Microarray analysis of salt-responsive genes in common wheat

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ABSTRACT

We developed oligo-DNA microarrays of common wheat. These microarrays were designed to include approximately 32,000 unique genes represented by a large number of expressed sequence tags (ESTs). In order to characterize the salt-responsive genes in common wheat, the expression profiles of transcripts that responded to salt-treatment were monitored using the microarrays. Two-week-old seedlings of Chinese Spring wheat were treated with 150 mM NaCl for 1 h, 6 h and 24 h, and total RNAs in their roots and shoots were separately subjected to microarray hybridizations. It was observed that 5,996 genes demonstrated a more than 2-fold change in expression. These genes were classified into 12 groups based on their expression patterns. These salt-responsive genes were assigned functions with Gene Ontology (GO) terms. Genes assigned transcription factor, transcription-regulator activity and DNA binding functions were preferentially classified as early-response genes. On the other hand, the genes assigned transferase and transporter activity were classified as late-response genes. These data suggest the existence of multiple signal transduction pathways in response to salt stress in wheat. The expression level of transcription factors (TFs), which have been reported to be involved in the salt-tolerance pathway, were observed to change in response to salt treatment. Only a few of those TFs demonstrated high sequence similarity with genes in rice. Furthermore, comparison of the microarray data for wheat and rice revealed a small number of commonly up- or downregulated genes in these plants. These investigations suggest that salt-responsive genes distinct from those observed in rice might exist in wheat. The wheat genes identified in this study are candidates for genes involved in salt-stress tolerance.

INTRODUCTION

Microarrays are a powerful tool for the high-throughput screening of stress responsive genes, including genes that respond to salt stress. Genome-scale microarrays harboring almost all the transcripts of model plants such as *Arabidopsis* and rice have been produced based on whole-genome sequencing. Using these microarrays, transcriptomes in response to salt stress have been monitored. Dynamic changes in gene expression in response to salt stress on a genome-wide scale have been reported. These salt-responsive genes have been grouped according to their putative functions; these groups include genes encoding osmoprotectants, reactive oxygen network components, transporters, regulators of primary energy metabolism, carbohydrates, cell wall components, protein metabolism modulators, signal transduction components, and hormones [1]. Furthermore, transcription factors (TFs) that enhance salt-stress tolerance have been identified; these TFs include members of the AP2/EREBP, MYB, NAC, and WRKY families [2].

Wheat is a staple food crop. The information that is currently available on the genome sequence of this crop is not sufficient for conducting whole genome transcript analysis. Recently, more than a million ESTs in wheat have been collected [3,4]. Based on these ESTs, we developed oligo-DNA microarrays that contained ca. 32,000 unique wheat genes. In this study, we monitored the transcriptomes of early salt response in wheat. Based on the expression patterns, the salt-responsive genes in wheat were grouped, annotated, and characterized. Furthermore, we discuss the characteristics of the salt response in wheat with the focus on the TFs.

MATERIALS AND METHODS

Plant materials

Triticum aestivum cv. Chinese Spring (CS) was grown in sterilized vermiculite in a growth chamber (16 h light/8 h dark; constant temperature 22°C). Two-week-old seedlings were transferred into distilled water and treated with a 150 mM NaCl solution. After treatment for 0, 1, 6, or 24 h, the plants were harvested and their roots and shoots were separately frozen in liquid nitrogen.

Microarray analysis

Two oligo-DNA microarrays, namely wheat 22k and 11k microarrays, were used[5]. Total RNA was labelled and used for microarray hybridization according to the manufacturer's instructions (Agilent Technologies).

Expression profiling

Salt-responsive genes were classified by hierarchical clustering[6] based on a log-transformed ratio. Gene functions were determined by the BLASTx (E value < 1E-20) to search the non-redundant (nr) database of the National Center of Biotechnology Information (NCBI), rap1-rep of the Rice Annotation Project Database (RAP-DB), and the rice pseudomolecules database of The Institute for Genomic Research (TIGR). Plant GOSlim terms that were assigned to rice genes annotated by TIGR were also assigned to the wheat counterparts of these genes.

