RNA-seq revealed the promoted expression of functional protein related to transposable element in rice exposure to low-energy ion beam bombardment

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Abstract: The low-energy N^+ ion beam was used to implant rice seeds which were cultured until to germinate, then after training seven days.The materials were divided into the non-growth inhibition (NGI: $1\times10^{17}N^+/cm^2$, $2\times10^{17}N^+/cm^2$) and growth inhibition group (GI: $6\times10^{17}N^+/cm^2$, $8\times10^{17}N^+/cm^2$) according to the germination percentage, height of seedling and root long determination. For the purpose of exploring the functional gene expression of transposable elements (TEs) in rice responding to nitrogen ion beam implantation, we used RNA-Seq to analyze the total RNA from 3-day rice seedlings in the control, GI and NGI group. The results showed that total 36382 transcriptions were obtained in the sequencing data, which included 11851 known rice transposon. It is 972, 818, 1271 genes of TEs expressed in control, NGI and GI group sample respectively in total 1655 types of TEs expression detected in the samples. These findings indicated that a certain dose of low-energy ion beam irradiation could promote transcription of transposons, enhance transpositional potential and increase chromosomal structural changes, this may be one mechanism of plant mutants induced by low energy ion beam.
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Key words: rice, Ion beam irradiation, RNA-seq, Transposalbe elements, DEGs

1. Introduction

The low energy ion beam is the effective radiation-induced mutation source and many mechanisms of mutant induced by low energy ion beam irradiation received extensive research. After more than 20 years of development, the research of the biological effect and mechanism of ion implantation on the different plant and microorganism has made very big progress, creating a larger economic and social benefits. The mutagenic effect of low energy ion beam on organism has little damage, high rate of mutation, broad mutational spectrum and other characteristics [1,2-3]. However, the low energy ion beam (heavy ion: 10-100, 200 keV) has very weakly penetrating capacity, such as based on Longitudinal static theory(LSS), the range of 60 keV$^{14}N^{1+}$ in the protein and 100 keV$^{14}N^{1+}$ in wheat is slightly larger than 0.2 and 110 keV$^{56}Fe^{1+}$ in the wheat germ with a range less than 0.2 μ m[5]. The study of Wei Zengquan showed that the maximum range of 110 keV$^{56}Fe^{1+}$ in wheat seed embryo was about 5 μ m with the most minimal range about 0.935 μ m[6]. In a word, the range of low energy ion beam in crop seeds is so shallow that could not reach the genetic material of the nucleus damaged growing point. But many studies indicate that energy deposition, momentum transfer and the charge exchange effect exist when the ion implanted into organisms, which caused distortion of chromosome, injury and break of DNA chain, further change or miss the genetic materials in gene or molecular level, at last improve the variation frequency [4]. According the differences between the theory and practice, we think that the mechanisms of the mutations induced by the low energy ion implantation rice/wheat and other crop seeds are not the direct effect but series of secondary process of composite role. We discovered that mutagenic effects induced by low energy ion beam implantation in plants may closely be related to the structure change of the cell nuclei and chromosome which caused by low energy ion beam implantation [7], and this is very similar with the results of the transposable element activity, so we speculate that low-energy ion beam bombardment is able to activate a number of transposable elements and cause the changes of chromosome structure. Previous studies showed that low-energy ion beam bombardment in rice can promote the transcriptional activity of reverse transcription factor [8,9]. But until now, the reports about the effects of the low-ion beam bombardment on the transposable elements from transcriptome level is not clear, and the research on the transcriptome level is very important to understand the mutagenesis mechanism induced by low energy ion beam exposure.

The development of high-throughput sequencing methods such as RNA-sequencing (RNA-seq)[10] has offered an opportunity to hasten a fuller characterization of the transcriptome[11]. RNA-Seq allows the analysis of several aspects of genome transcription, providing sequence data as well as the ability to detect alternative splicing events and quantify gene expression levels[12]. High-throughput

transcriptome sequencing based on the Illumina platform is considered the powerful tool for transcriptome analysis[13]. Transcriptome studies based on the RNA-seq technology provide new biological research to clarify the mechanisms of the biological effects about beam bombardment, but studies of the molecular mechanism based on the RNA-seq technology are relatively scarce. So we anglicized the gene from the transposons in rice treated with the low energy ion beam through the transcriptome studies based on RNA-seq technology in order to research the mechanism of mutant induced by low energy ion beam.

