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Identification and Characterization of Transcription Factors Regulating Arabidopsis *HAK5*

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Abstract

Potassium (K) is an essential macronutrient for plant growth and reproduction. *HAK5*, an Arabidopsis high-affinity K transporter gene, plays an important role in K uptake. Its expression is up-regulated in response to K deprivation and is rapidly down-regulated when sufficient K levels have been re-established. To identify transcription factors regulating *HAK5*, an Arabidopsis TF FOX (Transcription Factor Full-length cDNA Over-eXpressor) library containing approximately 800 transcription factors was used to transform lines previously transformed with a luciferase reporter gene whose expression was driven by the *HAK5* promoter. When grown under sufficient K levels, 87 lines with high luciferase activity were identified, and endogenous *HAK5* expression was confirmed in 27 lines. Four lines overexpressing *DDF2* (Dwarf and Delayed Flowering 2), *JLO* (Jagged Lateral Organs), *TFII_A* (Transcription initiation Factor II_A gamma chain) and *bHLH121* (basic Helix–Loop–Helix 121) were chosen for further characterization by luciferase activity, endogenous HAK5 level and root growth in K-deficient conditions. Further analysis showed that the expression of these transcription factors increased in response to low K and salt stress. In comparison with controls, root growth under low K conditions was better in each of these four TF FOX lines. Activation of *HAK5* expression by these four transcription factors required at least 310 bp of upstream sequence of the *HAK5* promoter. These results indicate that at least these four transcription factors can bind to the *HAK5* promoter in response to K limitation and activate *HAK5* expression, thus allowing plants to adapt to nutrient stress.

Keywords: Arabidopsis TFs FOX, *HAK5*, Potassium-deficient signaling

Abbreviations: ABRE, ABA-responsive element; ANOVA, analysis of variance; ARF, auxin response factor; bHLH121, basic helix–loop–helix 121; bZIP, basic leucine zipper protein; CE3, coupling element 3; CRF, cytokinin response factor; DDF2, dwarf and delayed flowering 2; DRE, drought-responsive element; ERE, ethylene-responsive element; ERF, ethylene response factor; GUS, β-glucuronidase; GR, glucocorticoid receptor; HAK5, high-affinity potassium transporter 5; HAK5pro::LUC, HAK5 promoter-fused luciferase reporter gene; IAAs, indole-3-acetic acid inducible genes; JLO, jagged lateral organs; LSM, low salt medium; ONPG, ortho-nitrophenyl-β-D-galactopyranoside; qRT–PCR, quantitative reverse transciption–PCR; RAP2.11, AP2/ERF transcription factor; ROS, reactive oxygen species; TFII_A, transcription initiation factor II_A gamma chain; TF FOX, Transcription Factor Full-length cDNA Over-eXpressor.

Introduction

Potassium (K) is an essential plant macronutrient and comprises 2–10% of plant dry weight. K is involved in the maintenance of turgor pressure, stomatal response and cell elongation (Marschner 1995). Although the importance of K uptake and low K signaling has been recognized, the mechanisms regulating these processes in plants is still largely unknown. Reactive oxygen species (ROS) (Shin and Schachtman 2004) and ethylene (Shin and Schachtman 2004, Jung et al. 2009) have been suggested as positive regulators of low K signaling, and auxin has also been linked to K availability (Philippar et al. 2006, Fujita et al. 2007, Osakabe et al. 2013).

