The Potassium Transporter SlHAK10 Is Involved in Mycorrhizal Potassium Uptake[1][OPEN]

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Most terrestrial plants form a root symbiosis with arbuscular mycorrhizal (AM) fungi, which receive fixed carbon from the plant and enhance the plant's uptake of mineral nutrients. AM symbiosis improves the phosphorous and nitrogen nutrition of host plants; however, little is known about the role of AM symbiosis in potassium (K^+) nutrition. Here, we report that inoculation with the AM fungus *Rhizophagus irregularis* improved tomato (*Solanum lycopersicum*) plant growth and K^+ acquisition and that K^+ deficiency has a negative effect on root growth and AM colonization. Based on its homology to a *Lotus japonicus* AM-induced K^+ transporter, we identified a mycorrhiza-specific tomato K^+ transporter, SlHAK10 (*Solanum lycopersicum High-affinity Potassium Transporter10*), that was exclusively expressed in arbuscule-containing cells. *SlHAK10* could restore a yeast K^+ uptake-defective mutant in the low-affinity concentration range. Loss of function of *SlHAK10* led to a significant decrease in mycorrhizal K^+ uptake and AM colonization rate under low-K^+ conditions but did not affect arbuscule development. Overexpressing *SlHAK10* from the constitutive cauliflower mosaic virus 35S promoter or the AM-specific *Solanum melongena Phosphate Transporter4* not only improved plant growth and K^+ uptake but also increased AM colonization efficiency and soluble sugar content in roots supplied with low K^+. Our results indicate that tomato plants have a SlHAK10-mediated mycorrhizal K^+ uptake pathway and that improved plant K^+ nutrition could increase carbohydrate accumulation in roots, which facilitates AM fungal colonization.

Potassium (K^+) is one of the three essential macronutrients, constituting 2% to 10% of plant dry matter (Leigh and Wyn Jones, 1984; Amrutha et al., 2007; Yang et al., 2009). As the most abundant cation in living plant cells, K^+ plays crucial roles in many fundamental processes, including photosynthesis, osmoregulation, membrane transport, enzyme activation, and anion neutralization, and consequently affects overall plant growth and development (Amrutha et al., 2007; Yang et al., 2009). As a result of leaching losses, chemical fixation, and the relatively low diffusion rate in soil, the availability of K^+ drastically fluctuates in large areas of agricultural land worldwide and is often a limiting factor for crop quality and yields (Rengel and Damon, 2008).

Plant cells need to maintain their cytoplasmic K^+ concentrations at approximately 100 mM (Leigh and Wyn Jones, 1984; Walker et al., 1996), but the K^+ concentration in the rhizosphere varies drastically and rarely exceeds 0.01 to 1 mM (Maathuis, 2009; White, 2013). This indicates the requirement for specialized transport systems for the uptake of K^+ and for its internal redistribution within plants. Transport of K^+ through plant membranes can be mediated either by K^+ channels, which use the membrane potential and electrochemical gradient to facilitate K^+ transport, or by secondary transporters with different affinities located in the plasma or organelle membranes (Gierth et al., 2005; Voelker et al., 2006; Li et al., 2018). Genes encoding plant K^+ transporters are classified into four major families: KT/HAK/KUP (K^+ Transporter/High-affinity K^+ transporter/K Uptake Permease), HKT (High-affinity K^+/Na^+ Transporter), KEA (K^+ Exchange Antiporters), and CHX (Cation/H^+ Exchanger; Uozumi

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et al., 2000; Cellier et al., 2004; Kunz et al., 2014; Aranda-Sicilia et al., 2016; Wang and Wu, 2017). The KT/KUP/HAK family comprises most of the plant K^+ transporters identified so far, and members of this family are further divided into four major clusters (I–IV; Rubio et al., 2000; Grabov, 2007; Gupta et al., 2008; Wang and Wu, 2013; Nieves-Cordones et al., 2016). Multiple transporters in cluster I, such as *AtHAK5*, *HvHAK1*, *OsHAK1*, and *OsHAK5*, have high-affinity K^+ uptake capacity and rapid up-regulation in response to K^+ deficiency (Schachtman and Schroeder, 1994; Santa-María et al., 1997; Gierth et al., 2005; Yang et al., 2014; Chen et al., 2015). The cluster II members of the KT/KUP/HAK family showed great divergence in sequence and function (Elumalai et al., 2002; Yang et al., 2009; Osakabe et al., 2013). Transporters in this cluster have been characterized to mediate both high- and low-affinity K^+ uptake. *AtKUP1* from Arabidopsis (*Arabidopsis thaliana*) is a typical cluster II member of the KT/KUP/HAK family that functions as a dual-affinity K^+ transporter (Fu and Luan, 1998). Much less has been reported regarding the potential functions or physiological roles of the cluster III and IV transporters (Han et al., 2016).

Arbuscular mycorrhizal (AM) symbiosis, formed between AM fungi of Glomeromycotina and roots of more than 80% of land plants, represents one of the most important symbiotic interactions in nature (Smith and Smith, 2011; Kobae et al., 2016). Formation of an AM symbiosis improves growth status for the plant partner, especially under nutrient-poor growing conditions (Govindarajulu et al., 2005; Javot et al., 2007; Jin et al., 2012; Casieri et al., 2013; Courty et al., 2016; Garcia et al., 2016). In the nonmycorrhizal (NM) plants, mineral nutrients can only be directly taken up from the rhizosphere via root epidermis and root hairs. Once colonized by AM fungi, the mycorrhizal plants thus possess another indirect uptake pathway, called the mycorrhizal pathway, via the AM fungal hyphae into root cortical cells (Kiers et al., 2011; Smith and Smith, 2012; Sieh et al., 2013; Giovannetti et al., 2014).

The role of phosphate (Pi) nutrition in AM symbiosis has always been a major focus. It has been repeatedly demonstrated that the mycorrhizal pathway is key for Pi uptake (Harrison et al., 2002; Paszkowski et al., 2002; Smith et al., 2003; Nagy et al., 2006; Chen et al., 2007). Multiple plant transporters responsible for transporting Pi across the intraradical symbiotic interfaces have been identified (Paszkowski et al., 2002; Nagy et al., 2005; Yang et al., 2012; Xie et al., 2013; Chen et al., 2014). Although the contribution of AM fungi to the transport of other nutrients is less clear, increasing evidence has shown that plants can acquire substantial amounts of nitrogen (N) and sulfur (S) through the symbiotic uptake pathway (Mäder et al., 2000; Allen and Shachar-Hill, 2009; Koegel et al., 2013; Giovannetti et al., 2014).

Compared with the relatively large number of reports on symbiotic Pi and N uptake, much less is known about the effects of AM symbiosis on plant K^+ acquisition. Nonetheless, enrichment of K^+ in different tissues of several mycorrhizal plants, including maize (*Zea mays*), lettuce (*Lactuca sativa*), and wheat (*Triticum aestivum*), could be traced in a small number of studies (Garcia and Zimmermann, 2014), leading to speculation that plants may also have a symbiotic K^+ uptake pathway. This hypothesis gained some support from the transcriptome analysis of both AM partners. On the fungal side, four sequences from an EST library of *Rhizophagus irregularis* were identified as K^+ transporters (Casieri et al., 2013). On the plant side, a KT/KUP/HAK transporter (Lj4g3v3116360.1; hereafter referred to as LjHAK) was shown to be up-regulated in AM roots of *Lotus japonicus* compared with the NM roots (Guether et al., 2009). A recent study using whole-genome RNA sequencing of *Medicago truncatula* mycorrhizal roots under K^+ deficiency revealed the up-regulation of several genes encoding putative transporters, including a putative K^+/H^+ exchanger, in mycorrhizal plants under K^+ deprivation (Garcia et al., 2017). However, in several transcriptome studies, no K^+ transporter was observed to be up-regulated in *M. truncatula* mycorrhizal roots (Gomez et al., 2009; Gaude et al., 2012). Thus, it is tempting to investigate whether the gene regulation involved in the transfer of K^+ from the AM fungus to the plant is conserved across different plant species.

In this study, we performed extensive searches of putative K^+ transporters in the recently released transcriptomes for several mycorrhizal plants (Güimil et al., 2005; Fiorilli et al., 2009; Gomez et al., 2009; Gaude et al., 2012; Garcia et al., 2017; Sugimura and Saito, 2017), which resulted in the identification of a member of the KT/KUP/HAK family from tomato (*Solanum lycopersicum*) named *SlHAK10* (Solyc03g097860.1.1) as the putative orthologue of the AM-induced *LjHAK*. *SlHAK10* was shown to be induced 500-fold in AM roots compared with the NM roots (Sugimura and Saito, 2017). Except for *LjHAK* and *SlHAK10*, none of the other K^+ transporters from the KT/KUP/HAK family or other families showed observable up-regulation in the transcriptomes of tomato and *L. japonicus* mycorrhizal roots. Genome-wide hunting and expression analysis of all the tomato KT/KUP/HAK genes confirmed that *SlHAK10* was the sole one having AM-inducible expression in this family. Tissue-specific and functional analyses revealed that *SlHAK10* was expressed exclusively in the cells containing arbuscules and could mediate K^+ uptake through the mycorrhizal pathway. These findings thus indicate that there might be a mycorrhizal K^+ uptake pathway in mycorrhizal plants, at least in tomato, through activating the AM-induced K^+ transporter(s), such as SlHAK10 and its potential orthologues.

