Evaluation of genetic effect on physiochemical properties changes of *Wx* near isogenic lines of Y58S in rice

Wentao Sheng[1], Lijie Zhou[2], Jun Wu[2], Bin Bai[2], and Qiyun Deng[2*]

The *Wx* gene primarily influences the physiochemical properties changes of rice (*Oryza sativa* L.) To improve the grain qualities of the cv. Y58S with low amylose content (AC) value, the genetic effect of *Wx* gene was explored on quality traits. With the BC₃F₂ genetic population of Y58S line associated with different AC value as the materials, this study reported the genetic effect of three different *Wx* alleles (*Wxᵃ*, *Wxⁱⁿ*, *Wxᵇ*) under near-isogenic background. It was shown that *Wx* had major effects on the quality traits, and the genetic effect value was in the order $Wx^a > Wx^{in} > Wx^b$. In addition, during the course of physiochemical properties changes of higher AC rice cultivars determined by *Wxᵃ* or *Wxⁱⁿ* allele, accompanied by the increasing of AC, gel consistency and head milled rice recovery would be decreased in combination with the rising of chalky traits, all of which resulted in substantial fluctuations on quality traits ($p < 0.01$). However, the genetic law was not evident for *Wxᵇ* allele. Therefore, except for the major gene *Wx*, the minor genes in the regulative network of starch-synthesis might be utilized for quality improvement.

Key words: Amylose content, *Oryza sativa*, physiochemical properties, quality improvement, *Wx*.

INTRODUCTION

The genetic effect evaluation of the major gene is very meaningful for quality improvement of rice (*Oryza sativa* L.) It was reported that *Wx* gene was a predominant determinant for the synthesis of amylose content (AC), which caused marked influence on the comprehensive physiochemical traits including AC, gel consistency (GC) and gelatinization temperature (GT) (Tian et al., 2010). In general, the cooking quality is a major criterion in evaluating rice grain trait, which is determined by the starch composition and correlated with the changes of AC, GC, and GT values. The rice grain with higher AC value demonstrates dry, fluffy and separated cooked rice features, representing poor cooking qualities. And in south of China, people tend to have cooked rice associated with intermediate AC, soft GC, and low GT.

Currently, a series of *Wx* alleles including *Wxᵃ*, *Wxⁱⁿ*, *Wxᵇ*, *Wxᵒᵖ*, and *wx* had been confirmed, which could bring about the AC change (Qiao et al., 2012). Mikami et al. (2008) found that these five alleles were directly related to high, intermediate, low, soft, and glutinous rice cultivars

individually, by analyzing the genetic rule of AC with *Wx* isogenic lines of 'T65'. And *Wx* gene had the pleiotropic roles, which not only controlled the changes of AC and GC, but also influenced the GT value (Tian et al., 2009). Ni et al. (2011) reported that the introduction of *Wxᵇ* allele into the parent genetic background of the hybrid 'Xieyou 57', resulting in AC reduced to lower level and the whole quality of the hybrids had been greatly optimized. Meanwhile, 'Manawthukha', a conventional high AC value cultivar, was obviously improved on quality traits, by importing *Wxⁱⁿ* from 'Basmati370'. Furthermore, little negative effect was caused on the yield characteristics (Myint et al., 2009). Thus, findings described herein showed that it might be feasible to improve grain quality by the method of substitution of *Wx* alleles. However, the study of Li et al. (2014) indicated that a complicated correlation existed on the whole qualities, where the value of chalkiness was associated with the change of AC, GC, and the head rice recovery in different introgressing lines of chalky gene. And we can also see that there was still short of the comprehensive analysis for the relationship between the selection of different *Wx* alleles and quality traits from the appearance, the milling and cooking qualities. Therefore, through the genetic material established using *Wx* near isogenic lines (*Wxᵃ*, *Wxⁱⁿ*, and *Wxᵇ*; NILs), the genetic effect would be assessed on *Wx* alleles and the comprehensive quality traits involving the relationship between AC and *Wx* genotype. The present study was conducted to provide theoretical clues for the quality improvement of Y58S line with low AC value by utilization of *Wx* alleles in rice.

