2011 International Conference on Environmental Science and Engineering (ICESE 2011)

Gene chip analysis of the retrotransposons in rice implanted by the N+ ion beam

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Abstract

In order to learn the retrotransposons expression profiles in rice implanted by low-energy ion beam and the effects on these retrotransposons adjacent genes, we analyzed expression features of the retrotransposons in rice with exposure to the nitrogen ion beam implantation (6×10¹⁷ N+/cm²), using the Agilent Rice Oligo Microarray (4×44K) Genome Array. Rice seeds were implanted by the nitrogen ion beam with 40Kev energy in dose 6×10¹⁷ N+/cm². Total RNA, from the seedlings 96 h after the germination, was used to genechip analysis. The vigor index was measured at 10 days after the germination. The results showed that the vigor index increased obviously than control. We also found that there were 43 retrotransposons transcripts detected by the chip, 4 out of these transcripts were up or down-regulated (≥2fold), including the gag, pol, and int. The differentially expressed genes (detected by the same gene chip), in the frame from up 1MB to down 1MB in the chromosomes at the differentially expressed retrotranscription, represented the same up or down regulated case. These findings suggested that the differential expression of retrotransposons in rice were related with the response to N+ ion beam implantation through the regulation of their adjacent genes.

Gene chips, also called DNA microarrays, is a molecular biology technique based on the theory of the hybridization sequencing. It has many features such as high parallel, multiplicity, miniature and automation. Gene chip technology is of high efficiency dozens to thousands of times than traditional methods, and thousands of genes can be measured simultaneously [1], so it is a powerful tool for DNA sequence and the gene expression information analysis [2]. This technology has been widely used in medical diagnostics, drug screening, the gene expression measurement, environmental monitoring, crop pest and detection etc., having a very broad application prospect [3].

Retrotransposons are mobile genetic elements which are widely distributed in eukaryotes. They copy themselves to RNA and then back to DNA that may integrate back to the genome. The second step of forming DNA may be carried out by a reverse transcriptase which the retrotransposon encodes [4].
Retrotransposons encode many proteins, including the major three genes, namely \textit{gag} (group specific antigen), \textit{pol} (polymerase) and \textit{int} (integrase). The proteins which are encoded by the \textit{gag} gene take part in the maturation and packaging of the retrotransposons RNA, and make it suitable for integration into the genome. \textit{Pol} gene which encodes reverse transcriptase and RNase H is necessary for the replication and transposition of the retrotransposons. Integrase enzymes which are encoded by \textit{Int} make the retrotransposons integrate into a new locus on chromosome \cite{5}. Commonly, retrotransposons keep silent in plants, but some still has the transposable potential. these retrotransposons activity can be induced by the Biotic and abiotic stress. Studies suggest that some stress (include: isolated protoplasts, tissue culture, chilling injury etc.) can activate many retrotransposons \cite{6-10}.

The low-energy \textit{N}+ beam irradiation treatment has already become an important method for studying plant genetics and breeding, growth and development, stress response and other aspects Since Yu Zeng-Liang used it to study the biological effects on crop seeds firstly \cite{11}. In this study, the rice was exposed to the low-energy nitrogen ion beam implantation, and then the total RNA of the seed after 96h of germination was extracted at 30°C. Agilent gene chip was used to screen the differentially expressed ESTs related retrotransposons.

1. **Materials and methods**

1.1 **Materials**

Rice cultivar Zhonghua 10 (\textit{Oryza sativa} L.ssp. japonica) was saved in the Key Laboratory of Ion Beam Bioengineering preservation, Zhengzhou University, Henan Province and the gene chip was customized in Shanghai National Engineering Research Center. The Low Energy Ion Beam implantation (UIL, 0.1512, TNV) was purchased from the Institute of strong electricity, Russia and its working vacuum was 2 × 10^-3 ~ 5 × 10^-3 MPa. Both the total RNA extraction kit (Takara D312) and DNaseI (D2215) were obtained from TaKaRa Biotechnology Co., Ltd. PCR instrument for the MJ Company (PTC-100), gene chip for the rice Agilent single gene chip microarray.

