Development of functional marker for GS3 gene controlling grain size in rice

Srisawat Khanthong1,2, Meechai Siangliw2 and Theerayut Toojinda2*

ABSTRACT: GS3 located on chromosome 3 is a major recessive gene controlling grain size in rice. The single point mutation from C to A on exon 2 results a premature stop codon in which it affect grain size. This study aimed to develop a functional marker for GS3 gene. GS3 HRM marker was successfully developed. The melting temperature of GS3 HRM marker was 81.437±0.076 °C for C/C allele, 80.679 ±0.061 °C for A/A allele and 80.220±0.942 °C for heterozygous C/A allele. This marker was used to identify genotype of GS3 in F2 population. It was clearly differentiate GS3 alleles and associated with grain size. Therefore, GS3 HRM marker is very useful for selection of grain size by marker-assisted selection.

Keywords: GS3, grain size, high-resolution melting (HRM), rice

Introduction

Grain size is an important trait for rice quality. Most premium rice is long gain and slender shape. Recently several genes controlling grain size including D1, D2, D11, D61, GS3, GW2, GW5/qGW5, Gif1, GS5, GW8, SPL16, SRS1, DEP2, SRS3 and SRS5 are identified and cloned (Huang et al. 2013). GS3 is a major gene controlling grain shape. It is located on chromosome 3. A single point mutation from C to A on exon 2 was discovered in which A/A allele results a premature stop codon while C/C allele results a normal expression. C/C and C/A alleles conferred a short grain and A/A allele conferred a long grain (Fan et al. 2009). For rice breeding, it is difficult to differentiate C/A allele with C/C allele by phenotype selection.

DNA markers can be used as indirect selection without interfered from environment. DNA markers also are allowed to select several target traits at once. However, DNA maker for marker-assisted selection (MAS) need to be easy to use, not complicate and friendly handling. Cleaved amplified polymorphic sequence (CAPS) markers for C/A SNP and digestion of PCR product with PstI restriction enzyme was developed for genotyping of GS3 (Fan et al. 2009).

Ramkumar et al. (2010) was also developed a DRR-GL tetra-primer PCR-based marker system in which it targets the functional nucleotide polymorphism at GS3. Single-nucleotide polymorphism (SNP) is a high throughput marker. It can be detected using CAP-marker, high-resolution melting (HRM), TaqMan™ and KASP™. HRM marker is one of the high-throughput post-PCR
analyses to detect mutation. The objective of this study is to develop functional HRM DNA marker for GS3 gene.

**Materials and Methods**

**Plant materials**

Rice varieties Paw San Hmwe (PSM), CT9993 and 15 F2 plant derive from PSM X CT9993 were used as material for checking short and long grain. PSM is short grain and CT9993 is long grain. The phenotype measurement of grain length was made by SmartGrain software (Tanabata et al. 2012).

**HRM marker development**

GS3 HRM marker was developed based on DNA sequences publishing on NCBI data base (http://www.ncbi.nlm.nih.gov/). Primer3 was used to design PCR primers (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi) by setting PCR product size 80-180 bp and Tm 70-85 °C, primer length 18-25 bp, primer Tm 55-60 °C, GC content 30-60% and analysis by OligoCalc (Kibbe, 2007). BLAST search was used to find regions of similarity and then verified the specificity of the markers for the GS3 gene.

**DNA extraction**

The total genomic DNA of population and parents were isolated from 100 ng of leaf tissue according to the DNA trap kit (DNA technology, Kasetsart university). Genomic DNA was measured concentration by NanoDrop 8000 UV-Vis Spectrophotometer and normalized to 20 ng/ μl with sterile water.

GS3 genotype

PCR amplification was conducted using 20 ng of DNA, 5 ul Ssofast pcr kit (Bio-Rad) 0.4 ul each 5 uM primer and adjust by DI water to 10 ul. PCR reaction was initiated by denaturation at 95°C for 3min followed by 35 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 2 min. Final 5 min incubation at 72°C was allowed for the completion of primer extension. The HRM marker products were visualized by QuantStudio™ 12K Flex system at temperature from 60 °C to 94 °C for +0.05°C/s.

**Results and Discussions**

The GS3 HRM marker consisted of forward primer - TTGCAGGGTGAAATA AATTCAATC and reverse primer - AACAGCAGGCTGGCTTACTC in which it produced a product size of 79 bp and Tm 76.6-77.1 °C (Table 1). The marker was genotyped PSM, CT9993 and 15 F2 plants. GS3 HRM marker showed significant difference between CT9993, a long grain carrying an A/A allele and PSM, a short grain carrying a C/C allele (Oo et al. 2015). In F2 plants, GS3 HRM marker showed differentiation among F2 plants carrying homozygous A/A, C/C and heterozygous C/A alleles (Figure 1). The melting temperature of each allele was 81.437±0.076 °C for C/C, 80.679 ±0.061 °C for A/A and 80.220±0.942 °C for C/A alleles (Table 2). Grain length of the F2 plants carried A/A C/C and C/A allele were 9.71±0.44, 8.21±0.28 and 8.60±0.89 mm. respectively. This result is agreed with Fan et al. (2009).

This study illustrated a successful development of functional GS3 HRM marker for GS3 gene. This marker is high throughput co-dominant marker that can be used in rice breeding program.
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**Table 1** description of functional GS3 HRM marker

<table>
<thead>
<tr>
<th>sequence (5’ - 3’)</th>
<th>length</th>
<th>Tm</th>
<th>%GC</th>
<th>size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F- TTGCAGGGTGAAATAAATTCAATC</td>
<td>24</td>
<td>58</td>
<td>33</td>
<td>79</td>
</tr>
<tr>
<td>R- AACAGCAGGCTGGCTTACTC</td>
<td>20</td>
<td>60</td>
<td>55</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** A GS3 HRM melting temperature mean and standard deviation of allele variance

<table>
<thead>
<tr>
<th>GS3 genotype</th>
<th>HRM (ºC)</th>
<th>sd (ºC)</th>
<th>Grain length (mm.)</th>
<th>sd (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous A/A</td>
<td>80.679</td>
<td>±0.061</td>
<td>9.71</td>
<td>±0.44</td>
</tr>
<tr>
<td>Homozygous C/C</td>
<td>81.437</td>
<td>±0.076</td>
<td>8.21</td>
<td>±0.28</td>
</tr>
<tr>
<td>Heterozygous C/A</td>
<td>80.220</td>
<td>±0.942</td>
<td>8.60</td>
<td>±0.89</td>
</tr>
</tbody>
</table>

**Conclusion**

GS3 HRM marker is high throughput co-dominance marker. It can be identified a single point mutation from C to A on exon 2 in GS3 gene.

**Acknowledgement**

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**References**


