Proteomic Analysis of Specific Proteins in the Root of Salt-Tolerant Barley

Manabu SUGIMOTO and Kazuyoshi TAKEDA

1Group of Cytomolecular Biochemistry, Research Institute for Bioresources, Okayama University, 2-20-1 Chuo, Kurashiki, Okayama 710-0046, Japan
2Barley Germplasm Center, Research Institute for Bioresources, Okayama University, 2-20-1 Chuo, Kurashiki, Okayama 710-0046, Japan

Received June 29, 2009; Accepted September 19, 2009; Online Publication, December 7, 2009
[doi:10.1271/bbb.90456]

Abstract

Salt stress is one of the most limiting factors in plant growth and the productivity of agricultural crops. A variety of genes and proteins have been reported to respond to salt stress, but it is important to confirm the type of stress-inducible genes and proteins and whether they function in salt tolerance or are simply induced without contributing to salt tolerance. Evaluation of genes and proteins as to whether specific/preferential expression in salt-tolerant plants as opposed to the salt-sensitive plants is necessary for identification of the genes and proteins that possibly play roles in conferring salt tolerance on plants. Although many genes responding to salt stress have been identified by expressed sequence tags (ESTs) and microarray analysis, the gene expression profile does not often reflect the protein expression profile, because expression of the protein is regulated at the translation rate, and some proteins are modified after translation for the function. Therefore, the protein profile is important to understand the biological process of salt tolerance. Furthermore, the identification and isolation of genes and proteins expressed specifically in many kinds of salt-tolerant plants provide more information to understand the plant salt-tolerant mechanism and the selection of strategies to develop stress-tolerant plants. In this study, we analyzed the protein profiles of salt-tolerant and salt-sensitive barley roots exposed to salt stress by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), and identified the proteins produced significantly in salt-tolerant barley, because the root is the organ of land plants most affected by salt stress.

Seeds of salt-tolerant barley (Hordeum vulgare L.), OUK305, and salt-sensitive barley, UI743, were germinated and cultured for 2 weeks in a hydropnic solution, as described previously. The plants were transferred to a culture solution containing 0.2 M NaCl and incubated for 5 d because the effect of osmotic stress was to be eliminated. No gross visual symptoms of stress were observed. The roots before and after NaCl treatment were pulverized by mortar and pestle under liquid nitrogen, and the powdered tissue was suspended in an extraction solution containing 8 M urea, 4% CHAPS, 40 mM Tris, 0.2% Bio-Lyte 3/10 ampholyte, 2 mM tributyl phosphine, 1 mM PMSF, 50 μg/ml of DNase I, and 20 μg/ml of RNase A. The suspension was centrifuged at 16,000 × g for 20 min at 4°C, and the supernatant was used as the protein solution. The protein solution (150 μg of protein) was loaded onto an immobilized pH gradient strip (pH 4–7, 18 cm, GE Healthcare, Little Chalfont, UK) for the first dimension, and subsequently in 12–14% gradient SDS-polyacrylamide gel for the second dimension. Protein spots were resolved in each gel loading OUK305 and UI743 protein solutions by silver staining using the Silver stain MS kit (Wako, Osaka, Japan) (Fig. 1), and were quantified on the basis of spot density/analysis features of the software (ImageMaster, GE Healthcare). In approximately 400 protein spots on the each gel, after discounting spots with undefined shapes and areas, four and three specific protein spots were detected in OUK305 and UI743 respectively, and 11 and 17 protein spots were increased after exposure to NaCl in OUK305 and UI743 respectively. Finally, six protein spots specifically and preferentially produced in OUK305 were detected under salt stress as compared with those in UI743.

Spot 1 appeared after exposure to NaCl, spots 3 and 4 were produced constitutively, and the protein levels of spots 2, 5, and 6 were increased by 4.9, 4.5, and 2.4-fold respectively after exposure to NaCl. Spots 3, 4, 5, and 6 were produced specifically in OUK305, and the protein levels of spots 1 and 2 in OUK305 after exposure to NaCl were significantly higher than those of UI743, while faint proteins of spots 1 and 2, of which the intensities were 1,700 and 3,000 respectively, appeared in UI743 after NaCl treatment (Fig. 2, Table 1).