[1]Nanchang Normal University, Department of Biological Technology, Nanchang 330032, Nanchang, China.
*Corresponding author (dqy900@gmail.com).
[2]Hunan Hybrid Rice Research Center, Changsha 410125, Hunan, China.

MATERIALS AND METHODS

Plant material

Four rice lines were used as the parents in this study: (1) Y58S, the backbone two-line sterile line for a lot of elite hybrids grown in China, was used as the receiver of Wx gene; (2) 'Teqing', 'Starbonnet99', and 'Yuejingsimiao 2' were used as donor of Wx alleles determining high, intermediate, and low AC, respectively. A cross was made between Y58S and three donors, followed by three backcrossing with Y58S as the recurrent parent and one self-pollination. In the generation of BC_3F_2 population, the materials were classified and the mean values of quality traits were compared according to the Wx genotype.

DNA marker analysis

On the basis of the diversity of $(CT)_n$ microsatellite in leader region and the first base G/T site (In1 (G/T)) polymorphism in the first intron of Wx (Ayres et al., 1997; Teng et al., 2012), the genotype of 'Teqing' $((CT)_{11}\text{-}G)$ was Wx^a associated with high AC value; the 'Starbonnet99' genotype $((CT)_{20}\text{-}G)$ belonged to Wx^{in} connected with intermediate AC value; 'Yuejingsimiao 2' $((CT)_{17}\text{-}T)$ owned Wx^b allele relevant with low AC value; the Y58S genotype $((CT)_{18}\text{-}T)$ was Wx^b correlated with low AC value. Utilization of the characteristics of In1 (G/T) site cleaved by restriction endonuclease AccI, the primer Wx-I (F:5′-GTTGGAAGCATCACGAGTTT-3′; R:5′-CGTCGTTGCAGACGAACA-3′) was devised to discriminate the genotype of Wx^a and Wx^{in} (Cai et al., 2002; Ni et al., 2011). Meanwhile, in order to distinguish the genotype of Wx^b between 'Yuejingsimiao 2' and Y58S, the Wx^b genotype of 'Yuejingsimiao 2' and Y58S was designated as Wx^{yj} and Wx^y, respectively. The polymorphic microsatellite markers found upstream and downstream of intragenic marker RM190, were used to differentiate the genotype of Wx^{yj} and Wx^y (Myint et al., 2009).

Measuring quality traits

In the light of the genotype, quality traits were determined using grain sample harvested from the genetic population at physiological maturity of Changsha Experimental Station (27°51' N, 111°53' E) in Hunan Province of south China in 2014. The dried grains were stored naturally in a greenhouse for 3-mo prior to the analysis. The quality traits were evaluated with three replicates following the procedures included in the Agricultural Industry Standard NY/T593-2013 (2013) issued by the Ministry of Agriculture, PR China. Briefly, for the milling quality, the rate of the whole rice after milling was a main parameter used to evaluate quality and efficiency of the milling process. For the appearance trait, it was calculated on the proportion between the area of chalkiness and the grain size. And for the cooking quality, AC of milled rice was measured by the colorimetric method with iodine and potassium iodide $(I_2\text{-}KI)$ staining. The GC was

assessed by measuring the flow length of rice glue. And GT was evaluated on the basis of the alkali spreading value (ASV), such that the higher GT is considered to be lower ASV value. All traits composed of brown rice ratio (BRR), milled rice ratio (MRR), head milled rice recovery (HMRR), milled rice length (MRL), milled rice width (MRW), chalky rice ratio (CRR), chalkiness (Ch), GT, GC, and AC, were subjected to statistical analysis using the SPSS 17.0 software program (SPSS, Chicago, Illinois, USA). ANOVA was performed using all the mean value of data in all traits at one percent probability level.

