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A proteomics approach for identifying osmotic-stress-related proteins in rice

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Abstract

Osmotic stress can endanger the survival of plants. To investigate the mechanisms of how plants respond to osmotic stress, rice protein profiles from mannitol-treated plants, were monitored using a proteomics approach. Two-week-old rice seedlings were treated with 400 mM mannitol for 48 h. After separation of proteins from the basal part of leaf sheaths by two-dimensional polyacrylamide gel electrophoresis, 327 proteins were detected. The levels of 12 proteins increased and the levels of three proteins decreased with increasing concentration or duration, of mannitol treatment. Levels of a heat shock protein and a dnaK-type molecular chaperone were reduced under osmotic, cold, salt and drought stresses, and ABA treatment, whereas a 26S proteasome regulatory subunit was found to be responsive only to osmotic stress. Furthermore, proteins whose accumulation was sensitive to osmotic stress are present in an osmotic-tolerant cultivar. These results indicate that specific proteins expressed in the basal part of rice leaf sheaths show a coordinated response to cope with osmotic stress.

Keywords: Rice; *Oryza sativa*; Graminacea; Proteomics; Osmotic stress; Mannitol; Rice; Basal part of leaf sheath

1. Introduction

Plants are continuously exposed to biotic and abiotic stresses that endanger their survival. Among abiotic stresses, osmotic stress is one of the most severe, caused by drought, high salinity and cold stresses in nature. Plants respond to osmotic stress at the morphological, anatomical, cellular, and molecular levels. To cope with osmotic-related stresses, plants have developed various responses such as production of osmolites for osmotic adjustment, synthesis of Na^+/H^+ antiporters for ion sequestration, and many other mechanisms (Bohnert et al., 1995). AtHKT1, which is a Na^+ transporter, mediates osmolality balance between xylem vessels and xylem parenchyma cells (Snarpi et al., 2005). The operation of these responses usually requires three steps: osmotic stress recognition, signal transduction, and production of components for the physiological response (Tamura et al., 2003). There have been many reports on osmotic stress recognition and signal transduction in bacteria and yeast. Both in *Escherichia coli* and yeast, osmotic stress is detected by an osmosensor (Maeda et al., 1994; Mizuno, 1998). In yeast, osmotic signals perceived by two osmosensors are transduced to a mitogen activated protein kinase through Pbs2 (Reiser et al., 2000). In plants, a hybrid-type histidine kinase (AtTHK1) functions as an osmosensor and transmits the stress signal to a downstream mitogen-activated protein kinase (MAPK) cascade (Urao et al., 1999).

Lu and Neumann (1999) reported that when rice seedlings were exposed to osmotic stress modulated by polyethylene glycol 6000, growth in emerging first leaves of

Abbreviations: ABA, abscisic acid; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis.

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the intact plant was inhibited. Early inhibition of leaf growth was not related to changes in root size, osmotic potential gradients, or cell wall-yielding characteristics in the leaf-expansion zone of stressed seedlings. In *Arabidopsis*, Deak and Malamy (2005) demonstrated that osmotic stress represses the formation of autonomous lateral roots from lateral root primordia, while lateral root initiation was not greatly affected. Abscisic acid (ABA) and a newly identified gene, LRD2, are involved in osmotic repression of lateral root formation. Further examination revealed that both ABA and LRD2 control root system architecture even in the absence of osmotic stress. This finding indicated that the same molecular mechanisms that mediated responses to environmental cues could also be regulators of intrinsic developmental programs in the root.

