TENOR:
Database for Comprehensive mRNA-Seq Experiments in Rice

O Yoshihiro Kawahara[1], Youko Oono[1], Hironobu Wakimoto[1,2], Jun Ogata[1], Hiroyuki Kanamori[1], Harumi Sasaki[1], Satomi Mori[1], Takashi Matsumoto[1] and Takeshi Itoh[1]

[1] Agrogenomics Research Center, National Institute of Agrobiological Sciences, [2] BITS. Co., Ltd.

National Institute of Agrobiological Sciences

TENOR
Transcriptome ENcyclopedia Of Rice

Abstract

Plant have the ability to adapt various growing conditions. To understand the genes and its regulatory networks responsible for the adaptation, we performed a time-course transcriptome analysis of rice reference cultivar (***Oryza sativa*** L. ssp. japonica cv. Nipponbare) under 10 abiotic stresses (high salinity, high-, low-phosphate, high-, low-, very-low-cadmium, drought, osmotic, cold and flood) and 2 plant hormone treatment conditions (abscisic acids and jasmonic acids) using mRNA-Seq. A large number of abiotic stress responsive genes, including previously unannotated genes predicted with the RNA-Seq data, were detected. A hierarchical clustering analysis revealed similarities in responsive transcriptome among environmental, nutrient, heavy-metal stresses and plant hormone treatments. Furthermore, tissue-specific and dose-dependent responsive profiles were also observed. A number of responsive genes encode transcription factors that likely control responsive transcriptome profiles, however the timing of induction of the genes were different. In addition, we found that a number of cis-regulatory elements significantly enriched in the promoter regions of responsive genes were shared between different conditions. Taken together, these results suggested that rice responsive transcriptome against various abiotic stresses and plant hormone treatments was regulated by transcriptional networks and a considerable number of components are shared between different stress signaling pathways. All resources (gene annotation, expression profiles, co-expressed genes, cis-regulatory elements, etc.) were available in the TENOR (Transcriptome ENcyclopedia Of Rice) database (http://tenor.dna.affrc.go.jp, Plant Cell Physiol (2015), doi: 10.1093/pcp/pcv179).

Materials & Methods

Table 1. Summary of samples and stress conditions

Conditions	Descriptions	Tissues	Time points	Read length
High salinity [a]	150mM NaCl	Shoot, Root	0h, 1h	36
High phosphate [b]	3 mM KH2PO4	Shoot, Root	0h, 1d, 5d, 10d, 10d+1d rec. [e]	51
Low phosphate [b]	0 mM NaH2PO4	Shoot, Root	0h, 1d, 5d, 10d, 10d+1d rec. [e]	51
High cadmium [c]	50 µM CdSO4	Shoot, Root	0h, 1h, 12h, 1d, 5d	76
Low cadmium [d]	1 µM CdSO4	Shoot, Root	0h, 1d, 4d, 10d	76
Very low cadmium [d]	0.2 µM CdSO4	Shoot, Root	0h, 1d, 4d, 10d	76
Drought [d]	grown without medium	Shoot, Root	0h, 1h, 3h, 6h, 12h, 1d	76
Flood [d]	submerged in medium	Shoot, Root	0h, 1h, 3h, 6h, 12h, 1d, 3d	76
Cold [d]	4ºC	Shoot, Root	0h, 1h, 3h, 6h, 12h, 1d	76
Osmotic [d]	0.6 M Mannitol	Shoot, Root	0h, 1h, 3h, 6h, 12h	76
ABA [d]	100 µM	Shoot, Root	0h, 1h, 3h, 6h, 12h, 1d	76
JA [d]	100 µM	Shoot, Root	0h, 1h, 3h, 6h, 12h, 1d	76
No treatment [d]	Grown in Yoshida's medium	Shoot, Root	0h, 1h, 3h, 6h, 12h, 1d, 3d, 4d, 5d, 10d	76

[a] Mizuno et al. (2010) BMC genomics, [b] Oono et al. (2011) Rice, [c] Oono et al. (2014) PLoS ONE, [d] Kawahara et al. (2015) Plant Cell Physiol.
[e] 10 days after high/low phosphate stress conditions followed by 1 day under normal condition for recovery. These are only for root sample.