RESULTS

Genotyping using Wx markers

The PCR product size of Wx-I amplification was a 423 bp fragment containing the splicing site in tested materials. Upon digestion with AccI, the amplified product of Wx^a or Wx^{in} types produced two fragments of 275 and 148 bp; the PCR product of Wx^b type could not be digested and only one fragment of 423 bp was revealed; the heterozygous genotype gave rise to three bands of 423, 275, and 148 bp (Figure 1). Justified by the physical position RM190 (1764661 ~ 1764696 bp), the microsatellite markers RM589 (1380931 ~ 1380978 bp) and RM19350 (1967182 ~ 1967205 bp) had been found to distinguish the genotype between 'Yuejingsimiao 2' and Y58S (International Rice Genome Sequencing Project, 2005). Polymorphisms were identified between alleles based on the number of microsatellite repeat, two kinds of DNA profiles ranging from 148 and 138 bp and 197 and 191 bp between Y58S and 'Yuejingsimiao 2' in RM589 and RM19350, respectively (Figures 2 and 3).

Comparison of quality traits among different genotypes

Analysis of quality traits from different genotypes is presented in Table 1. The result showed that these substitution lines caused dramatic influence on HMRR, chalky traits, AC, and GC, especially for these materials with Wx^a or Wx^{in} genotype. For the BC_3F_2 population (Y58S/Teqing), as compared to the homozygous dominate Wx^a/Wx^a and recessive Wx^b/Wx^b genotype, the obvious difference in these traits of AC, GC, chalky traits were found for the heterozygous
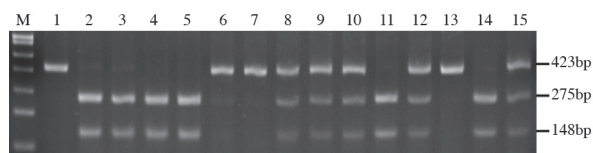

1: Y58S; 2: 'Teqing' ('Starbonnet99'); 3 ~ 5: homozygous genotype (Wx^a or Wx^{in}) of individual plant in BC_3F_2 population; 6 ~ 7, 13: homozygous genotype (Wx^b) of individual plant; 8 ~ 10, 12, and 15: heterozygous genotype of individual plant; M: 100 bp ladder fragment

Figure 1. Wx genotype was detected by Wx-I after AccI digestion in BC_3F_2 population of Y58S.
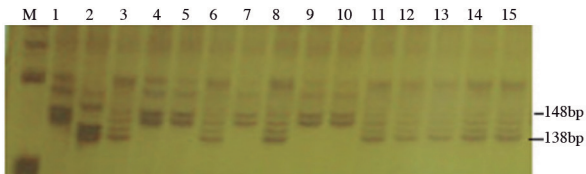
1: Y58S; 2: 'Yuejingsimiao 2'; 3 ~ 15: segregation individual of BC$_3$F$_2$ population; M: 100 bp ladder fragment.

Figure 2. BC$_3$F$_2$ segregation population of Y58S/Yuejingsimiao 2 associated with *Wx* linked marker RM586.
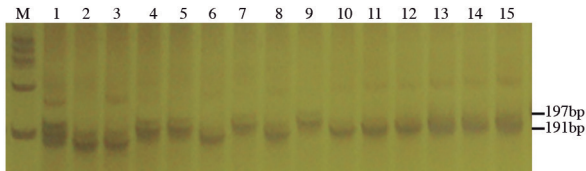
1: Y58S; 2: Yuejingsimiao 2; 3 ~ 15: segregation individual of BC$_3$F$_2$ population; M: 100 bp ladder fragment.
Note: The individual plant was synchronously detected with RM586 and RM19350. For the homozygous genotype (*Wx^y*), the makers RM586 and RM19350 had the same band type with Y58S; for the homozygous genotype (*Wx^{yj}*), the makers RM586 and RM19350 had the same band type with 'Yuejingsimiao 2'; for the heterozygous genotype (*Wx^y/Wx^{yj}*), the makers RM586 and RM19350 had heterozygous band types between 'Yuejingsimiao 2' and Y58S, respectively.

