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Comparative proteomic and physiological analyses reveal the protective effect of exogenous calcium on the germinating soybean response to salt stress

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ABSTRACT

Calcium enhances salt stress tolerance of soybeans. Nevertheless, the molecular mechanism of calcium's involvement in resistance to salt stress is unclear. A comparative proteomic approach was used to investigate protein profiles in germinating soybeans under NaCl-$CaCl_2$ and NaCl-$LaCl_3$ treatments. A total of 80 proteins affected by calcium in 4-day-old germinating soybean cotyledons and 71 in embryos were confidently identified. The clustering analysis showed proteins were subdivided into 5 and 6 clusters in cotyledon and embryo, respectively. Among them, proteins involved in signal transduction and energy pathways, in transportation, and in protein biosynthesis were largely enriched while those involved in proteolysis were decreased. Abundance of nucleoside diphosphate kinase and three antioxidant enzymes were visibly increased by calcium. Accumulation of gamma-aminobutyric acid and polyamines was also detected after application of exogenous calcium. This was consistent with proteomic results, which showed that proteins involved in the glutamate and methionine metabolism were mediated by calcium. Calcium could increase the salt stress tolerance of germinating soybeans via enriching signal transduction, energy pathway and transportation, promoting protein biosynthesis, inhibiting proteolysis, redistributing storage proteins, regulating protein processing in endoplasmic reticulum, enriching antioxidant enzymes and activating their activities, accumulating secondary metabolites and osmolytes, and other adaptive responses.

Biological significance

Soybean (*Glycine max* L.), as a traditional edible legume, is being targeted for designing functional foods. During soybean germination under stressful conditions especially salt stress, newly discovered functional components such as gamma-aminobutyric acid (GABA) are rapidly accumulated. However, soybean plants are relatively salt-sensitive and the growth, development and biomass of germinating soybeans are significantly suppressed under salt stress condition. According to previous studies, exogenous calcium counters the harmful effect of salt stress and increases the biomass and GABA content of germinating soybeans. Nevertheless, the precise molecular mechanism underlying the role of calcium in resistance to salt stress is still unknown. This paper is the first study employing comparative proteomic and physiological analyses to reveal the protective effect of

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exogenous calcium in the germinating soybean response to salt stress. Our study links the biological events with proteomic information and provides detailed peptide information on all identified proteins. The functions of those significantly changed proteins are also analyzed. The physiological and comparative proteomic analyses revealed the putative molecular mechanism of exogenous calcium treatment induced salt stress responses. The findings from this paper are beneficial to high GABA-rich germinating soybean biomass. Additionally, these findings also might be applicable to the genetic engineering of soybean plants to improve stress tolerance.

1. Introduction

Soybean (*Glycine max* L.) is a traditional edible legume. It is very popular in China, Korea, Japan, and other Southeast Asian countries. Large amounts of protein, minerals, vitamins, and functional compounds such as isoflavone, saponins and phosphatidylcholine exist in its seeds [1]. However, its nutritional value is limited by anti-nutritional factors like phytic acid, lectins, and tannins [2]. Studies have demonstrated that seed germination can improve nutritional composition and lower the levels of antinutritional components in legumes [3–5]. Newly discovered functional components such as gamma-aminobutyric acid (GABA) are generated after germination. GABA, a neurotransmitter in the mammalian brain and spinal cord, can prevent certain forms of cancer and reduce the risk of cardiovascular disease [6]. For this reason, GABA-enriched germinating soybeans have become popular as a source of therapeutic and salubrious functional ingredient. Germination under abiotic stress such as hypoxia and salt stress are the most common and effective ways of causing GABA to accumulate in soybeans [7–9]. However, the growth, development and biomass of germinating soybeans were found here to be significantly suppressed under stressful germination conditions, although the GABA content increased markedly.

Calcium has been widely examined for its protective role in most abiotic stresses: drought, cold, heat, heavy metals, oxidative stresses, and especially salt stress [10–13]. Although the in vivo role of calcium in plants subjected to abiotic stress or transgenic overexpression has been investigated in recent studies, the traditional and classical approach is the use of exogenous calcium [14–16]. Application of exogenous calcium can improve the inhibition of growth and development and maintain the integrity of cell function and structure of plants under abiotic stress [17,18]. Similarly, according to our previous studies, exogenous calcium countered the harmful effect of salt stress and increased the biomass and GABA content in germinating soybean plants [9]. All of the studies cited above indicate that calcium might be an ideal biotechnological target for improvement of GABA-enriched germinating soybeans grown under stressful conditions [9]. Nevertheless, the precise molecular mechanism underlying the role of calcium in resistance to salt stress is still unclear.

Proteomics is the best molecular tool available for describing the proteome profile and dissecting the complex molecular mechanism underlying plant physiology [19]. Shi et al. identified 36 differentially regulated proteins in Bermuda grass and proposed that polyamines could activate electron transport and energy pathways that facilitated the adaptation of Bermuda grass to salt and drought [20]. Hossain et al. suggested that β-aminobutyric acid pretreatment helped the soybean to combat cadmium stress by modulating the defense mechanism in plants and activating their cellular detoxification system [21]. Another previous study demonstrated that exogenous calcium increased the abundance of proteins involved in fermentative metabolism, the TCA cycle, glycolysis, defense against reactive oxygen species, and in nitrogen metabolism in cucumber roots under hypoxic conditions [22]. It was also found that the impact of salinization on metabolism, ripening process, and on inducing salt tolerance was limited by calcium in tomato fruit [23]. However, only a limited number of studies have investigated the effects of exogenous calcium on the proteome of soybean germinating in the dark and under salt stress.

The present study was to derive new insight into the molecular mechanism of calcium mediated salt stress response using exogenous calcium and the inhibitor of Ca^{2+} channels on soybean plants germinating in the dark. This might shed light on the role of calcium in physiological response of plants to salt stress in vivo. Comparative proteomic analysis was performed via two-dimensional electrophoresis (2-DE), and MALDI-TOF/TOF-MS was performed to identify differentially displayed proteins affected by calcium. The effect of exogenous calcium and the inhibitor of Ca^{2+} channels on protein level changes in the cotyledons and embryos of germinating soybeans were also compared. The results of physiological assays and comparative proteomic analyses might also provide information regarding the physiological and molecular mechanisms of calcium in the response of germinating soybean plants to salt stress.

2. Materials and methods

2.1. Plant materials and growth conditions

Dry soybean seeds (*Glycine max* L. cultivar *Yunhe*) were sterilized with 10 mM sodium hypochlorite for 30 min, washed, and steeped with distilled water at 30 ± 1 °C for 4 h. The soaked seeds were then placed in a soybean sprouting machine and germinated in a dark incubator at 30 ± 1 °C for 4 d with culture solution containing different additives: (a) NaCl: 50 mM NaCl; (b) NaCl-$CaCl_2$: 50 mM NaCl + 6 mM $CaCl_2$; (c) NaCl-$LaCl_3$: 50 mM NaCl + 5 mM $LaCl_3$. Based on a previous study and on pilot experiments, 50 mM NaCl, 6 mM $CaCl_2$, and 5 mM $LaCl_3$ were selected as effective concentrations [9]. During the 4 days of experiment, the culture solution was replaced daily. Three independent biological experiments were performed, and 4 d after treatment, the cotyledons and embryos were carefully collected for identification of differentially displayed proteins using the proteomic approach.