RESULTS

AM Symbiosis Improves the K^+ Accumulation in Tomato Plants

In this study, to investigate the potential roles of AM formation in plant K^+ acquisition, tomato plants were grown in a sand-based pot culture in the presence or absence of AM fungus (*R. irregularis*) supplied with

2 mM K^+ as a high-K^+ condition and with 0.2 mM K^+ as a low-K^+ condition. The plant samples were harvested at 5 weeks postinoculation for determining the shoot and root biomass, K^+ accumulation, and fungal colonization rates.

The plants treated with low-K^+ solution showed a significant growth suppression in both shoots and roots over than the plants supplied with high K^+ (Fig. 1, A and B). Under low K^+, root growth was more inhibited compared with shoot growth, leading to a typical decrease in root-shoot ratio (Supplemental Fig. S1A). Compared with the NM plants, the mycorrhizal plants (AM plants) showed increased shoot and root biomass under the high-K^+ condition (Fig. 1, A and B). The shoot K^+ content in AM plants grown under high K^+ did not differ significantly with that in the NM plants (Supplemental Fig. S1B), but the root K^+ content (Supplemental Fig. S1C) and the total K^+ accumulation in both shoots and roots (Fig. 1, C and D) increased by more than 20% in the AM plants. The shoot biomass of AM plants supplied with low K^+ had no significant difference compared with that of the NM control plants (Fig. 1A). By contrast, a significant increase in root biomass (Fig. 1B) and root-shoot ratio (Supplemental Fig. S1), as well as K^+ accumulation in both shoots and roots (Fig. 1, C and D), could be observed under the low-K^+ condition.

The plants supplied with low K^+ had a significantly lower AM colonization rate and arbuscule incidence compared with the plants grown under high K^+ (Fig. 1, E and F). An assessment of mycorrhization efficiency by different concentrations of K^+ application was performed, which led to the suggestion that the K^+-limiting growth condition might have a negative effect on AM colonization in tomato (Supplemental Fig. S2).

Identification and Characterization of a Tomato AM-Induced Potassium Transporter

A previous study based on microarray analysis revealed a putative AM-induced K^+ transporter gene (*Lj4g3v3116360.1*) of the KT/KUP/HAK family in *L. japonicus* (named *LjHAK* in this study; Guether et al., 2009). To identify the potential homologues responsive to AM symbiosis in tomato, the coding sequence

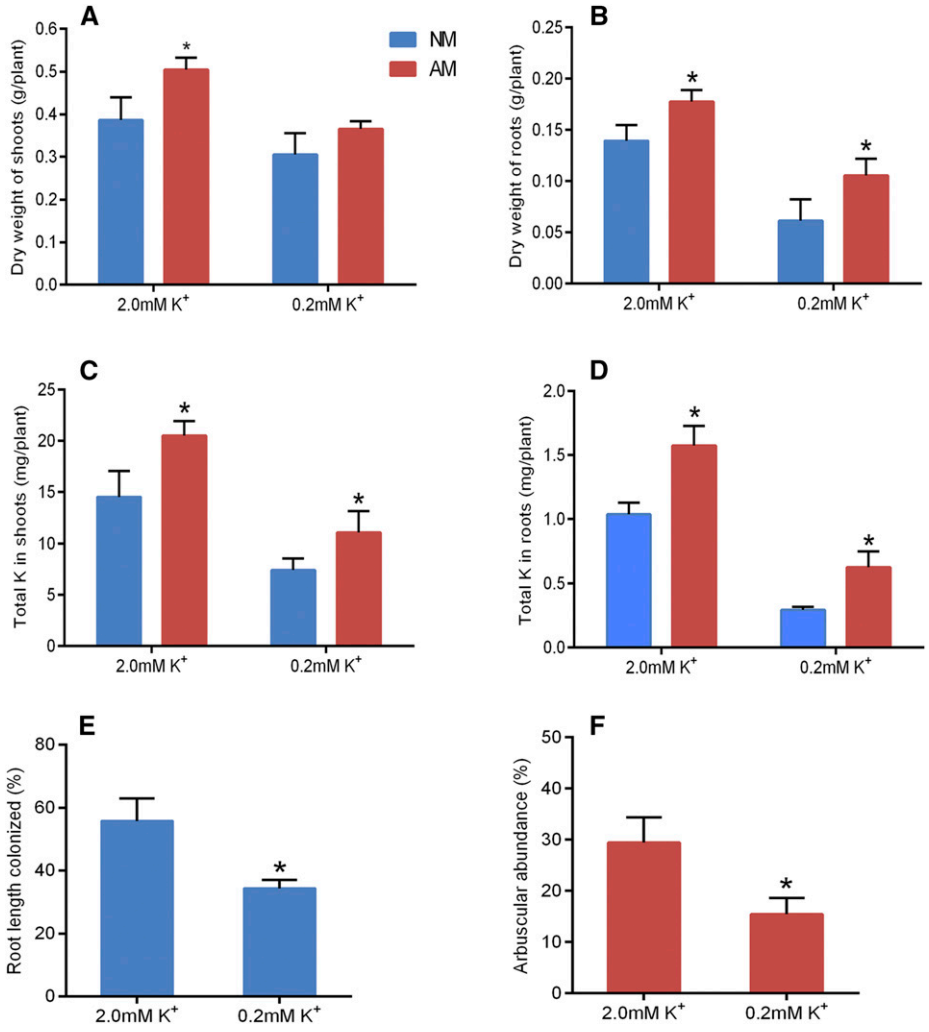

Figure 1. Impact of different K^+ applications and AM fungal colonization on tomato plant growth and K^+ acquisition. A and B, Shoot (A) and root (B) biomass of AM and NM plants. C and D, K^+ accumulation in shoots (C) and roots (D) of AM and NM plants. E and F, Total colonization rate (E) and arbuscule abundance (F) under high-/low-K^+ supply conditions. AM, AM fungus-colonized plants; NM, nonmycorrhizal plants. Error bars indicate SE ($n = 5$). Asterisks indicate significant differences ($P < 0.05$).

of *LjHAK* was employed for BLAST search against the tomato genomic database (http://solgenomics.net/), which led to the identification of 21 nonallelic sequences with substantial identities and similar structures to the tomato K^+ transporter genes (Supplemental Fig. S3).

A phylogenetic tree constructed with these putative tomato K^+ transporters (named SlHAK1 to SlHAK20 in this study) and homologues from several other plant species revealed a cluster of eight SlHAK transporters with the AM-induced LjHAK in group II of the KT/KUP/HAK family. Reverse transcription quantitative PCR (RT-qPCR) analysis revealed that, with the exception of *SlHAK10*, the closest orthologue of *LjHAK*, which was highly induced in the tomato mycorrhizal roots, none of the other *SlHAK* genes showed significantly up-regulated responses to AM symbiosis (Supplemental Fig. S4). The mycorrhiza-inducible expression property of *SlHAK10* was further confirmed by a recent study on comparative transcriptome analysis between *L. japonicus* and tomato, in which *SlHAK10* was shown to be induced 500-fold in AM roots (Sugimura and Saito, 2017). A time-course expression analysis further proved the high correlation between the transcript abundance of *SlHAK10* and colonization intensity (Fig. 2, A and B). The transcripts of *SlHAK10* were barely detectable in other tissues, including leaf, flower, and fruits, and showed no conspicuous induction to other environmental factors, such as K^+ and Pi deficiency, as well as high-Na^+ stress (Supplemental Fig. S5).

To investigate the *SlHAK10* expression pattern in detail, a 2,281-bp promoter fragment of *SlHAK10* was fused to the GUS reporter gene and introduced into tomato plants. The resulting transgenic plants were then inoculated with *R. irregularis*. Five weeks after inoculation, the GUS activity could be detected only in the mycorrhizal roots. Colocalization of GUS expression and AM fungal structure by overlay of Magenta-GUS with Trypan Blue staining revealed that GUS activity driven by the *SlHAK10* promoter was confined to distinct cells containing arbuscules (Fig. 2, C and D). No GUS staining could be observed in the neighboring noncolonized cells, consistent with the results reported for some other AM-specific nutrient transporters, such as Pi and NH_4^+ (Kobae et al., 2010; Chen et al., 2011).

To determine whether *SlHAK10* functions as a membrane-localized protein, its subcellular localization was investigated. The coding sequence of *SlHAK10* was fused in frame with the 5′ end of the eGFP reporter gene under the control of the 2× cauliflower mosaic virus 35S (CaMV35S) promoter (2×35S::*SlHAK10*::eGFP). The construct 35S::eGFP was used as a control. Both constructs were transiently expressed in the tobacco (*Nicotiana benthamiana*) leaf epidermal cells via *Agrobacterium tumefaciens*-mediated infiltration. Samples were stained using FM4-64FX, a plasma membrane-specific dye. Confocal microscopy showed an overlap signal (yellow) of the GFP fluorescence (green) and the FM4-64FX fluorescence (red) in the cells expressing the 2×35S::*SlHAK10*::eGFP chimeric gene (Fig. 3B), indicating that AM-specific *SlHAK10* might encode a plasma membrane-localized K^+ transporter.