Zonia and Mnnik (2004) investigated whether tobacco pollen tube cell volume changes in response to osmotic perturbation by activation of the phospholipid signaling pathway. Several intermediates in the phospholipid signaling pathway were detected during pollen tube growth. Hypo-osmotic stress induced a rapid increase in phosphatidic acid and a decrease in phosphatidylinositol phosphate. The fact that these signaling molecules are present during normal growth suggested that the mechanism for osmotic response involved components of the biomechanical networks driving pollen tube cell elongation. In osmotically stressed wheat coleoptiles, reduced rates of phenylalanine ammonia-lysase and tyrosine ammonia-lysase activities suppress phenylalanine biosynthesis, resulting in a reduced level of wall-bound ferulic acid. This decrease in wall-bound ferulic acid may lead to reduced levels of diferulic acid, an important contributor to maintaining cell wall extensibility (Wakabayashi et al., 1997). Clearly, there is much to learn at the biochemical and molecular levels about how plants respond to osmotic stress.

Proteomics is a powerful tool for separating complex protein mixtures, and has been employed to analyze protein changes in response to environmental changes. Abbasi and Komatsu (2004) have investigated rice proteins induced by salt stress using proteomics. The expression of superoxide dismutase was a common response to cold, drought, salt, and ABA stresses. Fructose bisphosphate aldolases, photosystem II oxygen evolving complex protein, and oxygen evolving enhancer protein 2 were expressed in leaf sheaths and leaf blades but not in roots. This result indicated that specific proteins, expressed in specific regions of rice, showed a coordinated response to salt stress. Riccardi et al. (1998) examined drought-responsive proteins of two maize lines and their first filial generation hybrid. There was significant quantitative variation in 78 out of 413 leaf proteins with 38 of them exhibiting differential expression in the two genotypes. In rice seedlings, phosphorylation of proteins induced by cold stress has been analyzed by 2D-PAGE (Komatsu et al., 1999). Although several proteins were found to be phosphorylated upon cold stress, a fragment originating from the RuBisCO large subunit accumulated to high levels after cold stress and was phosphorylated. These reports show that a proteomics approach is useful for analyzing the physiological function of stress-induced proteins.

Rice is not only a very important agricultural resource but also a model plant for biological research because its genome is smaller than those of other cereals, making it suitable for efficient genetic analysis and transformation. Because protein analysis is the most direct approach for defining the function of genes, analysis of the proteome linked to genome sequence information is a very useful strategy for functional genomics (Komatsu and Tanaka, 2004). To date, there are several reports about systematic proteomic analysis of rice protein abundance under abiotic stresses. In this study, proteins from the basal part of rice leaf sheaths were screened by a proteomics approach to investigate the response of rice to osmotic stress.

2. Results and discussion

2.1. Rice shoot elongation is inhibited by osmotic stress

The osmotic potential of soil alters the depth of root systems, the rate of root elongation, and the number of lateral roots (van der Weel et al., 2000); however, there are only a few reports about phenotypic changes in rice under osmotic stress (Lu and Neumann, 1999). To understand about phenotypic changes in rice under osmotic stress, treatment was done using two-week-old rice seedlings based on the sensitivity of rice plants. At the beginning of this study, rice seedlings were homogeneous in terms of shoot height. When two-week-old rice seedlings, cv. Nipponbare, were treated with mannitol at different concentrations for 48 h or at 400 mM for different intervals, shoot elongation was obviously inhibited and the leaf blade gradually withered from the tip to the bottom. This damage to rice seedlings was more severe as the mannitol concentration increased (Fig. 1a, left). Rice seedling heights were reduced with increasing concentrations of mannitol treatment for 48 h (Fig. 1b, left). The pattern of this damage was continuous when rice seedlings were exposed to mannitol for longer periods of time (Fig. 1a, right and Fig. 1b, right). Particular morphological adaptations may be vital in specific plant species under osmotic stress, but these adaptations are not common to all plants. In this study, inhibition of growth in emerging first leaves and inhibition of shoot elongation were shown to be dependent on mannitol dose and length of treatment. This result may be due to osmotic stress inducing hydraulic limitations to water uptake that limit water availability for the volume increase required for expanding cells. The damage to seedlings and inhibition of shoot elongation were started at 400 mM from 48 h after mannitol treatment, so in the following study, this condition was used.