Phytohormones have been considered important regulators of low K signaling. Arabidopsis MYB77 is a transcription factor involved in auxin signaling and interacts with auxin response factors (ARFs) (Shin et al. 2007). When grown in low K conditions, Arabidopsis plants showed a decrease in lateral root numbers, and the *myb77* mutant showed a decrease in lateral root density compared with the wild type. This phenotype is similar to the *tir1-1* mutant phenotype which has a mutation in the auxin receptor TIR1

(Shin et al. 2007). In maize, Philippar and colleagues (2006) reported that a K channel, ZMK1, plays an important role in auxin-regulated coleoptile elongation. These findings suggested that auxin is one of the key regulators of low K signaling. In addition to auxin, jasmonic acid is also involved in low K signaling. The expression of jasmonic acid-related genes is also linked to K limitation (Armengaud et al. 2004). Recently, Arabidopsis mutants with cytokinin deficiency and impaired receptors were used to demonstrate that cytokinin and cytokinin signaling are involved in low K-induced signal transduction (Nam et al. 2012). Arabidopsis DELLA proteins, which are negative regulators of gibberellin signaling, are involved in responses to phosphorus deprivation (Jiang et al. 2007). In wheat, the *Rht-B1* and *Rht-D1*-encoded DELLA proteins controlled the physiological responses in K deprivation (Moriconi et al. 2012).

Plants have many channels and transporters for the uptake and transport of ions and nutrients. In Arabidopsis, five major K transporter families have been identified, KUP/HAK/KT transporters (13 genes), Trk/HKT transporters (one gene), KCO K^+ channels (six genes), Shaker-type K^+ channels (nine genes) and K^+/H^+ antiporter homologs (six genes) (Maser et al. 2001). The KUP/HAK/KT transporter family is the largest, and gene members from this group are present in the genomes of all plants. Thirteen Arabidopsis and 17 rice KUP/HAK/KT transporters have been grouped into four clusters (clusters I–IV) (Rubio 2000, Banuelos et al. 2002). Cluster IV is only present in rice and includes OsHAK4 and OsHAK17. Even though many K transporters have been identified in plants, only a few of them have been studied in detail. The AtKUP2 mutant, *shy3-1*, showed a reduced cell size and a dwarf phenotype (Elumalai et al. 2002). High-affinity potassium transporter 5 (HAK5), an Arabidopsis cluster I transporter with a high affinity for K uptake (Rubio 2000, Gierth et al. 2005), was reported to be expressed in epidermal cells of roots (Gierth et al. 2005). Arabidopsis AKT1, an inward-rectifier K channel, also mediated the high-affinity K uptake in low K conditions (Pyo et al. 2010, Rubio et al. 2010). Transcript levels of *HAK5* increased in response to K starvation and rapidly decreased upon K resupply (Ahn et al. 2004). HAK5 mutants of Arabidopsis exhibited root growth inhibition under low K conditions (Qi et al. 2008). K transporters have also been linked to salt stress. Transcript levels of *AtHAK11* and *AtHAK6* were up-regulated during salt stress, while *AtHAK2* levels were reduced in salt-treated Arabidopsis (Maathuis 2006). These findings suggested that some K transporters are involved in abiotic stresses, especially salt stress (Nieves-Cordones et al. 2010).

Although multiple functional studies on HAK5 and *hak5* mutants have been carried out (Gierth et al. 2005, Qi et al. 2008, Rubio et al. 2008, Nieves-Cordones et al. 2010), the details of how HAK5 is regulated remain to be elucidated. Arabidopsis rare cold-inducible gene 3 (RCI3) and AP2/ERF transcription factor 2.11 (RAP2.11) were identified as positive regulators of HAK5 by an activation tagging method (Kim et al. 2010, Kim et al. 2012). The expression of *HAK5* was dramatically reduced in *rci3* knock-out plants (Kim et al. 2010). Kim and colleagues (2012) demonstrated that RAP2.11 can bind to a GCC-box (GCCGAC) element within the *HAK5* promoter and that overexpression of *RAP2.11* results in up-regulation of ethylene signaling-related genes, ROS production-related genes and calcium signaling-related genes in K-starved roots.

