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RESEARCH ARTICLE

GhKT2: a novel K^+ transporter gene in cotton (*Gossypium hirsutum*)

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Abstract Potassium is an essential nutrient for plant growth and productivity of crops. K^+ transporters are important for K^+ uptake and transport in plants. However, information on the function of K^+ transporters and K^+ channels in cotton is limited. The KT/KUP/HAK protein family is essential for a variety of physiological processes in plants, including nutrient acquisition and regulation of development. This study, identified a K^+ transporter gene, *GhKT2,* expressed in the roots of cotton (*Gossypium hirsutum*) cv. Liaomian17. The deduced transcript of *GhKT2* is highly homologous to Cluster II of KUP/HAK/KT K^+ transporters and is predicted to contain 11 transmembrane domains. *GhKT2* has been localized to the plasma membrane, and its transcripts were detected in roots, stems, leaves and shoot apices of cotton seedlings. Consistently, b-glucuronidase (GUS) expression driven by the GhKT2 promoter could be detected in roots, mesophyll cells, and leaf veins in transgenic *Arabidopsis*. In addition, the expression of *GhKT2* was induced by low K^+ stress in cotton roots and *pGhKT2*::GUS-transgenic *Arabidopsis* seedlings. The *GhKT2*-overexpression *Arabidopsis* lines plants were larger and showed greater K^+ accumulation than the wild type (WT) regardless of K^+ concentration supplied. The net K^+ influx rate, measured by the noninvasive micro-test technique, in root meristem zone of *GhKT2*-transgenic *Arabidopsis* lines was significantly greater than that of WT. Taken together, this evidence indicates that GhKT2 may participate in K^+ acquisition from low or high external K^+, as well as K^+ transport and distribution in plants.

Keywords cotton, *GhKT2*, potassium, transporter, uptake

1 Introduction

Potassium is one of the major nutrients with several critical roles in plant growth and productivity, including cell elongation, maintenance of turgor pressure, stomatal closure, protein synthesis, photosynthesis and photoassimilate transport[1,2]. Potassium concentration in plants usually ranges from 80 to 150 mmol·L^{-1}[3], accounting for about 2% to 10% of dry weight[4].

Cotton (*Gossypium hirsutum* L.) is more sensitive to low K^+ availability than most other field crops due to its sparse roots and high K requirements in cotton bolls[5]. In recent years, K stress has been a global problem in cotton production. Possible reasons for this deficiency include negative K^+ balance in the soil, adoption of modern cultivars characterized by faster fruit set and greater boll load[6], and popularization of transgenic *Bacillus thuringiensis* Berliner (Bt) cotton[7], which is more susceptible to K stress[8].

Plants have evolved a complex signaling and regulatory network to adapt to K-deficient environments[9]. Physiological studies have established the existence of different transport systems involved in K^+ uptake and transport, such as the Shaker, TPK and Kir-like K^+ channel families, and the KT/KUP/HAK, Trk/HKT, KEA and CHX K^+ transporter families[10,11]. The KT/KUP/HAK family is essential for a variety of physiological processes in plants, including nutrient acquisition and regulation of plant development. KT/KUP/HAK transporters are divided into four distinct clusters[12]. Cluster I transporters such as AtHAK5, CaHAK1, HvHAK1, LeHAK5, OsHAK1, OsHAK5 and ThHAK5 are characterized by high-affinity K^+ uptake, suggesting that these may probably have a key role in K^+ acquisition when external K^+ is low[13–18]. The role of Cluster II transporters is relatively diverse in terms of physiological processes; however, its role in K nutrition is not well defined. Some transporters of Cluster II are localized to the tonoplast, facilitating K^+ efflux from the vacuole to maintain K^+ homeostasis[19]. Other transporters in this cluster such as AtKUP1 mediate both high- and

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low-affinity K^+ uptake[20], whereas HvHAK2 and CnHAK1 act as low-affinity K^+ transporters[19,21]. In addition, the expression of some Cluster II genes, namely, *AtHAK6* and *AtHAK2*, is affected by salt stress; therefore, these may be involved in plant response to salinity[22]. Moreover, some of the cluster II K^+ transporters participate in different regulatory processes. Mutation of AtKUP4/TRH1 in *Arabidopsis* resulted in tiny root hairs[23], which is attributed to auxin transport impairment[24], whereas, mutations in AtKUP2 (*shy3-1*) decreases cell expansion in shoots[25]. Although potassium deficiency significantly decreases cotton production, information on the functions of K^+ transporters and K^+ channels in cotton is limited. Recently, Xu et al. cloned a novel Shaker-like K^+ channel gene, *GhAKT1*, which is involved in K^+ uptake in cotton[26]. Here, we report that *GhKT2*, a cotton KT gene, is widely expressed in different cotton tissues, and its expression is significantly induced by K starvation. Overexpression of *GhKT2* cDNA in *Arabidopsis* enhances K^+ accumulation and increases net K^+ influx. This report describes a novel cotton K^+ transporter that mediates K^+ uptake, transport and distribution in plants, and also possibly enhances the growth of new leaves.

2 Materials and methods

2.1 Plant materials and growth conditions

Liaomian17, a K^+-efficient cotton cultivar developed by Cash Crops Research Institute, Liaoning Academy of Agricultural Sciences, China, was used to isolate *GhKT2*. Seeds were surface-sterilized by soaking in 9% H_2O_2 for 20–30 min, and then germinated in K^+-free sand medium. After germination, uniform seedlings were cultured hydroponically by transferring them into 16 cm × 13 cm × 16 cm plastic pots filled with 2.2 L of modified Hoagland's solution[27]. Seedlings were grown in a chamber with 12 h light (30±2°C)/12 h dark (22±2°C).