Functional Characterization of *SlHAK10* in Yeast

The K^+ transport capacity of *SlHAK10* was initially evaluated by determining its ability to complement a yeast mutant. Full-length *SlHAK10* was introduced into

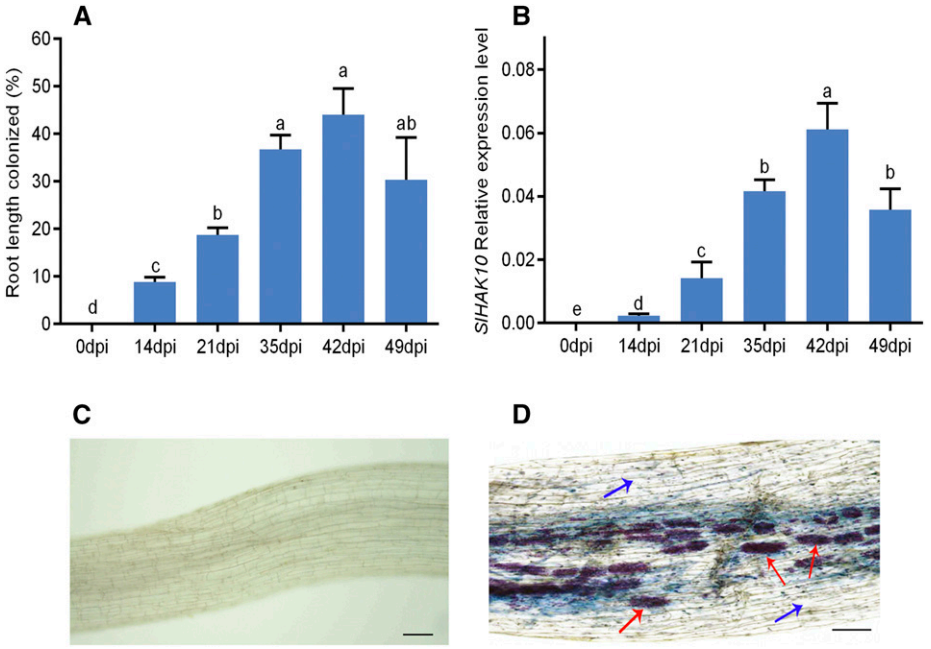

Figure 2. Expression analysis of *SlHAK10* in response to AM symbiosis. A, Quantification of AM fungal colonization at different sampling time points. dpi, Days postinoculation. B, Time-course expression of *SlHAK10* in mycorrhizal roots of tomato plants. Values are means of three biological replicates with SE. Different letters indicate significant differences ($P < 0.05$). C and D, Histochemical GUS staining of tomato roots expressing pSlHAK10::GUS in the absence (C) and presence (D) of *R. irregularis* inoculation. Red arrows indicate arbuscules. Blue arrows denote noncolonized cells in mycorrhizal roots. Bars = 50 μm.

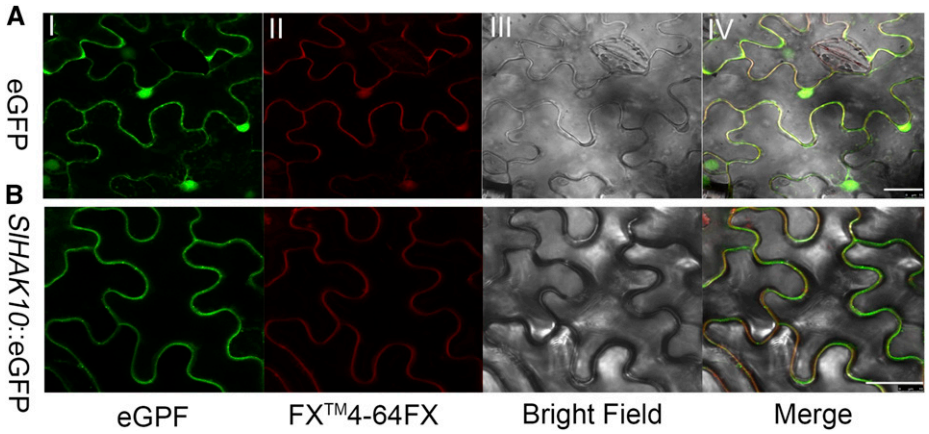

Figure 3. Subcellular localization analysis of *SlHAK10*. Confocal laser scanning microscopy images show tobacco leaf epidermal cells transiently expressing 35S::eGFP (A) or 35S::*SlHAK10*-GFP (B). I, Confocal images under GFP channel only. II, FM4-64FX dye-induced red fluorescence showing the position of the plasma membrane. III, Bright field. IV, Merged images of GFP fluorescence (green) and FM4-64FX fluorescence (red). Bars = 50 μm.

the auxotrophic yeast mutant strain R5421 (MATα ura3-52 leu2 trk1Δ his3Δ200 his4-15 trk2Δ1::pCK64). R5421 is defective in K^+ uptake and could not grow under low-K^+ culture conditions. *AtAKT1*, a K^+ channel from Arabidopsis, was also transformed into R5421 as a positive control (Hirsch et al., 1998; Wang and Wu, 2013). The yeast growth assays were carried out on arginine phosphate (AP) medium containing different concentrations of K^+. It was shown that, under high-K^+ conditions (5 and 10 mM), all the tested yeast strains could grow well, and no visible difference was observed between the yeasts transformed with empty vector (pYES2) versus the vector expressing *SlHAK10*. Along with the decline of K^+ concentration from 5 to 1 mM, the growth of R5421 harboring empty vector was almost entirely suppressed. Although the R5421 cells transformed with *SlHAK10* were also not able to grow at 0.5 mM K^+, the yeast mutant expressing *SlHAK10* could indeed rescue its growth defect at 1 mM K^+ (Fig. 4A). Growth kinetic analysis showed that the yeast R5421 cells expressing AtAKT1 and SlHAK10 grew much faster than the yeast cells harboring empty vector in liquid AP medium containing 1 mM KCl (Fig. 4B), suggesting that *SlHAK10* has K^+ transport activity.

Loss-of-Function Mutation of SlHAK10 Decreases Mycorrhizal K^+ Uptake But Does Not Affect Arbuscule Morphology

As *SlHAK10* is an AM-responsive K^+ transporter in tomato, it is reasonable to speculate that SlHAK10 might have a contribution to the symbiotic K^+ uptake and/or AM formation. To test this hypothesis, we first generated mutant lines of *SlHAK10* by the CRISPR-Cas9 system. Two independent homozygous mutant lines with one nucleotide deletion and insertion, respectively, in the coding sequence of *SlHAK10* were identified (Supplemental Fig. S6). The two mutant lines and wild-type plants were cultivated in a compartmented culture system containing a middle root/fungal compartment (RFC) and two hyphal compartments (HCs; Supplemental Fig. S7A). The RFC and HC were separated by 30-μm nylon mesh. All three compartments were filled with a 4:1 mixture of sterilized sand and low-K^+ soil (the soil contains 30.2 mg kg^{-1}

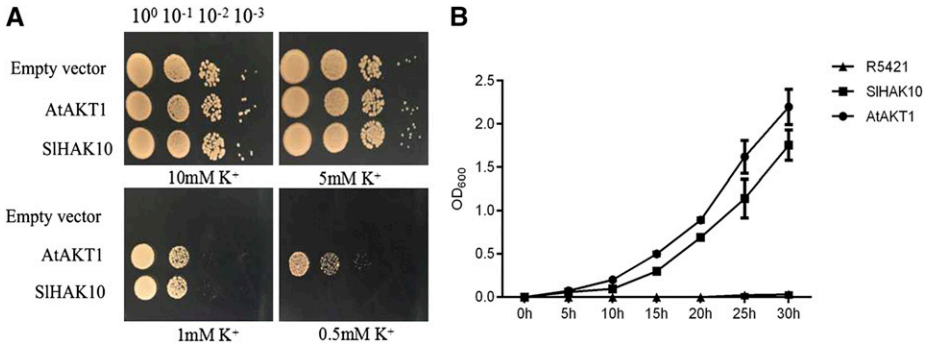

Figure 4. Functional complementation of *SlHAK10* for K^+ acquisition in the K^+ uptake-defective yeast strain R5421. A, Growth status of R5421 cells expressing AtAKT1, empty vector (pYES2), and SlHAK10 on AP medium supplemented with 10, 5, 1, and 0.5 mM KCl. Each transformant was precultured in liquid AP medium containing 100 mM KCl. The 1:10 serial dilutions of yeast cells were spotted on the AP medium and then incubated at 30°C for 6 d. B, Growth kinetic analysis of the yeast R5421 cells expressing AtAKT1, empty vector, and SlHAK10 cultured in liquid AP medium containing 1 mM KCl. Errors bars represent SD of five biological replicates.

