Mapping QTLs for the tissue culture performance of rice mature embryo using indica-japonica recombinant inbred lines

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Abstract

The tissue culture performance is the determinant factor of genetic transformation in indica rice (Oryza sativa L.). Quantitative trait loci (QTLs) associated with the tissue culture performance of mature embryo were detected by 202 genetic markers and 190 recombinant inbred lines (RILs) derived from the cross between two rice varieties, 93-11 (indica) as female parent and Nipponbare (japonica) as male parent. A composite interval mapping (CIM) was used to identify the QTLs for the tissue culture performance. The tissue culture performance of rice mature embryo were evaluated by six parameters, embryogenic calli induction rate (ECR), callus subculture capability (CSC), plantlet regeneration rate (RR), average number of regenerated shoots per callus (NRS), green plantlet regeneration rate (GRR) and average number of regenerated green shoots per callus (NRGS). Nipponbare has better tissue culture response than 93-11 because all indicators were higher in Nipponbare except CSC. Four QTLs for ECR, five QTLs for CSC, three QTLs for RR, two QTLs for NRS, four QTLs for GRR and three QTLs for NRGS were detected. These putative QTLs associated with tissue culture performance were distributed on eight rice chromosomes. These results demonstrated the possibilities of improving the tissue culture performance of indica rice by marker assisted selection (MAS) with those desirable alleles of japonica rice.

Keywords: mature embryo; quantitative trait loci (QTLs); rice; recombinant inbred lines (RILs); tissue culture performance.

Abbreviations: CAPS- cleaved amplified polymorphic sequence; CIM- composite interval mapping; CSC- callus subculture capability; DH- doubled haploid; ECR- embryogenic calli induction rate; GRR- green plantlet regeneration rate; LOD- logarithm of odds; MAS- marker assisted selection; NRGS- average number of regenerated green shoots per callus; NRS- average number of regenerated shoots per callus; QTLs- quantitative trait loci; RILs- recombinant inbred lines; RR- plantlet regeneration rate; SSR- simple sequence repeats; STS- sequence-tagged site.

Introduction

Rice (Oryza sativa L.) is the staple food that feeds half of the world’s population, especially in Asian countries. Genetic transformation is an important and indispensable method employed in gene function studies and crop improvement. Agrobacterium-mediated genetic transformation has become the mostly used transformation method in rice since 1994 (Hiei et al., 1994). The tissue culture performance of rice variety has a great influence on the transformation efficiency (Lin and Zhang, 2003), thus become the major factor in the improvement. There is an obvious difference of tissue culture ability between indica and japonica rice. Transgenic lines can be obtained very easily in japonica rice because an efficient tissue culture system was established. By contrast, the difficulty on tissue culture determined the lower transformation efficiency of indica rice (Aldemita and Hodges, 1996; Lin and Zhang, 2005). The immature embryo is the main explant used in indica rice transformation (Hiei and Komari 2008). But it is hard to obtain immature embryos without growth season limitation (Pons et al., 2000). And immature embryos are suitable for culture in the very narrow time window after harvest. The mature embryo is a better choice because it is easy to obtain and conserve. Nonetheless, the mature embryos of indica rice have not been widely applied in genetic transformation mainly due to the poor callus induction and regeneration ability. It is very urgent to study the genetic mechanism of the different tissue culture ability between indica and japonica rice. By isolating and identifying the quantitative trait loci (QTLs) or genes associated with tissue culture performance, we could improve the tissue culture ability and increase the genetic transformation efficiency of indica rice through marker-assisted selection (MAS) or gene transformation. Some QTLs associated with callus induction and plant regeneration in rice have been identified. Taguchi-Shiobara et al. (1997) detected five putative QTLs for average number of regenerated shoots per callus (NRS) and four QTLs for regeneration rate (RR) on chromosomes 1, 2 and 4 with 98 BC1F3 lines derived from Kasalath (indica) and Nipponbare (japonica), accounting for 38.5% and 32.6% of the total phenotypic variation, respectively. Kwon et al. (2001a) evaluated the tissue culture performance of mature seed using 164 recombinant inbred lines (RILs) derived from a cross between the tongil cultivar Milyang 23 and the japonica
cultivar Gihobyeo. Two QTLs for callus induction were located on chromosomes 1 and 2 and four QTLs for calli regeneration were located on chromosomes 2, 3, and 11. Taguchi-Shiobara et al. (2006) identified eight putative QTLs for callus induction and subculture on chromosomes 1, 4 and 9 using 183 BC$_2$F$_2$ lines derived from a cross between the indica cultivar Kasalath and the japonica cultivar Koshikihari. Zhao et al. (2009) detected two QTLs for the frequency of callus induction on chromosomes 2 and 11, two QTLs for the mean plantlet number per regenerated callus on chromosomes 1 and 3 in two culture systems using 139 chromosomal segment substitution lines derived from Zhengshan 97B (indica) and Nipponbare (japonica). Nishimura et al. (2005) isolated a major QTL for the plant regeneration ability of rice callus in Kasalath (indica) encoding ferredoxin-nitrite reductase (NiR) by positional cloning. This study also demonstrated that the NiR gene could be used as a selectable marker for rice transformation. All these studies indicated that the tissue culture ability of rice variety was controlled by genetic factors. In this study, we identified QTLs associated with calli induction from mature embryos and plantlet regeneration from calli using a RIL population derived from a cross between the indica variety 93-11 and the japonica variety Nipponbare. The whole genome of two parental varieties had been completed sequenced. The indica variety 93-11 has poor tissue culture performance, whereas the japonica variety Nipponbare has good calli induction and regeneration ability, and it has been utilized universally for transgenic experiments as a model variety. The tissue culture performance was evaluated by six indices including embryogenic calli induction rate (ECR), callus subculture capability (CSC), regeneration rate (RR), regenerated shoots per callus (NRS), green plantlet regeneration rate (GRR) and average number of regenerated green shoots per callus (NRGS).

