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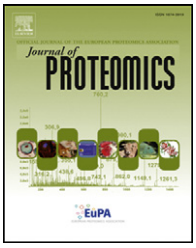

Proteomic study on the effects of silver nanoparticles on soybean under flooding stress

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ABSTRACT

Flooding negatively affects the soybean growth; however, silver nanoparticles (AgNPs) enhanced the growth under stress. To study the effects of AgNPs on soybean under flooding, a gel-free proteomic technique was used. The morphological analysis of early-stage soybean exposed to flooding with AgNPs of various sizes and concentrations revealed enhanced seedling growth by treatment with 15 nm AgNPs at 2 ppm. Differentially changed 107 root proteins were predominantly associated with stress, signaling, and cell metabolism. Hierarchical clustering divided these proteins into 3 clusters. Based on cluster analysis, the abundances of glyoxalase II 3 and fermentation related proteins were time-dependently increased under flooding stress, but decreased in response to AgNPs. Six enzymes involved in metabolic pathways were analyzed at the transcriptional level. The *alcohol dehydrogenase 1* and *pyruvate decarboxylase 2* genes were up-regulated under flooding stress while down-regulated in response to AgNPs. Moreover, comparatively low transcript level of *glyoxalase II 3* under AgNPs treatment implies that less cytotoxic by-products of glycolysis are produced in AgNPs exposed soybeans as compared to flooded soybean. These results suggest that the AgNPs treated soybeans might have experienced less oxygen-deprivation stress, which might be the key factor for better growth performance of AgNPs treated soybeans under flooding stress.

Biological significance
This study highlighted the effect of silver nanoparticles (AgNPs) on the soybean under flooding stress. Silver nanoparticles (2 ppm AgNPs, 15 nm in size) treatment facilitate the soybean under flooding stress enhancing seedling growth. A time-course comparative gel-free proteomic study was performed to analyze the changes in proteome profiles in response to AgNPs treatment under flooding. The 107 differentially changed root proteins were predominantly associated with stress, signaling, cell metabolism. The abundances of the glyoxalase II 3 and fermentation related proteins were significantly increased on exposure to flooding; however, decreased by

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

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AgNPs treatment. Comparatively low transcript level of glyoxalase II 3 under AgNPs treatment implies that less cytotoxic by-products of glycolysis are produced in AgNPs exposed soybeans as compared to flooded soybean. Moreover, the observed up-regulation of the alcohol dehydrogenase 1 and pyruvate decarboxylase 2 genes under flooding stress condition and its down-regulation in response to AgNPs treatment might be related to a metabolic shift towards normal cellular processes.

1. Introduction

Nanoparticles (NPs) are typically ultrafine particles with size range from 1 to 100 nm and have predictable physical, chemical, and biological properties [1]. The widespread use of silver and its compounds from ancient times could be attributed to their potent antimicrobial property against a wide range of pathogenic microorganisms [2]. There is also a growing demand of synthetic silver nanoparticles (AgNPs) as an antiseptic in health care and water treatment facilities [3], and as a major ingredient of several commercial agricultural products [4]. This indiscriminate release of AgNPs with varied physical and surface properties into the environment poses a serious threat to the ecological system including plant. Several studies have been conducted to evaluate the toxicity of AgNPs on animal [5], algae [6], bacteria [7], fish [8], and human [9]. However, research focusing on the impact of AgNPs on higher plant is limited.

The AgNPs negatively affect the growth of *Cucurbita pepo* [10] and root tip extension of *Allium cepa* [11]. In *Spirodela polyrrhiza*, exposure to AgNPs inhibits seed germination, root, and shoot growth [12]. Although AgNPs are often detrimental to plant growth, several studies have demonstrated the growth-stimulatory effects of AgNPs. For example, exposure to AgNPs enhances the root extension of *Brassica juncea* [13] and stimulates the plant growth of *Eruca sativa* [14]. Silver nanoparticles are also reported to enhance the growth of various wetland plant [15] and to increase the shoot and root lengths of *Phaseolus vulgaris* and maize [16]. The AgNPs alter the physiochemical properties and metabolic pathways of plant, although the underlying mechanisms and specific pathways involved remain unclear.

The AgNPs are reported to promote the growth of flooding-stressed *Crocus sativus* roots by blocking ethylene signaling [17]. In *Arabidopsis*, AgNPs induce the accumulation of reactive oxygen species and promote root growth, and also function as ethylene perception inhibitors [18]. Gene expression profiling of *Arabidopsis* treated with AgNPs has indicated that genes associated with responses to metal, oxidative stress, and the thalianol biosynthetic pathway are up-regulated, whereas genes associated with the ethylene signaling pathway are down-regulated [19]. In *P. vulgaris* and maize, increasing concentrations of AgNPs lead to corresponding increase in chlorophyll, carbohydrate, and protein levels [16]. Proteomic analyses have revealed that exposure to AgNPs results in differential changes in the levels of proteins related to oxidative stress tolerance, calcium regulation, and signaling in *Bacillus thuringiensis* [20] and proteins related to the endoplasmic reticulum and vacuoles in *E. sativa* [14]. However, the effect of AgNPs on the proteomic profiles of soybean under flooding stress has yet to be determined.

Flooding has devastating effects on crop growth and production, including soybean [21]. Soybean is particularly susceptible to flooding at the germination [22], early vegetative, and early reproductive growth stages [23]. Under flooding conditions, impaired root elongation and reduced hypocotyl pigmentation are observed in soybean seedlings [24], and ethylene synthesis-related genes are markedly up-regulated [25]. In contrast, AgNPs has been reported to inhibit ethylene biosynthesis in *Arabidopsis* [18]. Yin et al. [26] examined the effect of ethylene in soybean roots under flooding conditions. As flooding-responsive mechanisms are regulated by phytohormones, Oh et al. [27] examined gibberellic acid supplementation of soybean under flooding conditions and found that the abundance of proteins involved in secondary metabolism, cell cycle, and protein degradation/synthesis was predominantly affected. Komatsu et al. [25] reported that the genes associated with alcohol fermentation and ethylene biosynthesis are up-regulated in soybean seedlings under flooding stress. Although ethylene biosynthesis is inhibited by AgNPs treatment in many plant species, the effects of ethylene in soybean exposed to flooding stress remain poorly understood.

Because of the increasing use of AgNPs in agricultural products, the biological effects of these particles in plants, particularly soybean, which is cultivated worldwide and is highly susceptible to flooding, warrant intensive investigation. Although Rezvani et al. [17] reported that AgNPs treatment had positive effects by preventing the ethylene action in *C. sativus* under flooding stress, only a few proteomic studies have examined the effects of AgNPs on animal, bacterial, and plant cells. This study will aid understanding of the cellular processes that are altered by the application of AgNPs in soybean under flooding stress. In the present study, AgNPs-induced changes in the proteome profiles of roots and cotyledons of soybean exposed to flooding stress were evaluated using a gel-free proteomic technique to elucidate the underlying mechanism of AgNPs-mediated growth promotion of soybean under flooding stress.

2. Materials and methods

2.1. Plant material and treatments

Soybean (*Glycine max* L.) cultivar Enrei was used as the plant material in this study. Seeds were first surface sterilized in sodium hypochlorite solution and allowed to germinate on silica sand. Seedlings were maintained at 25 °C in a growth chamber illuminated with white fluorescent light (600 μmol m^{-2} s^{-1}, 16-h light period/day) and held at 70% relative humidity. To expose plants to flooding stress,