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GENE EXPRESSION PROFILING IN THE MANUFACTURING PROCESS OF “ORIENTAL BEAUTY”

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Summary

The famous Formosa Oolong tea “Oriental Beauty (Pom-Fong tea)” made in Taiwan has high quality flavor. Oriental Beauty is made from the tea leaves infested by green leafhoppers [Jacobiasca formosana], and the tea leaves are oxidized by high degree of the fermentation. These unique processes seem to induce synthesis of various volatile compounds in the leaves by utilizing plant-insect interaction and plant defense responses. To understand the biochemical process of the flavor formation during its manufacturing, we have investigated gene expression profiling in the manufacturing process. We made the tea samples not only from the leaves of cv. Chin-shin Dah-pan infested by the insects but also from healthy leaves without the insect feeding as a reference. The leaves were collected at each step of the manufacturing processes (from solar-withering to 4th turning-over and indoor withering) and immediately frozen before panning. Total RNA from each leaf sample was subjected to a gene expression profiling. We used a “DNA microbeads array” technique in order to identify a vast number of genes induced in response to the insect feeding and abiotic stresses. In the infested leaves treated with 1st turning-over and indoor withering, a huge number of genes involved in stress responses have been found to be induced.

Key wards

Oriental Beauty, green leafhopper, gene expression, tea aroma, stress response

Introduction

The famous Formosa Oolong tea “Oriental Beauty (Pom-Fong tea)” made in Taiwan has high quality flavor. Oriental Beauty is made from the tea leaves infested by green leafhoppers [Jacobiasca formosana], and the tea leaves are highly oxidized by the fermentation process. Because of the unique manufacturing process, it has rich aroma like a ripe fruit and honey. We are interested in the molecular mechanism by which the rich aroma is produced in Oriental Beauty. It is likely that attack by the green leafhopper effects on the high production of aroma compounds in the leaves. Both young and adult leafhoppers use their needle-like mouthpart to suck sap from the juvenile leaves. By feeding in this way, the green leafhoppers make wounds on the surface of tea leaves, and the leaves become covered with small yellow spots. The infested leaves finally become stunted, and especially under dry conditions, the upper part can dry out. The less seriously affected leaves can...
have a purple color. When manufacturing Oriental Beauty, the infested leaves with severe symptoms are used. It is very unique point in this tea product.

There are some different points in the manufacturing processes of Oriental Beauty from those of the other oolong teas. In Oriental Beauty, the step of solar withering is longer, turning over is deeper, and the additional step, wetting and softening, is done before rolling. Thus, the fermentation degree of Oriental Beauty becomes higher.

| Plant materials

We made the tea samples not only from the leaves infested by green leafhoppers but also from healthy leaves without sever symptoms as a control. We used the leaves of *Camellia sinensis* var. *sinensis* cv. Chin-shin Dah-pan. These leaves were collected at each step of the manufacturing processes from fresh leaves to 4th turning-over and indoor withering, and immediately frozen without panning.

| Table 1. Tea leaf samples

<table>
<thead>
<tr>
<th>Fresh leaves</th>
<th>Solar withering</th>
<th>Turning over and indoor withering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infested leaves</td>
<td>AF</td>
<td>ASW</td>
</tr>
<tr>
<td>Healthy leaves without sever symptom (control)</td>
<td>BF</td>
<td>BSW</td>
</tr>
</tbody>
</table>
Differential screening

Total RNA was isolated from the leaves with or without the insect infestation in each step of the manufacturing processes. To identify genes specifically induced in response to many stresses, we performed a differential screening. We used a “DNA microbeads array” technique. This microbeads array experiments were performed by Takara-Bio Inc. After the isolation of mRNA, the cDNAs were synthesized and immobilized onto the microbeads. One bead contains a single cDNA, and 400,000 beads were prepared from 400,000 of independent cDNAs derived from total RNA of the control and stressed leaves.

After fluorescence labeled probes were prepared by using the mRNA from control and stressed leaves, the Megaclone™ microbeads were sort out through Megasort™ by combining each two prepared probes. The beads derived from up-regulated genes and down-regulated genes were separately collected by a cell sorter machine.

Sequencing

The cDNAs on the beads were amplified by PCR, and the DNA sequences of the cDNAs were determined. Sequencing analyses were performed on an ABI Prism 377 DNA sequencer with the DigDye Terminator var. 1.1 Cycle Sequencing kit (Applied Biosystems). Using this technique, we have obtained a vast number of genes induced in response to insect infestation and abiotic stresses.

Results and Discussions

First, we did the differential screening between BF and AT1 (Fig.2). In this case, we can expect to obtain the cDNAs differently expressed in response to the insect infestation as well as many stresses during the manufacturing processes. We used 800,000 microbeads library derived from mRNA of the BF and AT1. There were about 90,000 cDNAs in the up-regulated genes among 800,000 cDNAs. On the other hand, there were about 110,000 cDNAs in the down-regulated genes (Table 2). Two hundreds of the cDNAs from each zone were randomly sequenced. The majority of the up-regulated genes were found to be heat shock proteins and dehydrin. On the other hand, the majority of down-regulated genes were photosynthesis related proteins.

There were too many genes differently expressed between BF and AT1, and therefore, we could not obtain the cDNAs involved in the biosynthesis of aroma and unique metabolites of Oriental Beauty.
Among them, we were interested in the induction of jasmonic acid induced protein, and so on. Between these two zones, namely highly induced and moderately induced genes to identify valuable gene, and so on. We greatly thank Mr. Xu, ex-member of Tea Research and Extension Station, and Mr. Kou, the farmer in Hsinchu County.