Results

Linkage map construction

A total of 225 pairs of polymorphism primers between the parents were selected from 693 pairs tested primers (659 pairs of SSR primers, 34 pairs of STS and CAPS markers), and were used to determine the genotype of each line. The PCR products of 202 pair primers revealed clear, stable and co-dominant result, which was used to build a polymorphic marker locus linkage map with Joinmap3.0 software. On average, 17 markers distributed on individual chromosome. The map covered entire rice genome, with 1640.25 centiMorgan (cM) in total (Fig. 1). The average genetic distance of markers was 8.12 cM, which could meet the requirement of QTL mapping.

Phenotypic variation in parents and RILs

The frequency distribution of ECR, CSC, RR, NRS, GRR and NRGS in the RIL population and their parents are shown in Figure 2. All indices of Nipponbare were higher than that of 93-11 except CSC (Fig. 2). This is consistent with the previous observation that Nipponbare has better tissue culture response than 93-11. It was shown that Nipponbare has better callus induction and plant regeneration performance than 93-11 (Fig. 3). Because albino plants regenerated from the calli of some lines, there were the similar but not uniform frequency distribution between RR and GRR, NRS and NRGS (Fig. 2). Transgressive segregations, continuous variations and normal distribution were observed among the parameters of the RILs population, according with the characteristics of quantitative traits, which suggested QTLs analysis was reliable (Fig. 2).

QTLs for the tissue culture performance of mature embryos

Four QTLs (qECR-1a, qECR-1b, qECR-4 and qECR-6) associated with ECR were detected on chromosomes 1, 4 and 6, accounting for 8.20%, 9.93%, 6.87% and 9.70% of phenotypic variation, respectively (Table 1, Fig. 1). Five QTLs (qCSC-3, qCSC-6a, qCSC-6b, qCSC-8 and qCSC-10) were detected for CSC on chromosomes 3, 6, and 8, and 10, explaining 50.50%, 9.29%, 5.90%, 46.17% and 46.87% of the phenotypic variation, respectively (Table 1, Fig. 1). Three significant QTLs (qRR-8, qRR-9 and qRR-10) were detected for RR on chromosomes 8, 9 and 10. These QTLs explained 70.59% of the total phenotypic variation. One QTL, qRR-10, accounted for 46.69% of the total phenotypic variation. The other two QTLs, qRR-8 and qRR-9, accounted for 9.90% and 14.00% of phenotypic variation (Table 1, Fig. 1). Two QTLs (qNRS-8 and qNRS-12) associated with NRS were detected on chromosomes 8 and 12, explaining 15.74% and 11.70% of the phenotypic variation (Table 1, Fig. 1). Four QTLs for GRR were identified and tentatively designated qGRR-1, qGRR-8, qGRR-9 and qGRR-10. Two QTLs were located on chromosomes 1 and 8, and the other two were located on chromosomes 9 and 10. These QTLs accounted for 74.79% of the total phenotypic variation. The effect of qGRR-10 explained 47.29% of the total phenotypic variation. The other three QTLs, qGRR-1, qGRR-8, qGRR-9, accounted for 5.40%, 9.83% and 12.27% of the phenotypic variation, respectively (Table 1, Fig. 1). Three QTLs (qNGRS-8, qNGRS-10a and qNGRS-10b) were detected for NRGS on chromosomes 8 and 10, explaining 13.06%, 6.45% and 7.35% of the phenotypic variation, respectively (Table 1, Fig. 1).