available K and 18.5 mg kg^{-1} available Pi). Two plants (mutant or wild type) were grown in the RFC in the presence or absence of *R. irregularis* for 5 weeks. All the plants were watered weekly with nutrient solution containing either 2 mM K^+ as a high-K^+ condition or 0.2 mM K^+ as a low-K^+ condition. To monitor whether fungal hyphae could reach and take up nutrients from HCs, additional ^{15}N-labled $Ca(NO_3)_2$ was introduced into the two HCs by watering. Compared with the inoculated wild-type and mutant plants, ^{15}N accumulation in all the noninoculated plants was extremely low (Supplemental Fig. S7B), suggesting that fungal hyphae can absorb nutrients from HCs and almost no ion diffusion across the nylon meshes occurred. In all the treatments, the mutant plants showed a comparable total biomass with the wild-type plants (Fig. 5, A and B). No significant difference in the K^+ accumulation could be detected between the NM wild-type and mutant plants grown under both the K^+ supply conditions (Fig. 5, C–F), while the K^+ contents decreased by nearly 20% in both shoots and roots of the mycorrhizal mutant plants compared with those in the mycorrhizal wild-type plants (Fig. 5, D and F). As it is well established that AM fungal colonization has a dominant contribution to plant Pi nutrition, the Pi contents in these plants were also determined. As expected, all the inoculated wild-type and mutant plants contained significantly higher Pi in both shoots and roots than those in the NM plants (Supplemental Fig. S8, A–D). The Pi contents in mycorrhizal mutant plants grown under low K^+ were significantly decreased compared with those in the mycorrhizal wild-type plants (Supplemental Fig. S8, B and D). The mycorrhizal mutant plants also contained a significantly lower Pi in their roots than the mycorrhizal wild-type plants grown under high K^+ (Supplemental Fig. S8C).

To determine whether *SlHAK10* might be involved in the regulation of AM symbiosis, the degree of AM colonization in the mycorrhizal roots of the wild type and mutant lines was assessed. Compared with wild-type plants, both the total colonization rate and arbuscule abundance were significantly reduced in the mutant lines grown under the low-K^+ supply condition (Fig. 6, A and B), while the arbuscule morphology in the mutant lines was not significantly impaired (Fig. 6, C and D). These findings suggest that SlHAK10 is involved in mycorrhizal K^+ uptake but is not essential for arbuscule development. Several recent studies have pointed to an important role of the host-derived fatty acids in maintaining AM symbiosis

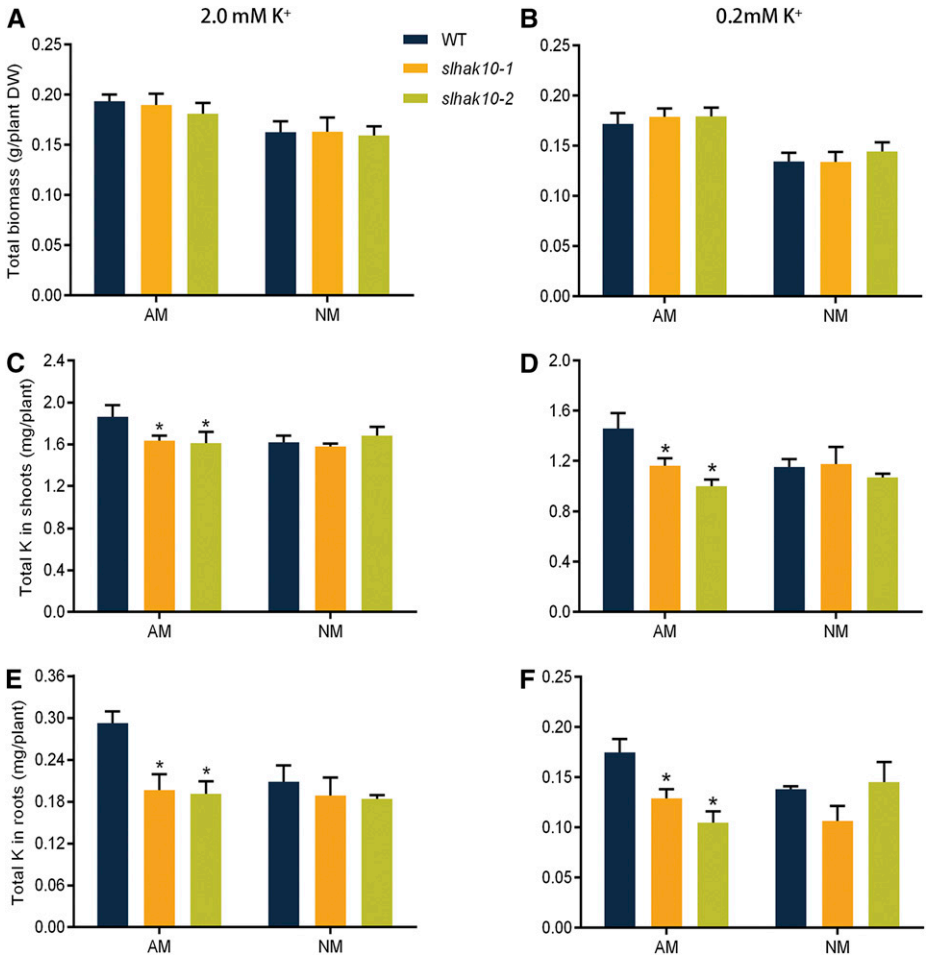

Figure 5. Effects of mutation of *SlHAK10* on the total biomass and K^+ accumulation in tomato grown under a compartmented culture condition. All the compartments were filled with a 4:1 mixture of sand and low-K^+ soil (the soil contains 30.2 mg kg^{-1} available K and 18.5 mg kg^{-1} available Pi). Wild-type (WT) and mutant plants were grown in the RFC for AM fungal inoculation. Plants were watered weekly with either high-K^+ (2 mM K^+) or low-K^+ (0.2 mM K^+) solution. The total biomass (A and B) and K^+ contents in shoots (C and E) and roots (D and F) of the wild-type and mutant plants were determined at 5 weeks postinoculation. AM, Mycorrhizal plants; DW, dry weight; NM, nonmycorrhizal plants. Error bars indicate SE ($n = 5$). The asterisks indicate significant differences ($P < 0.05$).

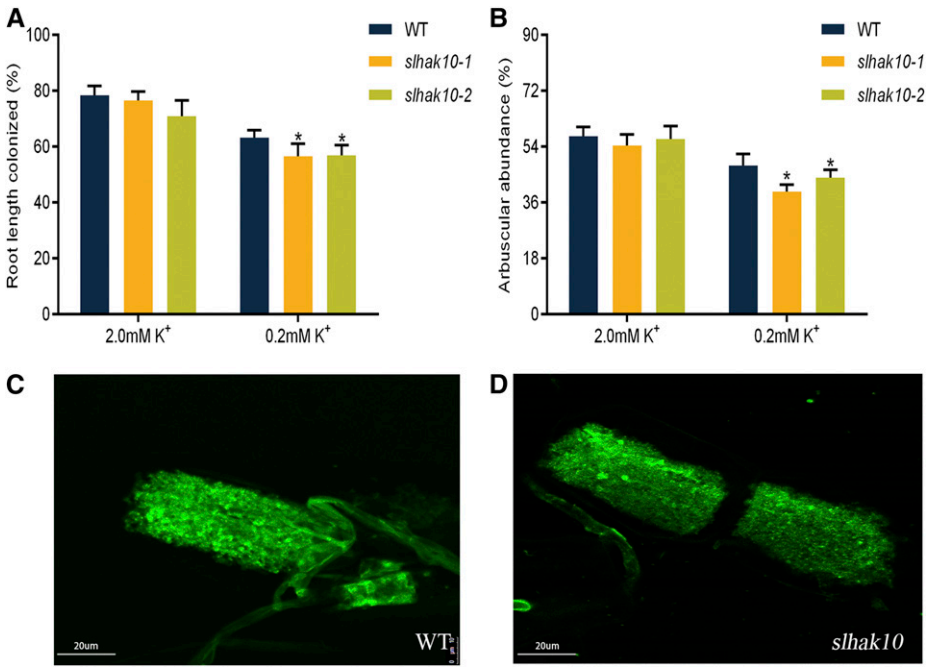

Figure 6. Effects of mutation of *SlHAK10* on the AM colonization and arbuscule morphology. Wild-type (WT) and mutant plants were grown in a compartmented culture system. Plants were watered weekly with either high-K^+ (2 mM K^+) or low-K^+ (0.2 mM K^+) solution. The total root length colonization (A) and arbuscule abundance (B) in the mycorrhizal roots of wild-type and mutant plants were assessed at 5 weeks postinoculation. Arbuscules were stained with WGA-AF-488 in the mycorrhizal roots of wild-type (C) and mutant (D) plants. Error bars indicate SE ($n = 5$). The asterisks indicate significant differences ($P < 0.05$).