Arabidopsis thaliana ecotype Columbia was also used. The seeds were surface sterilized and germinated on normal MS (Murashige and Skoog) or low K^+ (LK, 100 μmol·L^{-1}) medium. LK medium was modified from MS medium with KH_2PO_4 replaced by $NH_4H_2PO_4$, as well as partial KNO_3 replacement by NH_4NO_3 as described by Xu et al.[28]. For seed harvest, *Arabidopsis* plants were grown in a potting soil mixture (rich soil:vermiculite 2:1, v/v). The *Arabidopsis* growth chamber was kept at 22°C with illumination at 120 μmol·m^{-2}·s^{-1} for a 16-h light period. The relative humidity was 70%±5%.

2.2 Cloning and sequence analysis of the *GhKT2* gene

Total RNA was extracted from the roots of cv. Liaomian17 grown in a solution containing 2.5 mmol·L^{-1} K^+[27]. The amino acid sequence of AtKT2 was used as probe to search the G. *hirsutum* EST database in GenBank. The sequences of candidate ESTs were subjected to contig analysis using the SeqMan program. The full-length sequence of the *GhKT2* gene was obtained through the 5′- and 3′-rapid amplification of cDNA ends (RACE), following the user manual of the SMART RACE cDNA amplification kit (Clontech, Mountain View, CA, USA) with cDNA of Liaomian17 used as the template. The sequence of the gene-specific primers was as follows: QA-forward (5′-ATGGATCTTGAGTTTGGGAAGT-3′) and ZA-reverse (5′-TTACACCACATAAACCATGCCA-3′). The PCR product was cloned into the pGEM-T easy vector (Promega, Madison, WI, USA) and then sequenced. Phylogenetic analysis was performed using ClustalX version 1.83 and MEGA4 and the neighbor-joining method[29,30]. The amino acid sequence was analyzed with the SMART program[31]. Putative transmembrane spans were predicted using the TMPRED server.

2.3 Subcellular localization of the *GhKT2* gene

The full-length ORF of the *GhKT2* gene without the termination codon was amplified by PCR using primers containing a *Sac1* site (forward) and *XbaI* site (reverse): 5′-CGAGCTCGATGGATCTTGAGTTTGGGAAGT-3′ and 5′-GCTCTAGAGCCACCACATAAACCATGCCA-3′. The fusion construct of *35S-GhKT2-GFP* was transformed into *Arabidopsis*. The roots of seven-day-old transgenic *Arabidopsis* plants were soaked in 500 mmol·L^{-1} mannitol on glass slides for 20 min at room temperature to plasmolyze cells. Green fluorescence was observed under a microscope at excitation wavelengths of 488 nm. *Arabidopsis* roots were then examined under a laser scanning microscope (FV1000, Olympus, Tokyo, Japan).

2.4 Construction of vectors and transformation of *Arabidopsis* plants

2.4.1 Construction of *pGhKT2*::GUS

Genomic DNA was isolated from the roots of Liaomian17 by the CTAB method. To isolate the *GhKT2* promoter, an adaptor-ligated genomic library was constructed by ligating digested genomic DNA with adaptors from the Universal Genome Walker Kit (Clontech, Mountain View, CA, USA) following the manufacturer's instructions. Primers designed to amplify the putative promoter sequence corresponded to the 5′-untranslated region (UTR) and upstream sequences of the *GhKT2* gene. Two gene-specific primers, AGSP1 (5′-CTGCTGCTCTTTTG-GAAATGCTCTCTT-3′) and AGSP2 (5′-AGAGAGA-GAGGAACCAAAGGCTTTACC-3′) were derived from the mRNA sequence and used in PCR-based DNA walking. After obtaining the putative promoter fragment (2807 bp in length), it was amplified by using the common

downstream primers, PKL (5′-CCAGCCACCCTACATTACATTACAA-3′) and PKR (5′-TTAGAACAATCAAGCAAGTCCCCAC-3′). The *pGhKT2*::GUS construct was generated by fusing the promoter of the *GhKT2* gene upstream of the β-glucuronidase (GUS) coding sequence in the pBGWFS7.0 vector using the Gateway system. Plant CARE were used for promoter nucleotide sequence analysis[32].

2.4.2 Construction of *35S*::*GhKT2*

The *35S*::*GhKT2* construct was generated by cloning the coding sequence of the *GhKT2* gene into the binary vector, pBI121 under the control of the CaMV 35S promoter. The *SUPER*::*GhKT2* construct was generated by cloning the coding sequence of *GhKT2* into the pBI121 vector under control of the SUPER promoter[33].

2.5 Transformation of *Arabidopsis*

Arabidopsis wild type (WT) plants were transformed with the *35S*::*GhKT2* construct to generate *GhKT2* overexpression lines for phenotype assays, and also transformed with the *pGhKT2*::GUS construct to generate lines for GUS staining analysis. The transformations of *Arabidopsis* were conducted by the floral dip method using *Agrobacterium* (strain GV3101)[34].

