A One-Step PCR Method for Detecting the First Base of Splice Donor of Wx Intron 1 in Rice

MAO Xing-xue, LIU Yan-zhuo, XIAO Xin, CHEN Jian-wei, LUO Wen-yong, LI Xiao-fang
(Guangdong Key Laboratory of New Biotechnology in Rice Breeding; Rice Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China)

Abstract: A new method of one-step PCR was devised for detecting the first nucleotide in the splice donor site of Wx intron 1. Compared to the regular PCR-Acc I method, the method can produce the same result for detecting +1 nucleotide of Wx intron 1. The reliability of the new method was confirmed with 30 rice varieties. The new technique is more convenient and cheaper than the regular PCR-Acc I method, and could be widely deployed in rice molecular marker assistant selection.

Key words: rice; amylose content; polymerase chain reaction; research methodology

Rice eating quality is mainly controlled and affected by its starch properties. Gelatinization temperature, gel consistency and amylose content are three important properties of rice grain quality, and these three indexes are correlated significantly in a linear fashion [1,2]. Of them, amylose content plays a key role in rice quality, and it has an inheritable nature [3]. Rice amylose is synthesized by granule-bound starch synthase (GBSS), which is coded by Wx gene [4]. In addition, the amylose content is governed by the splicing efficiency of the first intron of Wx gene [5], and the splicing efficiency is subsequently determined by the type of +1 nucleotide of the intron 1 [6,7]. If the first nucleotide in the splice donor site of Wx intron 1 is G, the natural splicing will happen and more mature Wx mRNA will be appeared. In such case, the corresponding GBSS protein will be increased and rice amylose content will be higher in mature grains. In converse, if the first nucleotide of the splice donor site is T, an alternate low-efficiency splicing will happen instead, resulting in reduced mature Wx mRNA and reduced GBSS in endosperm and thereby finally resulting in reduced amylose content in grain. In general, the first nucleotide of Wx intron 1 is G for cultivars with intermediate or high amylose content [5]. Therefore, the detection of the first nucleotide type of Wx intron 1 will facilitate the distinction of rice varieties or breeding lines with varying amylose content. Method of PCR-Acc I is popularly used today, but it is quite complicated, costly and does not adapt to detect a large quantity of samples for rice breeding.

MATERIALS AND METHODS

Materials

Thirty leading rice cultivars in Guangdong Province, provided by Rice Research Institute, Guangdong Academy of Agricultural Sciences, were planted in Dafeng Experimental Farm, Guangdong Academy of Agricultural Sciences during the late season of 2003.

Leaf total DNA extraction

Total DNA in rice leaf was extracted as followed by Murray et al [8].

PCR-Acc I method

Based on the procedure described by Cai et al [9], the +1 nucleotide of Wx gene intron 1 was detected except that the PCR primer pair Wx-F (5′ CTTTTGCTATCTCAGAC 3′) and AC-2 (5′ TCAGCCTACAAACATAACGAA 3′) were adopted.

One-step PCR method

PCR amplification was performed using primers AC-1 (5′ TCAGGAAGAACATCTGCAAGG 3′) and AC-2 (5′ TCAGCCTACAAACATAACGAA 3′) in 15 µL reaction solution containing 1.5 µL 10×PCR buffer (Tris-HCl 10 mmol/L, pH 8.3, KCl 50 mmol/L, glutin 0.001%), 3 µL 25 mmol/L MgCl₂, 4 µL 2.5 mmol/L dNTPs, 50 pmol AC-1, 50 pmol AC-2, 1 µL total DNA, 1 U Taq polymerase and suitable water. Amplification was performed in PTC-100™ (MJ) as follows: pre-denatured at 94°C for 40 s, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 67°C for
60 s and extension at 72°C for 60 s, and finally post-extended at 72°C for 5 min. The PCR products were loaded on 1.5% agarose gel for electrophoresis.

**Amylose content assay**

Amylose contents in rice mature kernels were assayed according to Juliano et al [10].

**RESULTS**

**Comparison between one-step PCR and PCR-Acc I methods**

PCR-Acc I method was designed on the following mechanism. If the +1 site of Wx gene intron 1 is G, the sequence nearby will be a recognition site of restriction endonuclease Acc I. If the +1 nucleotide is other than G, there will be no Acc I recognition site. For one-step PCR method, if 3’end nucleotide of primer is G, the amplification efficiency will be 100 times higher when the corresponding site is C in template than when it is A in template [11]. The 3’end nucleotide of the primer AC-1 in our design was G, which equals to the +1 nucleotide of intron 1 of Wx gene. If the corresponding nucleotide in template DNA is C, the PCR product will be adequate and will be shown clearly in electrophoretogram. If the corresponding nucleotide in template DNA is A, only blurring image will be seen in electrophoretogram.

