JOURNAL OF PROTEOMICS 121 (2015) 15–27

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Proteomic analysis of soybean hypocotyl during recovery after flooding stress

Mudassar Nawaz Khan[a,b,1], *Katsumi Sakata*[c], *Setsuko Komatsu*[a,b,*]

[a]*Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan*
[b]*National Institute of Crop Science, National Agriculture and Food Research Organization, Tsukuba 305-8518, Japan*
[c]*Maebashi Institute of Technology, Maebashi 371-0816, Japan*

ARTICLE INFO

Article history:
Received 4 February 2015
Accepted 13 March 2015
Available online 27 March 2015

Keywords:
Proteomics
Soybean
Hypocotyl
Flooding
Recovery

ABSTRACT

Soybean is a nutritionally important crop, but exhibits reduced growth and yields under flooding stress. To investigate soybean responses during post-flooding recovery, a gel-free proteomic technique was used to examine the protein profile in the hypocotyl. Two-day-old soybeans were flooded for 2 days and hypocotyl was collected under flooding and during the post-flooding recovery period. A total of 498 and 70 proteins were significantly changed in control and post-flooding recovering soybeans, respectively. Based on proteomic and clustering analyses, three proteins were selected for mRNA expression and enzyme activity assays. Pyruvate kinase was increased under flooding, but gradually decreased during post-flooding recovery period at protein abundance, mRNA, and enzyme activity levels. Nucleotidylyl transferase was decreased under flooding and increased during post-flooding recovery at both mRNA expression and enzyme activity levels. Beta-ketoacyl reductase 1 was increased under flooding and decreased during recovery at protein abundance and mRNA expression levels, but its enzyme activity gradually increased during the post-flooding recovery period. These results suggest that pyruvate kinase, nucleotidylyl transferase, and beta-ketoacyl reductase play key roles in post-flooding recovery in soybean hypocotyl by promoting glycolysis for the generation of ATP and regulation of secondary metabolic pathways.

Biological significance

This study analyzed post-flooding recovery response mechanisms in soybean hypocotyl, which is a model organ for studying secondary growth, using a gel-free proteomic technique. Mass spectrometry analysis of proteins extracted from soybean hypocotyls identified 20 common proteins between control and flooding-stressed soybeans that changed significantly in abundance over time. The hypocotyl proteins that changed during post-flooding recovery were assigned to protein, development, secondary metabolism, and glycolysis categories. The analysis revealed that three proteins, pyruvate kinase, nucleotidylyl transferase, and beta-ketoacyl reductase, were increased in hypocotyl under flooding conditions and

Abbreviations: LC, liquid chromatography; MS, mass spectrometry; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

* *Corresponding author at: Setsuko Komatsu, National Institute of Crop Science, National Agriculture and Food Research Organization, Kannondai 2-1-18, Tsukuba 305-8518, Japan.* Tel.: +81 29 838 8693; fax: +81 29 838 8694.
E-mail address: skomatsu@affrc.go.jp (S. Komatsu).
[1] Additional address: Institute of Biotechnology and Genetic Engineering, University of Agriculture, Peshawar 25130, Pakistan.

http://dx.doi.org/10.1016/j.jprot.2015.03.020
1874-3919/

during post-flooding recovery. The proteins are involved in glycolysis, nucleotide synthesis and amino acid activation, and complex fatty acid biosynthesis.

1. Introduction

The hypocotyl plays an important role in both the primary and secondary growth of plants. The *Arabidopsis* hypocotyl, which is widely used as a model for studying secondary growth in plants [1], grows exclusively by cell expansion and does not undergo cell division [2]. Hypocotyl cell elongation in *Arabidopsis* is controlled by a central growth-regulation circuit that integrates hormonal, environmental, and developmental signals [3]. Phytohormones such as auxins, gibberellins, and brassinosteroids promote hypocotyl growth [4], whereas cytokinins, ethylene, and abscisic acid inhibit its growth [5]. In addition to its importance to plant growth and development, the hypocotyl is involved in plant tolerance to abiotic stresses, including salinity, drought, and flooding. In soybean, which is an important economic crop worldwide, calcineurin B-like protein enhances hypocotyl elongation during salt and drought stresses [6], and polyamine catabolism involving copper-containing amine oxidase promotes hypocotyl growth under salt stress [7].

