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ARTICLE

Investigation of the effect of drought stress on the rice transcriptome

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ABSTRACT Abiotic stresses such as drought are very important factors that endanger the crop production and stability. Plant root is the most important organ for uptake of water, therefore it plays important role in tolerance to osmotic and drought stresses. Plants developing stronger and deeper roots suffer less from water deficit. The aim of our work was to understand and improve the drought stress-tolerance in cereals by analyzing the transcriptional changes in the root system of different rice cultivars under drought stress conditions. According to the results of the DNA chip hybridizations the response of the genes to the drought stress is altered in roots during the day. Further experiments showed that the expression patterns of genes in cultivars with distinct levels of stress tolerance are different, and that may correlate to their stress tolerance.

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KEY WORDS

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The drought stress is a complex stress, it is usually combined with heat and oxidative stress. Plants usually respond to it at different levels. The evolutionary developed morphological alterations, such as leaf hairs, thickened cuticula, hidden stomata and different leaf modifications may reduce the water loss. Physiological answers such as stomata closure (Papp et al. 2004), twisted leafs, decreased photosynthetic activity are common short-term responses. Following an "escaper" strategy, the plant can step into the generative phase and produce seeds earlier. In longer term, increased root development can restore the water regime (Wu et al. 2002). The root length, the density of roots and the number of the thick roots are important parameters in the uptake of the limited water from the soil. Increased root-to-shoot ratios can facilitate the maintenance of water balance under stress conditions. A prolific root system can provide the advantage by exploiting the water and support accelerated growth in the early developmental stages (Shao et al. 2008). There are several drought stress-inducible genes that can improve the tolerance of the plants against the drought stress at molecular level. Their expression level can be modified via either abscisic acid (ABA)-dependent or ABA-independent signalling pathways (Yamaguchi-Shinozaki et al. 2002). The products of these genes are macromolecule protectors (late embryogenesis-abundant proteins (LEA), chaperons), osmoprotectors (prolin, glycine-betaine), transcription factors (MYC, MYB, DREB), transporters, detoxificating enzymes (GST, POD, CAT, APX), or proteins playing role in the signal transduction pathways (protein kinases and phosphatases). Taken together, the investigation of the transcriptional changes of the genes in the root system under drought condition can be a good method to understand and improve in the future the drought stress tolerance in crop plants.

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Materials and Methods

Plant Material

The rice plants were grown in soil mixture containing sand and perlite in the ratio of 2:1. The six rice cultivars we used in the experiments were the Bioryza, Sandora, Janka, Ábel, Marilla, and the Azsuka genotypes. The plants were kept in the same conditions for 2 weeks. The maximal field capacity (FC) of the soil in the pots was determined. After two weeks, the rice plants growing in the highly stressed pots were irrigated to the 20% of the FC, medium stressed to 60%, while the control ones to 100%. After 4 weeks, the shoots and the roots were harvested separately at three timepoints through the day (morning, mid-day, sunset), and frozen in liquid nitrogen immediately.

RNA isolation

RNAs were extraxcted according to the AGPC (acid guanidium thyocianate-phenol chloroform) method (Chomczynski and Sacchi 1987) using TRI reagent from Sigma.

Microarrays, probe preparation and hybridization

Rice arrays containing 20446 oligonucleotide from NSF Rice Oligonucleotide Array Project (www.ricearray.org) were used

