June 2016 Vol. 23 No. 2 28-44
Journal of Northeast Agricultural University (English Edition)

Constitutive Overexpression of *Myo*-inositol-1-Phosphate Synthase Gene (*GsMIPS*2) from *Glycine soja* Confers Enhanced Salt Tolerance at Various Growth Stages in *Arabidopsis*

Zaib-un-Nisa[1], Chen Chen[1], Yang Yu[1], Chao Chen[1], ALi Inayat Mallano[2], Duan Xiang-bo[1], Sun Xiao-li[3], and Zhu Yan-ming[1*]

[1] Key Laboratory of Agricultural Biological Functional Genes, Northeast Agricultural University, Harbin 150030, China

[2] Key Laboratory of Soybean Biology in Chinese Ministry of Education, Northeast Agricultural University, Harbin 150030, China

[3] Agronomy College, Heilongjiang Bayi Agricultural University, Daqing 163319, Heilongjiang, China

Abstract: The enzyme *myo*-inositol-1-phosphate synthase (MIPS EC 5.5.1.4) catalyzes the first step of *myo*-inositol biosynthesis, a product that plays crucial roles in plants as an osmoprotectant, transduction molecule, cell wall constituent and production of stress related molecule. Previous reports highlighted an important role of MIPS family genes in abiotic stresses particularly under salt stress tolerance in several plant species; however, little is known about the cellular and physiological functions of *MIPS*2 genes under abiotic conditions. In this study, a novel salt stress responsive gene designated *GsMIPS*2 from wild soybean *Glycine soja* 07256 was functionally characterized contained an open reading frame (ORF) of 1 533 bp coding a peptide sequence of 510 amino acids along with mass of 56 445 ku. Multiple sequence alignment analysis revealed its 92%-99% similarity with other MIPS family members in legume proteins. Quantitative real-time PCR results demonstrated that *GsMIPS*2 was induced by salt stress and expressed in roots of soybean. The positive function of *GsMIPS*2 under salt response at different growth stages of transgenic *Arabidopsis* was also elucidated. The results showed that *GsMIPS*2 transgenic lines displayed increased tolerance as compared to WT and *atmips*2 mutant lines under salt stress. Furthermore, the expression levels of some salt stress responsive marker genes, including *KIN*1, *RD*29*A*, *RD*29*B*, *P5Cs* and *COR*47 were significantly up-regulated in *GsMIPS*2 overexpression lines than wild type and *atmips*2 mutant. Collectively, these results suggested that *GsMIPS*2 gene was a positive regulator of plant tolerance to salt stress. This was the first report to demonstrate that overexpression of *GsMIPS*2 gene from wild soybean improved salt tolerance in transgenic *Arabidopsis*.

Key words: *Glycine soja*, *Arabidopsis thaliana*, MIPS, salt stress, functional analysis

CLC number: Q943.2 **Document code:** A **Article ID:** 1006-8104(2016)-02-0028-17

Introduction

Salinity is considered among major and most influential abiotic stresses that reduce growth and productivity of crops in vast proportion all around the world. Plant tolerance to saline conditions leads them to another level of complex physiological changes, activation of metabolic machinery and changes in expression level of gene networks (Gupta and Hunag, 2014; Parvaiz and Satyawati, 2008; Kumar *et al*., 2013). The phenomenon of salt tolerance relies upon three basic adaptations, Na^+ or Cl^- omission from shoot, osmotic stress tolerance and Na^+ tissue tolerance. Despite of

Received 12 October 2015
Supported by "863" Project (2008AA10Z153); the National Natural Science Foundation of China (31171578); Heilongjiang Provincial Higher School Science and Technology Innovation Team Building Program (2011TD005); the National Basic Scientific Talent Training Fund Projects (J1210069)
Zaib-un-Nisa (1987-), female, Ph. D student, engaged in the research of plant functional genomics. E-mail: Nisabiolab@gmail.com
* Corresponding author. E-mail: ymzhu2001@neau.edu.cn

several transporters and key ion channels discoveries, still there is a limited understanding of molecular pathways and influence of these proteins on Na^+ transport (Jha *et al*., 2010; Munns and Tester, 2008). In recent years, establishment of crop cultivars with useful traits is an impressive way to increase the crop yield and to develop sustainable agriculture worldwide (Li *et al*., 2014; Almeida *et al*., 2014).

