Rice Science, 2007, 14(2): 85-93

Genetic Polymorphism of *Wx* Gene and Its Correlation with Main Grain Quality Characteristics in Rice

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Abstract: The allelic variation of the *Wx* gene in 50 non-glutinous rice varieties (lines) was analyzed by using the microsatellite marker RM190 [for $(CT)_n$ simple sequence repeat (SSR)] and cleaved amplified polymorphic sequence(CAPS) marker 484/W2R-*ACC* Ⅰ[for G/T single nucleotide polymorphism (SNP)]. Six homozygous $(CT)_n$ types, namely $(CT)_{20}$, $(CT)_{19}$, $(CT)_{18}$, $(CT)_{17}$, $(CT)_{16}$, $(CT)_{14}$, $(CT)_{11}$ and $(CT)_{10}$, and a heterozygous genotype $(CT)_{11}/(CT)_{18}$ were detected for RM190, of which $(CT)_{11}$ and $(CT)_{18}$ were predominant. Two homozygous *Wx* genotypes (G/G and T/T) and one heterozygous (G/T) were detected using 484/W2R-*ACC* Ⅰ. Most of the materials with a RM190 of $(CT)_{11}$ were G/G for SNP of 484/W2R-*ACC* I, while T/T for SNP was predominantly appeared in materials with $(CT)_{18}$. The materials tested could be grouped into 10 categories using the two markers together. Results indicated that 59.3% variance of amylose content was attributed to the polymorphism of *Wx* gene revealed by RM190, while 56.1% and 24.6% of the variances in amylose content and gel consistency were respectively to the polymorphism of *Wx* gene revealed by 484/W2R-*ACC* I. Furthermore, with both SSR and CAPS markers, 72.4% of the variance in amylose content could be explained. In addition, the application prospects of the two markers in breeding were also discussed.

Key words: waxy gene; simple sequence repeat; cleaved amplified polymorphic sequence; single nucleotide polymorphism; gelatinization temperature; gel consistency; amylose content; rice

The rice quality mainly refers to appearance, cooking and eating qualities, and the parameters such as amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) are usually used to present eating and cooking qualities for rice. The proportion of amylose to amylopectin influences the structure and characteristics of the starch grain [1], whereas amylose content is the determinant to regulate the quality of rice [2]. It has been indicated that AC is mainly determined by the allelic variance of *Wx* gene and the single nucleotide polymorphism (G or T) at the first nucleotide of splice donor site of *Wx* gene intron 1, which affects the expression of *Wx* gene. If the first nucleotide of intron 1 is a G, the intron could be excised for external splicing normally and the expression level of mature mRNA is high, and then the AC is higher. On the contrary, if it is a T, the intron couldn't be excised to splice normally and the expression quantity of mature mRNA is restrained, so AC is lower [3-5]. Furthermore, there is a $(CT)_n$ repetition sequence [6] at 55 bp upstream of intron 1. These polymorphic alleles are significantly correlated to AC variation [6-11].

Beside the effect of AC, many other factors are also important in determining rice quality. It had been found that among the rice with similar AC, the cooking and eating qualities of rice were quite distinct due to different values of GT and GC and so on [12-13]. Recently, much research has been extensively made to investigate the genetic relationship between *Wx* gene and AC, GC and GT, but they failed to reach an agreement [14-15].

Hereditary studies concerning about the *Wx* gene are getting deeper and deeper, and 16 kinds of *Wx* alleles have been discovered by some researchers through G/T and $(CT)_n$ polymorphism analysis [9-11, 16]. These alleles are evidently related to AC, which contributed to 81.2-91.2% of AC variance [9-11]. However, many issues remain unknown such as the

Received: 22 October 2006; **Accepted:** 5 March 2007
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This paper was translated from its Chinese version in *Chinese Journal of Rice Science* Vol. 20, No. 6, 2006, Pages 603-609.

relationships between these multiple alleles and GC or GT, and the influences of these alleles interpreted by $(CT)_n$ and G/T markers on major quality characteristics of rice. Moreover, it still needs further study on how to breed high quality rice varieties and improve rice qualities by molecular marker-assisted selection.