2. Materials and methods

1.1 Plant Materials

In this experiment, we selected Xindao-18 (Oryza sotiva L.subsp.joponica Kato) as the material. After the cultivar is mature, we collected the panicle and removed the glume manually to avoid the injury of rice embryo. Then the complete seeds with similar size were selected as experimental materials.

1.2 Methods

1.2.1 Low energy ion beam irradiation

In low energy ion beam irradiation, the rice seeds (moisture content is 6.7%) are vertically fixed on a cork with embryo upward. Then we put this cork into a culture dish (with size of 10 cm×10 cm). Ion implantation is conducted in a vacuum (10^{-2}MPa) target chamber (implanter model: with the Ion Beam Bioengineering Facility (UIL.0.512, TNV, Russia) with current intensity of 2 mA, ion energy of 40 Kev, and ion fluence of 0×10^{17} N^+/cm^2, 1×10^{17} N^+/cm^2, 2×10^{17} N^+/cm^2, 4×10^{17} N^+/cm^2, 6×10^{17} N^+/cm^2, 8×10^{17} N^+/cm^2 respectively. Besides, we set a group without implantation as the blank control. Each sample group is set with three biological repetitions. Each repetition uses 150 rice seeds.

1.2.2 Cultivation of biomaterial and determination of germination rate, seedling height, root length

An aseptic filter paper is placed on the bottom of an aseptic culture dish after moistened with aseptic water. Put the implanted seeds and control materials on this filter paper, and then put the culture dish into a climate box (with12 h in light /12 h in dark) with constant temperature (28 ℃). For every 12 h, uniformly drip 10 ml aseptic water on the filter paper in culture dish to keep the filter paper be moist. After culturing for 96 h, randomly collect 100 buds (including bud and root, bud is about 3 cm now) in total from the three repetitions treated by each ion fluence. These 100 buds were stored in -80℃ and taken as the sequencing samples of the corresponding treatment and the materials for extracting the total RNA of mixed strains. The rest buds continue to be cultured to the seventh day (168 h). Then manually measure the germination rate (germination number / total number of seeds), seedling height (the length from hypocotyl to the top of the longest leaf), root length (the length of the longest root: the length from hypocotyl to root tip) of these buds.

1.2.3 Preparation of sequencing samples

Because a seven-day germination should provide ample time for early gene expression, rice buds cultured for 96 h were selected as material for RNA extraction and RNA-seq. Fifty of the above 100 buds cultured for 96 h and in similar height from each treated material group for the total RNA pool extraction. In experiments, we had one control RNA pool (the first sequencing sample) and five RNA pools from the five groups treated with ion fluences of 1×10^{17} N^+/cm^2, 2×10^{17} N^+/cm^2, 4×10^{17} N^+/cm^2, 6×10^{17} N^+/cm^2, 8×10^{17} N^+/cm^2 respectively. Based on measured biological effects, equal 10 μg RNA of two RNA pools from two ion-beam implanted groups with ion fluence of 1×10^{17} N^+/cm^2 and 2×10^{17} N^+/cm^2 were mixed to form the second sequencing sample (sample2, NGI: no significant growth inhibition differences compared to control). Next, equal 10 μg RNA of two RNA pool from two ion-beam implanted groups with ion fluences of 6×10^{17} N^+/cm^2 and 8×10^{17} N^+/cm^2 were combined to form the third sequencing sample (sample3,GI:significant growth inhibition compared to control).

1.2.4 RNA extraction, cDNA library preparation, sequencing

Total RNA was extracted from whole seedlingss using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and purified using RNAeasy Plant Mini Kit (Qiagen, Valencia, CA). The RNA quality was checked on a Bioanalyzer 2100 (Aligent, Santa Clara, CA); RNA Integrity Number (RIN) values were greater than 8.5 for all samples. Sequencing libraries were prepared according to the manufacturer's instructions (Illumina, San Diego, CA). Poly-A-containing mRNA was isolated from the total RNA, subjected to two purification rounds using poly-T oligo-attached magnetic beads, and fragmented using an RNA fragmentation kit. First strand cDNA was generated using reverse transcriptase and random primers. Following the second strand cDNA synthesis and adaptor ligation, 200-bp cDNA fragments were isolated using gel electrophoresis and amplified by18 cycles of PCR. The products were loaded onto an Illumina HiSeq2000 instrument and subjected to 100 cycles of paired-end (2 × 100 bp) sequencing. The processing of fluorescent images into sequences, base-calling and quality value calculations were performed using the Illumina dataprocessing pipeline (version 1.8). The sequence reads were submitted to GenBank GEO database under accession number GSE45908 (http://www.ncbi.nlm.nih.gov/ geo/query/ acc. cgi? acc=GSE45908).

