Changed-transcriptional activity of retrotansposons induced by implantation of low-energy ion beam effected the expression of genes adjacent to retrotransposons

LI Yong-hui[1], YU Xiang-li[1], LI Ya-he[2], GUO Xiang-meng[1], YA Hui-yuan[1*]

[1.]Life Science College, Luoyang Normal University, Luoyang, Henan 471022, China
[2.]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, 361005, China
huiyongli8209@126.com,yahuiyuan@yahoo.com.cn

Abstract: In order to study the expression profiles of the retrotransposons in rice implanted by low-energy ion beam and the effects on adjacent genes to these retrotransposons, we analyzed expression features of the retrotransposons in rice with exposure to the N^+ ion beam implantation (6×10^{17} N^+/cm^2), using the Agilent Rice Oligo Microarray (4×44K) Genome Array. The results showed that there were 43 probe sets in chip, 4 out of these transcripts were up or down-regulated (≥2fold), including the *gag*, *pol*, and *int*. These four transcripts were heterogeneous to the other members in the family by clustering analysis. We also found that this differential expression effects the genes expression were up 1MB to down 1MB from the differentially expressed retrotranscription ESTs, representing the same up or down regulated case. These findings suggested that retrotransposons in rice were related to the response to N^+ ion-beam implantation through the regulation of their adjacent genes.
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Introduction

Chip technology is of high efficiency dozens thousands of times than conventional methods, and thousands of genes can be analysed in parallel test[1], so it is a powerful tool for DNA sequence and information of gene expression analysis[2]. Gene chip technology has been used in medical diagnostics, drug screening, the gene expression measurement, environmental monitoring, crop pest and detection etc., showing a very broad application prospect[3].

Retrotransposons are a class of mobile genetic factors which are widely distributed in eukaryotes. Retrotransposons encode many proteins, including the major three genes, namely *gag* (species-specific antigen), *pol* (polymerase) and *int* (integrase). The proteins encoding by the *gag* gene take part in the maturation and packaging of the retrotransposons RNA, in order to make it suitable for integration into the whole genome. *Pol* gene encodes reverse transcriptase and RNase H, which is necessary for the replication and transposition of the retrotransposons. Integrase enzyme which is encoded by *Int* make the DNA state retrotransposons integrate into a new locus on chromosome[4]. Commonly, retrotransposons keep silent in plants, but some still has the potential to switch seats. Biotic and abiotic stress can induce the activity of these retrotransposons. Some studies suggest that many retrotransposons can be activated by the isolated protoplasts, tissue culture, chilling injury, and other stress[5-9].

Since Zeng-Liang Yu spearheaded used the low-energy nitrogen ion beam irradiation to study the biological effects on crop seeds, the low-energy N^+ beam irradiation treatment has become an important method for studying plant genetics and breeding, growth and development, stress response and other aspects[10]. In this study, the rice was exposed to the low-energy nitrogen ion beam irradiation, then we extracted the total RNA, by using the rice gene chip to scan the differentially expressed ESTs related retrotansposons.

1. Materials and methods

1.1 Materials

Rice cultivar Xindao-18 (*Oryza sativa L.ssp. japonica*) was obtained from the Key Laboratory of Ion Beam Bioengineering preservation, Zhengzhou University, Henan Province and the gene chip microarray was customized in Shanghai National Engineering Research Center. The equipment of the Low Energy Ion Beam implantation (UIL. 0.1512, TNV.) with the working vacuum of $2 \times 10^{-3} \sim 5 \times 10^{-3}$ MPa was purchased from the Institute of strong electricity, Russia. Both the total RNA extraction kit (Takara D312) and DNaseI (D2215) were purchased from TaKaRa Biotechnology Co., Ltd. PCR instrument for the MJ Company (PTC-100), for the rice Agilent single gene chip microarray.

