Gene expression analysis and data mining for the gene network analysis derived from drought stress treatments in rice

Kouji Satoh1, Koji Doi1, Toshifumi Nagata2, Aenzi Hosaka1, Kohji Suzuki2, Xumei Ji3, Mutazaj Raveendran3, Ramil Mauleon1, John Bennett3, Hei Leng3, Richard Bruskiewich3, Shoshi Kikuchi1

1 Plant Genome Research Unit Division of Genome and Biodiversity Research National Institute of Agrobiological Sciences (NIAIS) Kanon-dai 2-1-2 Tsukuba Ibaraki 305-8602 Japan
2 Hitachi Software Engineering Co., Ltd., 6-81 Onoe-cho, Naka-ku, Yokohama 223-0015, Japan
3 International Rice Research Institute (IRRI) DAPO Box 7777, Metro Manila, Philippines

Abstract

To elucidate global responses to drought stress in rice, a 60 mer oligomer microarray covering 22K unique genes based on the sequence of full-length cDNA clones was used to profile gene expression changes at the seedlings, using PEG and IR64 and in the peduncle at heading using IR64. As reported by Ji et al. (2005) Plant Mol Biol. 59: 945-964, peduncle elongation in rice is inhibited by drought stress. Cluster analysis of genes up- and down-regulated by drought stress in these two different growth stages revealed the possible combination of the transcription factors and their downstream genes and stage-specific gene expression profiles. Gene expression analysis using the metabolic pathway data in Rice Cyc (http://www.grc.nig.affrc.go.jp/cyc/index.html) showed that genes encoding many enzymes of sugar metabolism, such as degradation of sucrose, glucose and galactose, are down-regulated, along with genes encoding enzymes of cell-wall biosynthetic, while genes encoding enzymes of some amino acid biosynthetic pathways are up-regulated. Drought-induced ABA is clearly involved in antagonizing GA-dependent events underlying peduncle elongation, but the biosynthetic genes related to these hormones are not clearly affected by the drought stress treatment. Among 613 differentially expressed transcription factor-related genes, the C3H, AP2-EREBP, bHLH, NAC, MYB and WRKY types of TF-related genes showed differential expression during drought stress treatment and re-watering treatment. Finally, the promoter regions (1kb upstream sequence) of the genes clustered after microarray experiments were examined using a newly developed cis-element analysis tool; the results of this analysis will also be discussed. Data mining using gene annotation data (ex. GO term), pathway data, and genome mapping data suggests the existence of transcription network of drought stress responsive genes.

Method: Microarray system used in this paper

Agilent 22K rice Oligo Array

This array probes were designed from Rice (Nipponbare) Fl-cDNA sequences, and 21495 rice probes were spotted on a array. The annotation information of Fl-cDNA clones were presented in KOME site (http://cdna01.dna.affrc.go.jp/cdna/1) and these clones are distributed from Rice Genome Resource Center (http://www.rgrc.dna.affrc.go.jp/jp/index.html)

Table 1. Numbers of stress-responsive genes

Table 2. Genes respond to specific stress and respond commonly

Table 3. Eight physiological condition for microarray analysis

Table 4. 17 commonly down-regulated genes classified by gene families

Table 5. List of drought stress responsive TFs classified by gene families

Table 6. List of common drought responsive TFs

Table 7. Summary of cis-element search (using PLACE data)

Eight physiological condition for microarray analysis

NIAS (japonica, Nipponbare, 10 days old-seeding in hydroponic culture, control: non-treated seedling)
1: PEG treatment (25% PEG) three time course, 1 biological replicant (“drought stress” mimic)
2: Cold treatment (10 deg C) three time course, one biological replicant
3: Salt treatment (150mm NaCl) one time course, one biological replicant
4: Flood treatment four time course, one biological replicant
5: Osmotic treatment (260mm Mannitol) one time course, one biological replicant

IRRI (Drought treatment)
1: APO (indica, drought tolerant upland, leaf, no-treatment leaf) One time course, three biological replicants
2: IR64 (indica, drought susceptible lowland, leaf, no-treatment leaf) One time course, three biological replicants
3: IR64 (indica, drought susceptible peduncle, no-treatment peduncle) One time course, three biological replicants

Conclusion

Comparison of gene expression data through MA system in various stress treatments.
>> salt and osmotic stresses look specific to other stress
>> so many genes are differentially in cold stress, but very specific to cold stress
Specific and common responsive genes to various types of drought stress treatments
>> issue specifically differentially expressed genes are observed
Analysis of differentially expressed transcription factors and cis-element search in the promoter region of the clustered genes after microarray analysis
>> possible combination of the TF and its downstream genes

Fig 1. Frequency of appearance of cis-elements in the promoter regions

Comparison of gene expression data through MA system in various stress treatments.
>> salt and osmotic stresses look specific to other stress
>> so many genes are differentially in cold stress, but very specific to cold stress