Figure 3. BC$_3$F$_2$ segregation population of Y58S/Yuejingsimiao 2 associated with *Wx* linked marker RM19350.

genotype (*Wx^a/Wx^b*) (*p* < 0.01). As for the trait of HMRR, the difference between *Wx^a/Wx^a* and *Wx^a/Wx^b* genotype did not reach significant level (*p* < 0.01). It was postulated that they might be associated with higher AC producing similar effect on grain qualities, and the difference of the comprehensive effect were nearly identical for this trait of these materials without significance. For the BC$_3$F$_2$ population (Y58S/Starbonnet99), compared with the homozygous dominate *Wx^{in}/Wx^{in}* and recessive *Wx^b/Wx^b* genotype, noticeable differences were shown in these traits of AC, GC, chalky traits, and HMRR for the heterozygous genotype (*Wx^{in}/Wx^b*) (*p* < 0.01). But, the difference among these traits comprising of BRR, MRR, MRL, MRW, and ASV did not reach significant level in

the two populations. From data, it was confirmed that the alleles of *Wx^a* and *Wx^{in}* could have distinct impact on HMRR, chalky traits, GC, and AC, inducing the comprehensive negative effect on quality. For the BC$_3$F$_2$ population (Y58S/'Yuejingsimiao 2'), by comparing with the homozygous genotype between *Wx^{yj}/Wx^{yj}* and *Wx^y/Wx^y*, no dramatic difference was revealed on the quality characteristics for the heterozygous genotype (*Wx^{yj}/Wx^y*). The result indicated that different types of *Wx^b* alleles had the same genetic effect on the phenotype alteration of the comprehensive grain traits.

Correlation analysis among different quality traits
For the milling quality, HMRR was significantly positively correlated with MRL, the milled rice length/width ratio (L/B ratio), ASV and GC (0.794**, 0.771**, 0.805**, 0.952**), which showed that the increasing of MRL, ASV and GC occurred with the raising of HMRR, simultaneously (Table 2). However, HMRR was negatively linked with CRR, Ch, and AC (-0.972**, -0.984**, -0.988**), which revealed that along with the increasing of CRR, Ch, and AC, HMRR would be decreased remarkably. For the appearance quality, MRL was significantly positively related with L/B ratio, ASV and GC (0.910**, 0.941**, 0.699**), and also was negatively associated with CRR, Ch, and AC (-0.693**, -0.750**, -0.753**), which indicated that the increment of MRL and L/B ratio would result in lowering the chalkiness and AC values. For the chalky traits, CRR was significantly positively correlated with Ch and AC (0.980**, 0.960**), which suggested the chalky traits would be increased due to the augment of AC. And CRR was significantly negatively correlated with ASV and GC (-0.732**, -0.943**), which showed that the rice cultivars with low GT and soft GC had the tendency of owning the low chalky traits (Table 2). For the cooking traits, AC was significantly negatively relevant with ASV and GC (-0.795**, -0.975**), which revealed these traits of AC, GC, and GT might be controlled by *Wx* gene or else in a region very tightly linked to this gene (Table 2). As above mentioned, the present result illustrated a complex paradox existed between these quality traits. Therefore,

Table 1. Quality traits analysis of *Wx* gene in BC$_3$F$_2$ population of Y58S.