1.2 Methods

1.2.1 Low Energy Ion Beam

Select the same rice seeds (all seeds are from the same plant) putting on the dishes with the embryo upturned, then were implanted by low-energy N^+

(30keV) in dose 6×10^{17} N^+/cm^2 under the vacuum (3×10^{-3} Pa). After the exposure, part of the seeds immediately were carried into artificial climate chamber at 30 ℃ in dark conditions and germinated under proper humidity, with the seeds which had not been injected as the control N^+. There are three biological replicas for every treatment, and 130 seeds for each repetition.

1.2.2 RNA extraction

Total RNA was extracted from uniform thirty individual buds in each replicate, which were planted for 96 hours, using RNA plant reagents (Tiangen Biotech)and purified by use of the RNeasy Plant Kit (Qiagen) according to the manufacturer's instruction. The digestion of DNA with DNase I (Qiagen) was included for all RNA preparations after the extractions.

1.2.3 Determination of the vigor index

The vigor index were investigated after the seeds planted 10 days using the rest of the seeds.The whole-plant were weighed out after drying 12 h at 60 ℃. then the vigor index was calculated as:

Vigor index= Germination percentage * dry weight.

1.2.4 Agilent single microarray hybridization, scanning, data acquisition and processing

The Agilent Gene Chip hybridization and data analysis were carried out by the Shanghai Biochip National Engineering Research Center, including the scanning, data acquisition and processing.

1.2.5 Quantitative real time PCR

Select an increase of the gag EST (Os02g0514000) to do real time quantitative PCR to validate microarray. 2-ΔΔCT method was used to calculate the relative expression.

1.2.6 The cluster analysis of rice retrotransposons

According to common name, the nucleotide sequences of the genes (*gag*, *pol*, and *int*) were received from the NCBI. Then the nucleotide sequence analysis had been analyzed by the ClustalX, and the phylogenetic tree was obtained by the Mega5 software with the UPGMA method.

2. Results and Analysis

2.1 The effects of the N^+ irradiation on the vigor index

Compared the average dry weigh between the irradiation treatment and control groups using the t-test, the difference was significant (P = 0.042), and the same to the germination percentage, the P values was 0.017. From Table 1, we can calculate that the average vigor index was 8.59%, 13.07%, for the irradiated and control groups, respectively. And they had the significant difference (t-test, P = 0.024). In one word, the effects of N^+ irradiation on the rice seeds germination in 10 days, dry weight and average energy index were significant.

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

Batch processing	Germination ratio（%）	Dry weight (g)	Vigor index（%）
Control 1	76	0.11	8.36
Control 2	73	0.14	10.22
Control 3	72	0.10	7.20
6×10^{17} N^+/cm^2-1	80	0.18	14.40
6×10^{17} N^+/cm^2-2	80	0.16	12.80
6×10^{17} N^+/cm^2-3	80	0.15	12

2.2 Real-quantitative PCR

During the three samples, the relative expression levels of the Os02g0514000 at 6×10^{17} N^+ / cm^2 were 2.5, 2.0, 3.2, respectively, showing the increasing performance, 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 irradiation treatment using gene chip

Compared the differential expression of retrotrans -posons between the N^+ beam irradiation and control samples, more than 2-fold differentially expressed in the probe (Table 2) were filtered out. And the expression of samples with the exposure to N^+ beam irradiation was up-regulated mostly.

There were 21, 8, 14 gene probes for retrotransposon *gag*, *pol*, *int* respectively, which were detected by Chip. Compared with the control probe, all *gag* probes had 1.12 times (average value) in gene expression with the standard deviation of 0.46. As for the gene expression of the *pol*, *int* probes, the values were 1.22 (± 0.67), 1.28 (± 1.01), respectively.

Table 2. N^+ beam irradiation and control samples of rice differential expression of *gag*, *pol*, *int* gene conditions

Gene	ProbeName	FCAbsolute	regulation
gag	Os02g0514000	2.81	up
pol	Os08g0133100	2.74	up
	Os01g0116100	2.25	down
int	Os02g0309600	4.71	up

2.4 Cluster analysis for the genes (*gag*, *pol*, *int*)

In order to compare the nucleotide sequences of the probe which had a different expression between the N^+

beam irradiation treatment and control sample, cluster analysis had been done using the software named Mega5.1.