In the present study, non-glutinous rice varieties (lines) with different amylose contents from different resources were used as the materials to investigate the allelic variance of the *Wx* gene by using markers of 484/W2R-*ACC* I [for G/T single nucleotide polymorphism (SNP)] and RM190 [for $(CT)_n$ simple sequence repeat (SSR)], and the relationships between genetic effect of allelic variance and GT, GC or AC, and to evaluate the influence of *Wx* genotype on GT, GC and AC variance of rice for unveiling the connection between genetic polymorphisms of *Wx* gene and cooking and eating qualities of rice, aiming to provide reference for improving rice quality via marker-assisted selection.

MATERIALS AND METHODS

Rice materials

Fifty non-glutinous rice varieties (lines) with largely different amylose contents and genetic backgrounds were selected. The materials were planted at experimental fields of Rice Research Institute, Sichuan Agricultural University, Wenjiang, Sichuan Province.

GC, GT and AC determination

At the maturity, grains were harvested for testing rice quality. AC, GC and GT of rice were tested by Rice Product Quality Testing Center of the Ministry of Agriculture, China National Rice Research Institute, Hangzhou, China.

DNA extraction, polymerase chain reaction (PCR) and electrophoresis

Genomic DNAs were extracted from leaf tissue at the maximum tillering stage according to CTAB method described by Murray et al [17].

All the primers were synthesized by Invitrogen, and *ACC* Ⅰ enzyme was purchased from TaKaRa. The primer '484/485' designed by Blight et al [6] had even been used to detect $(CT)_n$ repetitive sequence of *Wx* gene, but its PCR products performed smearing and bad repetition, so we designed a pair of new primers, i.e. RM190 (forward: 5'-CTTTGTCTATCTCAAGAC AC-3', reverse: 5'-TTGCAGATGTTCTTCCTGATG-3'), with an amplification ability similar to primer '484/485'. Cleaved amplified polymorphic sequence (CAPS) marker '484/W2R' designed by Ayres et al [9] (forward: 5'-CTTTGTCTATCTCAAGACAC-3', reverse: 5'-TTTCCAGCCCAACACCTTAC-3') was used to test G/T polymorphism of *Wx* gene.

PCR was conducted with a M. J. PTC-220 DNA Engine Dyrad Cycler. The volume of reaction system was 20 μL containing 2 μL primers (50 μmol/L), 2.5 μL DNA (50 ng/μL), 2.0 μL dNTPs (2.5 μmol/L), 2.0 μL 10×buffer, 1 U *Taq*-DNA polymerase, and 11.3 μL ddH_2O. PCR profile included an initial pre-denaturation at 94℃ for 4 min, followed by 35 cycles of 94℃ for 1 min, 55℃ for 45 s and 72℃ for 1 min, and final extension at 72℃ for 10 min.

The PCR products of RM190 were separated by electrophoresis on 6% SDS-PAGE, followed by the fast silver nitrate staining. The target segments were recovered, purified and cloned. An ABI 3730XL DNA analysis system was used to detect products of clones. The products of marker 484/W2R were digested for 2 hours by *ACC* Ⅰ enzyme at 37℃ [reaction system including 10 μL of PCR products, 2 μL of 10×M Buffer, 2 U *ACC* Ⅰ enzyme (10 U/μL), and 7.8 μL of ddH_2O], and finally detected on agarose gels (3%).

Statistical analysis

The materials tested were classified by the results of RM190 and 484/W2R-*ACC* I at $P<0.05$ significance level. Multi-comparison amongst AC, GC and GT of rice in different genotypic materials was conducted using Duncan's method, and influences of genotypes on AC, GC and GT of rice were analyzed according to the method by Chen et al [19].