2.6 GUS staining analysis

Five-day-old or two-week-old *pGhKT2*::GUS transgenic *Arabidopsis* seedlings were incubated in a GUS staining solution containing 100 mmol·L^{-1} sodium phosphate buffer (pH 7.0), 1 mg·mL^{-1} 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-Gluc), 5 mmol·L^{-1} potassium ferrocyanide, and 0.03% Triton X-100 overnight at 37°C. For clearing the color of the chlorophyll in some tissues, 70% ethanol was used[35]. The tissues were observed and photographed under a stereomicroscope (SZ-16, Olympus). Furthermore, for detailed GUS staining analysis, the tissues were observed and photographed using bright-field microscopy.

2.7 RNA extraction and real-time PCR analyses

Total RNA was extracted from at least three seedlings of *Arabidopsis* and cotton using the RN38 EASY Spin Plus Plant RNA kit (Aidlab Biotech, Beijing, China). Two micrograms of total RNA were DNase I-treated and used for cDNA synthesis with oligo (dT) primers and reverse transcriptase (Promega). Real-time quantitative RT-PCR was conducted in an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems Inc., Foster City, CA, USA). The reaction volume was 15 μL, which contained 1.5 μL of diluted cDNA, 0.3 μL of ROX reference dye, 0.3 μL of both forward primer and reverse primers, 5.1 μL H_2O, and 7.5 μL SYBR Premier Ex Taq mix (Takara, Bio Inc., Kusatsu, shiga, Japan). PCR amplification was performed using two-step cycling conditions of 95°C for 30 s, followed by 40 cycles of 9°C for 5 s and 60°C for 35 s. The expression levels of *GhKT2* gene were calibrated to the expression of actin (*AtACT*, *Arabidopsis*) or ubiquitin (*GhUBQ7*, cotton) genes. The relative gene expression was calculated by using the $2^{-\Delta\Delta C_T}$ method[36]. The quantitative primer pairs were as follows: 5′-TCTTGGAGGACCTCACTGCT-3′ (forward) and 5′-CATCTGCTCGAAGAACCACA-3′ (reverse) for *GhKT2*; 5′-AAGAAGAAGACCTACACCAAGCC-3′ (forward) and 5′-GCCCACACTTACCGCAATA-3′ (reverse) for *GhUBQ7*, and 5′-GGCAAGTCATCACGATTGG-3′ (forward) and 5′-CAGCTTCCATTCCCACAAAC-3′ (reverse) for *AtACT*.

2.8 Identification of transgenic *Arabidopsis* phenotype

For *Arabidopsis* seedling phenotype assays, four-day-old seedlings grown on vertical normal MS medium were transferred onto either fresh normal or LK (100 μmol·L^{-1} K^+) plates and vertically positioned. After 7d, a batch of seedlings was harvested for determination of K^+ content. The remaining seedlings were photographed a few days later. For determination of K^+ content, the shoots and roots of the seedlings were separated and washed with ddH_2O, dried at 80°C for 2 d, and then weighed. The dried samples were dried to ash in a muffle furnace at 575°C for 5 h and then dissolved in 0.1 mol·L^{-1} HCl. K^+ was measured using an atomic absorption spectrophotometer (Z-2000, Hitachi, Japan). All assays were independently repeated three times.

2.9 K^+ flux measurement

Net K^+ fluxes in intact roots of *Arabidopsis* plants were measured by using noninvasive microtest technology (NMT) (BIO-001B, Younger USA Sci. and Tech. Corp., Amherst, MA, USA)[37]. Five-day-old seedlings grown on vertical MS medium were transferred onto fresh MS medium or LK (100 μmol·L^{-1} K^+) medium containing 2 mmol·L^{-1} CsCl for 3 d. Excessive Cs^+ (exceeding 200 μmol·L^{-1}) in the rhizosphere can inhibit K^+ uptake entirely, and thus induce K^+ starvation in plants[38,39]. The roots of *Arabidopsis* seedlings were equilibrated in a measuring solution (0.1 mmol·L^{-1} KCl, 0.1 mmol·L^{-1} $CaCl_2$, 0.3 mmol·L^{-1} MES, pH 6.0) for 10 min. Then, the roots were immobilized to the bottom of a chamber containing fresh measuring solution to measure K^+ flux at about 120 μm from the root tip. K^+ flux was calculated using the SIET software Mageflux (Younger USA Sci. and Tech. Corp., Amherst, MA, USA), with positive values

representing ion efflux and negative values influx. All measurements were independently repeated at least three times.

3 Results

3.1 Cloning and sequencing of the *GhKT2* gene

The ESTs of *GhKT2* were obtained by searching sequences similar to *AtKUP2* that encodes an *Arabidopsis* K^+ transporter protein in the *G. hirsutum* EST database[40]. These ESTs were aligned into a 1900-bp sequence. The full-length coding sequence was obtained using 5′-and 3′-RACE. Subsequently, a putative full-length cDNA sequence, named *GhKT2* (GenBank Accession No. KF658191) was cloned from cotton roots. The *GhKT2* cDNA is 2379 bp in length and encodes a protein of 792 amino acids, which shares sequence homology with members of Cluster II of the K^+ transporter family, particularly AtKUP2 (Fig. 1). It has 83.4% amino acid sequence similarity to KUP2 from *Arabidopsis*. GhKT2 is predicted to be a transmembrane protein with 11 transmembrane domains (Fig. 2), and is similar to HAK/KT/KUP transporters containing 10-14 transmembrane domains.