1.2.5 Mapping of short reads and assessment of differential gene expression

Raw reads were filtered to obtain high-quality reads by removing low-quality reads containing more than 50% bases with Q < 30. After trimming low-quality bases (Q < 30) from the 5' and 3' ends of the remaining reads, the resulting high-quality reads were mapped onto the Nipponbare reference genome (MSU v7.0) using Tophat v2.0.5. Differential expression was estimated and tested with the software package edgeR (R version: 2.14, edgeRversion: 2.3.52)[14]; we quantified gene expression levels in terms of RPKM （Reads Per Kilobase per Million mapped reads=total exon reads/mapped reads (millions) × exon length (Kb) ） [15], calculated FDR, and estimated FC (compared to control) and log2 values of FC. Transcripts that exhibited an FDR≤ 0.05 and an estimated absolute log2 (FC) ≥ 1 were determined to be significantly differentially expressed.

2. Results and Analysis

2.1 Descriptive statistics of seed germination rate, height of seedling and seedling root length.

The result showed （ Fig1-A ） that there is no statistically significant difference in the seed germination rate between implanted samples and control, despite the seed germination rate of all implanted samples are higher than the control.

According to the comparisons of the **height of seedling (Figure1-B)**, it suggests that there is a statistically significant difference in seedling height of the treatments with ion fluence of $4\times10^{17}N^{+}/cm^{2}$,$6\times10^{17}N^{+}/cm^{2}$ and 8×10^{17} N^{+}/cm^{2} comparing to the control was , while there is no statistically significant difference in seedling height of the treatments with ion fluence of $1\times10^{17}N^{+}/cm^{2}$ and $2\times10^{17}N^{+}/cm^{2}$ comparing to the control. these founding suggeste that the height of seedling is inhibited underlying the implantation with ion fluence of $4\times10^{17}N^{+}/cm^{2}$, $6\times10^{17}N^{+}/cm^{2}$ and $8\times10^{17}N^{+}/cm^{2}$.

According to the comparisons of the **seedling root length (Figure1-C),** we found that there is a statistically significant difference in height of seedling between treatments with ion fluence of $6\times10^{17}N^{+}/cm^{2}$, $8\times10^{17}N^{+}/cm^{2}$ and controls, while there is no statistically significant difference in height of root between treatments with ion fluence of $1\times10^{17}N^{+}/cm^{2}$, $2\times10^{17}N^{+}/cm^{2}$, $4\times10^{17}N^{+}/cm^{2}$and controls. It suggested that the root is inhibited underlying the implantation with ion fluence of $6\times10^{17}N^{+}/cm^{2}$, $8\times10^{17}N^{+}/cm^{2}$.