Figure 1. RNA-Seq analysis workflow

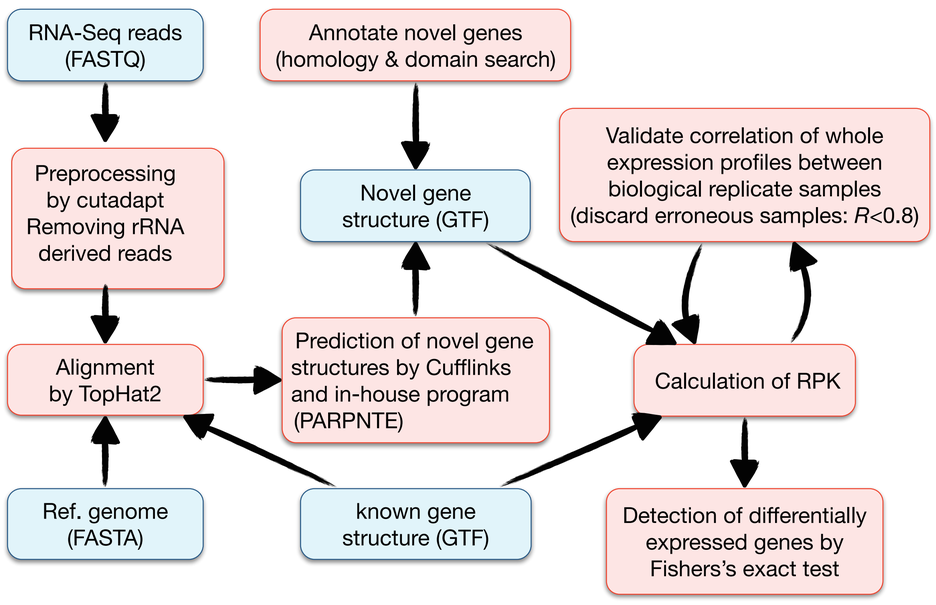

Table 2. Statistics of annotated (RAP-DB) and newly identified "RNA-Seq" genes

Category	# Loci	Mean transcript length (bp)	Mean CDS length (bp)	Mean # exons	Mean exp. level (RPK)
RAP-DB representative genes					
w/ mRNA support	35,469	1,580.0	912.0	4.3	327.9
ab initio prediction w/ EST support	2,400	1,067.0	1,067.0	3.0	59.2
RAP-DB predicted genes	8,121	541.3	541.3	1.6	4.7
Unannotated genes					
PARPNTE assembled protein-coding genes	742	2,069.1	552.8	2.3	40.1
PARPNTE assembled non-coding genes	2,274	1,281.2	-	2.0	48.5
Cufflinks assembled genes	1,263	943.4	-	1.8	22.5

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Database URL:
http://tenor.dna.affrc.go.jp

Contact:
y.kawahara@affrc.go.jp

Results of expression profile analyses

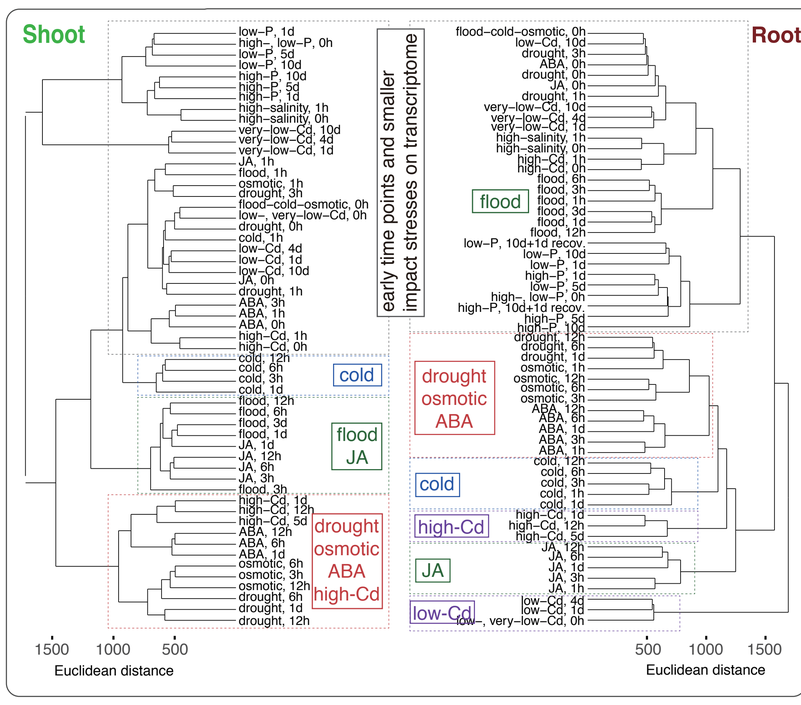

Figure 2. Hierarchical clustering of whole transcriptome profiles
Hierarchical clustering of whole transcriptome profiles for shoot and root under the abiotic stress and plant hormone treatment conditions based on euclidean distance and Ward' s clustering method. Representative clusters were shown in dashed rectangles. Several abiotic stresses and plant hormone treatments had a similar effect on the overall transcriptome profile. The result suggests that some of the responsive signaling cascades are shared among those stresses.