Discussion

Indica rice is the most widely cultivated subspecies of rice, and provides the staple food for more than half of the world population. In japonica rice, a high efficiency of Agrobacterium-mediated transformation was established and used widely in functional studies of genes (Hiei et al., 1994; Toki et al., 2006). However, Agrobacterium-mediated transformation is difficult in indica rice. There have been a few reports of successful Agrobacterium-mediated transformation of indica rice (Lin and Zhang, 2005; Kumar et al., 2005; Sahoo et al., 2011). But these results showed low transformation efficiency and were only applicable to specific genotypes. So these protocols could not be used in a large-scale genetic transformation of indica rice. The main bottleneck of indica rice transformation is poorer tissue culture response compared with japonica rice. Thus, it is very important to study genetic diversity and molecular mechanism about tissue culture performance between japonica and indica rice. In this study, we mapped 21 QTLs for the tissue culture performance of mature embryo using 190 RILs derived from a cross between 93-11 and Nipponbare (Table 1, Fig. 1). Some QTLs were highly concentrated in a few chromosomal regions. Two QTLs were simultaneously detected in the region around RM578 on chromosome 1. Two QTLs were simultaneously detected in the region around RM228 on chromosome 10 (Table 1, Fig. 1). Some QTLs were consistent with the previous studies. For example, qGRR-1, which was localized in the region between maker RM11910 and RM 414 on chromosome 1, have the same map position as that of Zhao et al. (2009). Two significant QTLs, qRR-10 and qGRR-10, which were detected on chromosome 10, were mapped to the chromosome position as those of Zhao et al. (2009). Nevertheless, novel QTLs for the tissue culture performance have been further located in this study (Table 1, Fig. 1), such as qECR-6, qRR-8, qRR-9, qGRR-8 and qGRR-9. The possible reason could be the...
Table 1. Putative QTLs for the tissue culture performance of mature embryo detected in the rice RIL population derived from 93-11 and Nipponbare.

