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Expression of *Arabidopsis thaliana SIK* (*Stress Inducible Kinase*) Gene in a Potato Cultivar (*Solanum tuberosum* L. 'Taedong Valley')

Jung-Ha Yoon, Yi Lan Fang, Eung-Jun Park, Hyejin Kim, Yun Jeong Na[1], Dong Hee Lee1, Deok-Chun Yang[2], Hak-Tae Lim[2]*

Deptartment of Plant Biotechnology, Kangwon National University, Chunchen, 200-701, Korea
[1]Genomine Co., Ltd. Venture dong 306, Pohang Techno Park, Pohang 790-834, Korea
[2]Department of Oriental medicinal materials and processing, KyungHee University, Youngin, 449-701, Korea

ABSTRACT

Osmotic stress is one of major limiting factors in crop production. In particular, seasonal drought often causes the secondary disease in the field, resulting in severe reduction in both quality and productivity. Recent efforts have revealed that many genes encoding protein kinases play important roles in osmotic stress signal transduction pathways. Previously, the *AtSIK (Arabidopsis thaliana Stress Inducible Kinase)* mutants have shown to enhance tolerance to abiotic stresses, accompanying with higher expression of abiotic stress-related genes than did the wild-type plants. In this study, we have transformed potato (cv. Taedong Valley) with the *AtSIK* expression cassette. Both PCR and RT-PCR using *AtSIK*-specific primers showed stable integration and expression of the *AtSIK* gene in individual transgenic lines, respectively. Foliar application of herbicide (Basta®) at commercial application rate (0.3% (v/v)) revealed another evidence of stable gene introduction of T-DNA which includes the bar gene for herbicide resistance. Overexpression of the *AtSIK* gene under dual CaMV35S promoter increased sensitivity to salt stress (300 mM NaCl), which was demonstrated by the reduction rate of chlorophyll contents in leaves of transgenic potato lines. These results suggest that possible increase of osmotic tolerance in potato plants may be achieved by antisense expression of *AtSIK* gene.

Key words : antisense expression chlorophyll, herbicide, protein kinase, salt stress,

INTRODUCTION

Various environmental stresses, including cold, drought and soils with changing salt and nutrient concentrations (i.e. abiotic stress), are the major limiting factors in the geographical distribution of plants, often adversely affecting crop development, growth, and productivity. These stresses together represent the primary cause of crop loss around the world, reducing average yields for most major crop plants by more than 50% (Bray *et al.*, 2000). In contrast, the estimated yield loss caused by pathogens is typically around 10% to 20%.

*Corresponding author : **Hak-Tae Lim,** E-mail : potatokorea@empal.com

Although salt, dehydration and cold stresses are clearly different from each other in their physical nature and each elicits specific plant responses, they also activate some common reactions in plants. The most widely studied common response is the induction of some plant genes by all three stresses (Shinozaki and Yamaguchi-Shinozaki, 1997). Because of this and other commonalities, these stresses are often considered together in molecular studies. It has often been commented that drought and cold stresses are also cause osmotic stress, and this is why salt, drought and cold stresses induce some common sets of plant genes. At the molecular level, the perception of extracellular stimuli and the subsequent activation of defense responses require a complex interplay of signaling cascades, in which reversible protein phosphorylation plays a central role (Yang *et al.*, 1997). Concerning the kinase-mediated signal transduction, the SOS (salt overly sensitive) pathway is probably the most documented and represents a major calcium-mediated pathway for the regulation of plant salt tolerance and ion homeostasis (Chinnusamy *et al.*, 2004). As a SOS-independent osmotic stress signaling pathway in plants, several MAPKs (Mitogen-activated protein kinase) have shown to be activated by hyperosmotic stress. In tobacco cells, a MAPK named SIPK (salicylic-acid-induced protein kinase) is activated by hypoosmotic stress, salicylic acid, or fungal elicitors (Droillard *et al.*, 2000). In addition, an *Arabidopsis* protein that cross-reacts with antibodies against the tobacco MAPK was also activated by hyperosmotic stress (Hoyos and Zhang, 2000). On the contrary, other kinase-mediated pathways are still poorly understood, although some components have been identified at the molecular level.

