Identification of Multiple Alleles at the Wx Locus and Development of Single Segment Substitution Lines for the Alleles in Rice

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Abstract: The microsatellite markers 484/485 and 484/W2R were used to identify the multiple alleles at the Wx locus in rice germplasm. Fifteen alleles were identified in 278 accessions by using microsatellite class and G-T polymorphism. Among these alleles, (CT)_{12}-G, (CT)_{15}-G, (CT)_{16}-G, (CT)_{17}-G, (CT)_{18}-G and (CT)_{21}-G have not been reported. Seventy-two single-segment substitution lines (SSSLs) carrying different alleles at the Wx locus were developed by using Huajingxian 74 with the (CT)_{11}-G allele as a recipient and 20 accessions containing 12 different alleles at the Wx locus as donors. The estimated length of the substituted segments ranged from 2.2 to 77.3 cM with an average of 17.4 cM.

Key words: rice; waxy gene; single segment substitution line; allelic variation; molecular marker-assisted selection

Amylose content (AC) is a key determinant of the cooking and processing quality of rice (Oryza sativa). It has been reported that the amylose content is mainly controlled by the Wx locus [1]. By using RFLP analysis, two alleles, largely corresponding to the indica and japonica subspecies of rice, have been identified at the Wx locus [2]; however, they are not adequate to explain all of the variation in apparent amylose content in rice. Bligh et al. [4] found a polymorphic microsatellite in the sequence of the Wx gene [3] and designed the pair of microsatellite primers '484/485'. Using the primers, nine (CT)$_n$ alleles have been identified [4-7]. It was found that the amylose content in rice endosperm was regulated at the transcript processing level, and more specifically, at the stage of intron I excision from the Wx pre-mRNA[8]. Ayres et al. [5] demonstrated that all of the cultivars with 18% or less amylose had the sequence AGTTATA at the putative leader intron 5' splice site, while all cultivars with a higher proportion of amylose had AGGTATA. This single nucleotide polymorphism could be assayed by Acc I digestion, viz. amplified fragments containing the sequence AGGTATA could be cleaved by Acc I, while the corresponding amplified fragments from cultivars having AGTTATA could not [9-12]. Thus far, 10 Wx multiple alleles had been identified using the polymorphic microsatellite class and the G-T polymorphism [4-7].

The Wx multiple alleles are prominently relative to the amylose content in rice endosperm, which together explained 82.9-91.2% of the variations in apparent amylose content of the rice [5-7]. Shu et al. [13] also confirmed that there was a high relativity between the (CT)$_n$ polymorphisms and the amylose content in a segregation population. Because the expression of the Wx gene is significantly affected by genetic background, it is necessary to precisely evaluate the genetic effect of the Wx multiple alleles using the materials with the same genetic background.

Chromosome segment substitution lines (CSSLs) or introgression lines (ILs) are substituted one or more chromosome segments from a donor in a genetic background of recipient [14-18]. The mapping populations comprised of single segment substitution lines (SSSLs), each containing a single homozygous marker-defined chromosome segment from a donor, could increase the ability of geneticists to dissect a quantitative trait [19-20]. In this study, a set of the multiple alleles at the Wx locus in rice germplasm were identified and then a series of the SSSLs
carrying different Wx alleles were developed by using advanced backcrosses and microsatellite marker-assisted selection.

**MATERIALS AND METHODS**

**Materials**

Two hundred and seventy-eight rice accessions involving the indica and japonica subspecies were employed to identify the alleles at the Wx locus. Twenty of them, carrying different alleles at the Wx locus, were used as donors to develop SSSLs in the genetic background of elite cultivar ‘Huajingxian 74’ (Table 1). All materials were planted in the field at the experimental farm of South China Agricultural University in Guangzhou.

**DNA extraction**

The DNA extraction of 278 rice accessions was conducted followed the CTAB method \[^{22}\]. Mini-scale DNA extraction for developing SSSLs was carried out according to the procedure described by Zheng et al \[^{23}\].

**SSR analysis**

SSR analysis was carried out according to the procedure described by Li et al \[^{24}\]. The microsatellite markers 484/485 and 484/W2R \[^{5}\] were used to identify the Wx multiple alleles. The PCR products amplified by 484/W2R were digested by the restriction endonuclease Acc I incubated at 37°C overnight. All samples were electrophoresed on 6% polyacrylamide gel. Five hundred and forty-nine SSR markers selected at an average marker interval about 2.8 cM on the rice microsatellite map \[^{25-26}\] were employed to survey the polymorphisms between the 20 donors and recipient ‘Huajingxian 74’. The polymorphism markers were used for detecting substitution segments and analyzing the recipient genetic background. All SSR markers were synthesized according to the design of Cornell University (RM primer) \[^{25}\], Akagi et al (OSR primer) \[^{27}\] and our laboratory (PSM primer), respectively \[^{26}\].

