# The use of microarrays to reveal the probabilistic gene network associated with the response of rice to low-energy ion beam bombardment

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**Abstract:** Bombardment with low-energy ion beams can induce various biological effects in plants including stimulation, damage, and mutation. However, the interactions in genes in plants in response to this stressor are not fully understood. We use a Rice Gene Expression Microarray to investigate the overlap differentially expressed genes (DEGs) in two independent bombardment experiments involving three ion fluences. The results show that 26 up- and 6 down-regulated overlap genes were observed. A RiceNet co-expressed network analysis of the overlap DEGs showed the direct and indirect co-expressed linkages, which suggests some signal transduction pathways should be involved in this response but the situation remains unclear. Our microarray data reveals the general and key genes responding to the stress of bombardment with low-energy ion beams during rice germination, and provides information on the candidate genes for further elucidation of the molecular mechanisms biological effects underlying the of ion-beam bombardment.
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**Key words:** Low-energy ion beam bombardment; biological effect; microarray; gene network; rice

## 1 Introduction

Bombardment with low-energy ion beams causes, directly or indirectly, severe cellular damage and stress in plants[1]. It seems likely that there are stress response elements in cells which are responsible for tolerance to bombardment with such low-energy ion beams. Researchers and scientists are interested in abiotic stress response sensing and the associated genetic and metabolic response pathways in plants[2,3]. However, the key genes and the network responding to bombardment with low-energy ion beams remain to be fully elucidated and few of these genes have been identified and recognized in plants. Recently, genome expression analysis has been used to identify the key functional genes involved in the response to abiotic stress because of its high throughput detection, especially the use of whole-genome microarrays.

Rice (*Oryza sativa*) is *the* most important staple food crop. As one of the best studied grasses, there is a wealth of accumulated knowledge about rice which makes it an attractive candidate as a reference for other important staple crops and emerging biofuel grasses. In previous (extensive) studies, many research groups have examined the effects of a wide variety of abiotic and biotic stresses in rice[4]. Thus, we selected rice seed as the experimental material in this study.

RiceNet is a probabilistic functional gene network for 41,203 non-TE related genes of *Oryza sativa*. It was constructed using modified Bayesian integration of many different data types from several different organisms, with each data type weighted according to how well it links genes that are known to function together in *Oryza sativa*. Each interaction in RiceNet has an associated log-likelihood score (LLS) that measures the probability of an interaction representing a true functional linkage between two genes [5].

In this study, we propose a strategy to discover the overlap differentially expressed genes (DEGs) in rice among multiple microarrays in two independent bombardment experiments using three ion fluences. Subsequently, we obtain the interaction network of these genes. The results shed light on the molecular mechanisms of the biological effects induced by ion-beam bombardment and enable identification of the genes regulating the response to bombardment with low-energy ion beams.

## 2 Materials and Methods

### *2.1 Rice seed and culture*

Dry seeds of rice cultivar Xindao-18 (*Oryza sativa L. ssp. japonica*) were used in the ion bombardment experiments. After bombardment with the ion beam, all seeds were planted on sterile medium with 0.8% agar (Sigma) in a climate chamber in the dark at 28°C. After the seeds had been incubated for 96 h, they were collected for RNA extraction.

### *2.2 RNA extraction*

In this study, in order to accurately investigate the commonly expressed DEGs responsive to the stress of ion-beam bombardment of rice, mixed RNA from the rice seedling populations is tested. Thus, 30 uniformed rice seedlings from each ion

fluence were mixed to prepare a mixed RNA sample and construct the RNA pool from three biological replicates. Total mixed RNA was isolated using RNA plant reagents (Tiangen Biotech) and purified by use of the RNeasy Plant Kit (Qiagen). The yield and purity of the RNA was determined spectrophotometrically (Nanodrop ND1000).

***2.3 Agilent microarray hybridization and data analysis***

Agilent microarray hybridization (Agilent-015241 Rice Gene Expression Microarray) and raw data analysis were carried out by the ShanghaiBio Company Ltd. A transcript was considered significantly up- or down-regulated if it met all of the following criteria: (1) it showed a statistically significant differential expression at the adjusted *p* value, $p < 0.05$; (2) it had a cut-off value at a 2-fold change; and (3) it had "present" calls on all of the three replicate samples for the controls and/or the bombarded sample.