The analysis for genetic distance had been obtained based on the expression of retrotransposons, and we could see that most of the genetic distance of the *gag*, *pol*, and *int* is greater than 0.3, 0.3, and 0.2, respectively (Fig.1, Fig.2, Fig.3). For the *gag* probe (Fig.1), the retrotransposons (Os02g0514000) which have different expression, belongs to this class. But the retrotransposons (*pol* probe; Os08g0133100) which also had different expression clusters as a single category, and others having the same expression multiples mass as a class (Fig.2). And for the *int* probe, there have the similar results as the *pol* probe. The important was strong sequence heterogeneitythat in the three probes. These results suggested that some specific-retrotransposons take part in response to the low-energy ion beam irradiation under this dose, but few in number.

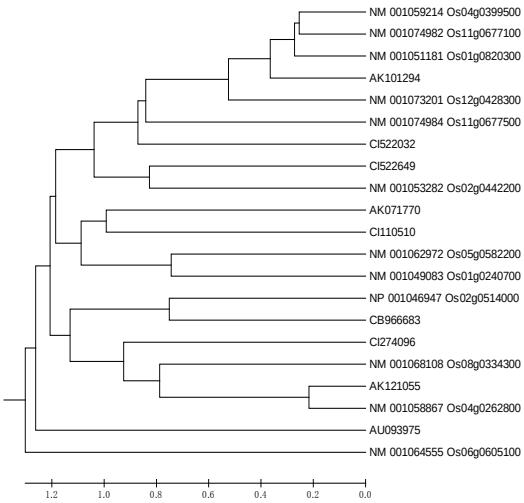

Fig.1 N^+ beam irradiation compared with control samples of rice expression *gag* probe dendrogram

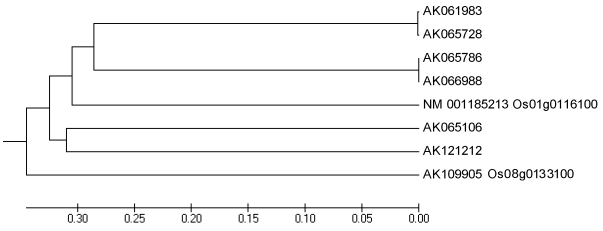

Fig.2 N^+ beam irradiation compared with control samples of rice expression *pol* probe dendrogram.

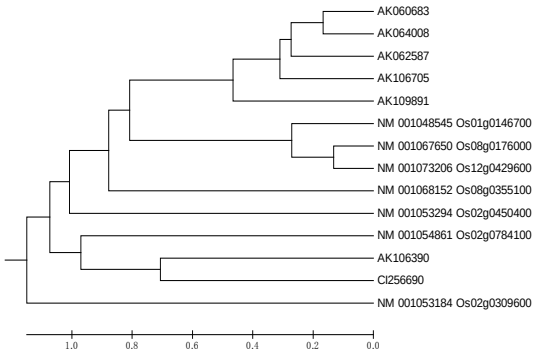

Fig.3 N^+ beam irradiation compared with control samples of rice expression *int* probe dendrogram

Through the NCBI, we found that Os02g0514000 located in between 18615320 and 18618386 on the second chromosome of the Oryza sativa (japonica cultivar group) genomic DNA. AK109905 (Os08g 0133100; short arm = 1844893, long arm = 18466) located in chromosome 8 (same genomic DNA). Os02g0309600 also had been found in chromosome 2 (same genomic DNA) between 12154821 and 12157317, and in Os02g0514000 upstream.

2.5 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 had more than 2.7 times in different expression had been selected. Then we also compared the probes having more than 1.7 times differential expression which located within 1MB of the chromosomal. We got the results through the NCBI as follows (Table 3,4).