Materials	Genotype	BRR	MRR	HMRR	MRL	L/B ratio	CRR	Ch	ASV	GC	AC
Y58S	*Wx^b/Wx^b*	78.1 ± 0.75	70.5 ± 0.2	56.5 ± 0.4	6.37 ± 0.02	2.95 ± 0.02	33.15 ± 0.25	6.02 ± 0.3	5.2 ± 0.2	60.0 ± 1.5	13.7 ± 0.3
Teqing	*Wx^a/Wx^a*	75.5 ± 0.55	69.1 ± 0.4	40.8 ± 0.7	4.51 ± 0.09	2.15 ± 0.05	60.6 ± 0.3	25.1 ± 0.5	3.7 ± 0.4	35.0 ± 2.5	25.1 ± 0.5
Y58S/Teqing BC$_3$F$_2$	*Wx^a/Wx^a*	77.83 ± 0.15a	70.1 ± 0.1b	38.6 ± 0.66Bc	6.37 ± 0.01a	2.95 ± 0.00a	66.33 ± 2.08Aa	15.3 ± 0.1Aa	4.93 ± 0.06Bb	30.6 ± 1.53Cc	25.03 ± 0.12Aa
	Wx^a/Wx^b	74.6 ± 3.98a	71.0 ± 0.1a	40.47 ± 0.64Bb	6.37 ± 0.01a	2.95 ± 0.01a	57.33 ± 2.08Bb	14.6 ± 0.1Bb	5.13 ± 0.06Aa	41.0 ± 1.00Bb	22.63 ± 0.06Bb
	Wx^b/Wx^b	77.0 ± 0.1a	70.3 ± 0.15b	50.63 ± 0.74Aa	6.37 ± 0.01a	2.95 ± 0.01a	32.67 ± 1.52Cc	5.57 ± 0.15Cc	5.13 ± 0.06Aa	57.3 ± 1.15Aa	16.17 ± 0.12Cc
Starbonnet99	*Wx^{in}/Wx^{in}*	80.3 ± 0.5	72.5 ± 0.8	51.8 ± 0.7	5.5 ± 0.09	2.4 ± 0.05	40.7 ± 0.8	13.6 ± 0.6	4.5 ± 0.4	47.0 ± 2.5	20.1 ± 0.5
Y58S/starbonnet99	*Wx^{in}/Wx^{in}*	79.3 ± 0.40Aa	71.6 ± 0.47Aa	40.7 ± 0.15Cc	6.41 ± 0.02a	3.04 ± 0.01a	60.7 ± 1.53a	12.2 ± 0.31a	5.46 ± 0.06a	41.3 ± 2.1Cc	21.7 ± 0.1Aa
BC$_3$F$_2$	*Wx^{in}/Wx^b*	76.3 ± 0.20Bb	71.0 ± 0.21Aa	47.5 ± 0.35Bb	6.42 ± 0.02a	3.02 ± 0.03a	37.3 ± 1.15b	8.1 ± 0.2b	5.53 ± 0.06a	47.7 ± 0.6Bb	18.5 ± 0.2Bb
	Wx^b/Wx^b	75.7 ± 0.45Bb	69.9 ± 0.36Bb	54.0 ± 1.00Aa	6.43 ± 0.03a	3.06 ± 0.02a	25.7 ± 0.58c	3.26 ± 0.45c	5.46 ± 0.06a	54.0 ± 1.0Aa	15.1 ± 0.3Cc
Yuejingsimiao 2	*Wx^b/Wx^b*	82.0 ± 0.5	75.3 ± 0.45	56.73 ± 0.85	6.45 ± 0.06	3.15 ± 0.02	16.5 ± 2.2	10.8 ± 0.25	6.2 ± 0.1	70.0 ± 2.5	15.2 ± 0.3
Y58S/Yuejingsimiao 2	*Wx^{yj}/Wx^{yj}*	76.8 ± 0.26b	70.4 ± 0.15Bb	57.3 ± 0.15Bb	6.56 ± 0.02b	3.12 ± 0.01a	24.67 ± 1.53a	2.77 ± 0.12Aa	6.03 ± 0.15a	63.67 ± 1.53Aa	13.37 ± 0.31
BC$_3$F$_2$	*Wx^y/Wx^{yj}*	77.4 ± 0.25a	71.3 ± 0.20Aa	56.7 ± 0.36ABc	6.61 ± 0.02ab	3.13 ± 0.01a	22.33 ± 1.15a	2.43 ± 0.06Bb	6.03 ± 0.06a	61.67 ± 1.15ABab	13.67 ± 0.12
	Wx^y/Wx^y	77.2 ± 0.10ab	70.9 ± 0.21Aa	57.9 ± 0.31Aa	6.53 ± 0.04a	3.10 ± 0.02a	24.33 ± 1.53Bb	2.53 ± 0.0ABb	6.0 ± 0.1a	59.67 ± 0.58Bb	13.37 ± 0.15