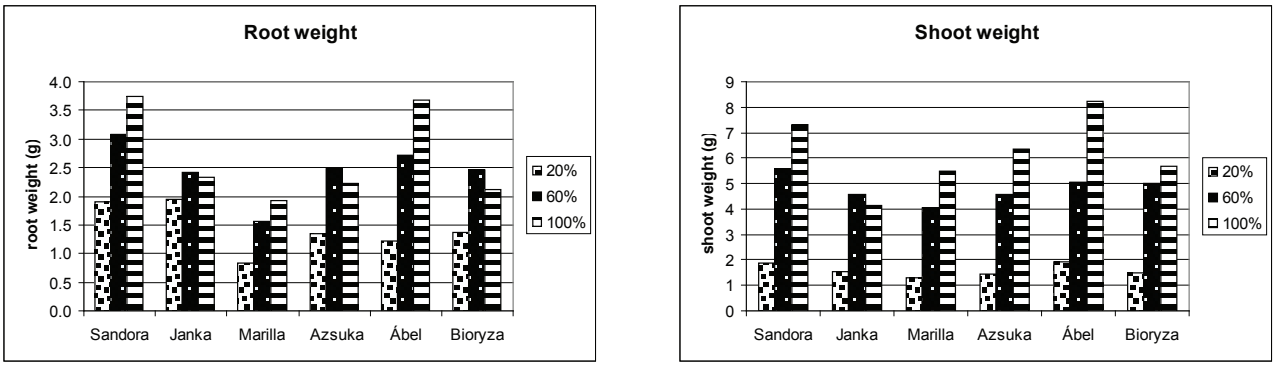

Figure 1. The freshweight of the shoot and the root samples in the genotypes.

to monitor gene expressions. For probe preparation, 3 µg of total root RNA was reverse transcribed using poly-dT and random primed Genisphere Expression Array 900 MPX Detection Kit system (Genisphere, Hatfield, PA, USA) according the manufacturer's instructions. cDNA with capture sequence was hybridized onto microarray in a Ventana hybridization station (Ventana Discovery, Tucson, AZ, USA) using the "antibody" protocol. The first hybridization was performed at 42°C for 12 hours in "FGL2" hybridization buffer, then 2.5 µl of Cy5 and capture reagents in 200 µl "Chiphybe" hybridization buffer (Ventana) were added to the slides and incubated at 42°C for 2 hours. The slides were washed twice in 0.2X SSC at RT for 10 min, then dried and scanned. Each array was scanned at 633 nm in Agilent Scanner at 100% laser power and 100% PMT with 10 um resolution. Output image analysis and feature extraction was done using GenePix 6.0 software. The mean pixel intensities were calculated at both wavelengths and pixel intensity ratios corrected for local background were determined for each spot. We used R script for integrated analysis of two-color microarrays including the global Lowess normalization method.

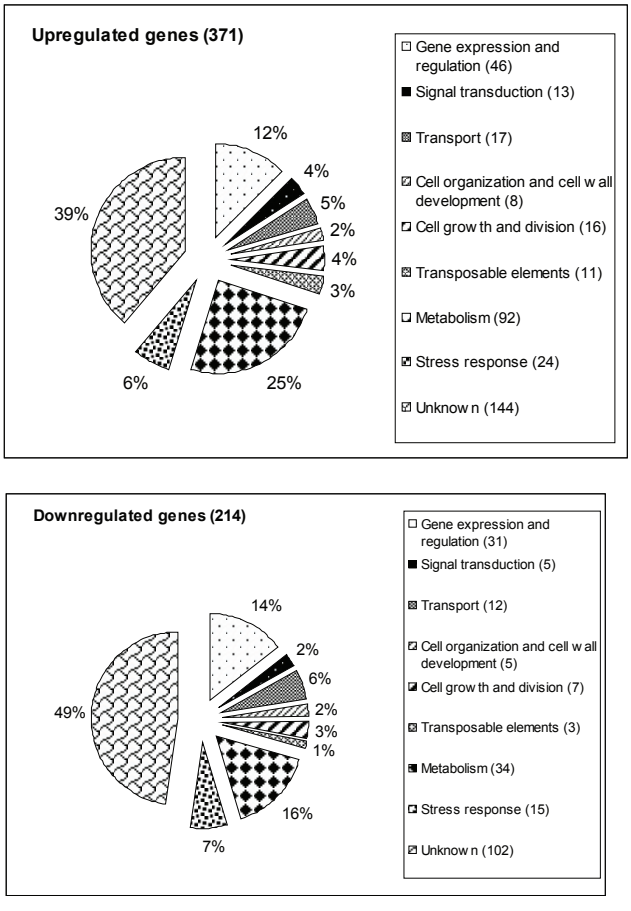

Figure 2. Functional clustering of the up- and downregulated genes.