Soybean is one of the most widely grown legumes in the world, due to its rich protein and oil contents. However, high soil salinity causes negative impacts on agronomical traits and ultimately leads to reduction in yield of soybean. Therefore, regulation and activation of specific stress related genes which participate in the whole series of stress responses, such as transcriptional control, signaling, proteins and membrane protection and scavenging of toxic compounds is in swing now days (Wang *et al*., 2003).

Many studies have revealed that phytic acid is a primary storage form of phosphorus that produces 60% to 80% of seed phosphorus in soybean followed by linear increase throughout its seed development. D-*myo*-inositol-3-phosphatase (MIPS3; EC 5.5.1.4) is an evolutionarily conserved protein that involves in conversion of D-glucose-6-phosphate in to 1L-*myo*-inositol-1-phosphate (MIP) that serves as first and crucial step in biosynthesis of phytic acid and other inositol producing complexes (Kumari *et al*., 2012; Majee *et al*., 2004; Wei *et al*., 2010; Hegemen *et al*., 2001; Majumder *et al*., 2003). This crucial enzyme involves in development and growth of all the living organisms with striking key features of cell wall biosynthesis, signaling pathways and membrane transferring, phytic acid biosynthesis, auxin transportation and storage along with construction of stress related molecules (Abreu *et al*., 2006; Chhetri *et al*., 2006). MIPS enzymes have been found in many living organisms, such as green algae, fungi, bacteria, parasites, higher plants and animals. Till now, more than 60 *MIPS* genes have been discovered (Majumdar *et al*., 2003). In plants, MIPS enzymes present in two forms chloroplastic and cytosolic (Ishitani *et al*., 1996). Whereas, activity of chloroplastic form increased in plants under light and salinity stress conditions (Majumdar *et al*., 1997). L-*myo*-inositol 1-phosphate synthase (INPS) and *myo*-inositol o-methyl transferase (IMT) are two derivatives of *myo*-inositol correlated with each other in terms of transcript levels, transcript rates and protein abundance. Under saline stress conditions INPS levels increase in leaves, while decrease in roots, in contrast IMT shows regulation in all the cell types, therefore, this tissue specific regulation of *myo*-inositol translocation leads towards sodium uptake and *myo*-inositol synthesis gradient and supports root growth, sodium sequestration and protection of photosynthesis (Nelson *et al*., 1998). Differential expression profiles of four MIPS family members named as MIPS1, MIPS2, MIPS3 and MIPS4 in vegetative tissues and developing seeds of soybean shows an up regulation with MIPS1 appeared with high transcript levels in early stages of development, whereas, MIPS3 and MIPS4 showed poor expression levels in developing tissues as compared to MIPS2 that displayed relatively high expression (Kumar *et al*., 2012). MIPS proteins are found to be cytoplasmic origin (Donahue *et al*., 2010). Recent studies has proved role of MIPS family genes in many physiological and biochemical processes, where *MIPS*1 is found most critical enzyme during early developmental stages of *Arabidopis thaliana*, soybean and cotton (Chen and Xiong, 2010; Tsui *et al*., 2009; Abid *et al*., 2009). These findings are further polished by genetic characterization of MIPS mutant lines (*mips*) to clarify its functions at cellular levels (Luo *et al*., 2011; Chen *et al*., 2010). However, the principal roles of *myo*-inositol and its other downstream derivatives (raffinose, galactinol) under abiotic stress conditions are still needed to be unveiled (Sato *et al*., 2011; Valluru *et al*., 2008). As hypothesized by Nelson *et al*. (1999) that *myo*-inositol served as a substrate for supply of compatible solutes along with leaf to root signal which boost sodium uptake in ice plants under salt stress conditions.

In this study, we analyzed the functional roles of a