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Analysis of proteomic changes in roots of soybean seedlings during recovery after flooding

Afshin Salavati[a,b,1], *Amana Khatoon*[a,c,1], *Yohei Nanjo*[a,1], *Setsuko Komatsu*[a,*]

[a]National Institute of Crop Science, National Agriculture and Food Research Organization, Tsukuba 305-8518, Japan
[b]Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources, University of Tehran, Karaj 31587-77871, Iran
[c]Department of Plant Sciences, Kohat University of Science and Technology, Kohat 26000, Pakistan

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ABSTRACT

A proteomic approach was used to identify proteins involved in post-flooding recovery in soybean roots. Two-day-old soybean seedlings were flooded with water for up to 3 days. After the flooding treatment, seedlings were grown until 7 days after sowing and root proteins were then extracted and separated using two-dimensional polyacrylamide gel electrophoresis (2-DE). Comparative analysis of 2-D gels of control and 3 day flooding-experienced soybean root samples revealed 70 differentially expressed protein spots, from which 80 proteins were identified. Many of the differentially expressed proteins are involved in protein destination/storage and metabolic processes. Clustering analysis based on the expression profiles of the 70 differentially expressed protein spots revealed that 3 days of flooding causes significant changes in protein expression, even during post-flooding recovery. Three days of flooding resulted in downregulation of ion transport-related proteins and upregulation of proteins involved in cytoskeletal reorganization, cell expansion, and programmed cell death. Furthermore, 7 proteins involved in cell wall modification and S-adenosylmethionine synthesis were identified in roots from seedlings recovering from 1 day of flooding. These results suggest that alteration of cell structure through changes in cell wall metabolism and cytoskeletal organization may be involved in post-flooding recovery processes in soybean seedlings.

1. Introduction

Legumes are autonomous plants that are able to fix nitrogen and carbon [1]. Soybean is the most important legume crop, and its production is increasing and is expected to continue to increase as a result of global demand for soybean oil for human consumption and biodiesel fuel production, and the demand for high-protein meal for animal feed [2]. The availability of the soybean genome sequence [3] provides unprecedented opportunities for investigations of the genes responsible for valuable traits in this important crop [4]. Although the molecular and cellular responses of soybeans to environmental stimuli have been extensively analyzed, the genetic basis of soybean stress responses is not well understood [5]. Soybean is sensitive to poor soil aerification caused by flooding or waterlogging [6], which significantly reduces growth and yield [7].

Abbreviations: 2-DE, two-dimensional polyacrylamide gel electrophoresis; CBB, Coomassie brilliant blue; ROS, reactive oxygen species; MS, mass spectrometry; pI, isoelectric point; IEF, isoelectric focusing; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; 1DFE, 1 day flooding experienced; 2DFE, 2 days flooding experienced; 3DFE, 3 days flooding experienced; FWR, flooded without recovery; CWR, 3-day-old seedling as control without recovery; Control, 7-day-old seedlings without flooding stress.

* *Corresponding author at:* National Institute of Crop Science, National Agriculture and Food Research Organization, 2-1-18 Kannondai, Tsukuba 305-8518, Japan. Tel.: +81 29 838 8693; fax: +81 29 838 8694.
E-mail address: skomatsu@affrc.go.jp (S. Komatsu).

[1] These authors equally contributed to this work.

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Flooding has a major negative impact on worldwide agricultural productivity. The water superfluity in root surroundings associated with flooding induces a decrease in the concentrations of cellular oxygen and of carbon dioxide, which leads to injurious effects on plants [8]. The yield of major crops is reduced by 50% annually as a result of damage caused by flooding stress [1]. Flooding stress is a complex phenomenon involving multiple stressors, such as hypoxic and light stress [9]. Flooding-induced increases in the production of reactive oxygen species (ROS) and a reduced capacity to detoxify ROS in susceptible plants lead to oxidative damage [10]. Flooding stress also decreases photosynthesis [11], nitrogen fixation [12], and carbon assimilation [13]. The evaluation of model systems can provide insights into low-oxygen sensing mechanisms and metabolic adjustments associated with controlling the use of carbohydrates and ATP [9].

It has been reported that the effects of flooding stress on soybean seedlings are manifested in every aspect of growth under stress lasting longer than 1 day [14,15]. Transcriptional and proteomic studies of flooded soybean seedlings have revealed that genes involved in alcohol fermentation, ethylene biosynthesis, and cell wall loosening are part of the anaerobic response of soybean to flooding stress [16]. Previous studies also demonstrated that flooding stress affects the expression of proteins involved in glycolysis [17], ROS scavenging [18], protein storage, disease resistance/defense [14], antioxidative systems, and signaling systems [19]. Furthermore, experiments involving nitrogen substitution have demonstrated that there are overlaps between the proteomic changes induced by flooding and those induced by low-oxygen stress [20].