In this study, an Arabidopsis TF FOX (Transcription Factor Full length Over-eXpressor) library was transformed into Arabidopsis lines in which a luciferase gene driven by the *HAK5* promoter had been previously inserted. The resulting transformants were used to identify transcription factors that regulate *HAK5* expression. Four transcription factors [dwarf and delayed flowering 2 (DDF2), jagged lateral organs (JLO), basic helix–loop–helix 121 (bHLH121) and transcription initiation factor II_A gamma chain (TFII_A)] induced the expression of *HAK5* via binding to the *HAK5* promoter when plants were grown under low K conditions. TF FOX lines containing these transcription factors showed better root growth under low K compared with control plants. Based on our results, we suggest that these four transcription factors are potential regulators of low K signaling through their ability to modulate *HAK5* expression.

Results

Identification of transcription factors that activate the expression of Arabidopsis *HAK5*

The Arabidopsis *HAK5* is one of the few KT/KUP/HAK-type K transporters whose expression is up-regulated in response to K deprivation (Wang et al. 2002, Ahn et al. 2004, Hampton et al. 2004, Gierth et al. 2005). We previously developed Arabidopsis lines containing a gene construct consisting of the *HAK5* promoter fused with a luciferase gene (HAK5pro::LUC) (Qi et al. 2008). Roots in the plants of these reporter lines exhibit high levels of luciferase activity in response to low K (Qi et al. 2008). An Arabidopsis TF FOX library was transformed into three independent HAK5pro::LUC homozygous lines (HAK5pro::LUC-2, HAK5pro::LUC-8 and HAK5pro::LUC-12) in order to identify transcription factors activating *HAK5* expression. Luciferase activity in all of these lines was fully characterized and shown to be highly induced in response to low K (–K) conditions, but was barely detectable in plants receiving sufficient K. After transformation with the TF FOX library, T_1 seeds (about 18,000 seeds) were screened for kanamycin resistance. Selected plants were transferred and grown for 5 d on low salt medium (LSM) without an antibiotic and their luciferase activity was compared with that of control lines (HAK5pro::LUC-2, HAK5pro::LUC-8 and HAK5pro::LUC-12) grown on the same medium. Luciferase activity was not detected in wild-type Columbia-0 (Col-0), plants and luciferase activity in control plants was very low under K-sufficient conditions (Figure 1A). A total of 168 TF FOX transgenic plants with high luciferase activity un-