RESULTS

Genetic polymorphism of *Wx* gene

$(CT)_n$ polymorphism of Wx gene

There were seven homozygous and one heterozygous

genotypes among the 50 materials tested by analyzing the polymorphism of *Wx* gene with RM190 (Fig. 1). Analysis of PCR products indicated that 10 and 23 materials exhibited 11 and 18 repetitive CTs, denoted as $(CT)_{11}$ and $(CT)_{18}$, respectively. In addition, 2, 2, 5, 1 and 3 materials showed $(CT)_{10}$, $(CT)_{14}$, $(CT)_{17}$, $(CT)_{19}$ and $(CT)_{20}$, respectively (Table 1). Forty-six materials were homozygous at the loci, while 4 materials, Xiangdalixuan, 2008R, Shuhui 205 and Hongcheng 20, were heterozygous at the loci including both alleles of $(CT)_{11}$ and $(CT)_{18}$.

Table 1. Distribution of *Wx* alleles detected by 484/W2R-*ACC* Ⅰ and RM190 in the tested materials.

Type	No. of varieties			
	G/G	G/T	T/T	Total
$(CT)_{20}/(CT)_{20}$	3	0	0	3
$(CT)_{19}/(CT)_{19}$	1	0	0	1
$(CT)_{18}/(CT)_{18}$	2	0	21	23
$(CT)_{17}/(CT)_{17}$	0	0	5	5
$(CT)_{14}/(CT)_{14}$	2	0	0	2
$(CT)_{11}/(CT)_{11}$	10	0	0	10
$(CT)_{10}/(CT)_{10}$	2	0	0	2
$(CT)_{11}/(CT)_{18}$	0	3	1	4

G/T polymorphisms of Wx gene

The CAPS marker (484/W2R-*ACC* Ⅰ) was used to detect G/T polymorphism of *Wx* gene (Fig. 2). Among all the materials, 20 were G/G types which showed two fragments with an approximate length of 120 bp (included *ACC* Ⅰ restriction site), 27 were T/T types with an approximate 240 bp fragment (no *ACC* Ⅰ restriction site) and 3 (2008R, Shuhui 205 and Hongcheng 20) were heterozygous G/T types with two different fragments.

Distribution of allelic variation of the *Wx* gene

The materials tested could be grouped into 10 categories using the two markers of RM190 and 484/W2R-*ACC* Ⅰ together (Table 1). The $(CT)_n$ repetition of *Wx* gene leader region was minor among

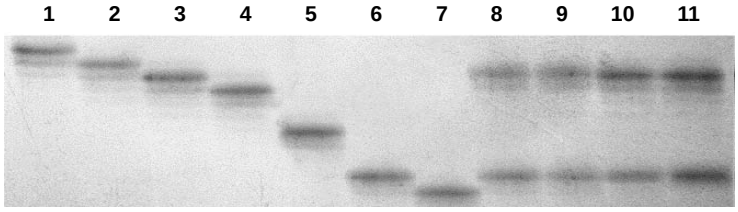

Fig. 1. $(CT)_n$ polymorphism of *Wx* gene unveiled by RM190.

Lane 1, $(CT)_{20}$; Lane 2, $(CT)_{19}$; Lane 3, $(CT)_{18}$; Lane 4, $(CT)_{17}$; Lane 5, $(CT)_{14}$; Lane 6, $(CT)_{11}$; Lane 7, $(CT)_{10}$; Lanes 8 to 11, $(CT)_{11}/(CT)_{18}$ (Lane 8, Xiangdalixuan; Lane 9, 2008R; Lane 10, Shuhui 205; Lane 11, Hongcheng 20).

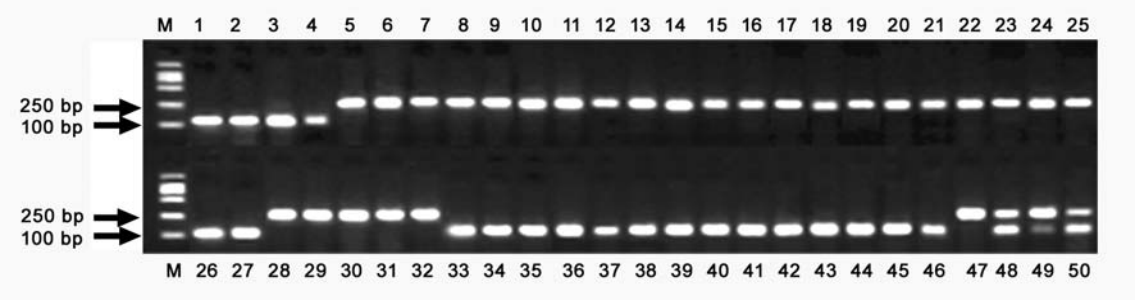

Fig. 2. G/T polymorphism of *Wx* gene unveiled by 484/W2R-*ACC* I.