**Development of single segment substitution lines**

The backcrossing and SSR marker-assisted selection technologies were adopted for the development of SSSLs \[^{18}\]. The Wx genotypes in individual plants of each backcross generation were examined using the microsatellite marker 484/485. Markers with an average interval about 5 cM on 12 chromosomes were selected to detect substituted segments on the whole genomes from BC2F1 until the SSSLs carrying different Wx alleles were developed.

**Estimation of the length of substituted segments in the SSSLs**

The length of substituted chromosome segments in SSSLs was estimated based on graphical genotypes \[^{28}\]. A chromosome segment flanked by two markers of donor type (DD) is considered as 100% donor type, flanked by two markers of recipient type (RR) as 0% donor type, and flanked by one marker of donor type

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**Table 1. Origin and type of the parents used for development of SSSLs.**

<table>
<thead>
<tr>
<th>Parent Origin Type</th>
<th>Recipient Huajingxian 74 China indica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td></td>
</tr>
<tr>
<td>Tetep</td>
<td>Vietnam indica</td>
</tr>
<tr>
<td>Zhong 4188</td>
<td>China indica</td>
</tr>
<tr>
<td>BG367</td>
<td>Bangladesh indica</td>
</tr>
<tr>
<td>Zihui 100</td>
<td>China indica</td>
</tr>
<tr>
<td>Katy</td>
<td>USA japonica</td>
</tr>
<tr>
<td>IR66897B</td>
<td>IRRI japonica</td>
</tr>
<tr>
<td>Suyunuo (Glutinous rice)</td>
<td>China japonica</td>
</tr>
<tr>
<td>IR64</td>
<td>IRRI japonica</td>
</tr>
<tr>
<td>Nanyangzhan</td>
<td>China japonica</td>
</tr>
<tr>
<td>Basmati 370</td>
<td>Pakistan indica</td>
</tr>
<tr>
<td>IR58025B</td>
<td>IRRI indica</td>
</tr>
<tr>
<td>Jiangxisiminiao</td>
<td>China indica</td>
</tr>
<tr>
<td>American jasmine</td>
<td>USA indica</td>
</tr>
<tr>
<td>Ganzhiangma(Glutinous rice)</td>
<td>China indica</td>
</tr>
<tr>
<td>IR66167-27-5-1-6</td>
<td>IRRI japonica</td>
</tr>
<tr>
<td>Chenglongshuijingmi</td>
<td>China indica</td>
</tr>
<tr>
<td>IR65598-112-2</td>
<td>IRRI japonica</td>
</tr>
<tr>
<td>Lemont</td>
<td>USA japonica</td>
</tr>
<tr>
<td>Star bonnet 99</td>
<td>USA japonica</td>
</tr>
<tr>
<td>IAPAR9</td>
<td>Brazil japonica</td>
</tr>
</tbody>
</table>

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and one marker of recipient type (DR) as 50% donor type. In other words, the length of DD plus the length of two half DR was considered to be the expected length of a substituted chromosome segment.

RESULTS

Identification of multiple alleles at the \(Wx\) locus

The \((CT)_n\) polymorphisms at the \(Wx\) locus

Six rice cultivars including Tetep, IR72, Teqing, Katy, IR64 and Lemont, which were used by Ayres et al.\(^5\) and Shu et al.\(^6\), were adopted as contrasts in the study. Twelve \((CT)_n\) alleles at the \(Wx\) locus were identified in 278 rice accessions using microsatellite markers \(484/485\). The amplified products contained \((CT)_n\) repeats between \(n = 8\) and \(n = 21\) (Fig. 1-A). About 82.4% of the accessions were of the alleles with \((CT)_{11}\), \((CT)_{17}\) or \((CT)_{18}\) while only one accession with the allele of \((CT)_{19}\) or \((CT)_{21}\) (Table 2).