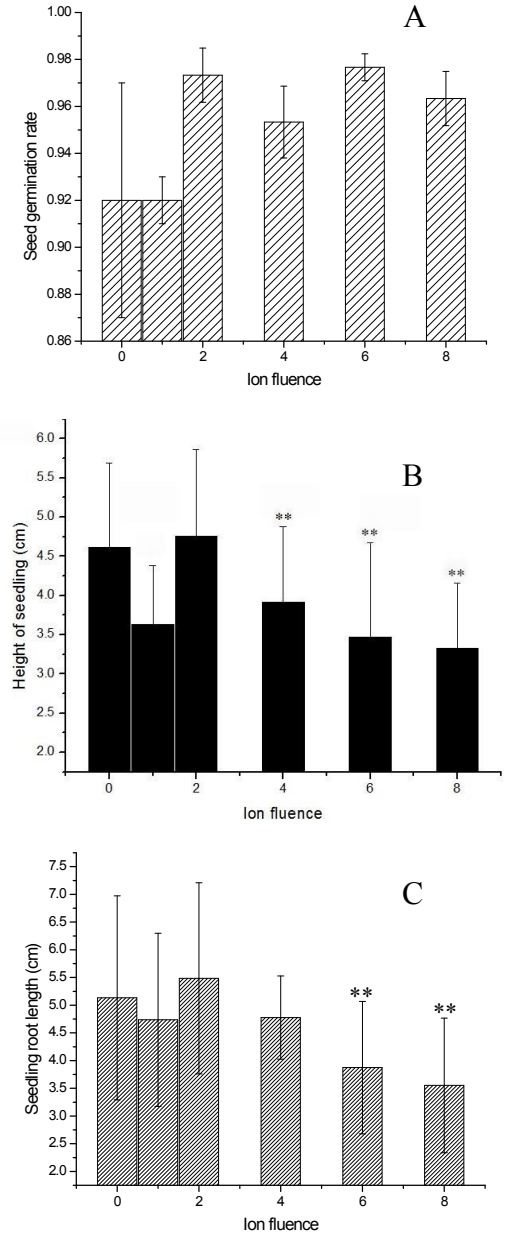

Figure 1 Comparisons of seed germination, Height of seedling and seedling root length among the different treatments. (A) The seed germination. (B) The height of seedling. (C) The seedling root length.
0, 1, 2, 4, 6, 8 refer to the treatments: blank control, $1\times10^{17}N^{+}/cm^{2}$, $2\times10^{17}N^{+}/cm^{2}$, $4\times10^{17}N^{+}/cm^{2}$, $6\times10^{17}N^{+}/cm^{2}$, $8\times10^{17}N^{+}/cm^{2}$, respectively.
**Significant difference with $p < 0.01$.
*Significant difference with $p < 0.05$.

According the above results, we considered that the larger ion fluence of $6\times10^{17}N^{+}/cm^{2}$ and $8\times10^{17}N^{+}/cm^{2}$ can induce the biological damage effect. While, ion fluence of $1\times10^{17}N^{+}/cm^{2}$ and

$2\times10^{17}N^{+}/cm^{2}$ can not induce the biological damage effect. So we mixed the RNAs from the ion-implanted samples with ion fluence of $1\times10^{17}N^{+}/cm^{2}$ and $2\times10^{17}N^{+}/cm^{2}$ as a sequencing sample (NGI: no growth inhibition), referring to the non-damaged sample, as well as, we mixed the RNAs from the ion-implanted samples with ion fluence of $6\times10^{17}N^{+}/cm^{2}$ and $8\times10^{17}N^{+}/cm^{2}$ as a sequencing sample (GI: growth inhibition), referring to the significantly damaged sample.

2.2 Identification of differentially expressed genes (DEGs)

According to the mapping of the reads and functional annotation of the rice genome (http://rice.plantbiology.msu.edu), we find out 36,382 annotated transcript from the control group, among which 544 transcript expression differences ($p<0.001$; NGI compared with control), 1.49% of the total transcript, and GI compared with control, 776 transcript expression of differences ($p<0.001$), 2.14% of the total transcript.

We found that 224 of these 262 up-regulated transcripts in NGI vs control were detected only in NGI, not detected in control (induced expression in NGI), and 38 of these 262 up-regulated transcripts were increased expressed in NGI comparing to samlp1. We also found that 38 of these 282 down-regulated transcripts in NGI vs control were only detected in control, but not detected in NGI, and 234 of these 282 down-regulated transcripts were restrainedly expressed in NGI comparing to control.

We also found that 111of these 283 up-regulated transcripts were detected only in GI vs control, not detected in control (induced expression in GI), and 172 of these 283 up-regulated transcripts were increased expressed in GI. We also found that 89 of these 493 down-regulated transcripts were only detected in control, but not detected in GI, and 404 of these 493 down-regulated transcripts were restrainedly expressed in GI comparing to control (GI vs control).

Compared GI with NGI, 726 transcripts expression differences, and 347 down-regulated genes, in which 192 were not detected in the GI, and 352 up-regulated genes, 248 of which were not expressed in NGI, but induced expression in the GI. And there were 44 transcripts not expressed in the control and NGI, but expressed in GI, meaning the genes only in GI (high dose, damage) were induced. These genes may be involved in the formation of damage, which protect against radiation damage.