The genes with different expression compared with control had been found located on the retrotransposons (Os02g0514000, Os08g0133100) probe (with in 1 MB) (Table 3, Table 4). For *gag* probe (Table 3), the expression of the upstream genes were down, implying the gag may up or down modulate the gene expression of the up or down-stream gene. As for Os08g0133100 (Table 4), the upstream probes were down, while the downstream probes mostly were up, suggesting *pol* gene may have different effects on the adjacent genes (with 1MB; up-regulated for downstream gene and down-regulated for upstream).

3. Discussion

Retrotransposons are widespread presence in the plant genome, and play an important role in the genome structure, evolution and function. Studies have shown that the genes near or located within the retrotransposon may have the potential transposon, when these retrotransposons were activated by certain

stimulation, this function can cause genetic variation. Whole rice genome draft sequence reveals that retrotransposons does not eliminate but exist with no active form. Inside a gene or genes into the nearby retrotransposons may affect the time of transcription and transcription model of the adjacent genes to control their expression or silence[11]. Kashkush K. et al. had reported that Wis2-1A in the new synthetic hexaploid wheat had a high activity and stability of expression. Transcriptional activation of Wis 2-1A could have far-reaching effects on adjacent genes, when induced, the adjacent genes transcript and shape chain or antisense strand, resulting in the corresponding gene expression or silencing[12]. In this study, we took the rice as materials and used the low-energy N^+ beam irradiation to study the differences in the expression of the retrotransposon-related EST and modulation. Microarray analysis revealed that differentially expressed genes located on the chromosomal (with 1MB) of the retrotransposon *gag* probe (Os02g0514000) with differential expression, suggesting that the differential expression of the retrotransposons EST might modulate the upstream or downstream genes (with 1MB) after the N^+-beam irradiation treatment. And for the pol probe, there also existed differentially expressed genes with less than 1MB chromosomal location, and down or up for the upstream, downstream probes, respectively.

All these suggested that differential expression of pol might down-regulated for the 1MB upstream genes and played the up-regulated role for downstream genes. So the increase of part of the retrotransposon EST expression may extend their probability of transposition, strengthen the regulation of certain genes (for upstream and downstream of the gene), and for its upstream and downstream gene expression there is a certain regularity. In summary, under the low-energy ion beam irradiation treatment, the retrotransposons have effects on the expression (increase or decrease), at least play the role of gene regulation. Of course, further studied about the accurate interpretation for these phenomena need to be researched.

Table 3. Differentially expressed the *gag* probe chromo- some position within 1Mb differentially expressed probe

Probe Name	Chromosome position	p-value	FCAbsolute	Regulation
Os02g0510400	upstream 18342477 -18343176	0.005	2.13	down
Os02g0512400	upstream 18514080 -18515103	0.009	1.73	down
Os02g0517700	downstream 18824891-18825982	0.202	1.84	down
Os02g0518400	downstream 18857193-18862864	0.070	2.95	up

Table 4. Differentially expressed the *pol* probe chromo- some position within 1Mb differentially expressed probe

Probe Name	Chromosome position	p-value	FCAbsolute	regulation
Os08g0122700	upstream1578794-1582551	0.012	2.30	down
Os08g0127900	upstream 1590894-1593153	0.194	2.25	down
Os08g0128000	upstream 1594506-1598551	0.020	2.96	down
Os08g0131100	upstream 1746781-1749181	0.023	2.17	down
Os08g0136600	downstream 2054411-2055286	0.067	2.32	down
Os08g0136700	downstream 2056871-2058397	0.011	2.48	up
Os08g0136800	downstream 2060089-2061374	0.004	1.83	up
Os08g0137300	downstream2101974-2105683	0.273	2.10	up

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Corresponding Author:
Dr. YA Hui-yuan
Luoyang Normal University
Life Science College
Luoyang Henan,China
E-mail: yahuiyuan@yahoo.com.cn

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