***2.4 Bioinformatics analysis***

Annotation of the transcripts represented by the microarray was described according to the following databases: Agilent probe name (http://www.ebi.ac.uk/microarray-as/aer/lob?Name= adss&id=2375208716), SAS web server, and the RAP database (http://rapdb.dna.affrc.go.jp/).

***2.5 Detection of the key DEGs***

The overlapping DEGs in multi-microarrays from the samples with different ion fluences and different experimental replicates were collected. The analysis reveals the key genes in rice responding to the bombardment with low-energy $N^+$ ion beams and is used for further validation.

***2.6 RiceNet analysis***

The gene identifiers (geneIDs) of the DEGs were input into the web service RiceNet v.1 to find out their interactions with others (www.functionalnet.org/ricenet).

## 3 Results and Discussion

***3.1 Profiles of the DEGs***

The DEGs were detected by comparing the expression signal values of the genes of the implanted samples and the blank controls. The microarray analysis showed that there are 821 DEGs underlying the ion-beam fluence at $6 \times 10^{17}$ $N^+/cm^2$ in the first bombardment experiment, 1256 DEGs underlying the ion-beam fluence at $2 \times 10^{17}$ $N^+/cm^2$, and 1136 DEGs underlying the ion-beam fluence at $8 \times 10^{17}$ $N^+/cm^2$ in the second bombardment experiment.

The number of DEGs differs greatly between the samples subjected to different ion fluences. This suggests that a single independent microarray cannot reflect the comprehensive and general key genes responsive to the stress of ion-beam bombardment. Therefore, we considered the genes that were differentially expressed in all microarrays underlying the three ion fluence bombardment experiments to date as the comprehensive and general DEGs associated with the response to the ion-beam bombardment stress in rice. By comparing the microarray data between the two independent bombardment experiments resulting in different biological effects, we obtained 26 up- (Table 1) and 6 down-regulated overlap general-key DEGs (Table 2) related to the response to ion-beam bombardment in rice. The short descriptions show that genes related to kinase, transporters, signals, resistance, etc. are involved in the response to ion-beam bombardment.

**TABLE 1**. The up-regulated general-key DEGs.

| Gene ID | Short Description | FC (*p*-value) | | |
|---|---|---|---|---|
| | | $2 \times 10^{17}$ | $6 \times 10^{17}$ | $8 \times 10^{17}$ |
| Os01g0959100 | Similar to abscisic stress ripening protein 1 | 4.02 | 4.51 | 4.68 |
| Os01g0723000 | Elongation factor 2 (EF-2) | 24.26 | 10.34 | 51.10 |
| Os01g0155000 | Esterase/lipase/thioesterase | 6.04 | 3.22 | 3.28 |
| Os01g0311800 | Pectin methylesterase isoform alpha | 2.67 | 2.0 | 2.44 |
| Os01g0660200 | (Chitinase) (EC 3.2.1.14) | 15.74 | 2.95 | 2.67 |
| Os01g0959200 | Similar to abscisic stress ripening protein 1 | 3.54 | 6.60 | 5.31 |
| Os02g0787600 | Ionotropic glutamate receptor | 3.63 | 2.06 | 2.10 |
| Os03g0575200 | $K^+$ potassium transporter family protein | 4.86 | 2.42 | 3.0 |
| Os03g0830500 | PGPS/D12 | 19.74 | 2.77 | 2.23 |
| Os04g0339400 | Aldo/keto reductase family protein | 6.34 | 3.47 | 9.50 |
| Os04g0368000 | Serine/threonine protein kinase | 2.18 | 2.35 | 3.16 |
| Os04g0524500 | Oligopeptide transporter | 2.47 | 2.03 | 2.16 |
| Os05g0276500 | Expansin Os-EXPA3 | 2.04 | 4.61 | 2.72 |
| Os06g0147300 | Conserved hypothetical protein | 3.30 | 3.0 | 2.42 |
| Os06g0521500 | Haem peroxidase | 5.02 | 2.40 | 3.0 |
| Os07g0493800 | Protein kinase domain containing protein | 3.65 | 2.24 | 2.20 |
| Os07g0638400 | Peroxiredoxin (B15C) | 4.31 | 5.63 | 3.53 |
| Os07g0690900 | Hytochelatin synthetase-like protein | 4.54 | 2.71 | 3.56 |