Flooding is a major constraint on the growth and productivity of crops [8]. Plant responses to flooding vary depending on the species and type of damage. At the metabolic level, most flooding-stressed plants change from aerobic respiration to glycolysis and fermentation for energy production [9]. In soybean, flooding has adverse effects on morphology and physiology. Under prolonged flooding conditions, soybean forms aerenchyma in the stem, roots, and nodules [10], which leads to the partial recovery of nitrogen metabolism [11]. The hypocotyl surface of soybean also contains hypertrophic lenticels that enable the entry of atmospheric oxygen into the secondary aerenchyma under flooding stress [12]. The metabolic and morphological changes that occur in flooding-stressed soybean are likely an attempt to cope with the anaerobic conditions caused by flooding.

Proteomic investigations of flooding stress-responsive mechanisms in soybean roots have demonstrated that numerous proteins and biological processes are involved. In particular, proteins that function in storage, transport, disease resistance, detoxification of reactive oxygen species, energy and cell wall metabolism, and cell signaling are induced by flooding in soybean roots [13]. Nanjo et al. [14] identified several cell wall-related proteins that serve as indicators for assessing the severity of flooding stress in soybean roots and hypocotyls. In addition, proteomic profiling for determining flooding tolerance of different soybean varieties revealed that the levels of RNA binding/processing-related proteins were positively correlated in untreated soybeans, whereas flooding stress indicator proteins were negatively correlated in flooded soybeans [15]. Although soybean root proteins have been extensively analyzed under flooding conditions, the proteins involved in hypocotyl responses to flooding stress and post-flooding recovery remain poorly characterized.

Plant responses to flooding are being widely researched; however, considerably less attention has been paid towards understanding the mechanisms linked to post-flooding recovery. Salavati et al. [16] analyzed the protein profiles in soybean roots during recovery after flooding and found that cell wall metabolism and cytoskeleton reorganization were predominantly affected. Similarly, Khan et al. [17] performed proteomic analysis of soybean roots during post-flooding recovery and concluded that the observed increase in peroxidases and cell wall-related proteins would minimize oxidative damage and promote cell wall integration, respectively, and thereby facilitate recovery from flooding stress. Despite these studies in roots, the protein changes that occur in soybean hypocotyls during post-flooding recovery have not been examined in detail. To determine the mechanisms involved in post-flooding recovery in soybeans, the temporal profiles of hypocotyl proteins were analyzed using gel-free proteomic technique.

2. Materials and methods

2.1. Plant material and treatments

Soybean (*Glycine max* L. cv Enrei) seeds were sterilized with 2% sodium hypochlorite solution and then thoroughly rinsed in water. The sterilized seeds were sown 4 cm inside surface of quartz sand (450 mL) wetted with 150 mL water in seedling cases and grown at 25 °C and 70% humidity in a growth chamber (Sanyo, Tokyo, Japan) under white fluorescent light (160 μmol m^{-2} s^{-1}, 16 h light period/day). Ten seeds were sown in each seedling case and the average seedling growth was estimated for each experiment. For proteomic analysis, two-day-old soybeans were flooded for 2 days and hypocotyl samples were collected at the day of removal of flooding and then at 2 more points during the 4-day recovery period. For mRNA expression and enzyme activity assays, the hypocotyl of soybean recovering after 2 and 4 days of flooding was analyzed. Three independent biological replicates were performed for each experiment.

2.2. Protein extraction

A portion (0.5 g) of collected hypocotyl was ground to powder in liquid nitrogen using a mortar and pestle. The powder was transferred to an acetone solution containing 10% trichloroacetic acid and 0.07% 2-mercaptoethanol, and the resulting mixture was vortexed and then sonicated for 10 min. The suspension was incubated for 1 h at −20 °C with vortexing every 15 min and then centrifuged at 9000 × *g* at 4 °C for 20 min. The supernatant was discarded and pellet was washed twice with 0.07% 2-mercaptoethanol in acetone. The pellet was dried using a Speed-Vac concentrator (Savant Instruments, Hickville, NY, USA) and then resuspended in lysis buffer, consisting of 7 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