1.2 **Methods**

**Low energy ion beam**

The rice seeds were implanted by the low-energy (40 Kev) \textit{N}+ ion beam in dose 6×10^17 \textit{N}+/cm² under the vacuum (3 × 10^-3 Pa). After the implant, part of the seeds immediately were incubated 96-hours in a dark climate at 30°C under proper humidity. All seeds were planted on sterile medium with 0.6% agar only. Three biological replications were done under each doses, and 100 seeds for each replicates. The untreated seeds were as the control.

**RNA extraction**

Total RNA was extracted from uniform thirty individual buds in each replicate using RNA plant reagents and purified by using the RNeasy Plant Kit according to the manufacturer's instruction. RNA extraction steps of the control samples were the same as above. The yield and purity of RNA were determined spectrophotometrically.

**Determination of the energy index**

The germination percentage and vigor index were investigated after the seeds planted 10 days using the rest of the seeds. The whole-plants were weighed out after drying 12 h at 60 °C. then:

\[
\text{Germination percentage} = \frac{\text{Number of the seedlings}}{\text{100 seeds}} \times 100\%
\]

\[
\text{Vigor index} = \text{drought weight} \times \text{Germination percentage}
\]

**Agilent single microarray hybridization and data analysis**

The Agilent Gene Chip hybridization and data analysis were carried out by the Shanghai Biochip
National Engineering Research Center, including the procedures for cDNA and cRNA synthesis, cRNA Cy3 fluorescence labeling, hybridization, washing, scanning, data collection and normalization.

**Quantitative real-time PCR**

An up-regulated of the gag EST (Os02g0514000) was selected to do real-time quantitative PCR to validate microarray. We used the 2-ΔΔCT method to calculate the relative expression.

1.2.6 Screening the differential expression of retrotransposons EST of the rice after the low-energy N\(^+\) beam irradiation treatment using gene chip

The differential expression of retrotransposons gag, pol, int were compared between the N\(^+\) beam irradiation and control samples, more than 2-fold differentially expressed probe were elected.

2. **Results and Analysis**

2.1 **Vigor index of the rice implanted by the low energy N+ ion beam**

Table 1. Two groups of rice budding 10 days after growth situation comparison

<table>
<thead>
<tr>
<th>Batch processing</th>
<th>Germination ratio</th>
<th>Dry weight (g)</th>
<th>Vigor index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-1</td>
<td>76</td>
<td>0.11</td>
<td>8.36</td>
</tr>
<tr>
<td>Control-2</td>
<td>73</td>
<td>0.14</td>
<td>10.22</td>
</tr>
<tr>
<td>Control-3</td>
<td>72</td>
<td>0.10</td>
<td>7.20</td>
</tr>
<tr>
<td>6×10(^{17}) N(^+)/cm(^2)-1</td>
<td>80</td>
<td>0.18</td>
<td>14.40</td>
</tr>
<tr>
<td>6×10(^{17}) N(^+)/cm(^2)-2</td>
<td>80</td>
<td>0.16</td>
<td>12.80</td>
</tr>
<tr>
<td>6×10(^{17}) N(^+)/cm(^2)-3</td>
<td>80</td>
<td>0.15</td>
<td>12.00</td>
</tr>
</tbody>
</table>

The difference of the average dry weigh between the radiation processing and control groups was singifican (\(P = 0.042\)) when we used the t-test to compare, and the same to the germination percentage, the \(P\) values was 0.017. From Table 1, we could calculate that the average vigor index of the radiation processing and control groups respectively was 8.59\%, 13.07\%. And it had the significant difference (t-test, \(P = 0.024\)). In short, after the rice seeds implanted by the low-energy N\(^+\)-beam germinated 10 days, germination percentage, dry weight and average energy index were significant.

2.2 **Quantitative real-time PCR**

The relative expression levels of the Os02g0514000 in the three group samples at 6 × 10\(^{17}\) N\(^+\) / cm\(^2\) were 2.5, 2.0, 3.2, respectively, showing the up-regulated, and consistent with the results of the chip.

2.3 **Screening the differential expression of retrotransposons EST of the rice after the low-energy N+ beam radiation treatment using gene chip**

The differential expression of retrotransposons gag, pol, int were compared between the N\(^+\) beam irradiation and control samples, more than 2-fold differentially expressed probe (Table 2,3,4)were elected. And the expression of samples with N\(^+\) beam irradiation were up-regulated mostly.

