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Quantitative proteomic analysis of post-flooding recovery in soybean root exposed to aluminum oxide nanoparticles

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ABSTRACT

Aluminum oxide nanoparticles (Al_2O_3 NPs) are used in various commercial and agricultural products. Soybean exhibits severe reduction in growth under flooding condition. To examine the effects of Al_2O_3 NPs on the recovery of soybean from flooding, proteomic analysis was performed. Survival percentage and weight/length of root including hypocotyl were improved after 2 and 4 days of flooding with 50 ppm Al_2O_3 NPs leading to recovery as compared to flooding. A total of 211 common proteins were changed in abundance during the recovery period after treatment without or with Al_2O_3 NPs. These proteins were related to protein synthesis, stress, cell wall, and signaling. Among the identified stress-related proteins, *S*-adenosyl-L-methionine dependent methyltransferases were recovered from flooding with Al_2O_3 NPs. Hierarchical clustering divided the identified proteins into three clusters. Cluster II exhibited the greatest change in proteins related to protein synthesis, transport, and development during the recovery from flooding with Al_2O_3 NPs. However, activity of enolase remained unchanged during flooding leading to subsequent recovery with Al_2O_3 NPs. These results suggest that *S*-adenosyl-L-methionine dependent methyltransferases and enolase might be involved in mediating recovery responses by Al_2O_3 NPs.

Biological significance: This study highlighted the role of Al_2O_3 NPs in recovery of soybean seedlings from flooding stress using gel-free proteomic technique. The key findings of this study are as follows: (i) survival percentage was enhanced at 50 ppm Al_2O_3 NPs during the recovery stage; (ii) seedling weight and weight/length of root including hypocotyl improved at 50 ppm Al_2O_3 NPs during the period of recovery; (iii) protein synthesis and stress related proteins were increased on recovery after flooding without or with Al_2O_3 NPs; (iv) the abundance of *S*-adenosyl-L-methionine dependent methyltransferases recovered from flooding with Al_2O_3 NPs; (v) glycolysis related proteins amplified under flooding with Al_2O_3 NPs; (vi) enolase enzyme remained unchanged during flooding leading to subsequent recovery from flooding with Al_2O_3 NPs. Collectively, these results suggest that *S*-adenosyl-L-methionine dependent methyltransferases and enolase are involved in response to flooding with Al_2O_3 NPs and might be helpful in recovery from flooding stress.

1. Introduction

Nanoparticles (NPs) have at least one dimension of <100 nm, and have attracted considerable attention due to their unique physiochemical properties [1]. Metal oxide NPs are industrially manufactured for use in electronics, medicine, cosmetics, and engineering. However, the large-scale production and use of NPs have resulted in their deposition in terrestrial and aquatic environments [2]. This has raised concerns of possible undesirable interactions, including toxicity, in biological systems [3]. Although the transport of NPs in the environment is influenced by many factors, such as NPs size, charge, and agglomeration rate [4], research on the behavior of NPs towards environment and biological systems at present is very limited.

NPs, being unique in their characteristics, are typically more toxic than those of their bulk counterparts [5]. Aluminum oxide (Al_2O_3) NPs are most profusely used in military and commercial applications [6]. The effects of NPs on plants are influenced by particle size, concentration, and plant species. For example, Al_2O_3 NPs inhibited seedling growth at a particle size of 13 nm [7], while 100 nm did not inhibit plant growth [8]. Treatment with Al_2O_3 NPs reduced the root length of corn seedlings, but promoted root growth in radish and rapeseed [9]. In wheat, treatment with high concentrations of Al_2O_3 NPs significantly reduced root length, whereas low concentrations promoted root

Abbreviations: LC, liquid chromatography; MS, mass spectrometry; Al_2O_3, aluminum oxide; NPs, nanoparticles.