The genes in the circle are all KT/KUP/HAK Cluster II members. The branch length is proportional to the evolutionary distance between the transporters.

3.2 Localization of *GhKT2*

Figure 3a shows that the fluorescence derived from GFP in the control experiments was distributed throughout the cell, including the nucleus, while the green fluorescence was found on the surface of root tip cells of GhKT2-GFP transgenic *Arabidopsis* plants (Fig. 3b). After being plasmolyzed by mannitol treatment, it was observed that the green fluorescence was located on the plasma member, indicating that the GhKT2-GFP fusion protein is localized on the plasma membrane in plant cells (Fig. 3c).

3.3 *GhKT2* expression patterns in cotton and *Arabidopsis*

The expression of *GhKT2* in cotton seedlings was detected by real-time PCR. The *GhKT2* transcripts were found in all organs tested, including roots, shoot apices, unexpanded leaves, stems, and three different expanded true leaves, the expanded leaves having higher expression levels than the other plant parts (Fig. 4).

In *Arabidopsis*, the promoter of *GhKT2* drove GUS expressed in both leaves and roots (Fig. 5a). In the leaves, *GhKT2* was mainly expressed in mesophyll cells and veins, as well as leaf trichomes (Fig. 5b). Strong staining was also observed in differentiated leaf primordia (Fig. 5c). In the roots, *GhKT2* was expressed in both primary and lateral root tips, as well as in mature regions. A total of 18 independent transgenic *Arabidopsis* lines carrying *pGhKT2*::GUS displayed the same pattern of GUS staining

