P123

Functional analysis of *AtHAK5*, a high affinity K^+ transporter, under K^+starvation and low temperature

Sun-Ju Ahn*, Gap-Chae Chung, Daniel P. Schachtman, Kyung-Soo Min, and Tei-Ik Whang
Agricultural Plant Stress Research Center, College of Agriculture & Life Science, Chonnam National University, Gwangju 500-757, Korea.
Donald Danforth Plant Science Center, 975 North Warson Rd., St. Louis, MO 63132

Objectives

Potassium (K^+) is essential for plant growth and is the most abundant cation in plants. Plants have multiple mechanisms for K^+ uptake from soil and translocation to various plant tissues. Previouse work showed that only *AtHAK5* was up-regulated upon K^+ deprivation among 13 genes named *AtKT/KUP*. However, relatively little is known about the physiological role of proteins in this family. Therefore, the present study is trying to understand several functions of *AtHAK5*.

Materials and Methods

Seeds were surface sterilized and then planted on plates with modified nutrient medium. Plants were grown under a 12-/12-h day/night cycle under 110 μmol m^{-2} s^{-1} light during the day. After K^+ deprivation, biomass was measured and total RNA was isolated and its quality was checked by agarose gel electrophoresis. Two micrograms of DNA free RNA was then reverse transcribed using First-Stand Synthesis System for RT-PCR. cDNA concentrations were then normalized using β-tubulin and ubiquitin primers. For the Rb^+ uptake experiments, plants were moved to beakers containing Rb^+ at 20, 100, and 1,000 μM concentrations after 2 d of starvation. Trace amounts of $^{86}Rb^+$ were added to start a 10-min uptake period and plants were moved to beakers containing 0.5 mM $CaSO_4$. Roots were then blotted, weighed, and placed in scintillation vials containing scintillant. Radioactivity in the roots was counted using a Beckman LS6500 scintillation counter.

Results and Discussion

Previousely, we found that the gene and protein structure of *AtHAK5* is distinct from the other *AtKT/KUPs* and induced under K^+ deprivation. AtHAK5 T-DNA insertion knock-out mutant was confirmed by RT-PCR (Fig. 1). AtHAK5 was lacking a component of high affinity K^+ uptake that was found in Col.(wild type) when compared the kinetics of $^{86}Rb^+$ uptake after K^+ starvation (Fig. 2). In addition, we found that a decrease in whole-plant biomass and growth rate at K^+-limiting conditions was related to the HAK5 gene present (Fig. 3). In addition, *AtHAK5* is up-regulated not only K^+ deprivation but also to low temperature (Fig. 4).

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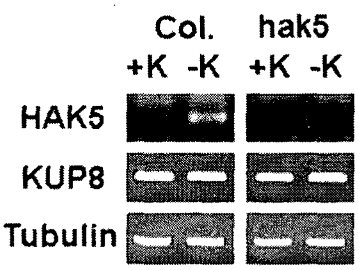
*Corresponding author : **Tel : 062-530-2052** **E-mail : asjsuse@chonnam.ac.kr**



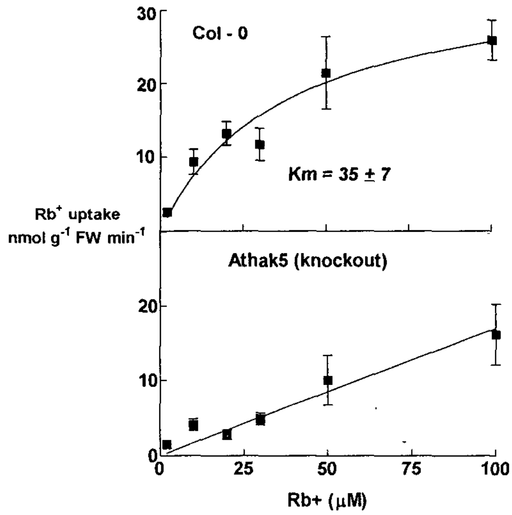
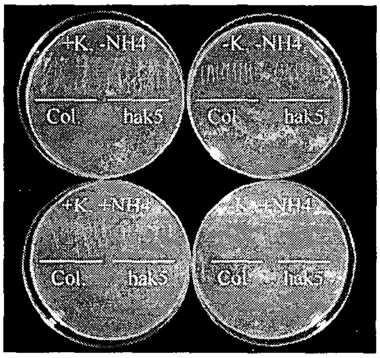


Fig. 1. Confirmation of hak5 T-DNA insertion line by RT-PCR

Fig. 2. Comparing the kinetics of $^{86}Rb^{+}$ uptake after K^{+} starvation



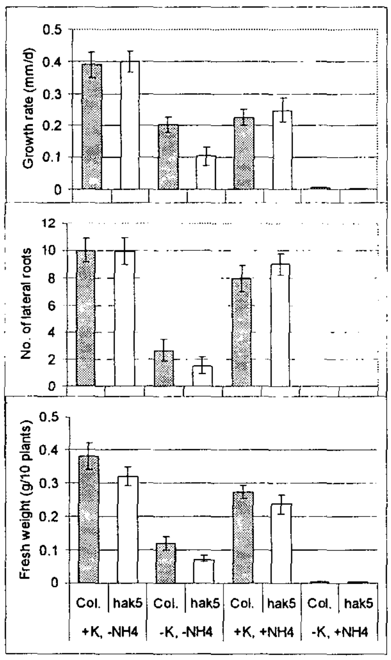


Fig. 3. Growth analysis of Col. and hak5 T-DNA insertion line on agar plates

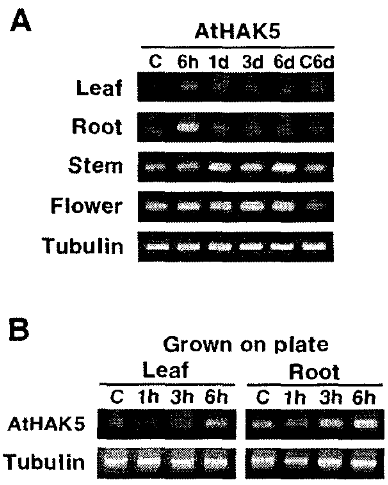


Fig. 4. Temporal expression of *AtHAK5* to low temperature