| | | | | |
|---|---|---|---|---|
| Os08g0136700 | Protein of unknown function DUF26 | 7.11 | 2.48 | 14.05 |
| Os08g0190100 | Germin-like protein (Germin-like 8) | 10.17 | 4.38 | 2.80 |
| Os09g0367700 | GST6 protein (EC 2.5.1.18) | 3.50 | 7.15 | 3.08 |
| Os09g0483200 | Ubiquitin domain containing protein | 2.45 | 18.18 | 32.52 |
| Os10g0452300 | Eggshell protein family protein | 4.07 | 6.26 | 3.11 |
| Os11g0227200 | NBS-LRR disease resistance protein homologue; nucleotide binding /protein binding | 6.68 | 2.32 | 3.11 |
| Os11g0482200 | Pathogenic type III effector avirulence factor | 5.43 | 2.31 | 2.73 |
| Os11g0669100 | Calmodulin binding protein | 5.91 | 2.94 | 2.14 |

Short descriptions were annotated according to the RAP-DB (Rice Annotation Project Database). Fold change (FC) in each gene is not expressed as log2. The p-value threshold of 0.05 was used for significant differential expression.

**TABLE 2.** The down-regulated general-key DEGs.

| Gene ID | Short Description | Fold changes | | |
|---|---|---|---|---|
| | | $2 \times 10^{17}$ | $6 \times 10^{17}$ | $8 \times 10^{17}$ |
| Os01g0800800 | Hypothetical protein | 2.13 | 2.23 | 5.26 |
| Os06g0654600 | Protein kinase | 2.10 | 2.0 | 2.60 |
| Os07g0543300 | Glycoside hydrolase, family 14B | 2.83 | 3.35 | 10.0 |
| Os12g0140700 | Zn-finger, RING protein | 2.26 | 2.10 | 2.30 |
| Os12g0640500 | $Na^+/H^+$ antiporter-like protein | 2.01 | 4.53 | 6.76 |
| Os12g0427600 | P69F protein | 11.50 | 2.83 | 5.84 |

Short descriptions were annotated according to the RAP-DB (Rice Annotation Project Database). Fold change (FC) in each gene is not expressed as log2. The p-value threshold of 0.05 was used for significant differential expression.

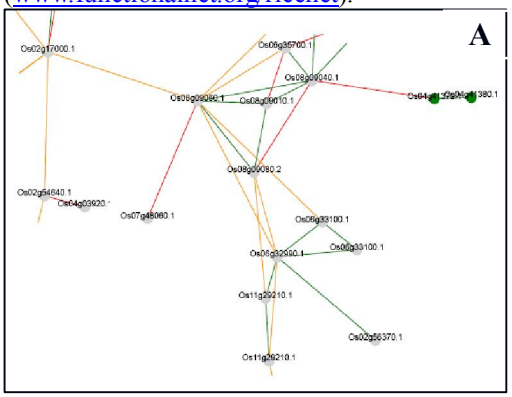
Microarray technology has been used with many plants, and functions as a powerful tool for high-thoughput screening of genes responsive to different abiotic stresses including salt, ABA (abscise acid ), cold, heat, and drought [6,7].