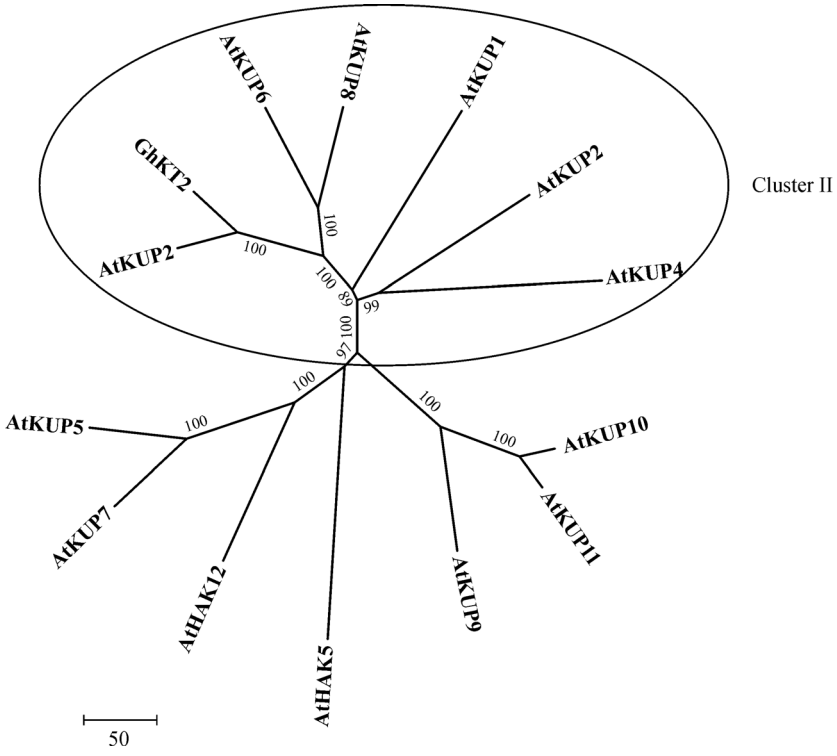

Fig. 1 Phylogenetic analysis of polypeptide *GhKT2* sequences

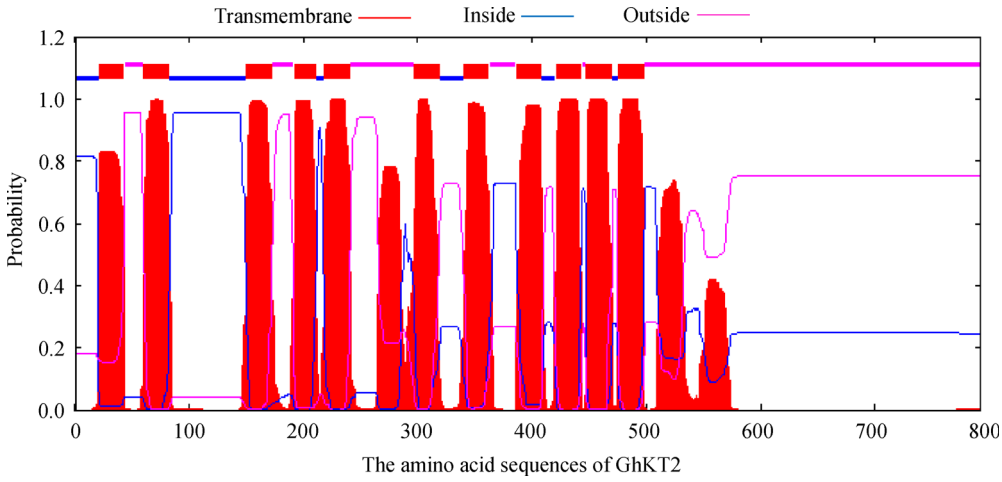

Fig. 2 The putative transmembrane structure of GhKT2

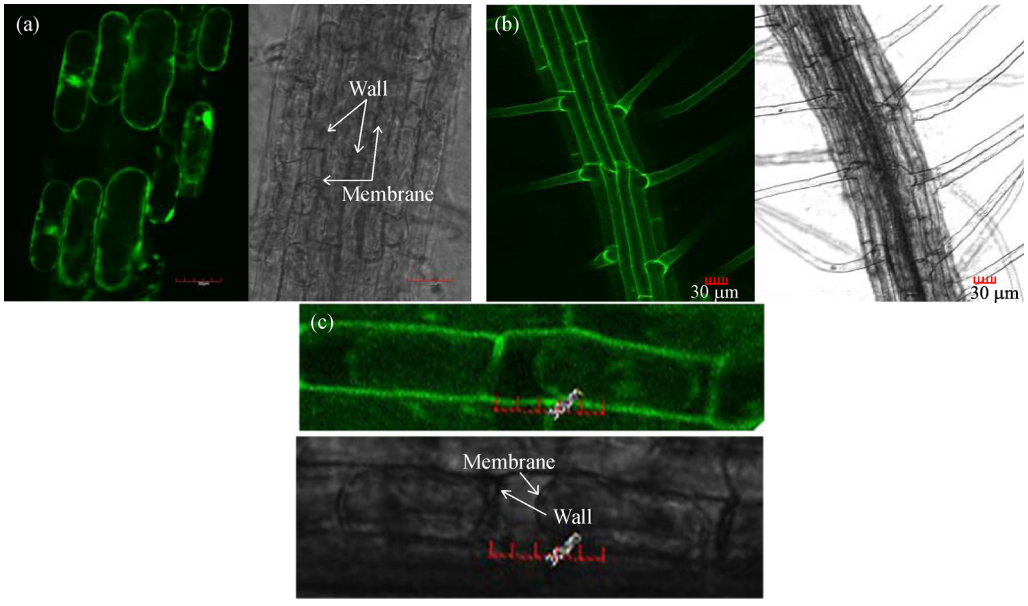

Fig. 3 Subcellular localization of the GhKT2-GFP fusion protein in transgenic *Arabidopsis* root cells. (a) Localization of control 35S-GFP fluorescence; (b) localization of 35S-GhKT2-GFP fluorescence; (c) localization of 35S-GhKT2-GFP fluorescence in root tip cells plasmolyzed with 500 mmol·L^{-1} mannitol.

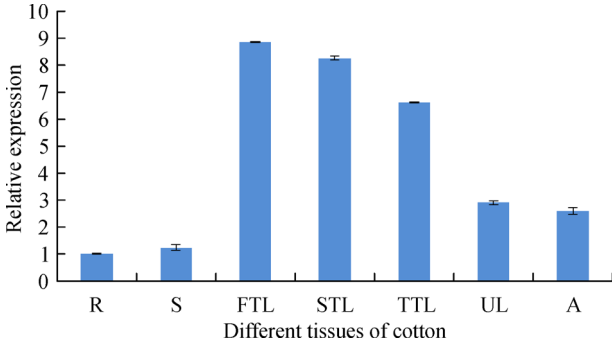

Fig. 4 Real-time relative-quantitative PCR analysis of *GhKT2* in various tissues of cotton seedlings. Tissues include roots (R), stems (S), first true leaf (FTL), second true leaf (STL), and third true leaf (TTL) unexpanded leaves (UL), shoot apices (A). The expression of *GhKT2* was calculated relative to *GhUBQ7* expression level. Data are means±SE (n = 3).

(data not shown).

The expression of *GhKT2* was induced by low K^+ in both *Arabidopsis* plants and cotton roots. GUS activity in transgenic *Arabidopsis* plants treated with low K^+ (100 μmol·L^{-1}) for 1 d substantially increased relative to control plants grown on normal MS (Fig. 5d, Fig. 5e). In addition, the expression of *GhKT2* in cotton roots showed more than a two-fold increase after 8 d K^+ starvation (Fig. 6).

3.4 Overexpression of *GhKT2* increases K^+ content and accumulation in *Arabidopsis*

To further analyze the *GhKT2* role in K^+ transport, we selected three individual *Arabidopsis* transgenic lines (K1, K2 and K3) that showed higher levels of *GhKT2* expression (Fig. 7a). We noted that ectopic expression of GhKT2 caused larger rosette leaves under normal or low