The six proteins were excised from the gel, incubated with DTT followed by iodoacetamide, and then the proteins were digested with trypsin. The peptides were applied to an integrated nano LC-MS/MS system and mass spectrophotometry to identify the specific proteins.
The MS/MS data were used to identify the protein by a search of the NCBInr databases with the MASCOT program. Hits were considered significant according to the score ($p < 0.05$).

Table 1 shows the identified proteins of six spots isolated from OUK305. All six proteins and their genes are known to be stress/defense-related proteins, the levels of which increase significantly under environmental stress. Spots 3 and 4 were identified as glutathione-$S$-transferase (GST), and spot 5 was dehydroascorbate reductase (DHAR). These are components of reactive oxygen species (ROS)-scavenging enzymes in plants. One role of plant GSTs is protection from oxidative stress by conjugating GSH to toxins producing oxidative damage in endogenous compounds. In addition, certain GSTs have DHAR activity. DHAR supplies ascorbate, which is not only the primary component of plant antioxidants, but also the reductant of ascorbate peroxidase (APX). While GST and DHAR do not scavenge ROS directly in a plant antioxidative system, our results confirm their contribution to salt tolerance, given reports that transgenic plants that overexpress GST and DHAR showed improvement in germination and growth under salt stress.

Spot 2, Caffeoryl-CoA $O$-methyltransferase (COMT), is one of the $O$-methyltransferase group for lignin biosynthesis, the gene of which was up-regulated significantly by salt...
stress not only in OUK305,\textsuperscript{3} but also in other stress-tolerant plants.\textsuperscript{10,11} Up-regulation of COMT in the root of OUK305 supports the suggestion that high intensity of lignification in the root of salt-tolerant plants helps to reduce the bypass water flow that allows Na\textsuperscript{+} ions to enter the roots via an apoplastic route.\textsuperscript{12,13} The function of peroxidase in salt tolerance has been reported to be help lignification in the root by catalyzing the cross-linking of pectins and non-cellulosic polysaccharides,\textsuperscript{14,15} in addition to reduction of ROS. Increases in peroxidase (spot 6) in OUK305 is implicated in the lignification with COMT or scavenging of ROS with GST and DHAR. Spot 1 was identified to be pathogenesis-related (PR) protein 10. Although the physiological function of PR10 in salt tolerance is not clear, there is a correlation between the expression of PR10 and salt tolerance, because the PR10 protein level increased significantly in the salt-tolerant callus of peanut,\textsuperscript{16} and a transgenic plant constitutively expressing the pea PR10 gene exhibited enhanced tolerance to salt stress.\textsuperscript{17} Increases in PR10 in OUK305 give additional evidence that PR10 is part of a defense mechanism in salt tolerance.

NaCl-treated Arabidopsis roots revealed changes in transcript abundance for at least 20\% of the genome, \textsuperscript{113} proteins increased,\textsuperscript{18,19} and 218 genes were up-regulated in the root of barley due to salt stress.\textsuperscript{20} However, only six protein spots were significantly produced in the root of salt-tolerant barley OUK305 as compared with those in the root of salt-sensitive barley OUI743, and these proteins have been reported to be up-regulated under salt stress. This suggests that a variety of genes up-regulated by salt stress does not reflect protein expression, and that a common salt tolerant mechanism might have developed in the plants. Salt stress induces an accumulation of ROS that are harmful to plant cells at high concentrations, and plants enhance ROS-scavenging enzymes, but no directly ROS-scavenging enzymes, such as superoxide dismutase, catalase, or APX were identified in the six proteins of OUK305. Our results suggest that salt-tolerant barley develops additive and synergistic biochemical strategies, such as selective accumulation or exclusion of ions, control of ion uptake by roots and transport into the leaves, and compartmentalization of ions at the cellular and whole-plant levels in addition to induction of ROS-scavenging enzymes in order to cope with salt stress.\textsuperscript{21} The results of this study provide candidates for proteins playing an important role in anti-salt stress systems to develop salt-tolerant plants.

Acknowledgment

This research was supported in part by the Ohara Foundation in Kurashiki, Japan.

References