RESULTS

Salt-responsive genes selected by microarray analysis

In this study, approximately 32,000 uniquely expressed genes were monitored using two microarrays. Genes whose expression changed by more than 2-fold in response to salt treatment were defined as salt-responsive genes. In total, 5,996 independent salt-responsive genes were selected. Most salt-responsive genes exhibited below a 3-fold change in expression. Root was more sensitive to salt treatment than shoot in gene expression response. This indicates that the genes in the roots and shoots exhibited differences in response to salt treatment.

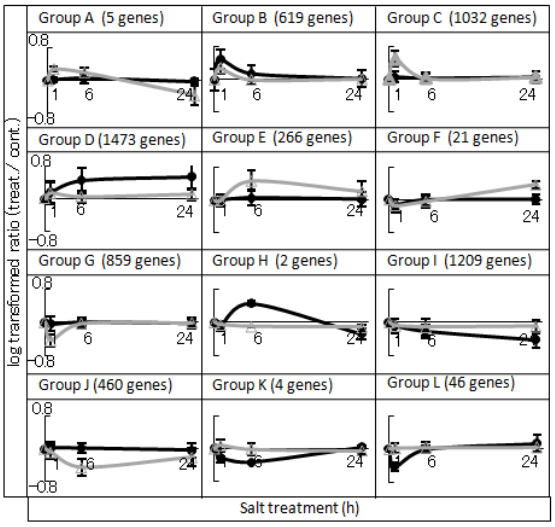

Figure 1 Expression patterns of 12 gene groups. Genes in each from group A to L are classified by hierarchical clustering. The average of the log-transformed ratios and SE are shown. Circles in black indicate the gene expression patterns in the roots. Triangles in gray indicate the gene expression patterns in the shoots.

Gene expression profile in response to salt treatment

The 5,996 genes were clustered into groups based on similarities in their expression patterns. These genes were clustered into 12 groups according to the correlation distance (Figure 1). The largest group was group D. The root genes in this group were upregulated after 6 h and 24 h of salt treatment. DHN/LEA/RAB-related genes, encoding various ion transporters, and genes involved in osmotic regulation were included in this group. Genes in groups B, C, G, and L showed early responses to salt stress; therefore, it is expected that these genes are located upstream of signal transduction pathways.

The function of the genes in each expression group was predicted on the basis of the plant GOSlim categories to which they were assigned. Of 5,996 salt-responsive genes, 3,075 were observed to be rice counterparts of wheat and were annotated with GOSlim terms. The number of genes annotated with GO terms for molecular function is presented. A relatively large number of genes in groups C, G, and I were assigned the function of DNA binding (GO: 0003677). Genes in groups C and G demonstrated an early response to salt treatment in the roots. Genes for kinase activity (GO: 0016301), receptor activity (GO: 0004872), and transporter activity (GO: 005215) were preferentially found in group D. The expression of these genes in the roots was upregulated after treatment for 6 h and 24 h. Genes assigned transcription factor activity (GO: 0003700), namely, those in groups B, C, and G, demonstrated an early response to salt treatment. Therefore, transcription-related genes may demonstrate an early response to salinity. On the other hand, genes assigned transferase activity (GO: 0016740) demonstrated a relatively late response to salinity in groups D and I.

Salt-responsive TF genes

TFs play key regulatory roles in stress tolerance, including salt stress in plants. By performing a BLASTn search against the wheat genes on the micorarrays, 59, 161, 53, and 48 genes were annotated as belonging to the AP2/EREBP, MYB, NAC, and WRKY families, respectively (Table 1). The expression patterns of these TF genes were monitored using microarrays. In all the four TF groups, the number of upregulated genes was greater than the number of downregulated genes. All AP2/EREBP genes that demonstrated a salt response were upregulated, except for one gene. Of the 161 MYB genes on the microarrays, 42 were salt-responsive. The majority of the salt-responsive MYB genes demonstrated transient up- or down-regulation. With regard to the NAC family, 23 of 53 genes on the microarrays belonging to this family were upregulated in response to salt treatment. Of these, five were consistently upregulated in the roots at 1 h, 6 h, and 24h following salt treatment. Two expression patterns were frequently demonstrated by the salt-responsive WRKY genes; downregulation in the shoots after 1 h of salt treatment, and the other was upregulation in the roots after both 6 h and 24 h of salt treatment. Consequently, TF genes demonstrated different responses to salt treatment, suggesting that TFs play various roles in pathways involved in the salt-stress response.