The G-T polymorphisms at the \(Wx\) Locus

PCR amplification of the DNA samples of 278 rice accessions were carried out by using of the primer pair \(484/W2R\). After digested by the restriction endonuclease \(Acc\) I, the amplified products were electrophoresed on 6% polyacrylamide gel (Fig. 1-B). The results indicated that the PCR products from the accessions with \((CT)_{16}\), \((CT)_{17}\), \((CT)_{19}\), \((CT)_{20}\) or \((CT)_{21}\) genotype could be digested by \(Acc\) I (Table 2). This suggested that the G-T polymorphism at the \(Wx\) locus in the accessions could be G. Most of the amplified products from the accessions with \((CT)_{16}\), \((CT)_{17}\) and \((CT)_{18}\) could not be digested by \(Acc\) I and their G-T polymorphism at the \(Wx\) locus could be T. As a result, the genotypes of \((CT)_{16}\), \((CT)_{17}\) and \((CT)_{18}\) could be further divided into \((CT)_{16}-G\), \((CT)_{17}-G\), \((CT)_{18}-G\), \((CT)_{16}-T\), \((CT)_{17}-T\) and \((CT)_{18}-T\) according to the G-T polymorphism.

By using of the \((CT)_n\) class and G-T polymorphism, fifteen alleles at \(Wx\) locus were identified in 278 accessions (Table 2).

Development of single segment substitution lines carrying different \(Wx\) alleles

Single segment substitution lines were developed by using of the indica cultivar Huajingxian 74 with \((CT)_{11}-G\) allele as recipient and the accessions with different \(Wx\) alleles as donors. Seventy-two SSSLs carrying different \(Wx\) alleles were developed using microsatellite marker-assisted selection. The substituted segments in the SSSLs came from 20

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*Fig. 1. Band patterns of multiple alleles at the \(Wx\) locus in rice.

A, \((CT)_n\) class at the \(Wx\) locus; B, G-T polymorphism at the \(Wx\) locus.

Lane 1, \((CT)_8\) (Tetep); Lane 2, \((CT)_{10}\) (IR72); Lane 3, \((CT)_{11}\) (Teqing); Lane 4, \((CT)_{12}\) (Zihui 100); Lane 5, \((CT)_{14}\) (Katy); Lane 6, \((CT)_{15}\) (IR66897B); Lane 7, \((CT)_{16}\) (Nutsuriwai); Lane 8, \((CT)_{17}\) (IR64); Lane 9, \((CT)_{19}\) (IR66167-27-5-1-6); Lane 10, \((CT)_{20}\) (IR65598-112-2); Lane 11, \((CT)_{21}\) (Lemont); Lane 12, \((CT)_{21}\) (Yuanzi 2).*
accessions with 12 Wx alleles. There were 17 SSSLs with (CT)$_{17}$-T allele while only one SSSL with (CT)$_{14}$-G or (CT)$_{18}$-G allele. The estimated lengths of the substituted segments in 72 SSSLs ranged from 2.2 to 77.3 cM with an average of 17.4 cM (Table 3 and Fig. 2).

**DISCUSSION**

Apparent amylose content has been found to be mainly controlled by the Wx locus. Using RFLP analysis, Sano et al.\(^2\) identified two alleles at the Wx locus: Wx\(^a\) allele which predominates in indica subspecies of rice. However, the two alleles are not adequate to explain all of the observed variation in apparent amylose content in rice. By using the (CT)$_n$ class and G-T polymorphism, 10 Wx alleles, (CT)$_8$-G, (CT)$_{10}$-G, (CT)$_{11}$-G, (CT)$_{14}$-G, (CT)$_{16}$-T, (CT)$_{17}$-T, (CT)$_{18}$-T, (CT)$_{19}$-G, (CT)$_{19}$-T and (CT)$_{20}$-G, had been identified at the Wx locus\(^3-7\). In this study, a total of 15 Wx alleles were identified in 278 rice accessions using microsatellite class and G-T polymorphism. Among the 15 Wx alleles, 6 alleles, (CT)$_{12}$-G, (CT)$_{15}$-G, (CT)$_{16}$-G, (CT)$_{17}$-G, (CT)$_{18}$-G and (CT)$_{21}$-G, had not been reported. The findings of those new

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Table 2. Distribution of Wx alleles detected by the microsatellite class and G-T polymorphism in rice germplasm.