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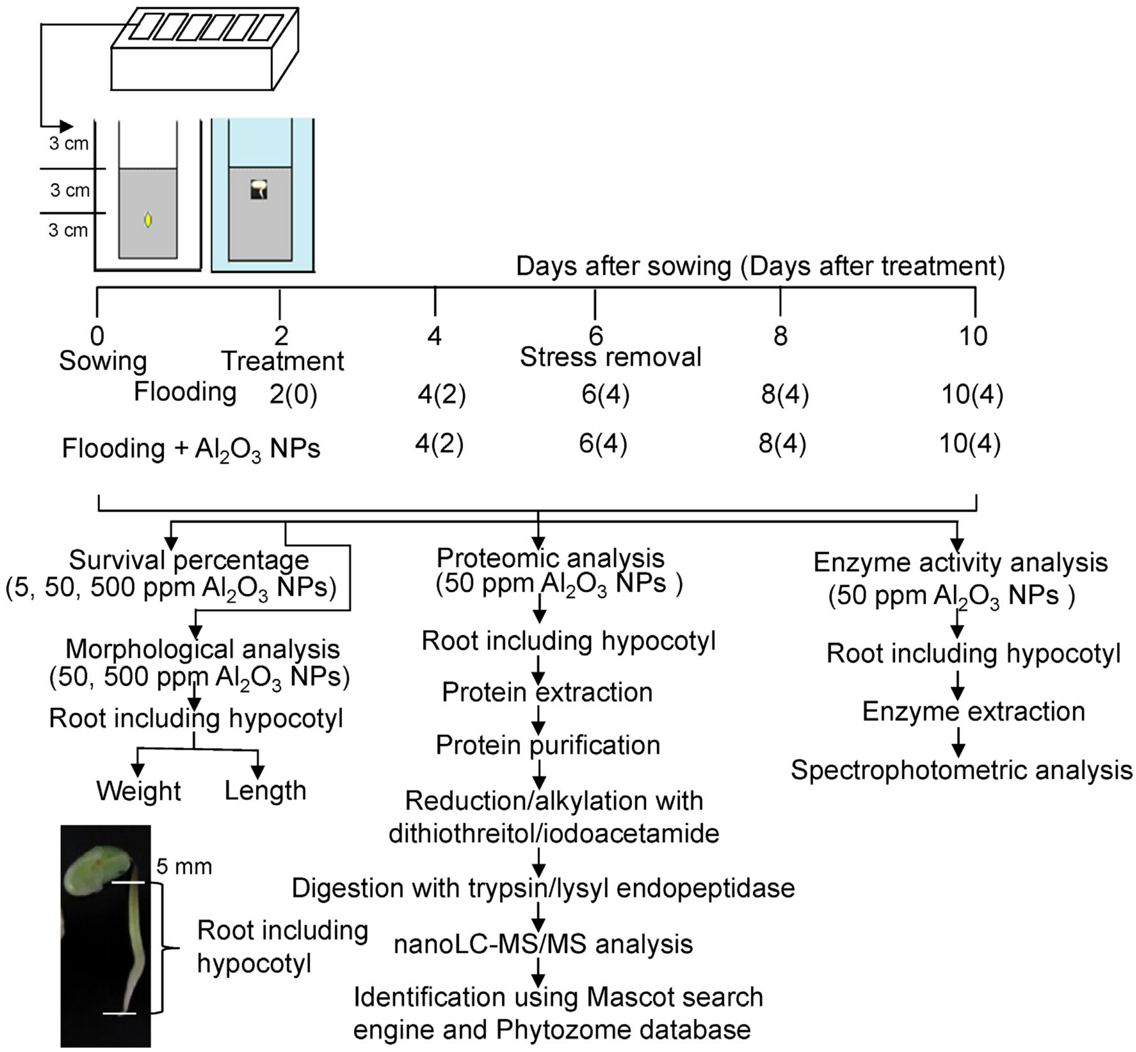

Fig. 1. Experimental outline for examining the effects of Al_2O_3 NPs on the recovery of soybean seedlings from flooding stress. Two-day-old soybeans were flooded without or with 5, 50, and 500 ppm Al_2O_3 NPs for 0, 2, 4 days and water was removed for 2, 4 days. Survival percentage, morphological, proteomic, and enzyme activity analyses were performed at indicated concentrations.

growth [10]. Based on these reports, additional studies intend at determining the suitable concentration and size of Al_2O_3 NPs for specific response to plants.

NPs have the potential to induce tolerance in plants against various stresses. For example, the exposure of rice seedlings to silver NPs resulted in the accumulation of a protein precursor involved in oxidative stress tolerance [11]. Silver NPs directly changed abundance of several proteins involved in primary metabolism and cell defense of germinating wheat seedlings [12]. Silver NPs have altered the proteins related to endoplasmic reticulum and vacuole indicating these organelles as target of silver NPs action in *Eruca sativa* [13]. In addition, silver NPs also enhanced soybean seedling growth under flooding conditions by mediating the detoxification of the cytotoxic by-products of glycolysis [14]. However, because NPs have variable modes of interaction with plants, the mechanism of action of NPs in stress response needs to be further explored.

Flooding, which is a major abiotic stressor for many plant species, resulted in reduced growth and death in maladapted species [15]. Plants typically respond to flooding by increasing plant height in an attempt to capture atmospheric oxygen for submerged tissues [16]. Duration of flooding affected the process of recovery as time taken for nitrogenase recovery increases with longer period of flooding [17]. However, the impact of submergence on vegetation can be minimized by establishing flooding depth and duration thresholds [18]. During recovery from flooding, soybean seedlings exhibited changes in cell wall metabolism and cytoskeletal organization [19] and increased peroxidase activity in roots [20]. The rate of recovery from flooding-induced damage was enhanced in soybean hypocotyl by increasing the rate of ATP generation and regulation of secondary metabolic pathways [21]. These responses to flooding conditions indicate that plants have various cellular and molecular mechanisms that are activated and modified to aid in the recovery from stressful environments.

The treatment of soybean in the germinating stage with Al_2O_3 NPs under flooding conditions promoted plant growth by altering proteins involved in energy metabolism and cell death [22]. Al_2O_3 NPs treatment optimized the soybean growth during the seedling stage by regulating proteins involved in oxidation reduction, stress, signaling, and hormonal pathways [23]. Although the effects of Al_2O_3 NPs on flooding-stressed soybean have been evaluated during the germinating and seedling stages; however, the plant proteins and pathways that are altered by Al_2O_3 NPs during the recovery stage have not been analyzed. To elucidate the role of Al_2O_3 NPs in the recovery of soybean from flooding stress, morphological and proteomic analyses were performed. In

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