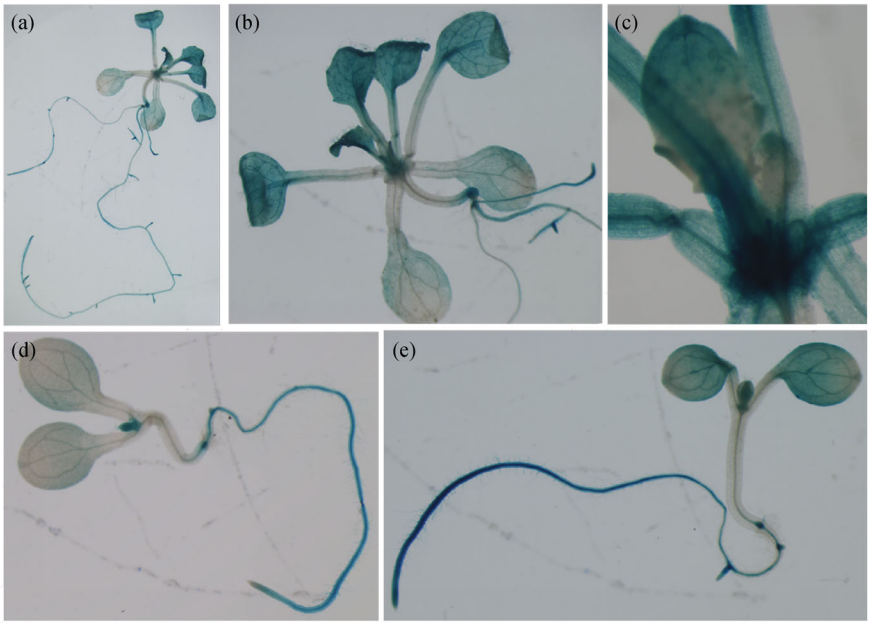

Fig. 5 Histochemical staining of *pGhKT2*::GUS transgenic *Arabidopsis* seedlings

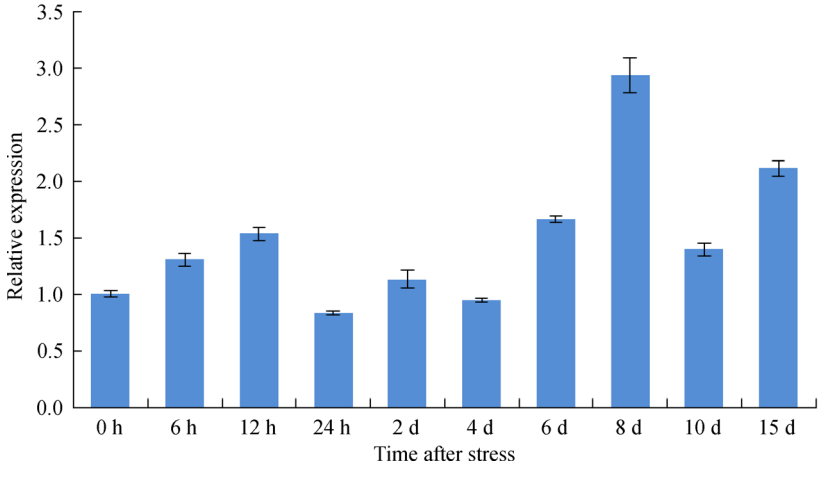

Fig. 6 Relative expression levels of *GhKT2* measured by real-time PCR in cotton roots exposed to 0.03 μmol·L^{-1} K^+. (a) Two-week-old plantlet; (b) leaves at higher magnification; (c) leaf primordia around shoot apex; (d) five-day-old seedling; (e) five-day-old seedling that was transferred to low K^+ (100 μmol·L^{-1}) MS medium for 1 day. Data are mean±SE for four independent experiments each with three independent seedlings (i.e., n = 12).

K^+ (LK) conditions. (Fig. 7b). The transgenic lines with or without LK treatment also had greater root and shoot biomass (Fig. 7c, Fig. 7d), shoot K^+ content (Fig. 7e, Fig.7f), and K^+ accumulation (Fig. 7g, Fig. 7h) compared with WT. However, significant increases in biomass and K^+ content were observed only under normal K^+ condition.

3.5 Net K^+ flux in roots of *GhKT2*-overexpression *Arabidopsis*

In *GhKT2*-overexpression lines and WT *Arabidopsis*, a net K^+ efflux in root meristematic zone (120 μm from the root tip) was observed under normal K^+ supply. However, under K^+ starvation conditions, caused by providing