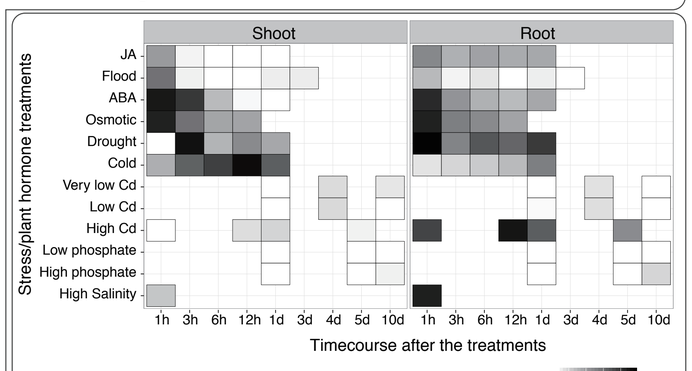

Figure 4. TF enrichment analysis of responsive genes
Significant enrichment of transcription factors in abiotic stress- and plant hormone-responsive genes was tested by Fisher' s exact test using enriched TFs in non-responsive genes as a background level. Enrichment scores (-log10(FDR)) were calculated based on corrected p-values by FDR. We found that the peaks of TF enrichment scores and peak time points were different among the different conditions. For drought, osmotic, flood, ABA and JA treatment conditions, the fraction of TFs was highest at relatively early time points, such as 1 or 3 hours after the treatments were applied. Even among the same cadmium stress conditions, the timing of TF induction varied depending on the concentration of cadmium. The result indicates that induction of TFs is a typical occurrence when environmental changes occur, but the timing of TF induction differs among different conditions.

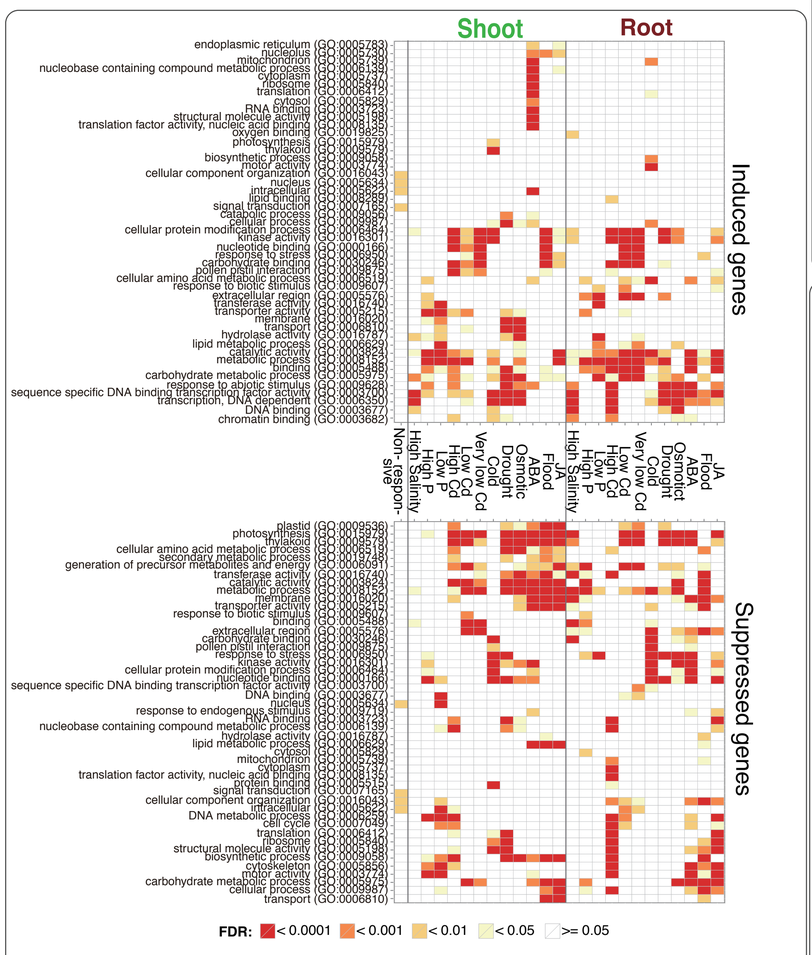

Figure 3. GO enrichment analysis of responsive genes
The functional categories of stress- and plant hormone-induced and stress- and plant hormone-suppressed genes were examined using the Gene Ontology (GO) classification. The stress- and plant hormone-induced genes tended to have DNA-binding TF activity, catalytic activity, transporter activity and kinase activity in the category 'molecular function' . Furthermore, a significantly large number of genes related to 'transcription' , 'response to abiotic stimulus' , 'metabolic process' , 'transport' , 'response to stress' and 'cellular protein modification process' were observed. The non-responsive genes related to 'cellular component organization' and 'signal transduction' differed from the responsive genes, and most of them could be housekeeping genes.

Figure 5. Enrichment of cis-regulatory elements in promoter regions of induced genes
Significantly enriched cis-regulatory elements in each condition were represented by colored boxes based on the significant levels. The TATA box element was significantly enriched in the promoter regions of almost all responsive genes, suggesting that transcription of abiotic stress- and plant hormone-responsive genes is strictly regulated by canonical TFs.

TENOR URL http://tenor.dna.affrc.go.jp