2.3 Identification of differentially expressed TEs

Moreover, according to the mapping of the reads and functional annotation of the rice genome (http://rice.plantbiology.msu.edu), there were 11851 TEs, and 1655 TEs were detected in all samples, among which 58.7% was found in control, 49.4% in NGI, and 76.8% in GI (Table 1). Fifty five of 1655 TEs were different expression, in which 14 transposons were differentially expressed in NGI compared to control (25.5%) including 6 induced expression, 6 fully suppressed and 2 up-regulated (Table 2). However, twenty four TEs were differentially expressed in GI compared to control, including 5 induced expression, 15 up-regulated, 2 fully stifling and 2 down-regulated (Table 3).

Table 1. The TEs of sample and rate

Sample	control	NGI	GI
Expressed TEs	972	818	1271
Number of expressed TEs/number of total TEs	58.7%（972/1655）	49.4%(818/1655)	76.8%(1271/1655)
The total known TEs in rice genome	11851		
The detected TEs	1655		

Table 2. The differentially expressed TEs of NGI

Gene_id	**log2(FC)**	**p_value**	**Description**
LOC_Os10g37160	2.38577	6.21E-07	protein|transposon protein, putative, unclassified, expressed
LOC_Os05g03120	induced	0.000811349	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os06g42640	induced	5.38E-07	protein|transposon protein, putative, unclassified, expressed
LOC_Os06g07010	induced	5.02E-08	protein|transposon protein, putative, unclassified, expressed
LOC_Os02g04924	fully suppress	6.26E-05	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os09g14960	fully suppress	1.74E-05	protein|transposon protein, putative, Pong sub-class, expressed
LOC_Os01g52690	fully suppress	2.93E-06	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os06g49090	fully suppress	3.51E-05	protein|transposon protein, putative, unclassified, expressed
LOC_Os04g56630	fully suppress	0.000113025	protein|transposon protein, putative, unclassified, expressed
LOC_Os11g43800	induced	0.00013963	retrotransposon protein, putative, Ty3-gypsy subclass
LOC_Os04g53660	induced	2.25E-05	transposon protein, putative
LOC_Os04g49780	fully suppress	1.05E-05	transposon protein, putative, CACTA, En/Spm sub-class
LOC_Os03g36130	2.95511	0.00033062	Retrotransposon, putative, centromere-specific, expressed
LOC_Os02g30530	induced	0.00027159	transposon protein, putative, unclassified, expressed

Table 3 The differentially expressed TEs of GI

Gene_id	log2(FC)	p_value	Description
LOC_Os05g03120	-1.87519	0.000433562	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os01g41120	-1.64471	2.86E-06	protein|retrotransposon protein, putative, Ty3-gypsy subclass, expressed
LOC_Os07g05440	1.68848	0.000614567	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os03g36130	2.05119	0.00037073	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os10g37160	2.26192	2.54E-06	protein|transposon protein, putative, unclassified, expressed
LOC_Os01g57960	2.71347	7.68E-13	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os06g10870	2.94361	8.59E-10	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os10g31460	3.19242	7.77E-15	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os10g31460	3.27251	6.97E-07	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os12g13890	3.37706	5.23E-09	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os06g09870	3.80964	2.50E-06	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os10g31440	4.31743	2.13E-08	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os10g31460	4.40532	0	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os01g67364	4.50139	0.0001093	protein|retrotransposon protein, putative, Ty3-gypsy subclass, expressed
LOC_Os10g31420	4.52987	6.66E-16	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os10g31490	5.46685	0.00063249	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os05g03120	induced	1.99E-05	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os02g24270	fully suppress	0.000215276	protein|retrotransposon protein, putative, unclassified, expressed
LOC_Os07g01214	fully suppress	1.35E-09	protein|transposon protein, putative, unclassified, expressed
LOC_Os11g43800	induced	0.000196145	retrotransposon protein, putative, Ty3-gypsy subclass
LOC_Os04g53660	induced	0.000323992	transposon protein, putative
LOC_Os04g49780	induced	0.000235153	transposon protein, putative, CACTA, En/Spm sub-class
LOC_Os03g36130	3.49934	3.20E-05	Retrotransposon, putative, centromere-specific, expressed
LOC_Os02g30530	induced	0.000291104	transposon protein, putative, unclassified, expressed

