JOURNAL OF PROTEOMICS 112 (2015) 1–13

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Gel-free quantitative proteomic approach to identify cotyledon proteins in soybean under flooding stress

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ARTICLE INFO

Article history:
Received 5 July 2014
Accepted 27 August 2014

Keywords:
Soybean
Flooding stress
Gel-free proteomics
Cotyledon

ABSTRACT

Flooding stress causes growth inhibition and ultimately death in most crop species by limiting of energy production. To better understand plant responses to flooding stress, here, flooding-responsive proteins in the cotyledons of soybean were identified using a gel-free quantitative proteomic approach. One hundred forty six proteins were commonly observed in both control and flooding-stressed plants, and 19 were identified under only flooding stress conditions. The main functional categories were protein and development-related proteins. Protein–protein interaction analysis revealed that zincin-like metalloprotease and cupin family proteins were found to highly interact with other proteins under flooding stress. Plant stearoyl acyl-carrier protein, ascorbate peroxidase 1, and secretion-associated RAS superfamily 2 were down-regulated, whereas ferretin 1 was up-regulated at the transcription level. Notably, the levels of all corresponding proteins were decreased, indicating that mRNA translation to proteins is impaired under flooding conditions. Decreased levels of ferritin may lead to a strong deregulation of the expression of several metal transporter genes and over-accumulation of iron, which led to increased levels of reactive oxygen species, resulting to detoxification of these reactive species. Taken together, these results suggest that ferritin might have an essential role in protecting plant cells against oxidative damage under flooding conditions.

Biological significance

This study reported the comparative proteomic analysis of cotyledon of soybean plants between non-flooding and flooding conditions using the gel-free quantitative techniques. Mass spectrometry analysis of the proteins from cotyledon resulted in the identification of a total of 165 proteins under flooding stress. These proteins were assigned to different functional categories, such as protein, development, stress, redox, and glycolysis. Therefore, this study provides not only the comparative proteomic analysis but also the molecular mechanism underlying the flooding responsive protein functions in the cotyledon.

Abbreviations: MS, mass spectrometry; HPLC, high-performance liquid chromatography; TCA, tricarboxylic acid cycle; qRT-PCR, quantitative real time-polymerase chain reaction.

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http://dx.doi.org/10.1016/j.jprot.2014.08.014
1874-3919

1. Introduction

Global climate change is one of the major factors affecting precipitation patterns and is expected to increase the frequency of long-term flooding in winter/spring and flash flooding in summer as a result of extreme rainfall events [1]. Flooding is a complex abiotic stress that influences species composition and productivity in various plant communities and is of particular concern for agricultural crops. Hydrological conditions strongly influence the vegetation that exists in natural and man-made wetlands, as it is dependent on the ecophysiological responses of species to flooding [2]. For most cereal crops, excess water is a major constraint to productivity [3] as it adversely affects plant growth and grain yields [4] through modification of the underlying biochemical and molecular biological pathways. Worldwide, approximately 10% of all irrigated farmland is estimated to experience frequent flooding, which may decrease crop productivity by up to 20% in affected areas [5].

In flooding conditions, a slowing of oxygen influx is the principal underlying cause of injury to roots and the shoot–cotyledon that they support [6]. The amount of oxygen dissolved in flood water in equilibrium with air is approximately only 3% of that present in air. During the early stages of flooding, this oxygen is rapidly consumed by aerobic microorganisms and roots. In addition to imposing oxygen shortage, flooding also impedes the diffusive escape and/or oxidative breakdown of gases such as ethylene [7] and carbon dioxide that are produced by plants and soil microorganisms, leading to the toxic accumulation of byproducts that adversely influence root growth and function. Damage caused by flooding is primarily due to a lack of oxygen and carbon dioxide, which have extremely slow rates of diffusion through water compared to those in air. In the case of soybean, which is an important oilseed and protein crop worldwide, accumulated ethylene may impair root extension, and excess carbon dioxide in the soil can severely damage roots [8], as trapped carbon dioxide may form bicarbonate ions in high-lime soils, leading to iron unavailability and chlorosis. Under such conditions, growth arrest and death predominantly occur because the demand for ATP exceeds the supply, and due to the accumulation of toxic byproducts of anaerobic metabolism. Under anaerobic conditions, roots produce ATP predominantly through glycolysis, which generates pyruvic acid that feeds into ethanolic fermentation [9].