(Bravo et al., 2017; Jiang et al., 2017, 2018; Keymer et al., 2017; Luginbuehl et al., 2017). The assay of fatty acid contents in the mycorrhizal roots of wild-type and *slhak10* mutant plants showed no significant difference under both the K^+ supply conditions (Supplemental Fig. S9). A further investigation of the soluble sugar content revealed that in the absence of AM fungal colonization, both the wild type and *slhak10* mutants supplied with low K^+ contained significantly higher soluble sugar in their shoots, but a lower amount in their roots, compared with plants grown under high K^+ (Fig. 7). The soluble sugar content in the mycorrhizal roots of mutant plants was significantly lower than that in the wild-type mycorrhizal roots under the low-K^+ supply condition (Fig. 7, C and D).

Overexpressing *SlHAK10* Improves K^+ Uptake and Mycorrhizal Colonization under K^+ Deficiency Conditions

To assess whether overexpression of *SlHAK10* can facilitate K^+ uptake, we further generated transgenic tomato plants overexpressing *SlHAK10* under the control of either the CaMV35S promoter or the AM-specific promoter *pSmPT4*. We have previously proven that *pSmPT4*, the promoter of an eggplant (*Solanum melongena*) Pi transporter, could drive GUS reporter expression exclusively in AM fungus-colonized cells (Chen et al., 2007, 2011). RT-qPCR analysis showed that the expression of *SlHAK10* was greatly increased in the mycorrhizal roots of the overexpression (OE) lines (Supplemental Fig. S10). Four independent transgenic lines (*35S-OE2* and *35S-OE6*, overexpression driven by the CaMV35S promoter; and *pSmPT4-OE1* and *pSmPT4-OE4*, overexpression driven by the *pSmPT4* promoter) were thus selected for analysis of K^+ absorption in sand-based pot culture inoculated with *R. irregularis* for 5 weeks. All the plants were irrigated with nutrient solution containing either 2 mM K^+ as a high-K^+ condition or 0.2 mM K^+ as a low-K^+ condition. In the presence of AM colonization, overexpressing *SlHAK10* could significantly increase the biomass of plants grown under the low-K^+ condition (Fig. 8, A and B). Compared with wild-type plants, all the 35S-OE lines showed a significantly higher K^+ accumulation in both shoots and roots under both K^+ supply conditions, irrespective of the presence or absence of AM inoculation (Fig. 8, C–F). It is worth noting that the NM 35S-OE plants showed a comparable K content in both shoots and roots to the mycorrhizal 35S-OE plants, suggesting that constitutive overexpression of *SlHAK10* may mask the contribution of the mycorrhizal K uptake pathway mediated by the native SlHAK10. This speculation seems to be reasonable, as the transcripts of *SlHAK10* were increased nearly 100-fold in the mycorrhizal roots of 35S-OE lines (Supplemental Fig. S10). With respect to the pSmPT4-OE lines, increased K^+ uptake, as indicated by significantly higher shoot and root K^+ contents compared with the wild-type plants, could be detected only in the mycorrhizal plants. As expected, no significant difference in either plant biomass or K^+ content could be detected between the NM wild-type and pSmPT4-OE plants grown under both the K^+ conditions (Fig. 8).

The wild type plants grown under low-K^+ conditions showed a significant decrease in AM colonization compared with the plants grown under the high-K^+ conditions (Fig. 9). Overexpressing *SlHAK10* from either promoter significantly improved the total fungal colonization and arbuscule abundance under the low-K^+

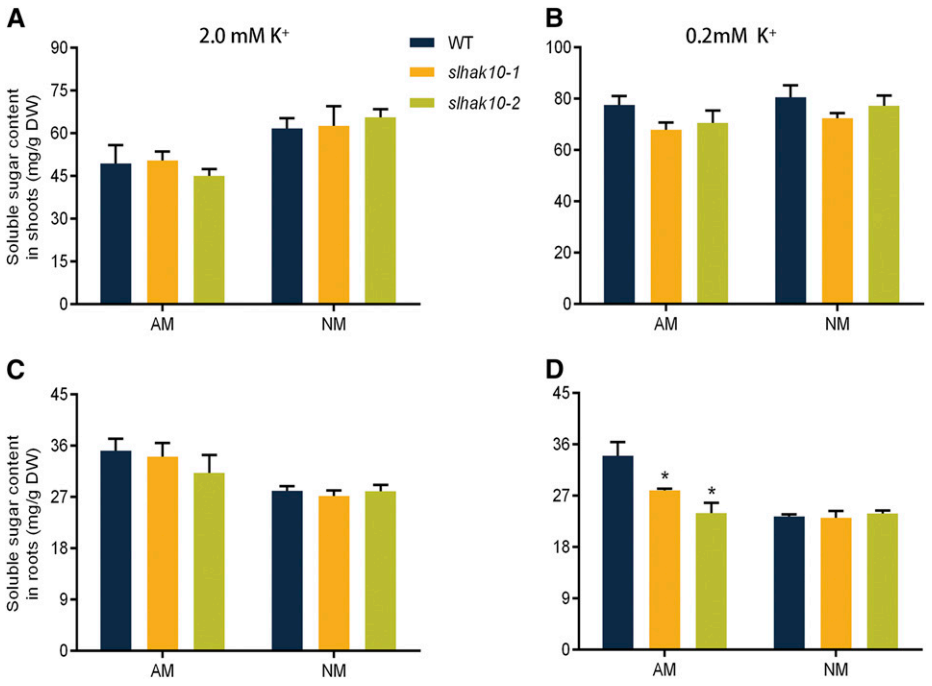

Figure 7. The contents of soluble sugar in the wild-type (WT) and *slhak10* mutant plants. Wild-type and mutant plants were grown in a compartmented culture system. Plants were watered weekly with either high-K^+ (2 mM K^+) or low-K^+ (0.2 mM K^+) solution. The soluble sugar concentrations in the shoots (A and B) and roots (C and D) of wild-type and mutant plants were determined at 5 weeks postinoculation. AM, Mycorrhizal plants; DW, dry weight; NM, nonmycorrhizal plants. Error bars indicate SE ($n = 5$). The asterisks indicate significant differences ($P < 0.05$).

condition. Consistent with this, the Pi contents in the mycorrhizal overexpressing lines grown under low K^+ were also significantly increased compared with the wild-type plants (Supplemental Fig. S11). The increased mycorrhizal colonization in the roots of SlHAK10-OE plants could also be seen in the increased expression of *SlPT4* and *RiTub* (Fig. 9, C and D), the two marker genes used for evaluating AM colonization (Floss et al., 2013; Liao et al., 2015). A further assay of the soluble sugar contents showed that the SlHAK10-OE plants grown under low K^+ contained significantly higher soluble sugar contents in their mycorrhizal roots compared with the wild-type plants (Fig. 10).

DISCUSSION

Contributing Effects of AM Fungal Colonization on Tomato Plant Growth and K^+ Acquisition

Improving the availability of mineral nutrients for plants is one of the major contributions of AM symbiosis. Although the role of AM symbiosis in plant K^+ nutrition received much less attention than Pi and N (Mäder et al., 2000; Nagy et al., 2006; Chen et al., 2007; Javot et al., 2007), an increased K accumulation in distinct tissues upon AM symbiosis has been reported related to diverse plant species (Perner et al., 2007; Baslam et al., 2013; Oliveira et al., 2016). Physiological analysis of the AM-associated responses of *M. truncatula* plants revealed a significant increase of root biomass and K^+ content in AM plants under the K^+ deprivation condition (Garcia et al., 2017). In this study, we also observed that the AM tomato plants had a higher root biomass by more than 20% than the NM plants under the low-K^+ condition (Fig. 1B). However, in the high-K^+ treatment, the AM plants showed more growth advantages, with shoot and root biomass increased by nearly 40% compared with the NM control plants (Fig. 1, A and B). Moreover, inoculation with AM fungus resulted in a much higher K^+ accumulation in AM plants than the NM plants, irrespective of K^+ regimen (Fig. 1, C and D). These findings highlighted the positive roles of AM fungal colonization in tomato plant growth and K^+ acquisition. However, it could not be ruled out that the increased K^+ accumulation in mycorrhizal tomato plants might be a partial consequence of an improved Pi uptake, as earlier studies have proposed K^+ to be one of the major counter-ions of polyphosphates, which forms a phosphate reserve for the host plant in AM symbiosis (Bücking and Heyser, 1999). A recent study of the K nutrition of ectomycorrhizal *Pinus pinaster* showed that altered K^+ transfer toward the host plant could affect fungal Pi translocation to the shoots, suggesting that interactions would also take place during the uptake of K^+ and Pi by ectomycorrhizal plants (Garcia et al., 2014).