Table 4 The differentially expressed TEs of GI vs control and NGI vs control

Gene_id	Log2(FC)		Description
	NGI vs control	GIvs control	
LOC_Os11g43800	1.79769e+308	1.79769e+308	retrotransposon, Ty3-gypsy subclass
LOC_Os10g37160	2.38577	2.26192	transposon protein, putative, unclassified
LOC_Os05g03120	1.79769e+308	1.79769e+308	protein|retrotransposon protein, putative, unclassified,
LOC_Os04g53660	1.79769e+308	1.79769e+308	transposon protein, putative
LOC_Os04g49780	-1.79769e+308	1.79769e+308	transposon protein, CACTA, En/Spm sub-class
LOC_Os03g36130	2.95511	3.49934	Retrotransposon, putative, centromere-specific.
LOC_Os02g30530	1.79769e+308	1.79769e+308	transposon protein, Transposase, MuDR

3. Disscussion

Integrated statistical comparison of germination, seedling height and root length, we conclude that the lower ion fluence, the less significant was the damage , but larger ion fluence produced significant damage, that means the height of seedling and root length growth significantly restrained under the larger fluence ion beam exposure, however, compared with other radiation, the damage effects of this larger dose of ion beam exposure on rice seed is relatively low, belongs to the low-damage effects [16-19].

In control 36,382 transcripts, among which NGI had 1.49% differentially expressed transcription compared with control and GI had 2.14% (0.65% higher than NGI), indicating higher dose of ion beam implantation ($6x10^{17}N^{+}$/cm2, and $8x10^{17}N^{+}/cm^{2}$) induced more difference expressed genes than low-dose ion beam implantation ($1x10^{17}N^{+}/cm^{2}$, and $2x10^{17}N^{+}/cm^{2}$), and 44 transcription were found not expressed in both the control and NGI but expressed in the GI, meaning that these genes may be involved in the formation of damage, which protect against radiation damage.

Compared with other physical, chemical exposure, ion beam irradiation had a low damage, high mutation (rates) and broad mutation spectrum and so on [20-22]. Effect of low energy ion beam on rice, tobacco, sugar beet plant also fully embodies the characteristics [23-26]. Here 14 DEGs in NGI, as well as 24 DEGs in GI were annotated as transposon or retrotransposon. Even, there were 7 DEGs related to TEs were differentially expressed both in NGI vs control and GI vs control, at the same time, 5 of these 7TEs were inducible expressed, and 2 of these were up-regulated. These findings mean that the activity of TEs were activated by low energy ion beam

irradiation. Transposable elements （ TEs ） namely transposon, are segments of DNA that may move around to different positions in the genome of a single cell. In the process, they can cause mutations or increase (or decrease) the amount of DNA in the genome. TEs are major components of most eukaryotic genomes and are particularly abundant in plants, representing 35% of rice genomes. TEs play an important role in genome and gene evolution. TE insertion can disrupt genes and mediate chromosome rearrangements, and can provide alternative promoters, exons, terminators and splice junctions. TEs change the expression of some genes due to the transcription of ncRNA from the transposon promoters, which contribute to the epigenetic regulation of neighboring genes through mechanisms such as RNAi. It has the high potential impact of the expression of the nearby genes[8，9,27]. For this reason, TE transcription was severely repressed in plants and only activated under certain precise circumstances. For example, pathogen infections, chemical mutagens, physical injuries or different abiotic stresses[28-33]. The ion beam implantation can re-activate a silent leaf color TE control in rice, so that instability of the mottled leaf rice seeds (treatmented by ion beam irradiation) changed to stable yellow leaves in M2 generation[36] and a silenced minimal Mutator TE in corn also was activated[35].

The more expression of genes from TEs have the potential to increase the potential of transposition, making gene restructuring and causing more genetic of mutations, maybe this is the reason for the low damage, high-mutation, broad mutation spectrum [23, 36]. In conclusion, our studies have shown that: low-ion beam bombardment stimulated the activites of transposons, leading to the missed, added and duplicated of DNA clip, causing chromosome structure change, and maybe this is one of the reasons for biological effects of the low ion beam bombardment.