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTLs</th>
<th>Chr</th>
<th>QTL region</th>
<th>LOD</th>
<th>PV (%)</th>
<th>Add</th>
<th>Positive allele</th>
</tr>
</thead>
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<tr>
<td>ECR</td>
<td>qECR-1a</td>
<td>1</td>
<td>RM5496-RM578</td>
<td>2.52</td>
<td>8.20</td>
<td>0.07</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>qECR-1b</td>
<td>1</td>
<td>RM578-RM10596</td>
<td>2.42</td>
<td>9.93</td>
<td>0.08</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>qECR-4</td>
<td>4</td>
<td>RM3466-RM1841</td>
<td>2.14</td>
<td>6.87</td>
<td>0.09</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>qECR-6</td>
<td>6</td>
<td>Wx-1-RS14</td>
<td>2.03</td>
<td>9.70</td>
<td>0.08</td>
<td>J</td>
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<tr>
<td>CSC</td>
<td>qCSC-3</td>
<td>3</td>
<td>RM7396-RM6266</td>
<td>3.43</td>
<td>50.50</td>
<td>2.41</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>qCSC-6a</td>
<td>6</td>
<td>Wx-1-RS14</td>
<td>2.69</td>
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<td>J</td>
</tr>
<tr>
<td></td>
<td>qCSC-6b</td>
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<td>RM22934-RM6917</td>
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<td>5.90</td>
<td>0.41</td>
<td>J</td>
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<tr>
<td></td>
<td>qCSC-8</td>
<td>8</td>
<td>RM22934-RM6699</td>
<td>2.72</td>
<td>46.17</td>
<td>2.28</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>qCSC-10</td>
<td>10</td>
<td>RM25032-RM1375</td>
<td>4.39</td>
<td>47.87</td>
<td>-1.84</td>
<td>J</td>
</tr>
<tr>
<td>RR</td>
<td>qRR-8</td>
<td>8</td>
<td>RM6683-RM22392</td>
<td>2.95</td>
<td>9.9</td>
<td>0.12</td>
<td>J</td>
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<tr>
<td></td>
<td>qRR-9</td>
<td>9</td>
<td>CS0900-RM7038</td>
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<td>14.00</td>
<td>0.10</td>
<td>J</td>
</tr>
<tr>
<td>NRS</td>
<td>qNRS-8</td>
<td>8</td>
<td>RS19-RM22934</td>
<td>2.37</td>
<td>15.74</td>
<td>1.65</td>
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<tr>
<td></td>
<td>qNRS-12</td>
<td>12</td>
<td>RM5479-RM12</td>
<td>2.04</td>
<td>11.70</td>
<td>1.07</td>
<td>J</td>
</tr>
<tr>
<td>GRR</td>
<td>qGRR-1</td>
<td>1</td>
<td>RM11910-RM414</td>
<td>2.04</td>
<td>5.40</td>
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<tr>
<td></td>
<td>qGRR-8</td>
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<td>RM6863-RM22392</td>
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<td>9.83</td>
<td>0.12</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>qGRR-9</td>
<td>9</td>
<td>CS0900-RM7038</td>
<td>2.23</td>
<td>12.27</td>
<td>0.09</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>qGRR-10</td>
<td>10</td>
<td>RM25032-RM1375</td>
<td>2.53</td>
<td>47.29</td>
<td>0.19</td>
<td>J</td>
</tr>
<tr>
<td>NRGS</td>
<td>qNRGS-8</td>
<td>8</td>
<td>RS19-RM22934</td>
<td>2.29</td>
<td>13.06</td>
<td>1.57</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>qNRGS-10a</td>
<td>10</td>
<td>RM3451-RM2288</td>
<td>2.15</td>
<td>6.45</td>
<td>0.83</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>qNRGS-10b</td>
<td>10</td>
<td>RM228-RM5494</td>
<td>2.15</td>
<td>7.35</td>
<td>0.89</td>
<td>J</td>
</tr>
</tbody>
</table>

* Trait abbreviations: embryogenic callus induction rate (ECR), callus subculture capability (CSC), regeneration rate (RR), regenerated shoots per callus (NRS), green plantlet regeneration rate (GRR), average number of regenerated green shoots per callus (NRGS).

* Chromosome on which the QTL was located. * Percent phenotypic variance explained by the QTL. * Positive allele of efficiency gene expressed from 9311(indica) with I, from Nipponbare (japonica) with J.

Fig 1. Chromosomal locations of the QTLs detected for the tissue culture performance of rice mature embryo in the RIL population derived from the cross of 93-11 × Nipponbare.

different mapping population with the different genetic background (Zhao et al., 2009). In order to improve the tissue culture performance of indica rice, we can transform the *japonica* alleles into indica variety by the utilization of DNA markers that are tightly linked to these QTLs (Kwon et al., 2001b). Different mapping populations were used to identify the QTLs associated with tissue culture ability. F2 populations were occasionally used in mapping tissue culture response genes (Bolibok and Rakocy-Trojanowska, 2006; Takeuchi et al., 2000), because it is difficult to obtain enough tissue amounts for evaluating the tissue culture ability and performing mapping analysis. Doubled haploid (DH) technology can rapidly develop homozygous and genetically fixed lines. Though DH lines are very useful for QTL mapping, DH lines can be produced only by anther culture where it is very genotype-dependent. Moreover, some DH lines show low callus induction efficiency and segregation distortion (Bolibok and Rakocy-Trojanowska, 2006; Taguchi-Shiobara et al., 1997). RILs, which are homozygous and genetically fixed lines, can be relatively easy developed by the single-seed descent method. Many studies have shown the suitability of RILs for QTL detection (Kwon et al., 2001a; Zhang et al., 2010; Yang et al., 2011; Yang et al., 2012). So utilizing the RILs in this study could overcome the shortcomings of F2 and DH population and improve the efficiency in QTL identification associated with tissue culture ability. Tissue culture is a very labor-intensive and time-consuming technology. Therefore, it is necessary to control deviation from operation and environment in the process of isolating QTLs associated with rice tissue culture ability. In this study, we used rice mature embryos to eliminate the unfavorable effects from endosperm composition during callus induction period. Our result also showed that embryogenic calli could be induced more rapidly. Moreover,
Table 2. Compositions of media for callus induction, subculture and plant regeneration.