DNase treatment and first-strand cDNA synthesis

DNase treatment was performed to prepare DNA-free RNAs prior to use in RT-PCR. The first-strand cDNAs were synthesized using random hexamers. The procedures were performed according to the protocols of Fermentas.

Real-time quantitative PCR

The real-time quantitative PCR analyses were performed using ABI Prism 7000 Sequence Detection System. The detection was based on SYBR Green fluorescence. The primers for the real-time PCR experiments were designed by the software Primer Express developed by Applied Biosystems.

Results and Discussion

According to the fresh weight of the roots and the shoots, the Sandora cultivar was chosen for the DNA chip hybridization experiment as the most adaptive genotype (Fig. 1). To follow the transcriptional changes, root samples from this genotype of the three different harvesting time were hybridized with rice oligonucleotide DNA chip. The results showed that 3200

of the genes represented on the chip gave measureable signal in the case of all of the six hybridizations. Among these genes, 11.6% were up-regulated, and 6.7% were down-regulated in the adaptive cultivar. Based on the expression profiles of genes during the day under drought-stress, eight clusters were built. Clusters of genes exhibiting various daily mRNA level change in roots were found. According to the data, not only the transcript levels of the genes were modified by the stress, but the daily expression patterns were altered, too. Furthermore, functional categorization was done based on the known or putative function of the encoded proteins, following the classification established by Yang et al. (2004). Comparing the ratios of the gene-classes between the induced and repressed genes it revealed that the ratio of the genes encoding proteins playing role in the signal transduction is two-fold higher, and the number of the genes involved in the primer metabolic processes is higher significantly among the induced genes. The ratio of the genes with unknown function was 49% in the group of the repressed genes, 10% higher than among the up-regulated ones (Fig. 2). Quantitative real-time PCR technique was used to validate the transcriptional changes observed in the chip hybridization experiments. The transcriptional alterations were investigated in two additional genotypes, in Marilla which is sensitive, and Azsuka which is medium tolerant. Based on the hybridization data, six genes were selected: two putative *LEA* gene, an abscisic acid/water deficit stress (*ABA/WDS*) induced gene, a putative protein kinase and two gene with unknown function. The chip hybridization results were confirmed in four cases: the *ABA/WDS induced* gene, the two putative *LEA*s, and one of the genes with unknown function. Furthermore, these genes` expression levels were determined in the shoot samples in all of the three genotypes, too. The alterations of the expression patterns reflected the differences in the stress-tolerance of the three cultivars. However all of them showed induction in roots, one of the *LEA* genes, the *LEA group 3* appeared to be the most stress-inducible and root-specific, becoming a candidate to investigate further its expression profile under drought stress.

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References

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156-159.

Papp I, Mur LA, Dalmadi Á, Dulai S, Koncz Cs (2004) A mutation in the *Cap Binding Protein 20* gene confers drought tolerance to *Arabidopsis.* Plant Mol Biol 55:679-686.

Shao H, Chu L, Jaleel CA, Zhao C (2008) Water-deficit stress-induced anatomical changes in higher plant. C.R. Biologes 331:215-225.

Wu Y, Cosgrove DJ (2000) Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins. J Exp Bot 51:1543-1553.

Yamaguchi-Shinozaki K, Kasuga M, Liu Q, Nakashima K, Shakiuma Y, Abe H, Shinwari ZK, Seki M, Shinozaki K (2002) Biological mechanism of drought stress response. JIRCAS Working Report 1-8.

Yang L, Zheng B, Mao C, Qi X, Liu F, Wu P (2004) Analysis of transcripts that are differentially expressed in three sectors of the rice root system under water deficit. Mol Gen Genomics 272**:**433-442.