SlHAK10 Is Involved in the Mycorrhizal K^+ Uptake at the Intraradical Symbiotic Interface

The increased K^+ accumulation in mycorrhizal tomato plants is a strong hint of the presence of an AM symbiotic pathway for K^+ uptake in tomato. SlHAK10, the AM-induced K^+ transporter, was proposed to be the suitable candidate for K^+ uptake at the symbiotic interface, as its expression was specifically confined in the cells containing arbuscules (Fig. 2). Its capacity in K^+ transport was demonstrated in both the heterologous

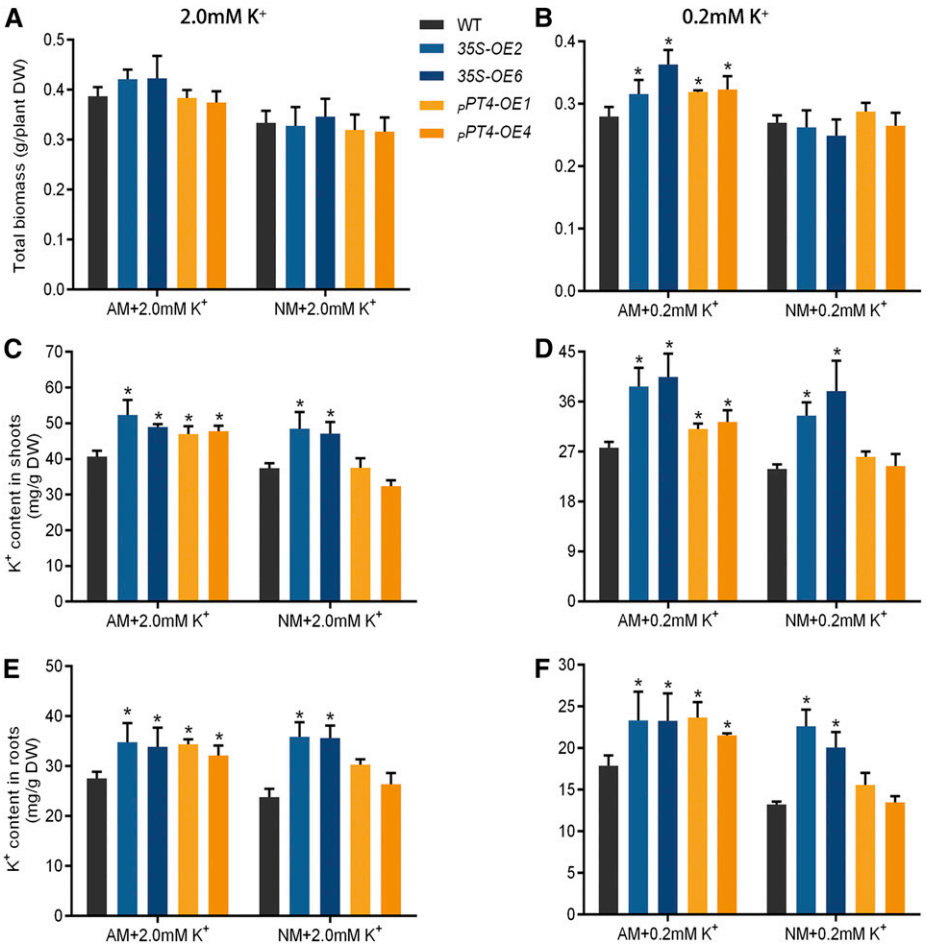

Figure 8. Effects of the overexpression of *SlHAK10* on tomato growth and K^+ acquisition under a sand-based pot culture condition. For 35S-OE, *SlHAK10* was overexpressed under the control of the constitutive CaMV35S promoter; for pPT4-OE, *SlHAK10* was overexpressed under the control of the mycorrhiza-specific Pi transporter promoter *pSmPT4*. Two-week-old seedlings were grown in sand-based pot culture for AM fungal inoculation for 5 weeks. The plants were treated with either high-K^+ (2 mM K^+) or low-K^+ (0.2 mM K^+) solution. AM, Mycorrhizal plants; DW, dry weight; NM, nonmycorrhizal plants. A and B, Total biomass. C and D, K^+ concentration in shoots. E and F, K^+ concentration in roots. Error bars indicate SE ($n = 5$). The asterisks indicate significant differences ($P < 0.05$).

yeast system and transgenic plants. Overexpression of *SlHAK10* with the constitutive CaMV35S promoter led to a significant increase of K^+ uptake under both the high-K^+ (2 mM) and low-K^+ (0.2 mM) conditions, regardless of the presence or absence of AM fungal colonization (Fig. 8).

The evidence for the action of *SlHAK10* in mycorrhizal K^+ uptake was obtained through the analysis of *slhak10* mutants in the compartmented culture in the inoculation condition. Knockout of *SlHAK10* significantly reduced the uptake of K^+ from the HCs, resulting in lower K^+ contents in both shoots and roots of the *slhak10* mutants relative to the wild-type plants (Fig. 5). More indirect evidence was obtained through the overexpression of *SlHAK10* in tomato under the control of a strong, AM-specific promoter, *pSmPT4*. Overexpressing *SlHAK10* exclusively in mycorrhizal roots resulted in a significant increase of K^+ content in both shoots and roots of the mycorrhizal transgenic plants compared with the wild-type plants (Fig. 8). Interestingly, the 35S-OE mycorrhizal plants seemed to have a higher K^+ content in shoots than the pSmPT4-OE plants grown under low K^+ (Fig. 8D). Such a discrepancy in K^+ accumulation might be caused by different expression efficiency and tissue localization of SlHAK10 controlled by the two kinds of promoters. A previous study demonstrated that blocking mycorrhizal Pi transport by silencing the AM-induced Pi transporter *MtPT4* in *M. truncatula* could severely impair arbuscule formation (Javot et al., 2007). The absence of defective arbuscular morphology in the *slhak10* mutants observed in this study (Fig. 5) suggests that transport of K^+ across the symbiotic interface might not be a requirement for arbuscule development. Even so, the identification of an orthologue of *SlHAK10* in *L. japonicus* that also showed an inducible response to AM symbiosis (Guether et al., 2009) is evidence that the regulatory mechanism regarding the AM-mediated K^+ uptake pathway might be conserved across different plant families.

Improving Plant K^+ Nutrition May Facilitate AM Colonization under the K^+ Deficiency Condition

The availability of mineral nutrients, in particular Pi, has a strong impact on AM colonization (Nagy et al., 2009; Breuillin et al., 2010; Nouri et al., 2014; Johnson et al., 2015). Pi and N starvation was also characterized as being able to induce a stress-responsive transcriptional reprogramming that is beneficial for AM colonization (Bonneau et al., 2013). However, the effect of

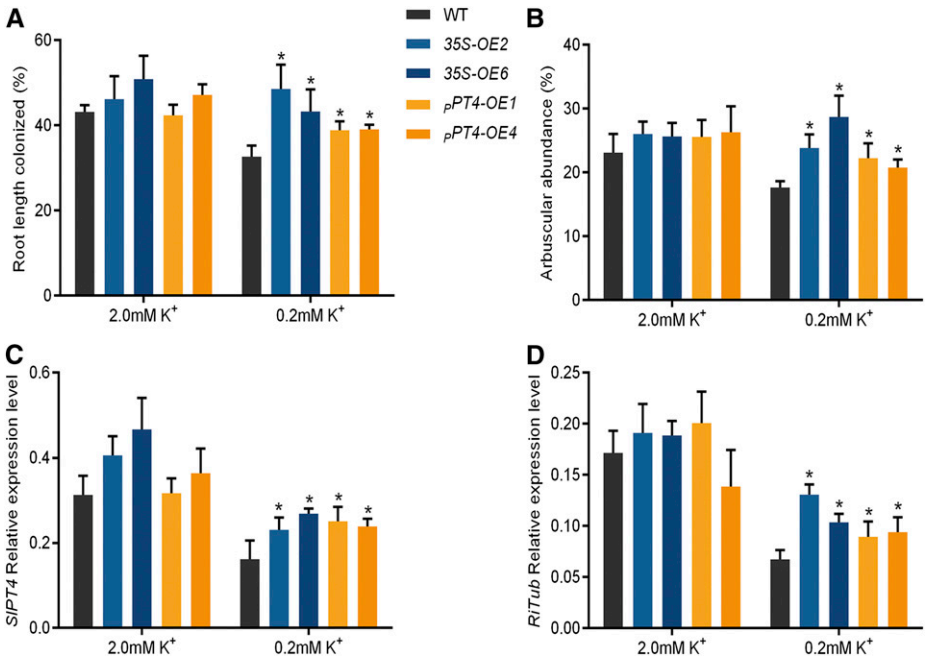

Figure 9. Effects of overexpression of *SlHAK10* on AM colonization efficiency under a sand-based pot culture condition. Two-week-old seedlings were grown in sand-based pot culture for AM fungal inoculation. The plants were treated with either high-K^+ (2 mM K^+) or low-K^+ (0.2 mM K^+) solution. A and B, The total root length colonization (A) and arbuscule abundance (B) in the mycorrhizal roots of wild-type (WT) and *SlHAK10*-overexpressing plants were assessed at 5 weeks postinoculation. C and D, The transcript levels of two AM-related marker genes, *SlPT4* (*LePT4*) and *RiTub* from tomato and *R. irregularis*, respectively. Error bars indicate SE ($n = 5$). The asterisks indicate significant differences ($P < 0.05$).

plant K^+ nutrition on AM colonization has not been well characterized thus far. A recent study regarding the mycorrhizal *M. truncatula* plants showed that K^+ availability did not affect the establishment of AM symbiosis (Garcia et al., 2017). However, the conclusions derived from different symbiotic systems were not always consistent, as in some other studies a positive effect on AM colonization by K^+ application has been proposed (Zhang et al., 2017).