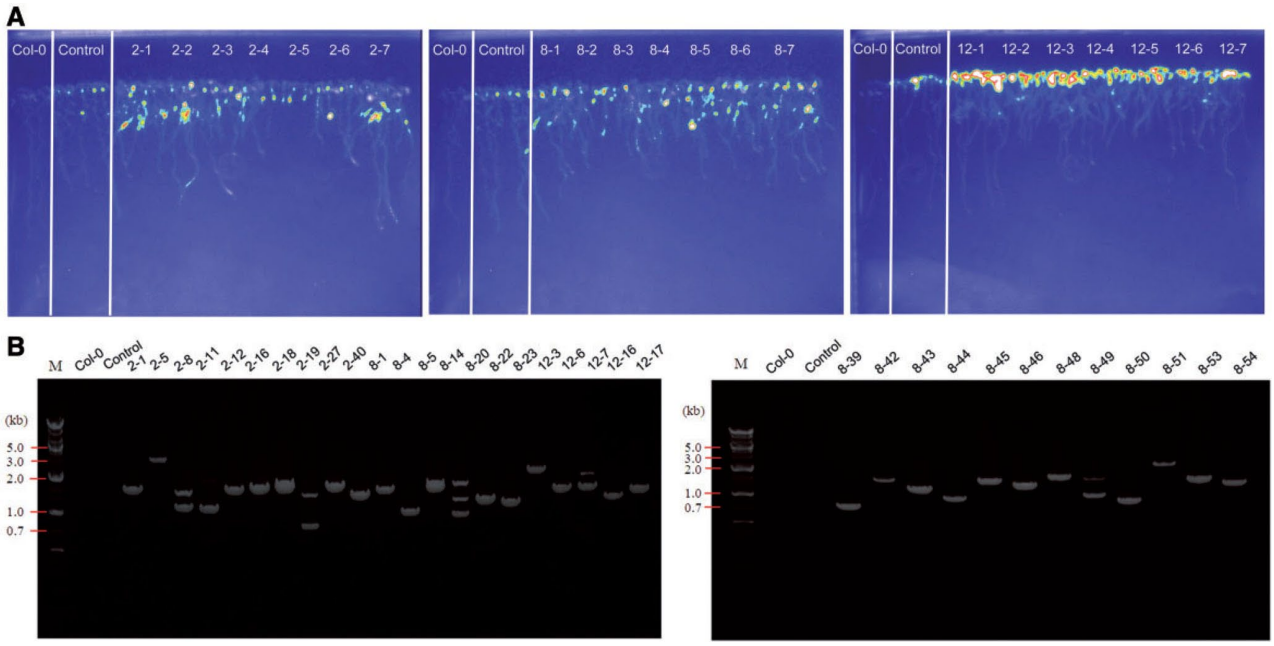


Figure 1. Luciferase activity and genomic DNA PCR analysis in selected TF FOX lines. (A) Luciferase activity in Arabidopsis TF FOX-transformed *HAK5* promoter::luciferase (HAK5pro::LUC) plants. Luciferase activity was measured in 8-day-old T_2 selected TF FOX plants. Blue and green colors indicate low luciferase activity, and red and white colors indicate high luciferase activity. Col-0, Columbia-0; Control, HAK5pro::LUC (HAK5pro::LUC-2, HAK5pro::LUC-8 and HAK5pro::LUC-12 lines). (B) Genomic DNA PCR analysis of selected TF FOX lines. Genomic DNA PCR analysis was performed using a vector-specific primer set (GR1 sense and GR1 antisense). PCR products were separated in a 1% agarose gel by electrophoresis. PCR products were eluted and analyzed for sequences. The sequence results are shown in Table 1 and Supplementary Table S1. Col-0 (Columbia-0) and control (HAK5pro::LUC-2 and HAK5pro::LUC-8) represent negative controls.

der +K were isolated. Among them, 116 TF FOX transgenic lines were selected for further characterization and reconfirmed in the next generation to have higher luciferase activity than Col-0 and control lines in +K medium (Figure 1A). Genomic DNA from the 116 selected lines was isolated and used as template for PCR-based identification of the TF FOX inserted genes using a specific primer set (GR1 sense and antisense primers) (Figure 1B). One PCR product was detected in 112 out of the 116 lines (Figure 1B). The identity of the inserted gene was confirmed in 87 lines by sequence analysis. As shown in Table 1 and Supplementary Table S1, many hormone-responsive transcription factors were identified, such as indole-3-acetic acid-inducible genes (IAAs), ethylene response factors (ERFs), ARFs and cytokinin response factors (CRFs). Also, multiple genes belonging to the same family of transcription factors were identified, such as two Myb domain proteins (At1g18570 and At1g48000), two LOB domain-containing proteins (At1g31320 and At2g45410), three alfin-like proteins (At1g14510, At3g42790 and At5g05610), 11 bHLH-type transcription factors (At1g35460, At1g32640, At2g18300, At2g20180, At2g41130, At3g19860, At3g21330, At3g47640, At3g61950, At4g02590 and At5g671110) and two basic leucine zipper proteins (bZIPs) (At4g37730 and At1g13600).

In order to avoid selecting false positives (i.e. genes not related to *HAK5* regulation), endogenous *HAK5* expression levels were analyzed in each of the selected TF FOX lines since the inserted TF FOX gene should be able to activate HAK5pro::LUC, as well as endogenous *HAK5* gene expression. Quantitative reverse transcription–PCR (qRT–PCR) analyses were performed using *HAK5*-specific primers to characterize the expression level of endogenous *HAK5* in the selected transgenic lines (Kim et al. 2012). The induction of endogenous *HAK5* expression, relative to expression levels in control plants, was confirmed in the selected lines. As shown in Table 1, endogenous *HAK5* expression levels in 27 lines were increased 1.5 to 62.5 times more than in control lines (HAK5pro::LUC-2, HAK5pro::LUC-8 and HAK5pro::LUC-12).