BRR: Brown rice ratio; MRR: milled rice ratio; HMRR: head milled rice recovery; MRL: milled rice length; L/B ratio, milled rice length/width ratio; CRR: chalky rice ratio; Ch: chalkiness; ASV: alkali spreading value; GC: gel consistency; AC: amylose content.
Data for the three combinations with a column followed by different lower (upper) case letters indicated significant difference among the three combinations at 5% (1%) probability levels, respectively.

Table 2. Coefficient of phenotypic correlation between grain quality characteristics in *Wx* isogenic lines of Y58S BC₃F₂ genetic population.

	BRR	MRR	HMRR	MRL	L/B ratio	CRR	Ch	ASV	GC
BRR									
MRR	0.210								
HMRR	-0.068	-0.129							
MRL	0.106	0.200	0.794**						
L/B ratio	0.184	0.216	0.771**	0.910**					
CRR	0.178	0.148	-0.972**	-0.693**	-0.708**				
Ch	0.038	0.134	-0.984**	-0.750**	-0.743**	0.980**			
GT	0.109	0.290	0.805**	0.941**	0.910**	-0.732**	-0.770**		
GC	-0.065	-0.032	0.952**	0.699**	0.739**	-0.943**	-0.950**	0.778**	
AC	0.073	0.075	-0.988**	-0.753**	-0.761**	0.960**	0.991**	-0.795**	-0.975**

**Significant at 1% probability level.
BRR: Brown rice ratio; MRR: milled rice ratio; HMRR: head milled rice recovery; MRL: milled rice length; L/B ratio: milled rice length/width ratio; CRR: chalky rice ratio; Ch: chalkiness; ASV: alkali spreading value; GC: gel consistency; AC: amylose content.

this complex linkage correlation should be broken for obtaining superior qualities during the course of rice breeding.

DISCUSSION

The *Wx* gene had been recognized as a major determinant of grain quality, which impacted the wide range of variation on AC value. As it is well known, the inheritance of AC is very complicated due to the influence of modified genes, cytoplasmic effects, the triploid nature of the endosperm, environmental temperature, and genetic background (Tian et al., 2009; Ardashir et al., 2012). To minimize the genetic background affecting the magnitude and quantity of the quality trait, we used *Wx* NILs in the population of BC₃F₂ generation, which had three divergent alleles effecting starch properties. Among these combinations, the range of AC values were as follows ($Wx^a/Wx^a > Wx^{in}/Wx^{in} > Wx^b/Wx^b$); the magnitude of AC variations in different types of Wx^b/Wx^b genotype was low and nearly the same; the genetic effect of heterozygous genotype was between two homozygous genotypes. It was just accorded with the expression ability of granule binding starch synthetase (GBSS) associated with different *Wx* genotypes (Mikami et al., 2008). And the study confirmed the complex relationship between AC and different genotypes furthermore.