Varieties represented by corresponding lanes: 1, ADAIR; 2, Balilla; 3, CALMAIL201; 4, Maintmolotsy 1226; 5, 5837; 6, BENGAL; 7, Baodali; 8, Dalidao; 9, Beijing 1; 10, Beijing 3; 11, Beijing 4; 12, Beijing 5; 13, Liaokai 97-3-1; 14, Shuhui 881; 15, Shuhui 202; 16, E540; 17, CDR22; 18, Duohui 1; 19, Xianghui; 20, Mianhui 725; 21, Shuhui 885; 22, Gui 630; 23, Shuhui 527; 24, Luhui 17; 25, D725; 26, Xinshan B; 27, G46B; 28, EARL; 29, Beijing 2; 30, Wuyujing; 31, Zhendao 99; 32, G201B; 33, New Bonnet; 34, Katy; 35, Lehui 188; 36, Xianguo; 37, Maylele; 38, COCODRIE; 39, Qimiaoxiang; 40, Guichao 2; 41, Yihui 1577; 42, IR24-3; 43, Guanghui 128; 44, IR24-2; 45, Diantun 502; 46, Yunhui 290; 47, Xiangdalixuan; 48, 2008R; 49, Shuhui 205; 50, Hongcheng 20.

the T/T genotypes. Among all the materials, 5 and 21 were $(CT)_{17}$ and $(CT)_{18}$, respectively, and $(CT)_{18}$ was predominant type. The materials of T/T-$(CT)_{18}$/$(CT)_{18}$ type accounted for 77.8% and 91.3% in all materials of T/T and $(CT)_{18}$/$(CT)_{18}$, respectively. The $(CT)_n$ repetition of *Wx* gene leader region was complicated in the G/G genotypes, in which 3, 1, 2, 2, 10 and 2 represented $(CT)_{20}$, $(CT)_{19}$, $(CT)_{18}$, $(CT)_{14}$, $(CT)_{11}$ and $(CT)_{10}$, respectively. The materials of G/G-$(CT)_{11}$/$(CT)_{11}$ type accounted for 50% and 100% in the materials of G/G and $(CT)_{11}$/$(CT)_{11}$ types, respectively, so the $(CT)_{11}$ genotype was predominant in G/G-type materials. The allelic genotypes of T/T-$(CT)_{18}$/$(CT)_{18}$ and G/G-$(CT)_{11}$/$(CT)_{11}$ of *Wx* gene were predominant, occupying 62% in all of the tested materials.

Relationship between the allelic variance of *Wx* gene and GC, GT and AC of rice

Relationship among GC, GT and AC of rice

The correlation analysis of AC, GC and GT of rice among the 50 materials indicated that correlations of AC and GC (r=-0.589**), GC and GT (r=-0.323*) were significant, whereas that of AC and GC was not.

AC and GC of rice with different Wx allelic genes

Allelic types of *Wx* gene and major rice quality indexes of the 50 materials were listed in Table 2. According to Table 2, the average value of AC in G/G-type varieties was about 21.5%, higher than that of T/T-type varieties (14.5%) apparently, while that of G/T-type varieties was about 19.9%, between the values of G/G-type and T/T-type varieties. Of the G/G-type varieties, 4 low-AC varieties, namely Yunnan soft rice Diantun 502 and Yunhui 290 belonged to G/G-$(CT)_{10}$/$(CT)_{10}$ type, American rice Maylele with G/G-$(CT)_{11}$/$(CT)_{11}$ type, and Italian rice Balilla with G/G-$(CT)_{20}$/$(CT)_{20}$ type, expressed a fluctuation of AC at 13.0-15.5%, similar to T/T-type materials, and the rest varieties at 20.0-27.4%, with very minor fluctuation rate in different $(CT)_n$-type materials. In all T/T-type materials, the AC fluctuation rate was the smallest in $(CT)_{18}$/$(CT)_{18}$-type varieties (lines) (12.8-15.8%), while the largest in $(CT)_{17}$/$(CT)_{17}$-type materials (10.6-19.0%). In all T/T-$(CT)_{18}$/$(CT)_{18}$-type materials, AC fluctuated gently but GC changed within a wider range from 38 to 78 mm. In all of the G/G-$(CT)_{18}$/$(CT)_{18}$ type materials, the mean of AC was 22.2%, obviously higher than 14.3%, the mean of ACs in T/T-$(CT)_{18}$/$(CT)_{18}$-type varieties (lines).