Selected TF FOX plants were grown under +K and –K conditions in order to determine the effect of overexpressed TF FOX genes on root growth under K deprivation. Under +K conditions, TF FOX transgenic plants exhibited similar root growth compared with both control and wild-type plants. However, in –K conditions, some of the TF FOX lines exhibited differences in primary root length and/or lateral root numbers (Supplementary Figure S1). These results indicated that the overexpression of specific transcription factors led to the induction of *HAK5* expression, which in turn may have resulted in better root growth in the –K condition. Although we cannot exclude the possibility that the better growth of the candidate lines is not due to overexpression of transcription factors and *HAK5*, it is still one of the best ways to narrow down candidates for further study. Based on the combined analysis of the gene sequence, the expression level of endogenous *HAK5*, and the resulting pheno-

Table 1. List of selected TF FOX lines showing >1.5-fold endogenous *HAK5* expression compared with control lines in +K conditions

Line No.	Locus No.	Gene description	Endogenous *HAK5* level
2-1	At1g63030	DDF2, Dwarf and delayed flowering 2	21.7
2-5	At4g00220	JLO, Jagged lateral organs	22.8
2-6	At5g18240	MYR1, MYB-related protein 1	2.8
2-7	At5g67110	ALC, Alcatraz	23.3
2-13	At2g37120	S1FA-like DNA-binding protein	21.9
2-14	At1g17310	MADS-box transcription factor	14.0
2-16	At5g10030	OBF4, OCS element binding factor 4	22.2
2-17	At3g61790	RING/U-box and TRAF-like domain protein	10.9
2-45	At1g30210	TCP24, Teosinte branched 1, cycloidea, and PCF family 24	1.5
2-51	At2g24500	FZF, C2H2 zinc finger protein	20.8
8-1	At1g64380	ERF/AP2 transcription factor	6.3
8-4	At3g23030	IAA2, Indole-3-acetic acid inducible 2	3.9
8-5	At1g18570	MYB51, Myb domain protein 51	44.5
8-6	At4g24440	TFII_A, Transcription initiation factor IIA gamma chain	13.4
8-9	At3g19860	bHLH121, Basic helix-loop-helix 121	8.3
8-14	At2g28710	C2H2-type zinc finger protein	2.3
8-18	At2g24790	COL3, Constans-like 3	62.7
8-20	At5g23280	TCP family transcription factor	14.1
8-21	At1g75410	BLH3, BEL1-like homeodomain 3	13.6
8-22	At5g50080	ERF110, Ethylene response factor 110	7.8
8-23	At4g37750	ANT, Aintegumenta	50.1
8-26	At2g20180	PIF1, PHY-interacting factor 1	2.0
8-31	At2g46530	ARF11, Auxin response factor 11	36.4
8-32	At3g61950	Basic helix-loop-helix (bHLH) DNA-binding protein	2.1
12-2	At3g24860	Homeodomain-like protein	1.7
12-6	At4g23750	CRF2, Cytokinin response factor 2	6.9
12-11	At3g60530	GATA4, GATA transcription factor 4	3.6

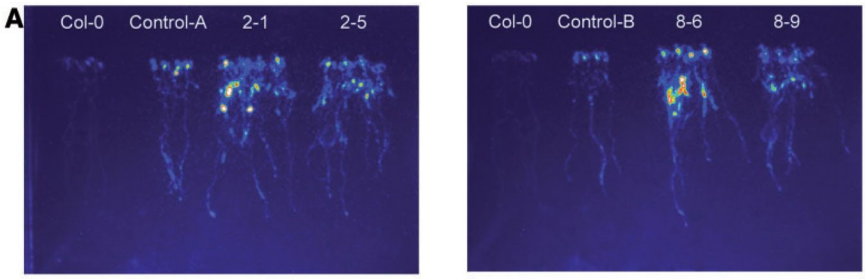
The endogenous *HAK5* level is the ratio of *HAK5* transcript in each selected FOX line compared with control lines as analyzed by qRT–PCR.

type, four lines (2-1, 2-5, 8-6 and 8-9) were chosen for further characterization.