A protein kinase gene, *AtSIK* (*Arabidopsis thaliana* Stress-Inducible protein Kinase, Korean Pat. Appln. No. 10-2001-0005097), is shown to be induced by various osmotic stress conditions such as NaCl, cold, dehydration, and exogenously applied ABA. *Arabidopsis* plants with a T-DNA insertion mutation in the *AtSIK* gene showed increased tolerance to several osmotic stresses, whereas normal expression of *AtSIK* showed negative regulation of stress-inducible genes. In the *AtSIK* mutant plants, a subset of the cold and NaCl stress-inducible genes was expressed at higher levels under cold and NaCl stress conditions, respectively, probably resulting in enhancing tolerance to those stresses. Furthermore, expression of *Cor15a* and *Cor47*, the cold stress-inducible genes, was induced at an earlier time point and at a higher level in the mutant upon cold stress. In addition, under high NaCl concentration the induction levels of *Cor47* were also much higher in the mutant than in the wild type.

Here, we report the expression of *AtSIK* in a potato cultivar (*Solanum tuberosum* L. 'Taedong Valley'). In this study, we transformed the *AtSIK* gene in potato, in order to know whether heterologous expression of *AtSIK* gene in potato also increases sensitivity to high salt stress as shown in the *AtSIK* mutant Arabidopsis plants.

MATERIALS AND METHODS

Plant materials

As a plant material for genetic transformation, we used a potato cultivar 'Taedong' (Patent No. 10-2002-000010), which was developed in 2002 by KPGR (Center for the Korea Potato Genetic Resources) and Potato Valley Co., Ltd., Korea.

Binary vector and *Agrobacterium* strain

The *AtSIK* expression cassette contained the *AtSIK* gene under the control of dual 35S promoter of cauliflower mosaic virus (CaMV35S), and a *NOS* terminator. The binary vector pNB96 (Genomine Co., Pohang, Korea), which include both *NPT II* and bar gene as plant selectable markers (Fig. 1a), was introduced into *Agrobacterium tumefaciens* EHA105.