Up to now, there was some disagreement about the role of *Wx* gene on GC. Huang et al. (2000) reported that GC was predominately determined by the gene on chromosome 3. Besides this major gene, an epistatic genetic site was found to affect GC. The study of Tian et al. (2010) demonstrated that not only AC and GC were controlled by *Wx* gene, but also GT was influenced to some extent. Through positional cloning, it was found that GC was determined by *Wx* gene (Su et al., 2011). In this study, from the genetic rule of quality traits in different substitution lines, it was further proved that *Wx* gene had pleiotropies and there was negative correlation between AC and GC. Moreover, the substitution of *Wx* allele would not produce obvious effect on GT value, but a distinct negative correlation existing between AC and ASV value. As *Wx* was a major gene controlling

both AC and GC but a minor gene affecting GT, while the *SSIIa* gene was a key determinant responsible for GT but a minor gene influencing AC and GC, all of which were tightly linked on the short arm of Chromosome 6 (Zhou et al., 2003; Tian et al., 2009). And this result was in agreement with the study of Tian et al. (2009), which indicated there was a positive relationship for the change of AC and GT. But surprisingly, the study of Sun et al. (2005) showed that there was no obvious relevance for AC and GT. Presumably, the difference was correlated with the genotype of the experiment materials, which might have the same haplotype combination for *Wx* and *SSIIa* gene.

Usually, rice grain quality is a comprehensive trait including the appearance, milling, cooking, and nutritional qualities. The high chalky traits influences the appearance, and the low HMRR decreases the milling quality, both of which results in poor rice quality. In this study, the *Wx* gene region associated with high or intermediate AC was successfully transferred into Y58S by selection of In1(G/T) site of *Wx* gene. The data showed that when AC was profoundly increased, two dramatic changes occurred with an accompanied reduction on GC and HMMR and an obvious increment of chalky traits. However, these genetic changes were not obvious for Wx^b types in the BC₃F₂ population. It was possible that rice strains with the Wx^b allele had low amylose, in contrast to rice strains of higher AC with the Wx^a or Wx^{in} allele, which had low genetic effects on the AC value (Teng et al., 2012). And Zhou et al. (2003) found that when Wx^a allele was substituted by Wx^b, it caused the decreasing of AC and chalky traits and the increasing of GC and HMRR, contributing the whole optimization of quality traits. Therefore, a complex correlation for *Wx* alleles was also defined among these quality traits. Given that the rice endosperm was mainly comprised with amylose and amylopectin, and the ratio between amylose and amylopectin was an important physiochemical indicator influencing rice quality. And it is well documented that the synthesis of amylose and amylopectin in endosperm was composed of a complex regulative network including ADP-glucose pyrophosphorylase, GBSS, soluble starch synthase, starch branching enzymes and starch

de-branching enzymes. Each enzyme had rich variant isoforms, which was synthesized by different alleles. And these alleles functioned in different stages during starch synthesis, suggesting that the genetic control of starch synthesis was very complicated (Tian et al., 2010; Ardashir et al., 2012). Meanwhile, there were obvious fluctuations in physicochemical properties arising from selection of any single gene (Tian et al., 2009). In view of other regulative genes have been identified to produce minor effect on the fine structure and characteristics of starch, optimization of different haplotype combinations of minor genes might be an ideal choice for improving grain quality of rice.

CONCLUSION

In this study, Wx as a major gene influencing the quality traits, we found that the genetic effect value was in the order $Wx^a > Wx^{in} > Wx^b$ among these alleles, and the genetic effect caused by heterogeneous genotype was among homogenous dominate and recessive genotype. Meanwhile, during the course of physiochemical indexes changes for Wx^a or Wx^{in} alleles, accompanied with the rising of amylose content, gel consistency and the head milling rice recovery would be decreased, resulting in the increasing of the chalky traits, all of which caused noticeable fluctuations on quality traits. But for Wx^b allele, the genetic effect was not influential on the comprehensive physiochemical properties.

ACKNOWLEDGEMENTS

LITERATURE CITED

Agricultural Industry Standard NY/T593-2013. 2013. Cooking rice variety quality. Rice Product Quality Supervision and Testing Center, Ministry of Agriculture, Beijing, People's Republic of China.

Ardashir, K.M., L.E.W. Daniel, F.R. Russell, W. Rachelle, and J.H. Robert. 2012. SNP in starch biosynthesis genes associated with nutrition and functional properties of rice. Scientific Reports 2:557-565.