In this study, we found that mycorrhizal tomato plants supplied with high K^+ had a significantly higher AM colonization rate and arbuscule incidence than the K^+-deficient plants (Fig. 1F; Supplemental Fig. S1). Previous studies have shown that in K^+ starvation plants, the export of carbohydrates from source leaves and the allocation to roots were heavily interrupted, leading to a severe repression in root growth and a typical decrease in root-shoot ratio (Cakmak et al., 1994a, 1994b; Deeken et al., 2002; Hermans et al., 2006; Kanai et al., 2007; Pettigrew, 2008; Cai et al., 2012). In tomato, it has been revealed that K^+ deficiency caused a decreased sugar accumulation in roots (Kanai et al., 2007). In this study, we also observed a significant increase of soluble sugar contents in shoots but a decrease in roots of both

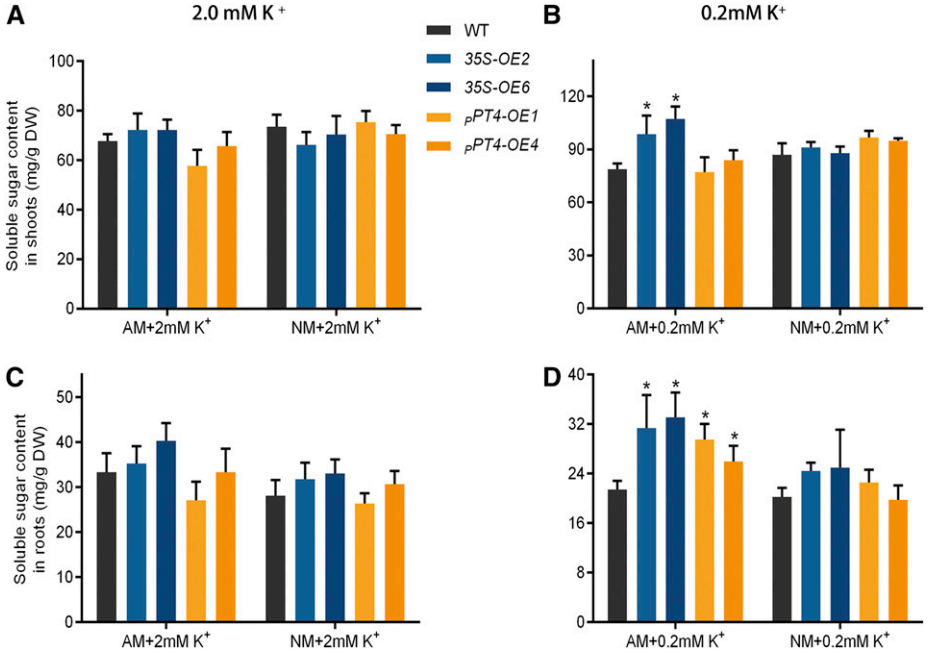

Figure 10. The contents of soluble sugar in the wild-type (WT) and *SlHAK10*-overexpressing plants. Two-week-old seedlings were grown in sand-based pot culture for AM fungal inoculation. The plants were treated with either high-K^+ (2 mM K^+) or low-K^+ (0.2 mM K^+) solution. The soluble sugar concentrations in the shoots (A and B) and roots (C and D) of wild-type and *SlHAK10*-overexpressing plants were determined at 5 weeks postinoculation. DW, Dry weight. Error bars indicate SE ($n = 5$). The asterisks indicate significant differences ($P < 0.05$).

the wild type and mutants grown under K^+ deficiency (Fig. 7). Additionally, overexpressing *SlHAK10* by either the constitutive CaMV35S promoter or the AM-specific *pSmPT4* promoter under K^+ deficiency led to not only an improvement in K^+ absorption but also a significant increase in AM colonization and soluble sugar accumulation in roots (Figs. 8–10). Considering that maintenance of AM symbiosis requires the transfer of substantial amounts of plant-fixed carbohydrates to AM fungi, it is reasonable to speculate that improving K^+ uptake under K^+ deficiency may promote carbohydrate allocation to roots, which facilitates root growth and AM colonization. Such a hypothesis could gain indirect support from an earlier study reporting that silencing a fungal monosaccharide transporter, MST2, with a broad substrate spectrum, impaired mycorrhiza formation in *M. truncatula* mycorrhizal roots (Helber et al., 2011).

In conclusion, in this study, we characterized the potential interactions between K^+ nutrition and AM symbiosis in tomato and assessed the function of a K^+ transporter, SlHAK10, in both the heterologous yeast system and transgenic plants. We demonstrated that there is a *SlHAK10*-mediated mycorrhizal K^+ uptake pathway in tomato and that improving plant K^+ nutrition under the low-K^+ condition may increase carbohydrate accumulation in roots and facilitate AM fungal colonization. As a mutually stimulating mechanism has been revealed to exist during the simultaneous exchange of C and Pi/N between the AM partners, it would be interesting to investigate in the future whether the free-market model is also applicable to the exchange of K and C during AM symbiosis.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Tomato (*Solanum lycopersicum* 'MicroTom') plants were used in this study. Seeds of wild-type and transgenic plants were surface sterilized with 10% (v/v) sodium hypochlorite for 10 min and then germinated in a growth chamber programmed for 16 h of light at 28°C and 8 h of dark at 20°C. The seedlings were then transplanted to sterilized quartz sand for a 2-week culture irrigated with full-strength nutrient solution containing the following: 1 mM NH_4^+, 4 mM NO_3^-, 2 mM K^+, 1 mM Pi, 0.75 mM Ca^{2+}, 0.5 mM Mg^{2+}, 0.25 mM Cl^-, 0.5 mM SO_4^{2-}, 20 μM Fe^{2+}, 9 μM Mn^{2+}, 46 μM BO_3^{3-}, 8 μM Zn^{2+}, 3 μM Cu^{2+}, and 0.03 μM MoO_4^{2-}. The plantlets were then transferred to either sand-based pot culture or a compartmented culture system for inoculation with AM fungi.

For sand-based pot culture, three plantlets were transplanted to a 3-dm^3 pot filled with sterilized sand. A sand-based mycorrhizal inoculum containing *Rhizophagus irregularis* (previously named *Glomus intraradices*) was used for colonization. Each plantlet was inoculated with 1.2 g of inoculum containing approximately 200 spores around the roots. The NM control plants were obtained by inoculation with autoclaved inoculum. The plants were supplied with nutrient solution including either 2 mM K^+ as a high-K^+ treatment or 0.2 mM K^+ as a low-K^+ treatment. To guarantee high mycorrhizal colonization, the plants in pot culture were watered with a relatively low concentration (50 μM) of Pi.

A compartmented culture system was employed to investigate the contribution of *SlHAK10* to mycorrhizal K uptake by using two loss-of-function *slhak10* mutant lines. The culture system contains an RFC and two HCs (Supplemental Fig. S7A). All three compartments were filled with a 4:1 mixture of sand and soil (the soil contains 30.2 mg kg^{-1} available K and 18.5 mg kg^{-1} available Pi). Two mutant lines or wild-type plants were grown in the RFC in the presence or absence, respectively, of *R. irregularis* inoculation for 5 weeks. All the plants were watered weekly with nutrient solution containing either 2 mM K^+ as a high-K^+ condition or 0.2 mM K^+ as a low-K^+ condition. To prevent ion diffusion between the RFC and HCs, the RFC was separated from the HCs by two iron gauzes with a 0.5-cm air gap. A 30-μm nylon mesh was placed between the two iron gauzes to prevent roots from entering the HCs. To monitor whether fungal hyphae could reach and take up nutrients from HCs, additional ^{15}N-labeled $Ca(NO_3)_2$ was introduced into the two HCs by watering, and ^{15}N accumulation in the mycorrhizal plants and NM plants was determined.

RT-qPCR

For performing RT-qPCR analysis, approximately 2 μg of total RNA from each sample was used to synthesize cDNA using a reverse transcription kit (Takara), and the synthesized cDNAs were used as templates for the following RT-qPCR analysis. The PCR was conducted on an Applied Biosystems Plus Real-Time PCR System using the SYBER premix ExTaq kit (Takara). The specific primer pairs for each of the HAK genes are listed in Supplemental Table S1. The relative transcript abundance of each target gene was standardized to the transcript level of a tomato constitutive *Actin* gene (Chen et al., 2014).

Identification of HAK Genes in Tomato

Members of the KT/KUP/HAK gene family in the tomato genome were identified using the BLASTN and TBLASTN algorithms. To identify the potential HAK genes in the tomato genome, the coding sequence of the AM-induced *Lotus japonicus* K^+ transporter (Lj4g3v3116360.1; Guether et al., 2009) was employed for the BLAST search against the tomato genomic sequence database (www.sgn.cornell.edu). Sequences with a query over 50% and *E* value less than −10 were taken as the candidates. All obtained sequences were submitted to the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for further confirmative analysis.