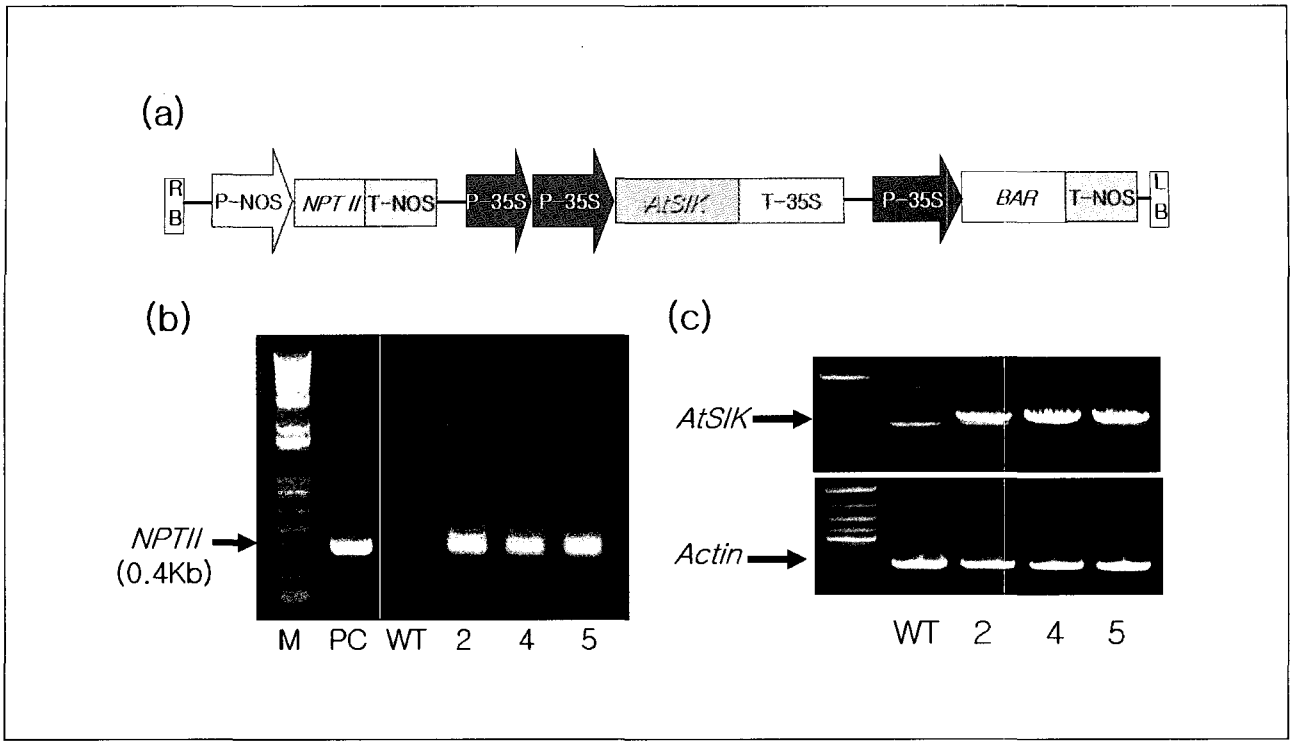

Fig. 1. Molecular characterization of the *AtSIK* transgenic potato plants. (a) T-DNA region of binary vector pNB96/*AtSIK*: P-NOS or T-NOS, *nos* promoter or terminator sequence; P-35S, CaMV 35S promoter; *AtSIK*, gene for stress inducible kinase from *Arabidopsis thaliana; BAR*, herbicide resistant gene for *phosphinothricin acetyltransferase*. (b) PCR analysis using *NPT II* gene specific primer: M, 1kb DNA ladder (Bioneer, Seoul, Korea); PC, positive control using pNB96/*AtSIK*; WT, wild-type; 2, 4, and 5, independent *AtSIK* potato transgenic lines. (c) RT-PCR analysis using *AtSIK* and *Actin* gene specific primer, respectively.

Molecular characterization of *AtSIK* transgenic potato plants

Potato (cv. 'Taedong Valle') was transformed with A. *tumefaciens* EHA105 (pNB96/*AtSIK*). Antibiotic-resistant (50 mg/l Kanamycin) shoots were selected and rooted *in vitro*, then transferred to soil and grown in a greenhouse. Integration of the *AtSIK* gene into the potato genome was confirmed by PCR using *AtSIK* gene specific primers (AtSIK-F: 5'-ATGGTTTTG-GGGTGTTTC-3' and AtSIK-R: 5'-TCAAAACC CTTCGAGGCT-3') (Fig. 1b). Expression of the *AtSIK* gene in those plants was verified by RT-PCR analysis, using *AtSIK* and actin specific primers (StACT-F: 5'-GTATTGTGCTGGATTCTGGTG-3' and StACT-R: 5'-CTGTTGGAAGGTGCTGAGA-G-3') (Fig. 1c).

Examination of herbicide resistance in transgenic plants

Herbicide Basta® at commercial application rate (0.3 %) was foliar-sprayed to both nontransgenic and three transgenic lines, which had grown in soil for over a month after acclimation. After the application of Bastar ®, the leaf area of treated-plants was visually monitored for subsequent four days.

Salt treatment and measurement of chlorophyll content

Both non-and transgenic plants grown in a tray with soil were submerged in a solution containing 300 mM NaCl for three hours and then placed under natural light (more than 1000 μmol/m^2/s) without watering. Salt

treatment had conducted every other day for seven days. Alterations of chlorophyll content after salt treatment was measured in 80 % (v/v) acetone according to the manufacturer's instructions (Chloroplast isolation kit, Sigma, St. Louis, USA).

RESULTS AND DISCUSSION

Expression of *AtSIK* gene in transgenic potato

We used an Agrobacterium-mediated transformation method to transform potato (cv. 'Taedong Valley') with the *AtSIK*-expression cassette (Fig. 1a). PCR analysis demonstrated that independently generated transgenic plants possessed a stable integrated *AtSIK* gene (Fig. 1b). Although we produced a number of *AtSIK* transgenic potato plants, only three independent transgenic lines were shown in this report. RT-PCR analysis revealed that all three transgenic plants expressed *AtSIK* gene, while little expression was detected in the nontransgenic (WT) plants (Fig. 1c).

AtSIK transgenic plants showed herbicide resistance

Both WT and three transgenic lines were sprayed with 0.3 % Basta which is known to be a commercial application rate. As shown in Fig. 2, WT control plants were severely necrotic within four days and completely died within seven to ten days, while no transgenic plants showed any damage on their leaves and consequently all transgenic plants were survived under this concentration of herbicide. This result clearly indicated that the *bar* gene to impart the herbicide Basta ® resistance was stably expressed in those transgenic plants.