<table>
<thead>
<tr>
<th>Media</th>
<th>Compositions</th>
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<tbody>
<tr>
<td>Induction and subculture medium</td>
<td>N6 majors, MS iron salts, B5 minors, B5 vitamins, 500 mg/L proline, 500 mg/L glutamine, 300 mg/L casein enzymatic hydrolysate, 30 g/L sucrose, 2 mg/L 2,4-D, 3 g/L phytagel, pH 5.8</td>
</tr>
<tr>
<td>Regeneration medium</td>
<td>N6 majors, MS iron salts, B5 minors, B5 vitamins, 1 g/L casein enzymatic hydrolysate, 30 g/L sucrose, 30 g/L sorbitol, 500 mg/L MES, 2.5 mg/L CuSO₄, 0.5 mg/L KT, AA amino acids, 2 g/L phytagel, pH 5.8</td>
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</table>

Fig 2. Frequency distribution of the six indices for the tissue culture performance of rice mature embryo in the RIL population derived from the cross of 93-11 × Nipponbare.

Fig 3. Callus induction (A) and plant regeneration (B) performance of 93-11, Nipponbare and some other representative lines.

contamination caused by bacterial or fungi from endosperm can be reduced remarkably. We dispensed all medium to the disposable petri dishes by a peristaltic pump. And we strictly maintained environmental conditions in our tissue culture room, such as light, temperature and humidity. By these ways and using the RILs, the data and identifying QTLs may be more believable in this study. In order to obtain the candidate genes corresponded for tissue culture ability, positional cloning is the most efficient tool. However, the data is limited using this method, partially because the huge workload on mapping population construction, phenotypic identification to fine mapping and confirming candidate gene. Up to now only one gene corresponded for rice callus regeneration, NiR, was cloned by positional cloning (Nishimura et al., 2005). However, the complete sequence of rice genome will accelerate positional cloning greatly. Since two parents of our mapping population, 93-11 and Nipponbare, have been sequenced, it could promote positional cloning and isolating candidate genes associated with the tissue culture performance or the other morphologic, yield and quality traits. Agrobacterium-mediated genetic transformation in rice is a very complicated process. Many factors can affect the efficiency of genetic transformation, such as the genotype of rice variety, the expression vector, the strain of Agrobacterium, the selectable marker genes (Hiei et al., 1997). Tie et al. (2012) reported that the lower transformation efficiency in indica rice was due to the low efficiency in T-DNA integration into the plant genome. And in the indica cultivar, some genes which may be necessary for the...
Transformation process were down-regulated. Tenea et al. (2009) reported that certain histones enhance transgene expression, protect incoming DNA during the initial stages of transformation, and subsequently increase the efficiency of Agrobacterium-mediated transformation. The expression of other genes has important promoting roles in rice tissue culture and genetic transformation, such as SERK1 (Hu et al., 2005), Os22A (Ozawa et al., 2003). Overexpression of OsSERK1 increased the shoot regeneration rate in rice (from 72% to 86%). However, suppression of OsSERK1 expression in transgenic calli by RNA interference resulted in a significant reduction of shoot regeneration rate (from 72% to 14%). The transformed calli of Koshihikari with overexpression of Os22A showed significantly better regeneration ability (from 4% to 99%). QTL mapping for the tissue culture performance will provide more candidate genes which may play an important role in genetic transformation. So in the near future, the transformation efficiency of indica rice should be improved remarkably since we had a more comprehensive and intensive understanding about each matter in Agrobacterium-mediated genetic transformation.