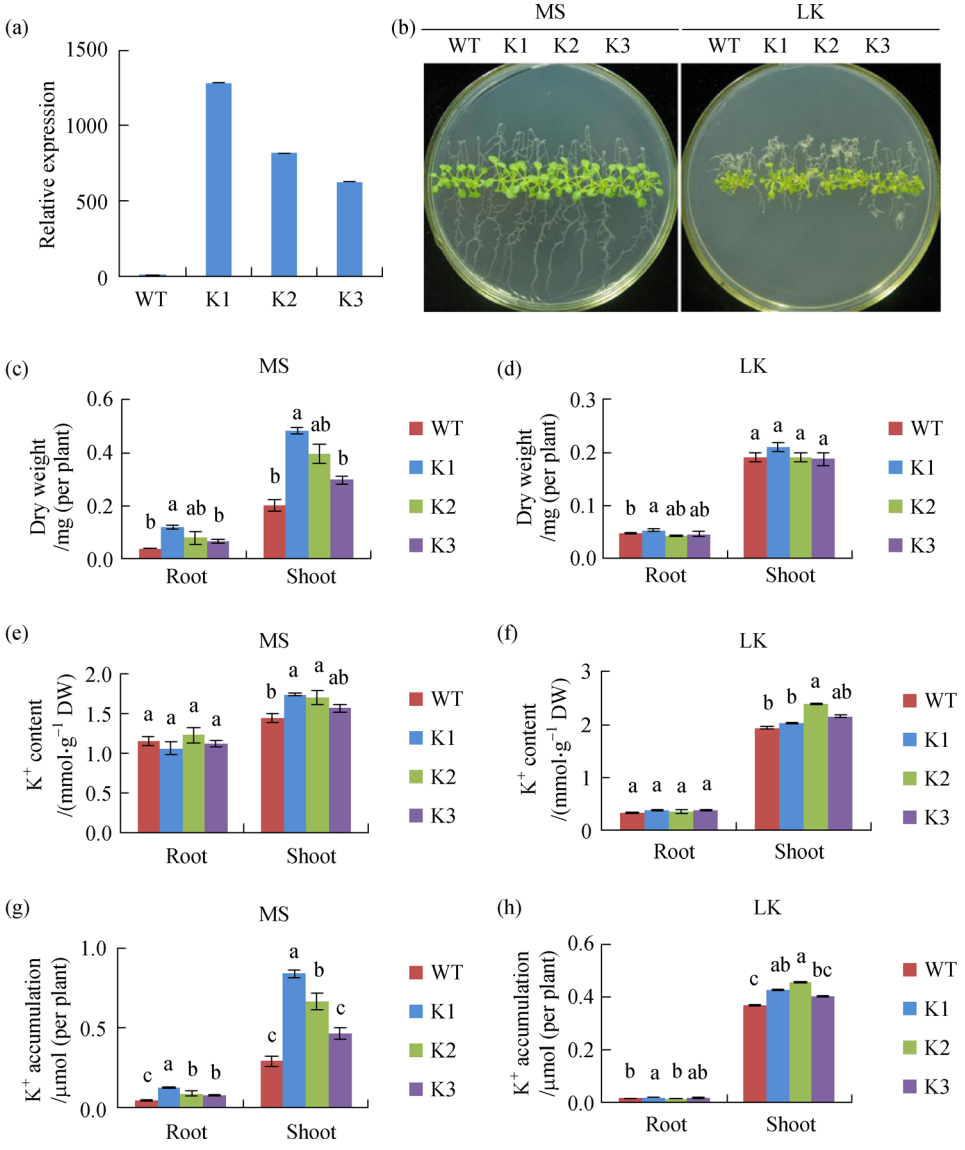

Fig. 7 Phenotype assays of *GhKT2*-overexpression *Arabidopsis* lines. WT is wild type (Columbia). K1, K2 and K3 are three independent transgenic lines with a WT background. (a) Real-time PCR analysis of WT and *GhKT2*-overexpression lines grown in MS medium for 7 d; (b) phenotype comparison between WT and transgenic plants grown on normal MS medium (left) for 10 d and on LK (low K^+, 100 μmol·L^{-1}) medium (right) for 26 d; (c–h) dry weight, K^+ content, and accumulation of 11-day-old seedlings (four-day-old seedlings grown on normal MS plates were transferred onto MS or LK medium for 7d). (c, d) Comparison of shoot and root dry weight on MS medium and LK medium, respectively; (e, f) comparison of shoot and root K^+ content on MS medium and LK medium, respectively; (g, h) comparison of shoot and root K^+ accumulation on MS medium and LK medium, respectively. Data are means±SE ($n = 4$). Different lower-case letters indicate significant differences at $P < 0.05$.

100 μmol·L^{-1} K^+ and 2 mmol·L^{-1} CsCl in the medium, the K^+ flux in the roots was reversed from efflux to influx (Fig. 8). Importantly, *GhKT2*-overexpression significantly increased the net K^+ influx in *Arabidopsis* roots by about 1.9–4.4 times compared to WT (Fig. 8).

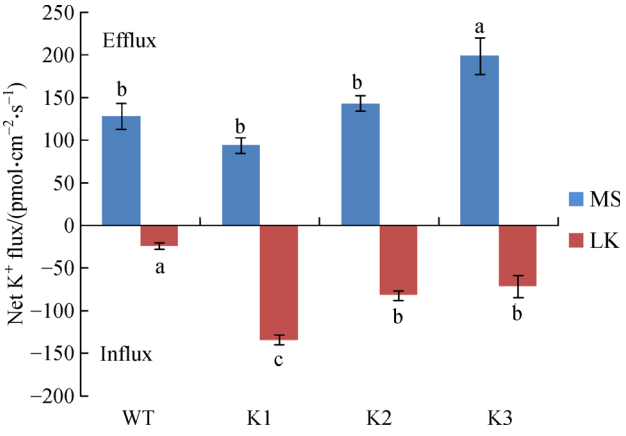

Fig. 8 Comparison of net K^+ flux as measured by the noninvasive microtest technique between *Arabidopsis* wild-type (WT) and *GhKT2*-overexpression transgenic lines (K1, K2, K3). All genotypes were starved of K^+ by growing on low K^+ (100 μmol·L^{-1}) medium containing 2 mmol·L^{-1} CsCl for 3 d, and then transferred to a measuring buffer with 100 μmol·L^{-1} K^+. Data are means±SE (n = 5 to 7). Different lower-case letters indicate significant differences at $P < 0.05$.

4 Discussion

In this study, we isolated and identified *GhKT2* from cotton cv. Liaomian17. The characteristic feature of KT/KUP/HAK transporters is the presence of the consensus motif GVVYGDLGTSPLY[41] (the amino acids conserved in all sequences are underlined), and *GhKT2* shows exactly the same conserved sequence as GVVYGDLSTSPLY. Phylogenetic analysis suggests that *GhKT2* belongs to Cluster II of the KT/KUP/HAK family, and the GFP reporter showed that *GhKT2* is localized to the plasma membrane. Moreover, overexpression of *GhKT2* in *Arabidopsis* increased biomass, K^+ accumulation and net K^+ flux, suggesting a specific role of GhKT2 in response to K^+ starvation. Our study identifies a novel K^+ transporter GhKT2 in cotton.