In order to confirm the feasibility of one-step PCR method, we randomly selected 10 samples of rice varieties to detect the +1 nucleotide with one-step PCR and PCR-Acc I methods, respectively. The results have been showed in Fig. 1. For PCR-Acc I method, two bands could be seen for samples 1, 3 and 10 and one single band for samples 2, 4, 5, 6, 7, 8 and 9 (Fig. 1-A), indicating that PCR products from samples 1, 3 and 10 contain Acc I recognition site and their +1 nucleotides were G while the +1 nucleotides of the other seven samples were not G. As for one-step PCR method, a clear single band could be observed in samples 1, 3 and 10 and only blurring shadow could be shown in the other seven samples (Fig. 1-B). The latter result also indicated that +1 nucleotides were G for samples 1, 3 and 10, and they were other than G for the other seven samples. Therefore, the same conclusion could be drawn from those two methods.

**Correlation between +1 nucleotide and amylose content**

We collected 30 indica rice samples from Guangdong Province, to detect the +1 nucleotide with one-step PCR method and determine their amylose content. As shown in Table 1, all the 30 varieties can be classified into two groups, i.e. Group G and Group T. The +1 nucleotides of Wx intron 1

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Amylose content (%)</th>
<th>Base</th>
<th>Cultivar</th>
<th>Amylose content (%)</th>
<th>Base</th>
<th>Cultivar</th>
<th>Amylose content (%)</th>
<th>Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruanxiangzhan</td>
<td>13.41</td>
<td>T</td>
<td>Tai 8-28</td>
<td>18.18</td>
<td>T</td>
<td>Aifengzhan</td>
<td>26.60</td>
<td>G</td>
</tr>
<tr>
<td>Shengtai 1</td>
<td>14.70</td>
<td>T</td>
<td>Jiafuzhan</td>
<td>18.44</td>
<td>T</td>
<td>Moliixinzhan</td>
<td>26.62</td>
<td>G</td>
</tr>
<tr>
<td>Luyouzhan</td>
<td>15.28</td>
<td>T</td>
<td>Teqing</td>
<td>23.43</td>
<td>G</td>
<td>Luyuanzhan</td>
<td>27.62</td>
<td>G</td>
</tr>
<tr>
<td>Wuxingsimiao</td>
<td>16.47</td>
<td>T</td>
<td>Xiaonongzhan</td>
<td>25.77</td>
<td>G</td>
<td>Texinzhan</td>
<td>27.63</td>
<td>G</td>
</tr>
<tr>
<td>Changsizhan</td>
<td>16.82</td>
<td>T</td>
<td>Tesanai</td>
<td>25.79</td>
<td>G</td>
<td>Yuexiangzhan</td>
<td>27.75</td>
<td>G</td>
</tr>
<tr>
<td>Fengbazhan</td>
<td>16.82</td>
<td>T</td>
<td>Fengmeizhan</td>
<td>25.81</td>
<td>G</td>
<td>Zhongmeizhan</td>
<td>27.80</td>
<td>G</td>
</tr>
<tr>
<td>Fengszhan</td>
<td>16.84</td>
<td>T</td>
<td>Xiangyuanzhan</td>
<td>25.89</td>
<td>G</td>
<td>Fengmeizhan</td>
<td>27.94</td>
<td>G</td>
</tr>
<tr>
<td>Fenghuazhan</td>
<td>17.10</td>
<td>T</td>
<td>Yuennongzhan</td>
<td>25.90</td>
<td>G</td>
<td>Guinongzhan</td>
<td>28.34</td>
<td>G</td>
</tr>
<tr>
<td>97 xiang</td>
<td>17.15</td>
<td>T</td>
<td>Fengguzhan</td>
<td>26.41</td>
<td>G</td>
<td>Fengnongzhan</td>
<td>28.42</td>
<td>G</td>
</tr>
<tr>
<td>Yuehang 1</td>
<td>17.56</td>
<td>T</td>
<td>Tai 10</td>
<td>26.43</td>
<td>G</td>
<td>Moliyouzhan</td>
<td>28.51</td>
<td>G</td>
</tr>
</tbody>
</table>

**Table 1. Amylose contents and the first bases of splice donor of Wx intron 1 in 30 indica rice cultivars.**
in Group G and in Group T are G and T, respectively. The amylose contents in Group G are higher than 18% while the amylose content in Group T is lower than 18% except for Tai 8-28 and Jiafuzhan, which are a bit more than 18%.

**DISCUSSION**

As shown in Fig. 1, PCR-Acc I method and one-step PCR method can produce the same conclusion and both of them could detect the difference of +1 nucleotide of Wx intron 1. Thus we can conclude that the one-step PCR method is reliable and can be adopted in rice molecular-assistant selection programs. Moreover, one-step PCR method doesn’t need Acc I enzyme and requires less time. Therefore, it is a quicker, more convenient and economical method.

The first nucleotide of Wx intron 1 is reported to be related with amylose content in mature rice kernels. In this experiment, 30 indica rice cultivars were assayed with one-step PCR method. According to their +1 nucleotide, the cultivars could be assorted into two groups. The cultivars in group G are higher in amylose content than those in group T. The result of this experiment is in accord with the previous research reports. Thus, the first nucleotide marker of Wx intron 1 could be used to assist rice quality improvement. Due to the restriction of conventional breeding, most of the current indica rice cultivars in Guangdong Province cannot reach the requirements for the grade one rice of national standard in amylose content. So it is hoped that marker aided selection such as the +1 nucleotide of Wx intron 1 would be helpful and efficient in rice breeding.

**REFERENCES**