Ayres, N.M., A.M. McClung, P.D. Larkin, H.F. Bligh, C.A., Jones, and W.D. Park. 1997. Microsatellite and a single-nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of US rice germplasm. Theoretical and Applied Genetics 94:773-781.

Cai, X.L., Q.Q. Liu, S.Z. Tang, M.H. Gu, and Z.Y. Wang. 2002. Development of a molecular marker for screening the rice cultivars with intermediate amylose content in *Oryza sativa* subsp. *indica*. Journal of Plant Physiology and Molecular Biology 28:137-144 (in Chinese).

Huang, Z.L., X.L. Tan, C.W. Xu, and A. Vanavichit. 2000. Molecular mapping QTLs for gel consistency in rice (*Oryza sativa* L.) Scientia Agricultura Sinica 33:1-5 (in Chinese).

International Rice Genome Sequencing Project. 2005. The map-based sequence of the rice genome. Nature 436:793-800.

Li, Y.B., C.C. Fan, Y.Z. Xing, P. Yun, L.J. Luo, B. Yan, et al. 2014. *Chalk5* encodes a vacuolar H$^+$-translocating pyrophosphatase influencing grain chalkiness in rice. Nature Genetics 46:398-404.

Mikami, I., N. Uwatoko, Y. Ikeda, J. Yamaguchi, H.Y. Hirano, Y. Suzuki, et al. 2008. Allelic diversification at the *wx* locus in landraces of Asian rice. Theoretical and Applied Genetics 116:979-989.

Myint, Y., T.N. Khin, V. Apichart, C.A. Witith, and T. Theerayut. 2009. Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha. Field Crops Research 113:178-186.

Ni, D.H., S.L. Zhang, S. Chen, Y. Xu, L. Li, H. Li, et al. 2011. Improving cooking and eating quality of Xieyou57, an elite *indica* hybrid rice, by marker-assisted selection of the *Wx* locus. Euphytica 179:355-362.

Qiao, W.H., Y.T. Chen, R.S. Wang, X. Wei, L.R. Cao, W.X. Zhang, et al. 2012. Nucleotide diversity in *Waxy* gene and validation of single nucleotide polymorphism in relation to amylose content in Chinese microcore rice germplasm. Crop Science 52:1689-1697.

Su, Y., Y.C. Rao, S.K. Hu, Y.L. Yang, Z.Y. Gao, G.H. Zhang, et al. 2011. Map-based cloning proves *qGC-6*, a major QTL for gel consistency of japonica/indica cross, responds by *Waxy* in rice (*Oryza sativa* L.) Theoretical and Applied Genetics 123:859-867.

Sun, Y.Y., Y. Lv, C.L. Dong, P.R. Wang, X.Q. Huang, and X.J. Deng. 2005. Genetic relationship among *Wx* Gene, AC, GC and GT of rice. Acta Agronomica Sinica 31:535-539 (in Chinese).

Teng, B., R.Z. Zeng, Y.C. Wang, Z.Q. Liu, Z.M. Zhang, H.T. Zhu, et al. 2012. Detection of allelic variation at the *Wx* locus with single-segment substitution lines in rice (*Oryza sativa* L.) Molecular Breeding 30:583-595.

Tian, Z.X., Q. Qian, Q.Q. Liu, M.X. Yan, X.F. Liu, C.J. Yan, et al. 2009. Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. Proceedings of the National Academy of Sciences of the United States of America 106:21760-21765.

Tian, Z.X., C.J. Yan, Q. Qian, S. Yan, H.L. Xie, F. Wang, et al. 2010. Development of gene-tagged molecular markers for starch synthesis-related genes in rice. Chinese Science Bulletin 55:2591-2601.

Zhou, P.H., Y.F. Tan, Y.Q. He, C.G. Xu, and Q. Zhang. 2003. Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. Theoretical and Applied Genetics 106:326-331.