Luciferase activity and transcription factor expression level in four selected TF FOX lines

Among the candidates, four lines were selected based on luciferase activity and endogenous HAK5 expression levels. The transcription factors identified by sequence analysis in the four selected lines were *DDF2* (At1g63030) in the 2-1 line, *JLO* (At4g00220) in the 2-5 line, *TFII_A* (At4g24440) in the 8-6 line and *bHLH121* (At3g19860) in the 8-9 line. T_2 seeds from each of the four lines were germinated on +K medium containing antibiotics, and luciferase activity was reconfirmed using the 6-day-old seedlings (Figure 2A). The four selected TF FOX lines showed higher luciferase activity compared with both the wild-type (Col-0) and control (HAK5pro::LUC-2 and HAK5pro::LUC-8) plants grown in K+ conditions. qRT–PCR analyses were also performed using gene-specific primer sets for each of the transcription factors in order to confirm their overexpression in the respective TF FOX line. The results indicated that overexpression of each inserted TF FOX gene in the 2-1 (*DDF2*), 2-5 (*JLO*), 8-6 (*TFII_A*) and 8-9 (*bHLH121*) lines was 26-, 3-, 15- and 10-fold higher than in the control lines (HAK5pro::LUC-2 and HAK5pro::LUC-8), respectively (Figure 2B).

Since the expression of *HAK5* is up-regulated in –K conditions (Ahn et al. 2004, Armengaud et al. 2004, Gierth et al. 2005) and this up-regulation of *HAK5* was suppressed by salt stress (Nieves-Cordones et al. 2010), expression levels of each transcription factor were analyzed in the respective line under both –K and salt stress conditions (Table 2). Five-day-old seedlings grown under adequate K were then subjected to either –K or salt stress (100 and 150 mM NaCl) for a period of 3 d. Data indicate that the transcript levels of *HAK5* were highly up-regulated in response to both –K and salt stress. The expression level of each of the four transcription factor genes also increased in leaves, as well as roots (with the exception of *TFII_A*), when plants were grown under –K conditions. All four genes (*DDF2*, *JLO*, *bHLH121* and *TFII_A*) were up-regulated in response to salt stress. The expression of *DDF2* was more highly induced under both –K conditions and salt stress compared with the other three genes (Table 2). These data suggested that these four transcription factors are linked to plant responses for both K deficiency and salt stress.



B

Fold change of their own gene expression compared to Col-0	
	Overexpressed
HAK5pro::LUC-2 or 8	0.98±0.05*
2-1 (DDF2)	26.49±12.5*
2-5 (JLO)	3.15±0.02*
8-6 (TF_IIA)	15.29±3.1**
8-9 (bHLH121)	9.84±5.2*

Figure 2. Luciferase activity and qRT–PCR analysis in four selected lines containing TF FOX constructs. (A) Luciferase activity in four selected TF FOX lines (2-1, 2-5, 8-6 and 8-9) under +K conditions. 2-1, *DDF2*-overexpressing line; 2-5, *JLO*-overexpressing line; 8-6, *bHLH121*-overexpressing line; 8-9, *TFII_A*-overexpressing line. Col-0 (Columbia-0) and control-A and B (HAK5pro::LUC-2 and HAK5pro::LUC-8) lines represent negative controls. Blue and green colors indicate low luciferase activity, and red and white colors indicate high luciferase activity. (B) qRT–PCR analysis of individual transcription factor gene expression levels in the four selected overexpression lines (each line has a different transcription factor). The expression level of each gene was compared with the expression levels measured in the wild type (Col-0). The mean fold change was calculated from three independent qRT–PCR analyses. Each independent experiment was performed with the different cDNAs as templates. Each cDNA was obtained from >15 seedlings. HAK5pro::LUC2 and HAK5pro::LUC8 are control lines.

