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Exploring the role of Inositol 1, 3, 4- trisphosphate 5/6 kinase-2 (*GmITPK2*) as a dehydration and salinity stress regulator in *Glycine max* (L.) Merr. through heterologous expression in *E.coli*

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Abstract

Phytic acid (PA) is implicative in a spectrum of biochemical and physiological processes involved in plant stress response. Inositol 1,3,4, Tris phosphate 5/6 kinase (*ITPK*), a polyphosphate kinase that converts Inositol 1,3,4 trisphosphate to Inositol 1,3,4,5/6 tetra phosphate, averting the inositol phosphate pool towards PA biosynthesis, is a key regulator that exists in four different isoforms in soybean. In the present study, *in-silico* analysis of the promoter region of *ITPKs* was done and among the four isoforms, promoter region of *GmITPK2* showed the presence of two MYB binding elements for drought inducibility and one for ABA response. Expression profiling through qRT-PCR under drought and salinity stress showed higher expression of *GmITPK2* isoform compared to the other members of the family. The study revealed *GmITPK2* as an early dehydration responsive gene which is also induced by dehydration and exogenous treatment with ABA. To evaluate the osmo-protective role of *GmITPK2*, attempts were made to assess the bacterial growth on Luria Broth media containing 200 mM NaCl, 16% PEG and 100 μM ABA, individually. The transformed *E.coli* BL21 (DE3) cells harbouring the *GmITPK2* gene depicted better growth on the media compared to the bacterial cells containing the vector alone. Similarly, the growth of the transformed cells in the liquid media containing 200 mM NaCl, 16% PEG and 100 μM ABA showed higher absorbance at 600 nm compared to control, at different time intervals. The *GmITPK2* recombinant *E.coli* cells showing tolerance to drought and salinity thus demonstrated the functional redundancy of the gene across taxa. The purity and specificity of the recombinant protein was assessed and confirmed through PAGE showing a band of ~35 kDa on western blotting using Anti- Penta His- HRP conjugate antibody. To the best of our knowledge, the present study is the first report exemplifying the role of *GmITPK2* isoform in drought and salinity tolerance in soybean.

Key words: Abiotic stress, *Glycine max*, Phytic acid, ITPK2, Liquid Assay, Real time, Spot assay

1. Introduction

Plants being sessile in nature are often challenged with a variety of abiotic stresses like drought, salinity and adverse temperatures throughout their lifespan. The molecular mechanisms underlying the phenomenon of stress tolerance are well understood through genome sequencing and microarray analysis. Genes and their related signaling pathways controlling the physiological and biochemical responses to abiotic stress have been identified and characterized in plant species (Xiong et al., 2002; Zhu, 2002). Among the plethora of signaling molecules involved in combating abiotic stress, inositol phosphates and phytic acid (PA) too are critical players. Phytic acid (myo inositol 1,2,3,4,5,6 hexa kis phosphate) is a cyclic compound derived from D-glucose with phosphorus moieties bound to the carbon atoms in the ring. Around 75-85% of total seed phosphorus is found in the phytate form (Raboy, 2009) and therefore PA mainly acts as a phosphate reservoir in plants. The phosphate groups on PA impart a negative charge to the compound and form stable salts called phytins, on binding with the divalent metal ions like Zn^{+2}, Fe^{+2}, Ca^{+2} and Mg^{+2} in the cytosol. Ultimately the