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Reference

1. YU Zeng-liang.Ion beam application in genetic modification. IEEE Trans on Plasma Science, 2000, 28 (1):128-132.
2. Yusuke Kazama, Liqiu Ma, Tomonari Hirano, Sumie Ohbu, Yuki Shirakawa,Shin Hatakeyama, Shuuitsu Tanaka,Tomoko Abe.Rapid evaluation of effective linear energy transfer in heavy-ion mutagenesis of Arabidopsis thaliana.Plant Biotechnology.2012, 29:441–445.
3. Abe T, Ryuto H, Fukunishi N. Ion beam radiation mutagenesis in: Q.Y. Shu, B. P. Forster, H. Nakagawa (Ed.). Plant Mutation Breeding and Biotechnology, Oxford: CABI,2012:99-106.
4. LI Shi-chang, ZHANG Peng-peng, YANG Na, ZHANG Jing, WANG Yong-jie. Screening for Polyunsaturated Fatty Acid- Producing Strain Rhodotorula glutinis by Low-Energy Ion Implantation. Acta Biophysica Sinica, 2013, 29(2): 118-124.
5. WEI Zeng-quan, XIE Hong-mei, HAN Guangwu, LI Wen-jian. Physical Mechanisms of Mutation Induced by Low Energy Ion Implantation[J]. Nucl Instr and Meth, 1995,B95:371.
6. WEI Zeng-quan, YUAN Shi·bin, XIE Hong-mei, ZHAO Li-min, MAO Shu-hong. Range Distribution of Low Energy Ions Implanted into Embryo of W heat Seed. Nuclear Physics Review.
7. Feng H Y, Liu X L, Yuan H, Kong M G, Wu L J, Wu Y J, Yu Z L. Utilizing low-energy ion beams to study living organisms. Surface and Coatings Technology, 2007,201:8034-8038.
8. LI Yong-hui, YU Xiang-li, LI Ya-he, GUO Xiang-meng, YA Hui-yuan. Changed-transcriptional activity of retrotansposons induced by implantation of low-energy ion beam effected the expression of genes adjacent to retrotransposons. Life Science Journal, 2011, 8(4):575- 579.
9. YA Hui-yuan,GU Yun-hong, WANG Wei-Dong, QIN Guang-yong. The Low-energy N^+ beam implantation promoting transcription of Wis2-A and affecting the expression of its flanking genes. Journal of Nuclear Agricultural Sciences, 2008,22(5): 595-599.
10. WANG Zhong. MARK Gerstein, MICHAEL Snyder. RNA-Seq: a revolutionary tool for transcripto- mics. Nature Reviews Genetics, 2009, 10, 57–63..
11. Carninci,P. Is sequencing enlightenment ending the dark age of thetranscriptome? Nat. Methods, 2009,6**:**711 –713.
12. Richard W. Francis, Katherine Thompson-Wicking, Kim W. arter, Denise Anderson, Ursula R. Kees, Alex H. Beesley. A Software Tool to Identify Expressed Gene Fusion Candidates from RNA-Seq Data. PLOS ONE. 2012,7 (6):1-12.