Table 2 N\(^+\) beam irradiation and control samples of rice differential expression of gag gene conditions

<table>
<thead>
<tr>
<th>ProbeName</th>
<th>FC Absolute</th>
<th>regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Os02g0514000</td>
<td>2.81</td>
<td>up</td>
</tr>
</tbody>
</table>

Table 3 N+ beam irradiation and control samples of rice differential expression of pol gene conditions
We detected 21, 8, 14 gene probes for retrotransposon gag, pol, int respectively with gene chip. Compared with the control probe, the average value of all gag probes were 1.12 times in gene expression with the standard deviation of 0.46. And the gene expression of the pol and int probes, the average values were 1.22 (± 0.67), 1.28 (± 1.01), respectively.

2.4 Analysis for the probes with differentially expressed located with 1MB chromosomal

Genetic analysis for the retrotransposons with differential expression had been done, and the probes having more than 2.7 times in different expression were selected. Then we also analysed the probes having more than 1.7 times in differential expression which located within 1Mb of the chromosomal. The results were got through the NCBI as follows (Table 3,4):

The differentially expressed genes compared with control had been found located on the retrotransposons (Os02g0514000, Os08g0133100) probe (within 1 MB of the chromosomal) (Table 5, Table 6). For gag probe(Os02g0514000) (Table 5), the upstream genes expression were all down, suggesting the gag gene might up or down modulate the gene expression of the up or downstream genes. As for pol probe (Os08g0133100) (Table 6), the expression of the upstream probes were down-regulated while the downstream probes mostly were up-regulated, implying pol gene might had different effects on the adjacent genes (within 1Mb; up-regulated for downstream gene and down-regulated for upstream).

3. Discussion

Retrotransposons are widely distributed in plant genomes, and play an important role in the genome
structure, evolution and function. Studies have shown that the retrotransposons located near or within the genes may have the transposition potential, when these retrotransposons are activated by certain stimulation, the transposition will cause genetic variation. The whole rice genome draft sequence reveals that retrotransposons don't eliminate but exist with inactive form. The retrotransposons inserting nearby or into genes may affect the transcription time and transcription model of the adjacent genes to control their expression or silence\(^{[12]}\). Studies had found that \textit{Wis2-1A} in the new synthetic hexaploid wheat had a high activity and stable expression. Transcriptional activation of \textit{Wis 2-1A} can had far-reaching effects on adjacent genes, when induced, \textit{Wis 2-1A} LTRs made the adjacent genes transcribed into antisense or sense RNA, resulting in the corresponding gene activation or silencing\(^{[13]}\). In this study, we used the low-energy N\(^+\) beam irradiation to study the differential expression of the rice retrotransposon-related EST, and the effects on these retrotransposons adjacent genes.

Microarray analysis showed the low-energy N\(^+\) beam radiation treatment using gene chip could lead to the differential expression of the part retrotransposons EST. Real-time quantitative PCR validation results are consistent with the gene chip. Germination percentage, dry weight and average energy index were significant after the rice seeds implanted by the low-energy N\(^+\)-beam germinated 10 days, and it might also be associated with the differential expression of the retrotransposons EST in rice implanted by low-energy N\(^+\)-beam beam. Studies also showed that differentially expressed genes located on the chromosomal (within 1MB) of the retrotransposon \textit{gag} probe (Os02g0514000), suggesting that the differential expression of the retrotransposons \textit{gag} probe might modulate the upstream or downstream genes after the low-energy N\(^+\)-beam irradiation processing. And for the \textit{pol} probe, there also existed differentially expressed genes within 1MB chromosomal location, and the differential expression of \textit{pol} genes might down-regulated for the 1MB upstream genes and up-regulated for downstream genes. So the rise of part of the retrotransposons EST expression might extend their transposition potential, strengthen the certain genes regulation, and for theirs upstream and downstream genes expression had certain regularity. In summary, under the low-energy N\(^+\)-beam irradiation treatment, the differential expression retrotransposons EST have effects on the expression of the adjacent genes (increase or decrease), at least play a certain role to gene regulation. Of course, the accurate interpretation for these phenomena need to be studied in depth.

Acknowledgement

This work was supported by The National Natural Science Fund of China (grant no.30800204) and the opening foundation of the Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Science (2009B004), most of the work was performed in the key lab of the ion-beam biotechnology in Henan Province.

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