Correlation analysis of GT, GC and AC of rice with allelic variance interpreted by 484/W2R-ACC Ⅰ

Statistical analysis of polymorphism among materials tested by 484/W2R-*ACC* Ⅰ (Table 3) indicated that GT showed no prominent difference in different genotypic varieties (lines), while GC and AC were significant. The mean of ACs in T/T-type materials (lines) was 14.5%, obviously lower than that in G/C-type materials (lines), 21.5%. However, the mean of GCs in T/T-type materials (lines) was 52.2 mm, obviously higher than that of G/G-type materials (lines), 40.6 mm. Genotypes interpreted by 484/W2R-*ACC* Ⅰ (r=-0.749**) was significantly and negatively correlated to AC, but significantly and positively correlated to GC (r=0.496**). In other words, 24.6% of GC variance and 56.1% of AC variance could be explained by genetic polymorphism of *Wx* gene unveiled by 484/W2R-*ACC* Ⅰ.

Relationship between GT, GC and AC and allelic variance of Wx gene interpreted by RM190

Statistical analysis of polymorphism among materials tested by RM190 (Table 4) indicated that GT and GC were no obvious difference in various varieties (lines) with different genotypes, while AC was significantly different among genotypes. The ACs of $(CT)_{11}$/$(CT)_{11}$, $(CT)_{14}$/$(CT)_{14}$, $(CT)_{19}$/$(CT)_{19}$ and $(CT)_{11}$/$(CT)_{18}$-type varieties were significantly higher than those of $(CT)_{10}$/$(CT)_{10}$, $(CT)_{17}$/$(CT)_{17}$ and $(CT)_{18}$/$(CT)_{18}$-type varieties. Genotypes unveiled by RM190 were significantly and negatively correlated with AC (r=-0.770**), and 59.3% of AC variance was attributed to genetic polymorphism of *Wx* gene.

Correlation analysis of GT, GC and AC with allelic variance of Wx gene interpreted by RM190 and 484/W2R-ACC Ⅰ together

As shown in Table 5, GT and GC were not obviously different in various varieties or lines with different genotypes, while AC was significantly

Table 2. Allelic genotypes and their values of GT, GC and AC of rice.