Table 2. Expression of four selected TF genes in response to potassium deficiency in roots and shoots or salt stress (100 and 150 mM NaCl) in roots as quantified by qRT-PCR

Fold change compared with −K/+K			Fold change compared with NaCl treatment/no treatment		
	Leaves	Root		100 mM NaCl	150 mM NaCl
HAK5	1,864.1 ± 715.1*	243.9 ± 83.9*	*HAK5*	189.4 ± 19.9***	433.0 ± 77.2***
DDF2	5.6 ± 0.8*	3.2 ± 0.6*	*DDF2*	55.4 ± 8.2**	138.1 ± 27.9**
JLO	3.1 ± 0.8*	1.6 ± 0.1*	*JLO*	1.7 ± 0.1*	1.6 ± 0.1*
TF_IIA	3.7 ± 0.9*	1.3 ± 0.04	*TF_IIA*	1.9 ± 0.09*	2.5 ± 0.03**
bHLH121	2.3 ± 0.3*	1.6 ± 0.2*	*bHLH121*	1.9 ± 0.03**	3.2 ± 0.004**

The mean fold change was calculated from three independent quantitative PCR analyses. Each independent experiment was performed with different cDNAs as templates. Each cDNA was obtained from >15 seedlings.

Asterisks indicate values that are significantly different from wild-type plants ($P < 0.05$).

HAK5, At4g13420; *DDF2*, At1g63030; JLO, At4g00220; *TF_IIA*, At4g24440; *bHLH121*, At3g19860.

Root growth analysis of plants from four selected TF FOX-overexpressing lines under potassium deficiency

Since K deficiency inhibits root growth, this feature was analyzed in the four TF FOX lines under +K and −K conditions. Four-day-old seedlings grown on +K medium were transferred onto either +K or −K medium. After 2 weeks, primary root length and lateral root numbers were recorded. Primary root length in control seedlings was reduced under the −K condition and the number of lateral roots was dramatically reduced (Figure 3A). Primary root length in the four TF FOX lines grown under +K conditions was similar to the control but was greater than in the control lines under −K conditions (Figure 3A, B). In addition, the number of lateral roots in the selected TF FOX lines was greater in both the +K- and −K-grown plants (Figure 3C). These results indicate the possibility that overexpression of the four selected transcription factor genes enabled the roots to grow more under conditions of K deprivation, most probably due to an induction of *HAK5* expression or other genes in the transcription factor gene expression network.