Ub/proteasome-mediated proteolysis of enzymes is involved in glycolysis and fermentation pathways and may be negatively controlled under the hypoxic conditions induced by flooding stress in soybean [10]. Eukaryotic translation initiation factor 5A-1 (eIF-5A) was anticipated to function as a biomodular protein capable of binding both RNA and protein, and involved in multiple phases of cellular signaling activities [11]. eIF-5A is regulated by dephosphorylation, while several unknown proteins are highly phosphorylated in soybean roots in response to flooding [12]. Eukaryotic translation initiation factor 5A may be phosphorylated by protein kinase CK2 in the nucleus [13]. However, the functions of several other candidate genes involved in the glycolytic and fermentative networks are not completely clear in relation to plant defense against flooding stress. Thus, the identification of the genes encoding key proteins responsible for providing flooding tolerance is required to better understand the mechanisms by which soybean resists flooding stress.

It is well recognized that proteomics are powerful and effective tools for identifying proteins associated with physiological processes, including osmotic adjustment, ion homeostasis, and detoxification of reactive oxygen species [14]. Proteomic studies with plants grown under flooding stress conditions have identified many differentially regulated proteins, including those related with protein transport, protein storage, ATP synthesis, metabolism, and signal transduction pathways [10]. Komatsu and Hossain [15] used a gel-based proteomic technique to examine cotyledon-specific responses in soybean plants flooded for 2 days and showed that the abundance of 20 proteins, which were mostly involved in transport and seed storage, changed significantly in response to flooding stress in the cotyledon. In particular, heat shock protein 70 and glycinin G3 were increased, whereas glycinin G2 and ferrous iron transport protein b were decreased. Interestingly, the levels of sucrose-binding protein and the beta-conglycinin alpha subunit were changed unevenly. Recent advances in the field of proteomics offer a better opportunity for dissecting quantitative traits in more comprehensive and meaningful way. Flooding stress of soybean is a serious problem because it inhibits the growth; however, flood-tolerant cultivar has not been identified. Here, a quantitative comparison of the proteins in the cotyledon proteome of soybean plants under flooding stress was performed using a gel-free quantitative proteomic approach. The findings from this study may help to better understand the response mechanisms at the protein level in soybean plants under flooding stress.

2. Experimental procedures

2.1. Plant materials and stress condition

Seeds of soybean (*Glycine max* L.) cv. Enrei were surface sterilized with 25% sodium hypochloride solution for 2 min, rinsed twice with distilled-deionized water for 2 min, and then germinated on silica sand under illumination with white fluorescent light (photosynthetic photon density of 200 μmol m^{-2} s^{-1}; 12/12 h light/dark cycle) at 25 °C and 70% relative humidity. Two-day-old soybean plants were exposed to flooding for 4 days. Flooding conditions were maintained that reached 3 cm above the sand surface. To identify flooding-responsive proteins in the cotyledons, cotyledons were collected at days 1, 2, 3, and 4 under flooding conditions. For all experiments, non-flooded plants were collected as controls on the same days as treated plants, and triplicate experiments were performed as biological replicates.

2.2. Protein extraction

Total proteins were extracted for gel-free proteomic analysis according to Komatsu and Hossain [15]. Briefly, 500 mg of fresh cotyledon samples was ground to a powder in liquid nitrogen with a mortar and pestle. The powder was transferred to Solution I (10% trichloroacetic acid and 0.07% 2-mercaptoethanol in acetone) and vortexed. The resulting