Subcellular Localization Analysis

For subcellular localization analysis of SlHAK10 by agroinfiltration of tobacco (*Nicotiana benthamiana*) leaves, the full-length coding sequence of SlHAK10 without the stop codon was first ligated to an intermediate vector, pSAT6AEGFP-N1. The SlHAK10-GFP fusion construct as well as GFP alone were then subcloned into the expression vector pRCS2-ocs-nptII with the rare restriction enzyme PI-*Psp*I. The plasmids were then transformed into the *Agrobacterium tumefaciens* strain EHA105. The bacterial cells were harvested by centrifugation and resuspended in a solution (pH 5.7) containing 10 mM MES, 10 mM $MgCl_2$, and 200 μM acetosyringone. Cell suspensions at an optical density (600 nm) of 0.1 were infiltrated into the leaves of tobacco using a needle-free syringe. GFP fluorescence in the transformed leaves was imaged using confocal microscopy (Leica Confocal TCS-SP8) after 48 to 72 h.

Construction of a Binary Vector and Plant Transformation

To construct the *SlHAK10* promoter vector, a 2,281-bp-long promoter fragment of *SlHAK10* immediately upstream of the translation start ATG was amplified by PCR to introduce *Hin*dIII and *Bam*HI restriction sites at the end of the 5′ and 3′ regions, respectively. After digestion with the two restriction enzymes, the amplified fragments were cloned into binary vector pBI121 to replace the CaMV35S promoter in front of the GUS reporter gene. The generated vector was used for tomato transformation by the EHA105 strain.

To construct the *SlHAK10* constitutive overexpression vector, the coding sequence of *SlHAK10* was amplified and cloned into the binary vector pCAMBIA 1300 using the ClonExpress II One Step Cloning Kit (Vazyme Biotech). The resulting construct (named *35S-SlHAK10*), in which *SlHAK10* was controlled by the CaMV35S promoter, was introduced into *A. tumefaciens* EHA105 strain for transformation of tomato and rice (*Oryza sativa*) plants. To construct the *SlHAK10* AM-specific overexpression vector, an 865-bp-long promoter fragment of the AM-specific Pi transporter gene *SmPT4* (Chen et al., 2011) was amplified and cloned into the *35S-SlHAK10* construct to replace the CaMV35S promoter. The recombinant construct (named *pSmPT4-SlHAK10*) was also introduced into the EHA105 strain for transformation of tomato plants.

The CRISPR/Cas9 gene knockout constructs were generated using the commercial vector VK005-03 (Viewsolid Biotech). Two spacers were designed

to target two different sites in exons of *SlHAK10*. For site 1, we used the oligonucleotide pair 5′-TGGATCCTTGCCATATCAAATC-3′ and 5′-AACGAT TTGATATGGCAAGGAT-3′, and for site 2, we used the oligonucleotides 5′-TGGACAGACCTAAGTCCCGGACT-3′ and 5′-AACAGTCCGGGACTTAGG TCTGT-3′. The oligonucleotide pairs were first annealed to produce a double-stranded fragment with three-nucleotide 5′ overhangs at both ends and then ligated into the linearized vector VK005-03.

A. tumefaciens-mediated transformation of tomato plants was performed according to the protocol described by Sun et al. (2006). The transformation of rice plants was carried out as described previously (Upadhyaya et al., 2000).

Functional Characterization of *SlHAK10* in Yeast

The full-length coding sequences of *SlHAK10* and *AtAKT1* were inserted into yeast expression vector p-YESII under the control of the Met promoter (Bernstein et al., 2013). The resulting vectors were transformed into the yeast mutant strain R5421 (MATα ura3-52 leu2 trk1Δ his3Δ200 his4-15 trk2Δ1::pCK64), in which two endogenous K^+ transporter genes (*TRK1* and *TRK2*) were deleted (Li et al., 2014). The yeast cell transformation was performed using the LiAC/single-stranded DNA/polyethylene glycol method, and the growth assays on AP medium were performed as described by Horie et al. (2011).

Determination of the Concentrations of K, P, and Soluble Sugars

The dried plant material was digested with 98% H_2SO_4 and 30% hydrogen peroxide as described previously (Chen et al., 2007). The K^+ concentration in the solution was measured by an ICP emission spectrometer (Optima 2100DV; Perkin-Elmer) as described by Cai et al. (2012). The total Pi concentration was measured using the Molybdate Blue method as described previously (Chen et al., 2007). The measurement of soluble sugar concentrations was conducted based on the method of Hansen and Møller (1975), as described in detail by Chen et al. (2015).

Analysis of Fatty Acid Contents

Extraction of fatty acid methyl esters from tomato roots was performed using the modified method of Browse et al. (1986) and Jiang et al. (2017). Briefly, 50 mg of root samples was finely ground in liquid N and then transferred into 2-mL centrifugation tubes containing 1 mL of 5% (v/v) H_2SO_4 in methanol. The samples were then heated to 80°C for 1 h. After cooling, extraction solution containing 30 μL of methyl nonadecanoate, 0.3 mL of hexane, and 1 mL of 0.9% NaCl was added to the samples and then shaken vigorously for 5 min. After centrifugation, the hexane phase containing the fatty acid methyl esters was collected and used for determination by gas chromatography-mass spectrometry (GC-FID, 7890A Plus GC; Agilent). The parameters for the gas chromatography-mass spectrometry assay were used as described by Jiang et al. (2017).

Detection of Mycorrhizal Colonization and Histochemical GUS Staining

The examination of mycorrhizal fungal colonization was performed using the magnified line intersection method (McGonigle et al., 1990). Root samples were cut randomly into 1-cm pieces and cleared for 3 h at 70°C in 10% (w/v) KOH solution, and then the root segments were stained in 0.3% Trypan Blue at 90°C for 2 h. Ten 1-cm root segments were mounted on microscope slides, and each of these segments was investigated at 10 randomly chosen spots, which were scored for the presence of hyphae, arbuscule, vesicle, or none (absence of any fungal structures). For each root system, three to four microscope slides were employed. The total colonization level (root length colonization percentage) was calculated by the formula (number of intersections cutting through any fungal structures)/(total number of intersections examined); and the arbuscular colonization level (arbuscule abundance percentage) was calculated by the formula (number of intersections cutting through at least one arbuscule)/(total number of intersections examined). At least three different root systems were evaluated for each genotype. Histochemical staining of the GUS activity in transgenic plants was performed as described previously (Karandashov et al., 2004).

Statistical Analysis

The data were analyzed by ANOVA (SPSS 16.0; SPSS), followed by Tukey's test ($P < 0.05$) to test differences between different plant genotypes and treatments. The data represent means ± SE of five independent biological replicates.

Accession Numbers

Sequence data from this article can be found in the aramemnon data libraries (http://aramemnon.botanik.uni-koeln.de/) under the following accession numbers: SlHAK1 (Solyc08g015680), SlHAK2 (Solyc06g050170), SlHAK3 (Solyc12g096580), SlHAK4 (Solyc12g017910), SlHAK5 (Solyc12g005670), SlHAK6 (Solyc02g087000), SlHAK7 (Solyc04g025880), SlHAK8 (Solyc05g005740), SlHAK9 (Solyc10g047270), SlHAK10 (Solyc03g097860), SlHAK11 (Solyc01g105150), SlHAK12 (Solyc05g005720), SlHAK13 (Solyc09g074800), SlHAK14 (Solyc04g008450), SlHAK15 (Solyc09g074790), SlHAK16 (Solyc12g005680), SlHAK17 (Solyc06g051830), SlHAK18 (Solyc02g031840), SlHAK19 (Solyc02g068590), SlHAK20 (Solyc04g025990), and SlHAK21 (Solyc09g074820).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Effects of different K^+ applications and AM fungal colonization on root-shoot ratio and K^+ uptake.

Supplemental Figure S2. Effects of different K^+ applications on AM colonization.

Supplemental Figure S3. Phylogenetic analysis and exon/intron organization of tomato HAK transporters.

Supplemental Figure S4. Expression analysis of tomato HAK genes in response to AM symbiosis.

Supplemental Figure S5. Tissue-specific expression analysis of *SlHAK10*.

Supplemental Figure S6. Screening the homozygous mutant lines of *SlHAK10* by PCR sequencing.

Supplemental Figure S7. Effects of mutation of *SlHAK10* on tomato growth under a compartmented culture condition.

Supplemental Figure S8. Effects of mutation of *SlHAK10* on mycorrhizal Pi uptake in tomato plants grown under a compartmented culture condition.

Supplemental Figure S9. Fatty acid contents in the mycorrhizal roots of wild-type and *slhak10* mutant plants.

Supplemental Figure S10. *SlHAK10* is overexpressed in mycorrhizal roots of transgenic tomato plants.

Supplemental Figure S11. Effects of overexpression of *SlHAK10* on tomato Pi acquisition under a sand-based pot culture condition.

Supplemental Table S1. List of the primers used for RT-qPCR analysis in this study.

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