Expression of *AtSIK* increased the sensitivity to salt stress

To examine whether the overexpression of *AtSIK* gene increas sensitivity to salt stress, both WT and transgenic plants were treated in a solution containing 300 mM NaCl. Sfhersalt treatment, no severe chlorosis on leaves of WT plants was appeared within a few days, although most transgenic plants showed distinct visible damage on their leaves (Fig. 3a). Seven days after NaCl treatment, the reduction rate of chlorophyll content in leaves of all three transgenic lines was much higher

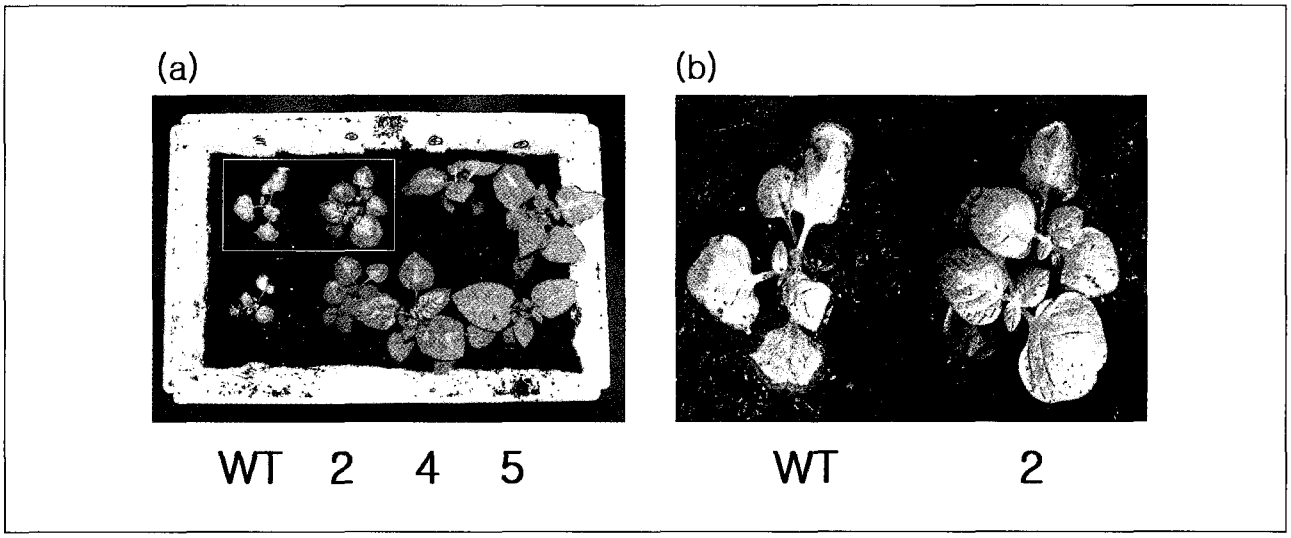

Fig. 2. Herbicide resistance in potato transgenic lines. (a) Herbicide Basta® tolerance of transgenic potato plants at commercial application rate (0.3% (v/v)). Plants were photographed two days after the foliar-application of Basta®. (b) The box area in panel (a) was magnified in four days after the foliar-application of Basta®.

than that in WT plants (Fig. 3b). Particularly, a transgenic line No. 4 retained only 47 % of the original chlorophyll content which measured before salt treatment, while WT plants maintained 67 % of the original value. This result suggested that the expression of the *AtSIK* gene increased the sensitivity of potato transgenic plants to salt stress, resulting in higher reduction of chlorophyll content than that in WT plants, under given stress condition. It has been shown that strong light induces photodamage to PS II (Photosystem II), whereas salt stress inhibits the repair of the photodamaged PS II and does not accelerate damage to PS II directly, via suppression of the activities of the transcriptional and translational machinery (Allakhverdiev *et al.*, 2002). This impairment of the PS II complex is associated with interruption of the transport of electrons that is mediated by PS II, resulting in increased accumulation of toxic ROS (Reactive Oxygen Species) in salt-stressed plants (Nishiyama *et al.*, 2001).

Although signal transduction cascades induced by water stress have not been fully understood, phosphorylation processes are now considered to have important roles in various signal transduction pathways in plants as well as in yeasts and animals. Recently, a number of transgenic approaches using protein kinases have shown to improve plant tolerance to water stress. For example, overexpression of a rice calcium-dependent kinase (OsCDPK7) results in increased chilling-and osmotic-stress tolerances in rice (Saijo *et al,* 2000). Transgenic tobacco plants that express a constitutively active tobacco mitogen-activated protein kinase kinase kinase (NPK1) display elevated tolerance to multiple environmental stress conditions. Transgenic Arabidopsis plants that overexpress *Arabidopsis* NDPK2 (AtNDPK2) have fewer ROS than the WT plants does (Moon *et al.*, 2003). In contrast, mutants that lack AtNDPK2 have higher levels of ROS than do WT plants. Furthermore, those AtNDPK2 transgenic *Arabidopsis* plants confer tolerance to multiple environmental stresses that elicit ROS accumulation *in situ* (Moon *et al.*, 2003).