Table 1 Number of salt-responsive TF genes on microarrays.

TF	On arrays	Salt-resp.	Expression group						
			B	C	D	E	G	I	J
AP2/EREBP	59	21	3	3	13		1	1	
MYB	161	42	6	5	11	2	7	7	4
NAC	53	23	5		14		2		2
WRKY	48	23	2		15		6		

Comparison of the salt-responsive genes in wheat and rice

Rice genes similar to wheat-gene encoding TFs were searched using RAP-DB. Of the four families of salt-responsive wheat TF genes, four AP2/EREBP, nine MYB, nine NAC, and two WRKY genes demonstrated high sequence similarity with the corresponding rice genes (E-value < 1E-60). The remainder of salt-responsive wheat TF genes might be divergent and unique to wheat.

In addition to performing a comparison between the TF sequences in wheat and rice, the global expression patterns of the salt-responsive genes in wheat and rice were compared. The expression datasets for the salinity response genes in rice are available from the Gene

Expression Omnibus (GEO) of NCBI. In the GEO datasets (GDS1383)[7], the control dataset was compared with the dataset of salt-responsive genes available at the NCBI website. Sets of 2,690 and 3,404 rice genes were observed to be up- and down-regulated, respectively. Of 5,996 salt-responsive genes in wheat, 3,649 were assigned to probes on the rice microarray. It was observed that in both wheat and rice, 141 genes were upregulated and 186 genes downregulated in response to salt treatment. Genes that are known to be involved in stress responses, such as dehydrin, EREBP, and glutathione S-transferase, were upregulated in both wheat and rice. On the other hand, genes encoding survival-related proteins, such as histones, DNA-replication-related elements, and ATPase complex, were frequently downregulated in wheat and rice. The functions of these genes in salt stress might be conserved in wheat and rice.

DISCUSSION

Using microarrays, a transcriptome of early salt-stress responsive genes in wheat was investigated. The expression levels of 5,996 of ca. 32,000 wheat genes changed in response to salt treatment for 1 h, 6 h, and 24 h. This means that approximately 19% of the wheat genes are salt-responsive. In *Arabidopsis*, at least 20% of the genome showed a dynamic change in expression following salt treatment[1]. On the other hand, Ueda et al. reported that, in response to salt stress, 67 of 460 genes were up-regulated in barley; however, only five genes were upregulated in rice[7]. These lines of evidence suggest that the number of salt-responsive genes in wheat and barley is comparable to that in *Arabidopsis* and larger than that in rice.

The salt-responsive genes in wheat were grouped into 12 groups based on their expression patterns (Figure 1); however, their functions in salt tolerance remain to be clarified. We examined the functions of these genes based on their GOSlim categories to which they were assigned. Genes that act upstream of stress pathways, such as those involved in DNA binding, transcription factor activity, and transcription regulator activity, were preferentially assigned to early responsive groups. On the other hand, genes whose putative functions are transferase and transporter activities were more frequently assigned to late-response groups. Based on the gene expression patterns revealed by the microarrays, it can be hypothesized that various salt-stress response pathways exist in wheat. In fact, QTL analyses suggest that a number of genes regulate salt tolerance in wheat[9]. In rice, several QTLs are involved in salt tolerance. Genes encoding ion transporters were isolated from QTLs by map-based cloning. Furthermore, many transformed plants have enhanced salt tolerance. Therefore, numerous genes that were newly characterized in this study may be involved in salt stress tolerance not only in wheat but also in other crops.

Salt-responsive genes in wheat selected by the microarrays only partially overlapped with those in rice. Genes commonly upregulated by salt treatment in wheat and rice have been previously reported to be involved in abiotic-stress tolerance. Although the rice expression data used in this study were collected under different salt-treatment conditions, the functions of salt-responsive genes in wheat might be different from those of rice. Furthermore, only a few salt-responsive wheat TF genes demonstrate high sequence similarity with corresponding rice genes. As TFs are generally located upstream of environmental-stress response pathways, wheat TFs whose functions differ from those of rice TFs could be utilized as novel genetic resources for developing salt tolerant plants. These key genes could contribute to the improvement of salt tolerance in crops.

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