Microarray-based Classification of Seed-specific Gene Expression for Pigmentation in Colored Rice

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1 Introduction

Anthocyanin is a major class of flavonoids that tissues produce in response to environmental signals. The pigmentation of colored rice was analyzed by oligo microarray based on two factors, cultivar and developmental stage. As many as 250 and 350 genes were identified to be significantly up- and down-regulated. Using hypergeometric analysis for transcription factor function, the Myb and GT families, PBP, PBF, RAV, and STF factors were identified have potential anthocyanin-specific functions.

We also obtained seventeen unknown genes which display novel functions. Among the genes within the GT-1 and Myb1 groups, three unknown genes were found to be up-regulated significantly in both combinations of cultivar and developmental stage. These results showed functional diversity of TF families and that the biological functions of the particular TFs may be activated in a pigmentation pathway in rice.

2 Method and Results

2.1 Rice
The samples used to the three different genotype.
- White (Manmibyeo : Milyang162), Red (Jinjubyeo : Milyang194), Black (Hukcholbyeo : Suwon451)
The rice were harvested by three different developmental stages
- heading, heading+7day, heading+14day during falls 2006 in Suwon.

2.2 Statistical analysis and scanning of oligo- microarray
The microarray experiments were performed using Agilent Rice 60-mer 22K arrays which were designed and validated by the National Institute of Agrobiological Sciences (NIAS, http://www.nias.affrc.go.jp/) in Japan. The microarray includes oligonucleotide probes against 21,495 genes from the genome of Oryza sativa L. ssp japonica (cultivar Nipponbare).

All experiments were run using only Cy3 in order to eliminate the dye-swap error value. Spot intensity was calculated as the median value of the spot compared to the background median value. Samples were separated into two groups, cultivar and developmental stage, in order to test our hypothesis. For gene expression clustering, eigen values of the data were first generated with SAS Enterprise software and then statistically analyzed with TM4 software developed by the J. Craig Venter Institute.

3 Discussions

3.1 An evaluation of selected genes
To evaluate the pigmentation of colored rice, we performed a two-stage screen. First, we performed hypothesis testing to find a common gene from various combinations of colored rice, such as black/white, red/white, and black/red, and verified the significance of the data. Then, we evaluated the transcription
factors (TFs) involved in pigmentation by average linkage clustering and the hypergeometric analysis method. From this second step, we retained those genes whose expression was related to color production without differences in developmental stage and cultivar. The data was screened to select genes with a greater than 2.5 average upregulation (or downregulation) using three cultivars across three developmental stages.

The selected genes were compared to the International Rice Genome Sequencing Project (IRGSP, http://rgp.dna.affrc.go.jp/E/IRGSP/) database and annotated by the rice genome system supported by National Academy of Agricultural Science (NAAS). During comparison to the IRGSP database, genes related to tannin were removed. Ultimately, 304 candidates were found to be significant genes related to anthocyanin.

3.2 Potential transcription factors associated with pigmentation

The selected 304 genes were analyzed by average linkage clustering to group genes with similar function. Clustering analysis was performed according to the median intensity measured from the array. To prevent a complicated model, we assigned a value of 0.35 as the average distance between clusters. From this, seven clusters were identified. To identify the best candidate genes, transcription factor analysis was performed on genes consistently found to be associated with rice pigmentation. Table 1 shows the transcription factor groups predicted by the cumulative hypergeometric distribution analysis method utilized in this study.

<table>
<thead>
<tr>
<th>TFs</th>
<th>Description</th>
<th>Gene</th>
<th>P-value</th>
<th>TF ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYB1</td>
<td>A flower-specific related to Myb protein</td>
<td>69</td>
<td>0.0323</td>
<td>48.25</td>
</tr>
<tr>
<td>MYB26</td>
<td>A Myb-like protein of flowers</td>
<td>16</td>
<td>0.0095</td>
<td>11.18</td>
</tr>
<tr>
<td>MYB305</td>
<td>Gene related to flavonoid-biosynthesis</td>
<td>30</td>
<td>0.0298</td>
<td>20.98</td>
</tr>
<tr>
<td>GT-1</td>
<td>Nuclear factors related to trans-acting</td>
<td>91</td>
<td>0.0180</td>
<td>63.63</td>
</tr>
<tr>
<td>GT-2</td>
<td>A trans-acting factor in GT-motif</td>
<td>27</td>
<td>0.0217</td>
<td>18.89</td>
</tr>
<tr>
<td>PBF</td>
<td>The regulates cereal storage protein gene</td>
<td>31</td>
<td>0.0319</td>
<td>21.67</td>
</tr>
<tr>
<td>PBP</td>
<td>Flower-specific gene related to Myb305</td>
<td>15</td>
<td>0.0087</td>
<td>10.48</td>
</tr>
<tr>
<td>RAV</td>
<td>Negative growth regulator gene</td>
<td>8</td>
<td>0.0273</td>
<td>5.59</td>
</tr>
<tr>
<td>STF</td>
<td>Auxin-responsive gene</td>
<td>6</td>
<td>0.0173</td>
<td>4.19</td>
</tr>
</tbody>
</table>

There were seventeen unknown genes among the 304 genes selected and categorized. Within the GT-1 and Myb1 groups, 6 and 5 unknown genes were identified in the black/red rice combination, respectively. Among these two groups, the expression of three unknown genes differed dramatically between the black/red rice and developmental stages with great statistical significance. These three genes may potentially play either a regulatory role in the developmental process or be related to anthocyanin metabolism. Functional studies are necessary to clarify the nature of these three new candidates as possible regulatory factors of the anthocyanin pathway in colored rice.

References