Materials and methods

Plant materials

The F2:11 recombinant inbred line population was developed from a cross between the indica variety 93-11 and the japonica variety Nipponbare (Yang et al., 2012). The indica variety 93-11 was female parent with poor tissue culture performance. Nipponbare was male parent with good tissue culture performance. The eleventh high-generation group consisting of 190 lines was generated by the single-seed descent method. The agronomic traits of the plants from every line were identical in field.

Tissue culture procedure

Callus induction

Surface sterilization of the dehulled mature seeds of the 190 lines and the two parents (93-11 and Nipponbare) was conducted by immersing in 70% ethanol for 1 min, followed by soaking in 50% sodium hypochlorite (with final available chlorine of 2%) for 40 min. After washing with sterile distilled water, seeds were immersed in sterile distilled water at 28°C dark overnight. The mature embryos were isolated from the endosperms with a scraper along the aleurone layer and plated with scutella upward on induction medium. Twelve embryos were plated into one disposable petri dish (100 × 25 mm) containing 50 mL induction medium, and four dishes were prepared for each line and the parents as one replication. Callus was induced at 28°C in continuous darkness. ECR, the ratio of the embryos producing embryogenic callus to the number of the embryos germinated, was recorded after 4 weeks. The transformed calli of Koshihikari with overexpression of Os22A showed significantly better regeneration ability (from 4% to 99%). QTL mapping for the tissue culture performance will provide more candidate genes which may play an important role in genetic transformation. So in the near future, the transformation efficiency of indica rice should be improved remarkably since we had a more comprehensive and intensive understanding about each matter in Agrobacterium-mediated genetic transformation. The distribution of the target characters in RIL population was visualized by silver staining according to the method of Lu et al. (1992). Six hundred and fifty-nine pairs of simple sequence repeats (SSR) markers, 34 pairs of sequence-tagged site (STS) markers and cleaved amplified polymorphic sequence (CAPS) markers were used for parental polymorphism selection. The markers with rich polymorphisms and stable amplification were selected for genotyping analysis of 190 RILs. PCR analysis was performed using the protocol of Chen et al. (1997). PCR products were separated by 3% agarose gel with ethidium bromide staining or 8% non-denaturing polyacrylamide gel and visualized by silver staining according to the procedure of Sanguinetti et al. (1994). The linkage map was constructed with Joinmap3.0 software (Van Ooijen and Voorrips, 2002).

Data analysis and QTL detection

The distribution of the target characters in RIL population was analyzed by DPS software (Tang and Feng, 1997). The related QTLs of mature embryo tissue culture performance were detected in rice genome by MapQTL5.0 software for composite interval mapping (CIM) (Li et al., 2008). The phenotypic explainable variation of each QTL contribution was calculated by MapQTL5.0 software (Li et al., 2008). A logarithm of odds (LOD) score of 2.0 was set as a threshold for declaring the presence of QTL. The nomenclature of QTLs was following Sanguinetti et al. (1994). The linkage map was constructed with Joinmap3.0 software (Van Ooijen and Voorrips, 2002). The distribution of the target characters in RIL population was visualized by silver staining according to the method of Lu et al. (1992). Six hundred and fifty-nine pairs of simple sequence repeats (SSR) markers, 34 pairs of sequence-tagged site (STS) markers and cleaved amplified polymorphic sequence (CAPS) markers were used for parental polymorphism selection. The markers with rich polymorphisms and stable amplification were selected for genotyping analysis of 190 RILs. PCR analysis was performed using the protocol of Chen et al. (1997). PCR products were separated by 3% agarose gel with ethidium bromide staining or 8% non-denaturing polyacrylamide gel and visualized by silver staining according to the procedure of Sanguinetti et al. (1994). The linkage map was constructed with Joinmap3.0 software (Van Ooijen and Voorrips, 2002).

Conclusion

In the present study, 21 QTLs associated with tissue culture performance of rice mature embryos were localized. This information would benefit the understandings of the genetic mechanism of the different tissue culture ability between indica and japonica rice. The genotypes with good tissue culture response can be selected by using DNA markers that are tightly linked to these QTLs. And some recombinant inbred lines with good tissue culture performance could be the recipient material in genetic transformation. By secondary mapping population construction and positional cloning, we will confirm the efficiency of major QTLs and candidate genes in future study.

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