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Quantitative proteomics of nuclear phosphoproteins in the root tip of soybean during the initial stages of flooding stress

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ABSTRACT

Soybean is sensitive to flooding stress, which has affected many proteins in roots. To identify the upstream events controlling the regulation of flooding-responsive proteins, nuclear phosphoproteomics of soybean-root tip was performed. Nuclei were isolated from the root tip of 2-day-old soybeans treated with flooding for 3 h. The purity of nuclear fractions was confirmed by Western blotting and enzyme-activity assays for subcellular-specific enzymes. Phosphopeptides in the fractions were enriched and analyzed using gel-free proteomic technique. Fourteen phosphoproteins significantly changed in root tip in response to flooding stress. Of these phosphoproteins, 10 proteins including 5 protein synthesis-related proteins were predicted to be localized in the nucleus. In particular, zinc finger/BTB domain-containing protein 47, glycine-rich protein, and rRNA processing protein Rrp5, which are related to abscisic acid (ABA) response, were clearly phosphorylated in response to flooding stress. The mRNA expression levels of these nuclear phosphoproteins were down-regulated in root tip exposed to flooding stress with ABA. In addition, the fresh weight of soybean decreased under flooding stress with ABA, although the fresh weight of plant increased during the initial stage of flooding stress. These results suggest that ABA may affect the flooding response of early-stage soybean through the regulation of nuclear-localized phosphoproteins.

Biological significance
This study reported nuclear phosphoprotein analysis of root tip under initial flooding stress using gel-free quantitative proteomics. The main findings of this study are as follows: (i) Fourteen nuclear phosphoproteins in soybean root tip cells were significantly changed in the initial stages of flooding stress; (ii) Zinc finger protein, glycine-rich protein, and Rrp5 were phosphorylated in the nuclei of root tip in response to flooding; and (iii) The mRNA expression levels of these genes were down-regulated by ABA under flooding conditions. These results suggest that ABA may be involved in the initial responses of early-stage soybean to flooding stress by altering the phosphorylation of nuclear-localized phosphoproteins. This study

Abbreviations: LC, liquid chromatography; MS, mass spectrometry; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; ABA, abscisic acid; PAGE, polyacrylamide gel electrophoresis.

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provides not only the nuclear phosphoproteomic analysis but also the molecular mechanism underlying the initial flooding responsive nuclear phosphoproteins functions in the root tip of soybean.

1. Introduction

Flooding is a widespread environmental stress that negatively affects the growth of nearly all crops and impedes agricultural development in many parts of the world [1]. Flooding causes a decrease in the oxygen concentration of farmland soil and restricts energy production by plants through the impairment of oxidative phosphorylation [2]. Among agricultural crops, the growth and grain yields of soybean are particularly reduced under flooding conditions [3]. In the early germination stage, flooding stress leads to the physical disruption of cells due to the rapid uptake of water [4]. During the first 3 h of flooding stress, the fresh weight of germinating soybeans rapidly increases, whereas the growth rate exhibits a marked decrease in response to flooding [5]. In addition, the cellular ATP content is clearly reduced in soybean roots after 24 h of flooding stress [6,7]. Together, these findings indicate that flooding stress impairs the growth and development of soybean by changing the physiological status of plants.

The response mechanisms of soybean to flooding have been examined using proteomic techniques, which have revealed that proteins involved in signal transduction, transcriptional regulation, energy metabolism, and proteasome-mediated protein degradation are differentially regulated by flooding stress [7–10]. For example, alcohol dehydrogenase, the key enzyme involved in alcohol fermentation, was increased [8], whereas cytosolic ascorbate peroxidase, which functions in active oxygen scavenging, was decreased in response to flooding stress [10]. In the initial stages of flooding stress, calcium-related signal transduction has been shown to play important roles in regulating soybean growth [7]. The findings from these studies indicate that numerous cellular mechanisms are activated in soybean under flooding conditions. However, the organ-specific mechanism remains unclear.

To further investigate the cellular events that are initiated in soybean in response to flooding stress, subcellular proteomic approaches have been applied [11–13]. Under flooding conditions, plasma membrane proteins related to the antioxidative system mediate resistance to oxidative damage [11]. These proteomic analyses also demonstrated that mitochondrial proteins related to the tricarboxylic acid cycle are increased by flooding; however, proteins comprising electron transport chain complexes are decreased [13]. In the cell wall, proteins related to reactive oxygen species scavenging system and jasmonate biosynthesis were decreased under flooding stress [12], which was also shown to affect protein synthesis and glycosylation in the endoplasmic reticulum [14]. Together, the findings from these studies suggest that different groups of subcellularly localized proteins play important roles in regulating flooding stress-responsive pathways in soybean.

Phosphorylation of plant proteins is a reversible modification process, and is a common signaling event that occurs upon plant exposure to abiotic and biotic stresses [15]. In soybean, the phosphorylation of proteins during flooding stress leads to changes in the translational or post-translational regulation of proteins involved in carbohydrate metabolism [16]. Furthermore, energy-related metabolic processes are particularly sensitive to changes in protein phosphorylation during flooding stress [6]. Recently, it was reported that the ethylene signaling pathway of soybean played critical roles with phosphorylation to the initial stages of flooding stress [5]. These results indicate that adaptive responses of soybean to flooding conditions are regulated at least in part by protein phosphorylation.

Proteomic studies of flooding-stressed soybean have shown that flooding induces a number of cellular signaling cascades [9]. In particular, nuclear proteomic analyses of soybean under flooding stress have indicated that levels of the receptor for activated protein kinase C1 are increased [17]; however, the abundance of poly-ADP-ribose polymerases is decreased [18]. Recently, when abscisic acid (ABA) was added during flooding treatment, survival ratio was improved compared with those soybeans flooded without ABA [19]. Furthermore, the nuclear proteins were analyzed; resulting that the flooding tolerance of soybean was enhanced through down-regulating zinc finger and cell division cycle 5 proteins in flooding condition [19]. Although these reports indicate that nuclear proteins serve important roles in the regulation and expression of genes involved in flooding stress responses in soybean, further nuclear proteomic analyses of regulatory proteins and transcription factors in roots under flooding conditions are needed to develop strategies for improving the flooding tolerance of soybean. Here, to examine the regulatory mechanisms controlling the expression and function of flooding-responsive proteins in the early stages of soybean growth, a nuclear phosphoproteomic approach was used. Specifically, nuclear proteins were extracted from the root tips of flooding-stressed soybeans and phosphopeptides were identified using a gel-free proteomic technique.

2. Materials and methods

2.1. Plant material and treatment

Seeds of soybean (*Glycine max* L. cultivar Enrei) were sterilized with 1% sodium hypochlorite solution, rinsed in water, and sown on 500 mL silica sand saturated with 150 mL water in a plastic case (180 × 140 × 45 mm). Soybeans were cultivated in a growth chamber illuminated with white fluorescent light (160 μmol m^{-2} s^{-1}, 16 h light period/day) at 25 °C and 70% relative humidity. For proteomic analysis, 2-day-old soybeans were treated without (control) or with 500 mL water for 3 h. For mRNA expression analysis, 2-day-old soybeans were flooded for 3 h with 500 mL water supplemented without or with 100 μM ABA. After treatment, root tips were collected as samples for protein and mRNA extraction. For morphological and ATP content analyses, 2-day-old soybeans were flooded