<table>
<thead>
<tr>
<th>Type</th>
<th>(CT)$_n$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digested by Acc I</td>
<td>8 10 11 12 14 15 16 17 18 19 20 21</td>
<td>410</td>
</tr>
<tr>
<td>Undigested by Acc I</td>
<td>0 0 0 0 0 2 4 12 6 1 17 1</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>2 13 105 5 4 2 4 4 83 1</td>
<td>278</td>
</tr>
</tbody>
</table>

Table 3. Origin and length of the substituted segments in the SSSLs with multiple alleles at the Wx locus.

<table>
<thead>
<tr>
<th>Wx genotype</th>
<th>Donor</th>
<th>Number of SSSLs</th>
<th>Length range of substituted segments (cM)</th>
<th>Average length of substituted segments (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CT)$_{8}$-G</td>
<td>Tetep</td>
<td>6</td>
<td>8.0-38.7</td>
<td>20.9</td>
</tr>
<tr>
<td>(CT)$_{10}$-G</td>
<td>Zhong 4188</td>
<td>7</td>
<td>2.2-14.8</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>BG367</td>
<td>4</td>
<td>2.2-41.7</td>
<td>15.9</td>
</tr>
<tr>
<td>(CT)$_{12}$-G</td>
<td>Zihui 100</td>
<td>2</td>
<td>2.8-6.8</td>
<td>4.8</td>
</tr>
<tr>
<td>(CT)$_{15}$-G</td>
<td>Zaty</td>
<td>1</td>
<td>8.2</td>
<td>8.2</td>
</tr>
<tr>
<td>(CT)$_{16}$-G</td>
<td>IR66897B</td>
<td>3</td>
<td>5.6-36.0</td>
<td>19.0</td>
</tr>
<tr>
<td>(CT)$_{17}$-G</td>
<td>Suyunuo</td>
<td>4</td>
<td>6.5-9.5</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>BG367</td>
<td>2</td>
<td>2.2-11.5</td>
<td>6.1</td>
</tr>
<tr>
<td>(CT)$_{17}$-T</td>
<td>IR66167-27-5-1-6</td>
<td>1</td>
<td>16.3</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>BG367</td>
<td>4</td>
<td>2.2-11.5</td>
<td>25.3</td>
</tr>
<tr>
<td>(CT)$_{18}$-G</td>
<td>IR58052B</td>
<td>7</td>
<td>2.2-11.5</td>
<td>25.3</td>
</tr>
<tr>
<td>(CT)$_{18}$-T</td>
<td>Jiangxisimiao</td>
<td>4</td>
<td>2.2-11.8</td>
<td>25.3</td>
</tr>
<tr>
<td>(CT)$_{20}$-G</td>
<td>American jasmine</td>
<td>2</td>
<td>2.2-11.8</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>Nanyangzhan</td>
<td>105</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>(CT)$_{21}$-G</td>
<td>Basmati 370</td>
<td>1</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>(CT)$_{21}$-T</td>
<td>Nanyangzhan 2</td>
<td>2</td>
<td>8.5-48.0</td>
<td>28.2</td>
</tr>
<tr>
<td></td>
<td>IR65598-11-2</td>
<td>4</td>
<td>16.3</td>
<td>16.3</td>
</tr>
<tr>
<td>(CT)$_{23}$-G</td>
<td>Chenglongshujiangmi</td>
<td>2</td>
<td>8.8-50.3</td>
<td>29.5</td>
</tr>
<tr>
<td>(CT)$_{24}$-G</td>
<td>Star bonnet 99</td>
<td>4</td>
<td>10.8-42.0</td>
<td>19.6</td>
</tr>
<tr>
<td>(CT)$_{25}$-G</td>
<td>Lemont</td>
<td>7</td>
<td>2.2-26.0</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>IAPAR9</td>
<td>4</td>
<td>9.5-26.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>2.2-77.3</td>
<td>17.4</td>
<td></td>
</tr>
</tbody>
</table>
alleles will promote the study on the genetic evolution of the Wx locus and play important role in the improvement of amylose content in rice.

The relationship between Wx gene and amylose content had been investigated using rice germplasm as materials. The results revealed that the genotype at the Wx locus accounts for most of the variation in amylose content [5-7, 12-13]. Nevertheless, the relationship needs to be further evaluated by using of the materials with the same genetic background because the expression of the Wx gene was obviously affected by genetic background and environment. In the present study, 72 SSSLs with 12 different alleles at the Wx locus coming from 20 accessions were developed. This will provide useful tools for more accurately evaluating the genetic effects of the multiple alleles at the Wx locus and the relationship between the Wx gene and amylose content in rice.

ACKNOWLEDGEMENTS

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REFERENCES