Allelic genotype		Variety	Gelatinization temperature (grade)	Gel consistency (mm)	Amylose content (%)
G/T	$(CT)_n$				
G/G	$(CT)_{20}/(CT)_{20}$	ADAIR	5.0	36.0	22.9
		Balilla	4.6	41.0	13.6
		CALMAIL201	5.0	35.0	20.0
	$(CT)_{19}/(CT)_{19}$	Maintmolotsy 1226	5.0	42.0	21.3
	$(CT)_{18}/(CT)_{18}$	Xinshan B	6.0	35.0	22.7
		G46B	6.0	34.0	21.7
	$(CT)_{14}/(CT)_{14}$	New Bonnet	5.0	34.0	20.9
		Katy	6.0	46.0	22.5
	$(CT)_{11}/(CT)_{11}$	Lehui 188	6.0	40.0	24.0
		Xianguo	6.0	34.0	24.2
		Maylele	4.5	46.0	13.0
		COCODRIE	5.0	68.0	23.6
		Qimiaoxiang	7.0	31.0	23.7
		Guichao 2	7.0	31.0	27.4
		Yihui 1577	7.0	41.0	26.0
		IR24-3	7.0	48.0	25.3
		Guanghui 128	6.0	32.0	23.1
		IR24-2	7.0	38.0	23.9
	$(CT)_{10}/(CT)_{10}$	Diantun 502	7.0	58.0	13.7
		Yunhui 290	7.0	42.0	15.5
T/T	$(CT)_{18}/(CT)_{18}$	5837	4.5	68.0	12.8
		BENGAL	7.0	45.0	14.5
		Baodali	7.0	40.0	15.0
		Dalidao	7.0	41.0	15.4
		Beijing 1	7.0	50.0	15.1
		Beijing 3	5.7	38.0	15.8
		Beijing 4	5.2	48.0	15.6
		Beijing 5	7.0	46.0	15.3
		Liaokai 97-3-1	7.0	45.0	14.6
		Shuhui 881	6.5	54.0	14.1
		Shuhui 202	4.5	68.0	13.8
		E540	7.0	50.0	13.9
		CDR22	4.5	72.0	13.9
		Duohui 1	7.0	44.0	13.3
		Xianghui	5.0	56.0	13.4
		Mianhui 725	7.0	53.0	15.1
		Shuhui 885	5.0	78.0	13.7
		Gui 630	7.0	57.0	14.3
		Shuhui 527	5.0	57.0	13.5
		Luhui 17	7.0	49.0	13.8
		D725	4.5	54.0	12.9
	$(CT)_{17}/(CT)_{17}$	EARL	7.0	52.0	14.2
		Beijing 2	7.0	38.0	17.5
		Wuyujing	6.8	40.0	19.0
		Zhendao 99	7.0	58.0	15.7
		G201B	4.2	62.0	10.6
	$(CT)_{11}/(CT)_{18}$	Xiangdalixuan	7.0	45.0	15.7
G/T	$(CT)_{11}/(CT)_{18}$	2008R	5.4	31.0	21.3
		Shuhui 205	7.0	42.0	22.1
		Hongcheng 20	7.0	50.0	16.2

Table 3. Means of gelatinization temperature (GT), gel consistency (GC) and amylose content (AC) of rice among varieties (or lines) with different genotypes classified by 484/W2R-*ACC* Ⅰ.

Genotype	Number of varieties	Gelatinization temperature (Grade)	Gel consistency (mm)	Amylose content (%)
G/G	20	6.0 a	40.6 b	21.5 a
G/T	3	6.5 a	41.0 b	19.9 a
T/T	27	6.2 a	52.2 a	14.5 b

Mean values within a column followed by the common letters are not significantly different at $P<0.05$.

Table 4. Means of gelatinization temperature (GT), gel consistency (GC) and amylose content (AC) of rice among varieties (or lines) with different genotypes classified by RM190.

Genotype	Number of varieties	Gelatinization temperature (Grade)	Gel consistency (mm)	Amylose content (%)
$(CT)_{10}/(CT)_{10}$	2	7.0 a	50.0 a	14.6 b
$(CT)_{11}/(CT)_{11}$	10	6.3 a	40.9 a	23.4 a
$(CT)_{14}/(CT)_{14}$	2	5.5 a	40.0 a	21.7 a
$(CT)_{17}/(CT)_{17}$	5	6.4 a	50.0 a	15.4 b
$(CT)_{18}/(CT)_{18}$	23	6.1 a	51.4 a	15.0 b
$(CT)_{19}/(CT)_{19}$	1	5.0 a	42.0 a	21.3 a
$(CT)_{20}/(CT)_{20}$	3	4.9 a	37.3 a	18.8 ab
$(CT)_{11}/(CT)_{18}$	4	6.6 a	42.0 a	18.8 a

Mean values within a column followed by the common letters are not significantly different at $P<0.05$.

Table 5. Means of gelatinization temperature (GT), gel consistency (GC) and amylose content (AC) among varieties with different genotypes classified by markers of 484/W2R-*ACC* Ⅰ and RM190 jointly.