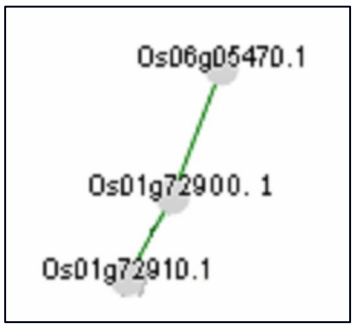
In the present study, a total of 32 overlap DEGs (26 up- and 6 down-regulated) are observed. These comprise 3.90% (32/821), 2.55% (32/1256), and 2.81% (32/1136) of the total DEGs underlying the three independent ion-fluence bombardment experiments, respectively. This observation means that less than 10% of the transcripts were overlap differentially expressed. These 32 overlap DEGs represent target genes for the study of the molecular mechanism for the biological effects induced by bombardment with low-energy ion-beams.

Stress-regulated proteins can be classified into two groups, namely, those that take part in signal transduction and those that directly play a role in plant survival under stress conditions. Proteins of the first group include transcription factors, RNA-binding proteins, protein kinases, and phosphatases [8]. It is seen that 10 out of 26 of the overlap up-regulated transcripts were involved in the response process. Further, out of the 26 up-regulated DEGs 5, 4, and 4 are related to transport, signaling, and protein modification (including protein kinases and phosphatases), respectively. This finding suggests that rice responds to the bombardment with ion beams through unknown signal pathways.

### *3.2 RiceNet analysis*

RiceNet is a platform of web-tools dedicated to visualization of the transcriptomic co-expression networks in rice. The platform combines the available expression and sequence data together with Mapman ontology analysis (http://aranet.mpimp-golm.mpg.de/ricenet), constructed using modified Bayesian integration of many different data types from several different organisms. Each data type is weighted according to how well it links genes that are known to function together in *Oryza sativa* (www.functionalnet.org/ricenet).




B

FIG. 1. Partial co-expressed linkages in Ricent

Networks, or graphs, consisting of nodes and edges. The colors of the edges indicate the strength of the co-expression, wherein green, orange, and red edges indicate that the HRR value between any two genes is HRR $\leq$ 10, 10 < HRR $\leq$ 20, and 20 < HRR $\leq$

30, respectively. The colors of the nodes indicate the description of the knock-out of a given gene, wherein red, yellow, green, and gray nodes represent embryo lethal, gametophytic lethal, described non-lethal phenotype, and no available phenotype, respectively. (A) shows the partial co-expressed network in cluster 364 showing the direct linkage of three up-regulated genes: Os01g0959200, Os01g0959100, and Os06g0147300. LOC_Os01g72910.1 is the gene identifier in RiceNet representing the gene with ID in RAP-DB: Os01g0959200. Similarly, LOC_Os06g05470.1 represents Os06g0147300 and LOC_Os01g72900.1 represents Os01g0959100. (B) shows the partial co-expressed network in cluster 462 showing the indirect linkage of three up-regulated genes: Os02g0787600, Os06g0521500, and Os11g0482200. LOC_Os02g54640.1 represents Os02g0787600, LOC_Os06g32990.1 represents Os06g0521500, and LOC_Os11g29210.1 represents Os11g0482200.

RiceNet analysis (Fig. 1) shows that three genes including Os01g0959200, Os01g0959100, and Os06g0147300 present the direct co-expressed network (Fig. 1-A) in cluster 364 which is involved in the secondary metabolism of flavonoids and flavonols and signaling (http://aranet.mpimp-golm.mpg.de/ricenet/rc364). Also, there is a direct co-expressed network linkage between Os11g0482200 and Os06g0521500 (Fig. 1-B) in cluster 462 which is involved in lipid metabolism, abiotic stress, and signaling, but an indirect co-expressed network linkage between Os02g0787600, Os11g0482200, and Os06g0521500 (http://aranet.mpimp-golm.mpg.de/ricenet/rc462). These findings suggest there are complicated biological processes occurring in response to bombardment with low-energy ion beams in the plants.

Our microarray data on the different biological effects occurring in the low-energy ion beam bombardment experiments reveals the general and key genes responsive to the stress of bombardment during rice germination. It suggests that the differentially expressed transcripts clustered into many groups and were associated with the requirements of the different events occurring while adapting to ion bombardment. The data provides information on the candidate genes for further elucidation of the molecular mechanisms for the biological effects caused by ion-beam bombardment and for its use regarding the improvement of corn yield.

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