4.1 K^+ uptake

GhKT2 transcripts were detected in the roots of cotton and *Arabidopsis*, particularly in the root tips and root hairs of *pGhKT2*::GUS transgenic *Arabidopsis*, thereby pointing to the involvement of *GhKT2* in K^+ uptake.

In the present study, three lines of evidences indicate that *GhKT2* participates in K^+ uptake with high-affinity. First, the expression of *GhKT2* was clearly upregulated under K stress, K^+ starvation induced *GhKT2* transcription, and low K^+ (100 μmol·L^{-1}) enhanced GUS staining in the whole roots of *pGhKT2*::GUS transgenic *Arabidopsis* grown with 100 μmol·L^{-1} K^+ for 1 d. These responses to K stress are similar to those shown by the members of Cluster I of the KT/KUP/HAK family such as *AtHAK5*, *OsHAK1*, and *HvHAK1*[14,42], which performs high-affinity K^+ uptake. Second, while grown under K starvation, the roots of *GhKT2*-overexpression *Arabidopsis* lines showed a greater K^+ influx rate (as measured by the NMT technique at a low external K^+ concentration of 100 μmol·L^{-1}) than WT. Third, the *GhKT2*-overexpression transgenic *Arabidopsis* grown on LK (100 μmol·L^{-1} K^+) medium for 26 d showed a 15% increase in K^+ accumulation compared with WT.

However, *GhKT2*-overexpression in *Arabidopsis* also resulted in an average of 122% greater K^+ accumulation than WT while grown on normal MS medium containing 20 mmol·L^{-1} K^+ for 10 d, suggesting that GhKT2 also participates in K^+ uptake with low-affinity.

Taken together, GhKT2 apparently participated in high-affinity K^+ uptake within a short time (less than 1 d) or after transient (20–25 min) K stress. When K stress lasted for a longer time (26 d), the function of GhKT2 appeared to be weaker, as shown by a 15% increase in K^+ accumulation compared with control and GhKT2 clearly showed low-affinity K^+ uptake when sufficient K was supplied for a long time (⩾2 d). In fact, transporter affinity showed no obvious boundaries. For example, *AtKUP1* encodes a high-affinity K^+ transporter protein, whereas it also has the capacity for low-affinity K^+ uptake[20].

4.2 K^+ transport and distribution

Most studies on K^+ transport in different plant parts have focused on the HKT family[43]. It has also been reported that several KT/HAK/KUP members are crucial for K^+ transport. For example, *OsHAK5* expression predominantly occurs in mesophyll and parenchymal cells of the vascular bundle, and OsHAK5 mediates root-to-shoot transfer of K^+ at low external K^+ concentration[18]. Using the K^+ selective fluorescent dye, the K^+ concent in stelar tissues was reduced in *kup7* under K deficient conditions. AtKUP7 might also be involved in K^+ transport into xylem sap, affecting K^+ translocation from root toward shoot especially under LK conditions[44]. In the present study, *GhKT2* was markedly expressed in the true leaves of cotton seedlings, and GUS activity driven by promoter of *GhKT2* was also strong in the expanded leaves of *Arabidopsis*, in virtually all mesophyll cells and vascular bundles. Therefore, we infer that *GhKT2* may have a general function in regulating K^+ transport and distribution in leaves.

4.3 Growth and K^+ utilization

K^+ is a major cellular solute, and impairment in K homeostasis reduces cell turgor and thus restricts cell

expansion, eventually inhibiting plant growth and development[45]. Some members of Clusters I and II of the KT/KUP/HAK family are important for plant growth[10]. OsHAK1, a member of the KT/HAK/KUP family in rice, is essential in K-mediated rice growth[46]. In the present study, the activity of the *GhKT2* promoter in the leaf primordia of *pGhKT2*::GUS transgenic *Arabidopsis* and *GhKT2* transcripts were detected in shoot apex of cotton plants, suggesting the involvement of *GhKT2* in cell elongation and leaf expansion as with *AtKUP2*[25]. However, we did not observe any changes in cell size in the root meristem zone and hypocotyl in *GhKT2*-overexpression *Arabidopsis* grown on either normal MS or LK medium (data not shown).

The potassium utilization index (KUI), the dry matter produced according to the K^+ concentration in plants, reflects the efficiency of internal K utilization. The *GhKT2*-overexpression *Arabidopsis* lines showed an average of 110% greater KUI in roots and 65% greater KUI in shoots than WT while grown on MS medium, suggesting that GhKT2 enhances the efficiency of internal K utilization in plants. However, the underlying mechanism involved remains unclear. Checchetto et al. reported that the thylakoid K^+ channel is required for efficient photosynthesis in cyanobacteria[47]. However, no similar reports involving K^+ transporters from the KT/KUP/HAK family have been published.

5 Conclusions

In the present study, we characterized a K^+ transport gene, *GhKT2*, from *G. hirsutum* roots. It was localized on the plasma membrane, and possibly participates in K^+ acquisition, as well as K^+ transport and distribution in plants. In addition, it is likely that *GhKT2* also enhances the growth of new tissues. These results may facilitate the elucidation of the mechanism underlying the acquisition and utilization of K^+ in cotton, which may assist in the development of K^+-efficient cotton genotypes using biotechnological approaches.

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Compliance with ethics guidelines Yiru Wang, Juan Xu, Mingcai Zhang, Xiaoli Tian, and Zhaohu Li declare that they have no conflicts of interest or financial conflicts to disclose.

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