Genotype	Number of varieties	Gelatinization temperature (Grade)	Gel consistency (mm)	Amylose content (%)
G/G-$(CT)_{20}/(CT)_{20}$	3	4.9 a	37.3 a	18.8 abc
G/G-$(CT)_{19}/(CT)_{19}$	1	5.0 a	42.0 a	21.3 a
T/T-$(CT)_{18}/(CT)_{18}$	21	6.1 a	53.0 a	14.3 c
G/G-$(CT)_{18}/(CT)_{18}$	2	6.0 a	34.5 a	22.2 a
T/T-$(CT)_{17}/(CT)_{17}$	5	6.4 a	50.0 a	15.4 bc
G/G-$(CT)_{14}/(CT)_{14}$	2	5.5 a	40.0 a	21.7 a
G/G-$(CT)_{11}/(CT)_{11}$	10	6.3 a	40.9 a	23.4 a
G/G-$(CT)_{10}/(CT)_{10}$	2	7.0 a	50.0 a	14.6 bc
T/T-$(CT)_{11}/(CT)_{18}$	1	7.0 a	45.0 a	15.7 bc
G/T-$(CT)_{11}/(CT)_{18}$	3	6.5 a	41.0 a	19.9 ab

Mean values within a column followed by the common letters are not significantly different at $P<0.05$.

different among genotypes. Mean of ACs in G/G-type varieties (lines) [$(CT)_{19}/(CT)_{19}$, $(CT)_{18}/(CT)_{18}$, $(CT)_{14}/(CT)_{14}$ and $(CT)_{11}/(CT)_{11}$] was apparently higher than that of T/T-type varieties (lines) [$(CT)_{18}/(CT)_{18}$, $(CT)_{17}/(CT)_{17}$, $(CT)_{11}/(CT)_{18}$] and G/G-type varieties (lines) [$(CT)_{10}/(CT)_{10}$]. Genetic polymorphism of *Wx* gene interpreted by these two markers was significantly related to AC (r=0.851**, R^2=0.724), or 72.4% of AC variance was attributed to allelic variance of *Wx* gene unveiled by RM190 and 484/W2R-*ACC* Ⅰ together.

DISCUSSION

Genetic polymorphism of *Wx* gene

It has been reported that *Wx* gene is multi-allelic, and 16 separate *Wx* alleles have been identified through allelic analysis of *Wx* gene by using polymorphisms of $(CT)_n$ and G/T. In the present study, fifty rice materials from different origins had been used to perform allelic variance analysis of *Wx* gene,

and 10 alleles were detected, including eight homozygous genotypes [G/G-$(CT)_{20}/(CT)_{20}$, G/G-$(CT)_{19}/(CT)_{19}$, T/T-$(CT)_{18}/(CT)_{18}$, G/G-$(CT)_{18}/(CT)_{18}$, T/T-$(CT)_{17}/(CT)_{17}$, G/G-$(CT)_{14}/(CT)_{14}$, G/G-$(CT)_{11}/(CT)_{11}$ and G/G-$(CT)_{10}/(CT)_{10}$], and two heterozygous ones [T/T-$(CT)_{11}/(CT)_{18}$ and G/T-$(CT)_{11}/(CT)_{18}$]. Two heterozygous genotypes might generate a new allelic genotype T/T-$(CT)_{11}/(CT)_{11}$ at homozygotic state. This will enrich classes of allelic variance of *Wx* gene, and may play a pivotal role in studying genetic effect of *Wx* gene and improving rice quality.