13. QI Yun-xia, LIU Yong-bin, RONG Wei-heng. RNA-Seq and its applications:a new technology for transcriptomics. Hereditas 2011,33(11): 1191—1202.
14. Robinson MD, McCarthy DJ, Smyth GK: edge R: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010, 26:139–140.
15. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature Methods 2008,5: 621–628.
16. Mpoloka S. W. Effects of prolonged UV-B exposure in plants. Afr. J. Biotechnol. 2008,7(25), 4874-4883.
17. Zlatko S Zlatevl, Fernando J.C.Lidon, M. Kaimakanova. Plant physiological responses to UV-B radiation. Journal of Food Agriculture and Environment, 2012. 24 (6): 481-501.
18. E.Kovcs,. keresztes. Effect of gamma and UV-B/C radiation on plant cells.Micron.2002,33:199-210.
19. Takano N, Takahashi Y, Yamamoto M, Teranishi M, Yamaguchi H, Sakamoto AN, Hase Y, Fujisawa H, Wu J, Matsumoto T, Toki S, Hidema J. Isolation of a novel UVB-tolerant rice mutant obtained by exposure to carbon-ion beams. Journal of Radiation Research, 2013:1–12.
20. Atsushi TANAKA, Naoya SHIKAZONO, Yoshihiro HASE. Studies on Biological Effects of Ion Beams on Lethality, Molecular Nature of Mutation, Mutation Rate, and Spectrum of Mutation Phenotype for Mutation Breeding in Higher Plants. Journal of Radiation Research, 2010,51:223–233.
21. Hiroyasu Yamaguchi, Akemi Shimizu,Yoshihiro Hase, Atsushi Tanaka, Naoya Shikazono, Konosuke Degi, Toshikazu Morishita. Effects of ion beam irradiation on mutation induction and nuclear DNA content in chrysanthemum. Breeding Science. 2010, 60: 398–404.
22. Yamaguchi H; Nagatomi S; Morishita T, Degi K, Tanaka A, Shikazono N, Hase Y. Mutation induced with ion beam irradiation in rose. Nuclear Instruments and Methods in Physics Research Section B.2003, 206: 561-564.
23. FENG Hui-yun, YU zeng-liang, Paul K C. Ion implantation of organisms. Materials Science and Engineering: R:Reports, 2006,54(15):49-120.
24. ZHANG Xiang-sheng, YA hui-yuan. RAPD Analysis on the Stimulation and Mutational Effects of Low Energy Ion Beam Implantation in Liquorice (*Glycyrrhiza uralensis* Fisch).Journal of Henan Agricultural Sciences.2007,12:83-86.
25. HUANG Ming-jing, MA Bu-zhou, LU Zhi-jun, WANG Zi-qiang. Simulative Effects of Ion Beam Injection upon Tobacco Seed on M1.Journal of Shanxi Agricultural Sciences. 2004, 32(4): 49-51.
26. ZENG Xian-xian,WU Bao,WANG Yan-fei.March of the Study on Sugar Beet Seeds by Ion Implantion to Gain Precocity and High Yield and High Sugar Parent.Material Seed,2003,6:3-5.
27. Mikel Zaratiegui, Danielle V.Irvine, Robert A. Martienssen. Noncoding RNAs and gene silencing. Cell 2007,128:763-776.
28. YA Hui-yuan, ZHANG Huan-li, FENG Wei-sen, CHEN Qiu-fang, WANG Wei-dong, ZHEN Jiao. Implantation with low-energy N^+ beam changes the transcriptional activation of the expression sequencetags (ESTs) from the transposable elements in rice.Journal of Agricultural Research. 2011,6(18): 4318-4326.
29. Zehava Eichenbaum, Zvi Livneh. UV Light Induces IS10 Transposition in Escherichia coli. Genetics.1998, 149:1173-1181.
30. QIAN Ye-xiong, CHENG Xiao, LIU Yan, JIANG Hai-yang,ZHU Su-wen, CHENG Bei-jiu. Reactivation of a silenced minimal Mutator transposable element system following low-energy nigrogenion implantation in maize. Plant Cell Reports, 2010; 29:1365–1376.
31. Ce´cile Pasternak, Bao Ton-Hoang, Genevie`ve Coste, Adriana Bailone, Michael Chandler, Suzanne Sommer. Irradiation- Induced Deinococcus radiodurans Genome Fragmentation Triggers Transposition of a Single Resident Insertion Sequence. PLOS Genetics.2010, 2(1):1-10.
32. Georgi Bonchev, Lubomir Stoilov,Zorniza Angelova, Sevdalin Georgiev. Genomic diversity of Ac-like transposable elements in sphaerococcum mutant forms of common wheat (*Triticum aestivum* L.) and triticale (X *Triticosecale* Witt.).Journal of Applied Genetics, 2012,53:9–17.
33. Salazar M, Gonza´lez E, Casaretto JA, Casacuberta JM, Ruiz-Lara S. The promoter of the TLC1.1 retrotransposon from Solanum chilense is activated by multiple stress-related signaling molecules. Plant Cell Reports, 2007, 26(10):1861–1868.
34. M. Maekawa, Y. Hase, N. Shikazono, A. Tanaka. Induction of somatic instability in stable yellow leaf mutant of rice by ion beam irradiation. Nuclear Instruments and Methods in Physics Research Section B.2003, 206: 579-585.
35. Yexiong Qian, Xiao Cheng, Yan Liu, Haiyang Jiang, Suwen Zhu, Beijiu Cheng. Reactivation of a silenced minimal Mutator transposable element system following low-energy nitrogen ion implantation in maize. Plant Cell Reports,2010, 29:1365-1376.
36. T.Abe, Y. Kazama, H. Ichida, Y Hayashi, H. Ryuto, N. Fukunishi. Plant Breeding Using the Ion Beam Irradiation In Riken. Cyclotrons and Their Applications,2007:222- 224.

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