating quality of japonica rice

The semi waxy japonica rice shows milky white and cloud-like opaque phenotype because of its low AC, which has an adverse impact on the appearance quality of rice. Because the AC is varied with different semi waxy genes, the selection of semi waxy genes is of great significance for the development of japonica rice with good eating quality (Ni et al. 2011; Caffagni et al. 2013). Our breeding practice shows that although the taste quality of rice with semi waxy gene is generally good, but the semi waxy japonica rice is not that the lower the AC, the better. Too low AC (below 8%) may lead to opaque rice grain, bad appearance quality, soft eating quality, and lack of elasticity. Under the background of semi waxy gene, the appearance quality can be improved by properly increasing the AC, while the taste quality remains good. When the AC reaches more than 12%, the cooked rice is elastic, good in texture when it is cold, and

smooth in taste, which meet the sensory requirement from people in Yangtze River Delta.

The AC of EMS mutant *Wx*<sup>mp</sup> derived from japonica Koshihikari is approximately 9%, but the AC of different semi-waxy lines derived from the same combination with *Wx*<sup>mp</sup> gene was still very different, which indicates that there were other genes besides *Wx* locus that affected the synthesis of amylose. This study found that the *SSIIa* and *SSIIIa* alleles of Wujing13 could increase AC by 1.87 and 1.23%, respectively, under the background of semi-waxy gene *Wx*<sup>mp</sup>. Meanwhile the two genes can increase AC by 3.1%, reaching approximately 12%. These kind of semi waxy lines not only have good eating quality, but also have bright appearance. Therefore, the selection of different allelic genotypes of *SSIIa* and *SSIIIa* could make semi waxy japonica rice both delicious and good-looking. Of course, there are many genes that affect the synthesis of amylose. Through the study of the genetic effects of these genes, we can provide theoretical basis for breeding more ideal genotypes with AC that meet the needs of consumers.

#### Conclusion

Under the same *Wx*<sup>mp</sup> gene background, the allelic variation of *SSIIa* and *SSIIIa* genes had significant effects on AC, GT, GC and RVA profile characteristics, and the two genes have interactive effects. *SSIIa* and *SSIIIa* alleles derived from non-semi waxy parent had a tendency

**Table 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									
Milky Princess	0.03 <sup>b</sup>	-0.61	-3.40 <sup>b</sup>	197.20 <sup>b</sup>	185.60 <sup>b</sup>	191.70 <sup>b</sup>	11.70 <sup>b</sup>	6.10 <sup>b</sup>	-5.60 <sup>b</sup>
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									
Milky Princess	0.67 <sup>b</sup>	-0.57	7.24 <sup>a</sup>	559.00 <sup>b</sup>	322.20 <sup>b</sup>	290.20 <sup>b</sup>	236.80 <sup>b</sup>	-32.00 <sup>b</sup>	-268.80 <sup>b</sup>
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

<sup>a</sup>, <sup>b</sup> Significance at 5 and 1% level, respectively

to increase AC. The GT was not significantly affected by single *SSIIa* and *SSIIIa* genes but remarkably influenced by the interaction of the two genes. The GC were significantly varied among different genotypes. The effect of *SSIIa* on the RVA characteristic value was greater than that of *SSIIIa*. The allelic variation and interaction effect of *SSIIa* and *SSIIIa* genes have important reference value for improving cooking and eating quality of semi waxy *japonica* rice.

#### Abbreviations

AC: Amylose content; ACLA: American Association of Grain Chemistry; ANOVA: Analysis of variance; ARMS-PCR: Amplification refractory mutation system PCR; BDV: Breakdown viscosity; bp: Base pair; cP: Centipoise; CPV: Cool viscosity; CSV: Consistence viscosity; CTAB: Cyltrimethylammonium Bromide; CV: Coefficient of variation; DBE: Starch Debranching enzyme; DNA: Deoxyribonucleic acid; dNTP: Deoxyribonucleotide triphosphate; DSC: Differential scanning calorimeter; ECQ: Eating and cooking quality; EDTA: Ethylene Diamine Tetraacetic Acid; GBSS: Granule bound starch synthase; GC: Gel consistency; GT: Gelatinization temperature; HPV: Hot viscosity; *Isa*: Gene encoding isoamylase; PaT: Pasting temperature; PCR: Polymerase chain reaction; PeT: Peak time; PKV: Peak viscosity; RVA: Rapid visco-analyser; SBE: Starch branching enzyme; SBV: Setback viscosity; SD: Standard deviation; SPSS: Statistical Product and Service Solutions; SS: Starch synthase; SSS: Souble starch synthase; STS: Sequence-tagged site; *Wx*: Waxy gene

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#### Authors' contributions

Cailin Wang designed the experiments, Shu Yao, Ya dong Zhang, Chun fang Zhao, Li hui Zhou, Zhen Zhu, Tao Chen and Qing Yong Zhao prepared laboratory for experiments and analysed the results; Shu Yao and Cailin Wang wrote the manuscript, Cailin Wang and Balakrishna Pillay reviewed and modified the manuscript. The author(s) read and approved the final manuscript.

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#### Availability of data and materials

All data is available in this manuscript.

#### Competing interests

The authors declare that they have no competing interests.

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