While the responses of plants to flooding stress are generally well-known [21], relatively little work has focused on understanding the mechanisms associated with plant recovery after flooding. The processes involved in the recovery of plants from flooding stress have not been fully elucidated. In the present study, a proteomic approach was used to ascertain the changes that occur in the soybean seedling root proteome during recovery from flooding stress.

2. Materials and methods

2.1. Plant growth condition

Soybean (*Glycine max* L. cultivar Enrei) seeds were sterilized with sodium hypochlorite solution and rinsed in water. The sterilized seedlings were sown in a plastic case (180×140×45 mm) containing 400 mL of quartz sand wetted with 100 mL of water and grown at 25 °C and 70% humidity in a growth chamber (Sanyo, Tokyo, Japan) under white fluorescent light (600 μmol m^{-2} s^{-1}, 12 h light period/day). For flooding treatments, two-day-old seedlings were flooded with 700 mL of water. The flooding condition was maintained at 2 cm of water above the quartz sand surface. The roots of soybean seedlings were collected and were used for analyses. In order to study the expression trend of proteins in recovery from flooding, seven-day-old soybean seedlings with or without experience of flooding stress were used. Seven-day-old seedlings grown for 7 days without flooding treatment were used as control (Control, Fig. 1). Seven-day-old soybean seedlings with experience of flooding for 1, 2 and 3 days were used as 1, 2 and 3 days flooding experienced (1, 2 and 3DFE), respectively. Furthermore, three-day-old soybean seedlings with or without flooding treatment were also used for analysis. Three-day-old soybean seedlings with flooding were used as flooded without recovery (FWR, Fig. 6). Three-day-old soybean seedlings without flooding were used as control without recovery (CWR).

2.2. Protein extraction

A portion (500 mg) of fresh roots was ground to powder in liquid nitrogen with a mortar and pestle. The powder was transferred to 10% trichloro acetic acid and 0.07% 2-mercaptoethanol in acetone and the mixture was vortexed. The suspension was sonicated for 5 min and then incubated for 45 min at −20 °C. After incubation, the suspension was centrifuged at 9000×*g* for 20 min at 4 °C. The supernatant was discarded and resulting pellet was washed twice with 0.07% 2-mercaptoethanol in acetone. The resulting pellet was dried using a Speed-Vac concentrator (Savant Instruments, Hicksville, NY, USA) and resuspended with 8 M urea, 2 M thiourea, 5% CHAPS and 2 mM tributylphosphine by vortexing for 1 h at 25 °C. The suspension was centrifuged at 20,000×*g* for 20 min at 25 °C. Supernatant was collected as protein extract. Protein contents were determined using the Bradford [22] method with bovine serum albumin as the standard.

2.3. Two-dimensional polyacrylamide gel electrophoresis

Protein samples in a final volume of 180 μL of lysis buffer containing 0.4% Bio-Lyte pH 3/10 (Bio-Rad, Hercules, CA, USA) were directly loaded into a focusing tray. The immobilized pH gradient strips (3-10NL, 11 cm, Bio-Rad) were rehydrated for 14 h at 50 V. Isoelectric focusing (IEF) was carried out with the Protean IEF Cell (Bio-Rad) using following conditions: 250 V for 15 min with a linear ramp, 8000 V for 1 h with a linear ramp, and finally 8000 V at 35,000 V/h with a rapid ramp at 20 °C. After IEF, the strips were equilibrated with 6 M urea, 2% SDS, 0.375 M Tris–HCl (pH 8.8), 20% glycerol and 130 mM dithiothreitol for 30 min. The last equilibration step was done with 6 M urea, 2% SDS, 0.375 M Tris–HCl (pH 8.8), 20% glycerol, and 135 mM iodoacetamide for 30 min. The equilibrated strips were placed onto 15% SDS-polyacrylamide gels with 5% stacking gels and sealed with 1% agarose. Electrophoresis in the second dimension was performed at a constant current of 35 mA. After the electrophoresis, gels were stained with Coomassie brilliant blue (CBB).

2.4. Gel image analysis

2-DE images were obtained using a GS-800 calibrated densitometer scanner (Bio-Rad) and the position of individual proteins on gels was evaluated with PDQuest software (version 8; Bio-Rad). The isoelectric point (pI) and molecular mass of each protein were determined using 2-DE standard marker (Bio-Rad). The amount of protein in a spot was estimated using the PDQuest software with local regression model normalization.

2.5. Peptide preparation for mass spectrometry analysis

To identify proteins in protein spots using MS, protein spots were excised from 2-DE gels and washed with water. Proteins