Correlation between genetic polymorphisms of *Wx* gene and rice quality

Four varieties or lines with low AC were selected from G/G-type materials via analyzing AC. Two of them were Yunnan soft rice Diantun 502 and Yunhui 290 whose genotypes were G/G-$(CT)_{10}/(CT)_{10}$, and AC were 13.7% and 15.5%, respectively, apparently lower than the mean value of 21.5% in G/G-type varieties, suggesting that $(CT)_{10}/(CT)_{10}$-type mutation might be one factor resulting in low AC in G/G-type materials. Zeng et al [20] mapped a recessive gene controlling soft rice trait in F_2 generation of IR36/Babaomi, with a genetic distance of 18.8 cM to RM190. Thus, it needs further research to determine the causal genetic factor resulting in low AC by polymorphic mutation of $(CT)_n$ in *Wx* gene or by other unknown factors in Yunnan soft rice varieties Diantun 502 and Yunhui 290. The AC of the other low AC genotypes, American rice Maylele with G/G-$(CT)_{11}/(CT)_{11}$ and Italian rice variety Balilla with G/G-$(CT)_{20}/(CT)_{20}$ were 13.0% and 13.6%, respectively, lower than the mean values of the other materials with G/G-$(CT)_{11}/(CT)_{11}$ and G/G-$(CT)_{20}/(CT)_{20}$ genotypes (23.4% and 18.8%). Sato et al [21] found that low AC was controlled by a *Wx-mq* gene through genetic analysis of low AC varieties. Recently, some researchers reported that low AC was also controlled by other allelic *Wx* genes such as Wx^{OP} [22], and non-allelic genes such as *du* gene and *lam*(*t*) gene [23] located on chromosome 9 in rice. Therefore, it was presumed that the low AC of these two materials is controlled by other factors along with the allelic *Wx* gene unveiled by RM190 and 484/W2R-*ACC* Ⅰ.

Moreover, AC of T/T-$(CT)_{17}/(CT)_{17}$-type distributed equally between 10.6% and 19.0%. Ge et al [24] found that an upstream fragment with a length of 31 bp could increase the expression level of the genes, indicating that AC variance might be controlled by some minor factors. As a result, the AC was possibly controlled by allelic *Wx* gene, non-allelic *Wx* gene and minor factors except for allelic *Wx* gene unveiled by RM190 and 484/W2R-*ACC* Ⅰ.

Previous study showed that GC is controlled by seed genetic effect, cytoplasmic effect, endosperm genotype and interactions [25], and the seed genetic effects are mainly controlled by a number of multi-allelic genes and minor genes. For instance, the dominant effect of GC exhibited hard against medium hard or soft, and medium hard against soft [15]. In the current study, GC is related to polymorphic G/T loci of *Wx* gene, but AC variance of genotypes [T/T-$(CT)_{18}/(CT)_{18}$ and G/G-$(CT)_{11}/(CT)_{11}$] was small and the GC variance was large. This indicated that GC was controlled by other genes except for *Wx* gene. He et al [26] mapped two QTLs on chromosomes 2 and 7, which explained 20.2% and 14.2% of GC variance, respectively. Huang et al [27] pointed out that GC was controlled by two linked loci on chromosome 3. Therefore, we could conclude that heredity of GC in rice is presumably controlled by a few major genes, cytoplasmic effect, endosperm effect and interactions jointly.

In terms of GT, it has no relationship with genetic polymorphism of *Wx* gene. The previous study had showed that *ALK* on the short arm of chromosome 6 acts as the major gene to control GT, which had been cloned [28], indicating that the major gene controlling GT in rice is not the *Wx* gene locus. This is in agreement with the current research.

Feasibility by MAS to improve rice quality

The current study found that GC variance was significantly correlated with G/T polymorphism but not with $(CT)_n$ polymorphism, suggesting CAPS marker 484/W2R-*ACC* Ⅰ could be used in improvement of rice quality by MAS. Based on AC analysis, *Wx* genes were heterozygous in $(CT)_n$ and G/T loci in some advanced generations (lines) or commercial varieties, such as 2008R, Shuhui 205 and Hongcheng 20. In addition, $(CT)_n$ of *Wx* gene was not completely

linked to G/T, thus in breeding practice, breeders should concern about AC more strictly and carefully and pay more attention to the homozygosity of *Wx* gene, due to that the heterozygous *Wx* gene will result in segregation of AC and GC in new generations.

Moreover, neither of the two markers in this study could be used to explain AC variance, for instance, in $(CT)_{18}$/$(CT)_{18}$-type varieties (lines), AC of G/G-type was apparently higher than that of T/T-type. In G/G-type varieties (lines), AC varied in different $(CT)_n$-repetitive varieties. Statistic data indicated that AC variance could be well explained by the two markers together (72.4%), being 20% higher than by either of the two markers. Therefore, the quality indicator AC could be screened more effectively by RM190 and 484/W2R-*ACC* Ⅰ jointly than by either of the two markers.

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