Previously, it had shown that a *SIK* mutant *Arabidopsis* exhibited enhanced tolerance to both cold

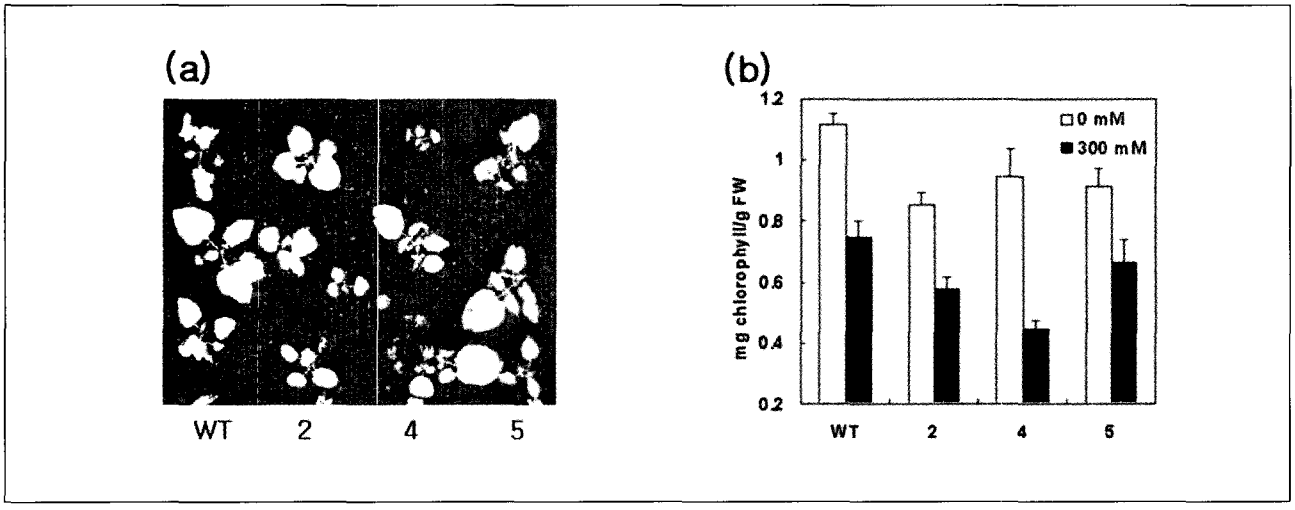

Fig. 3. Increased sensitivity to salt stress in the *AtSIK* transgenic potato lines. Both non- and transgenic plants grown in a tray containing soil were submerged in a solution containing 300 mM NaCl for three hours and then placed in a greenhouse without watering. Salt treatment has conducted every other day for seven days. (a) Plants were photographed a week after the treatment. (b) Measurement of chlorophyll contents a week after salt treatment.

and osmotic stresses. In contrast, a WT plant without a mutation in the *AtSIK* gene exhibited sensitivity to osmotic stress. Analysis of this phenomenon at molecular level revealed that the expression levels of COR (cold-regulated) genes, including *COR15a* and *COR47*, were much higher in the mutant plants than in WT plants, indicating that *AtSIK* functioned to repress genes induced by osmotic stress. However, as for *RD29A* which is known to be induced by dehydration, expression was induced higher level in the WT upon exposing to both a low temperature and a high concentration of salt, compared to the mutant plants. This result suggested that *AtSIK* did not repress *RD29A* gene expression, unlike other osmotic stress-inducible genes. Therefore, the *AtSIK* gene is not only involved in a negative regulation pathway by which the protein represses osmotic stress-inducible genes in plants, but that it is also involved in a positive regulation pathway. Furthermore, complementation analysis of the *AtSIK* mutants regained sensitivity to salt stress at a level similar to the WT plants.

In conclusion, we showed that the introduction of the *AtSIK* gene into potato increased sensitivity to salt stress. Although we don' t know yet whether overexpression of the *AtSIK* gene reduced abiotic stress inducible genes in transgenic potato plants, our results suggest that inactivation of the *SIK* gene in potato plants may enhance tolerance to osmotic stress, whereby overexpressing the antisense *AtSIK* gene.

ACKNOWLEDGMENT

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