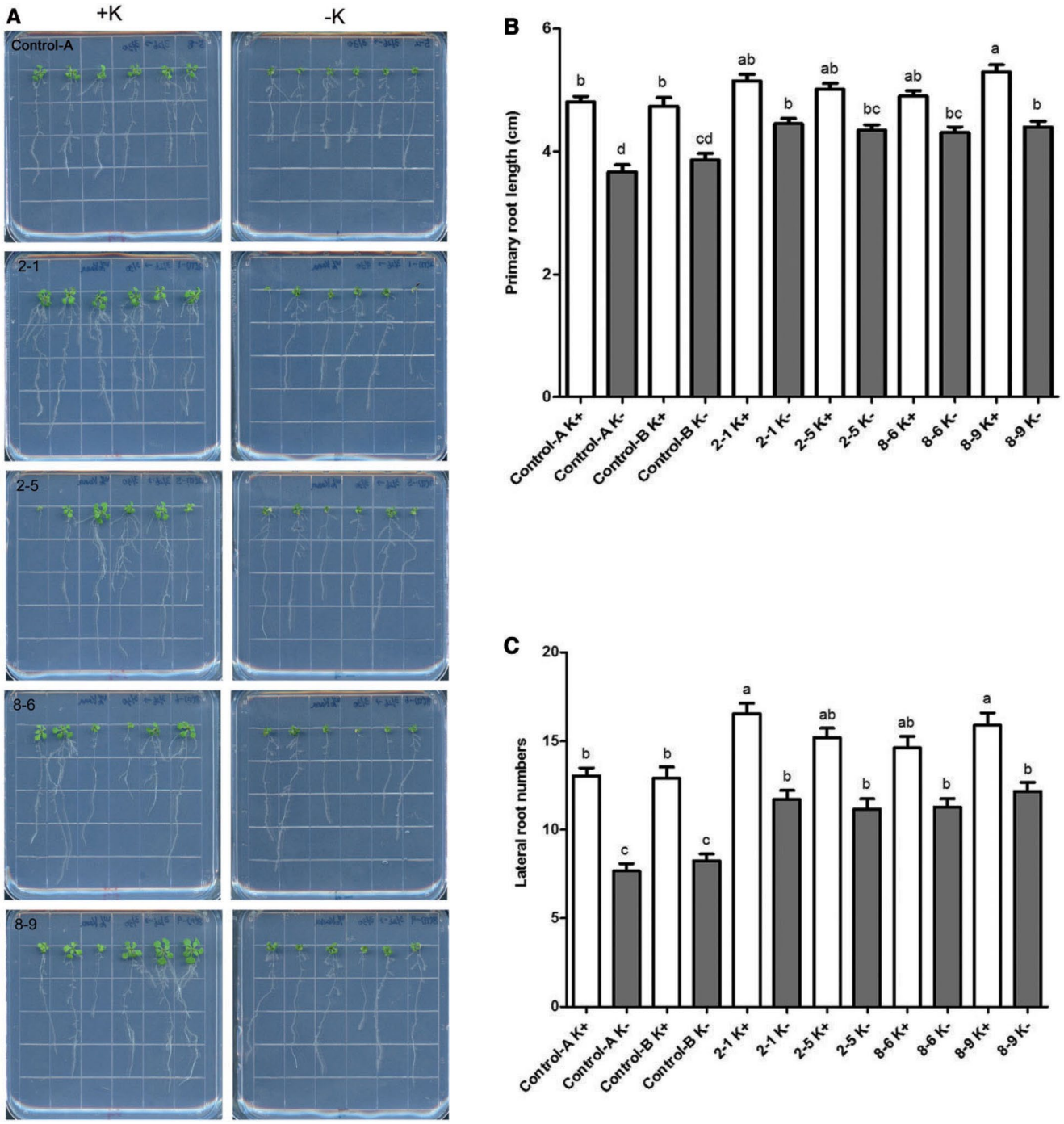


Figure 3. Root growth analysis in four TF FOX lines under +K and –K conditions. Four-day-old seedlings grown on +K medium were transferred to either +K or –K medium and grown for 14 d. After 2 weeks, plates were scanned (A) and primary root length (B) and lateral root number (C) were recorded. Control-A and B (HAK5pro::LUC-2 and HAK5pro::LUC-8 lines) were used as controls. Control-A and B did not show any difference. Control-A is presented as a representative image of control (A). Significant differences between means are indicated by different letters as determined using a one-way ANOVA ($P < 0.05$) and a Tukey's mean separation test. Values in (B) and (C) are the mean ± SE ($n = 50$).

Binding of the four selected transcription factors to the *HAK5* promoter

Transcription factors bind to specific elements within promoter sequences and impact the transcription of downstream genes. Therefore, to identify regions of the *HAK5* promoter sequence to which the transcription factors bind, a series of *HAK5* promoter deletions (Kim et al. 2012) were constructed and used in subsequent analyses (Figure 4A). The upstream region >910 bp of the

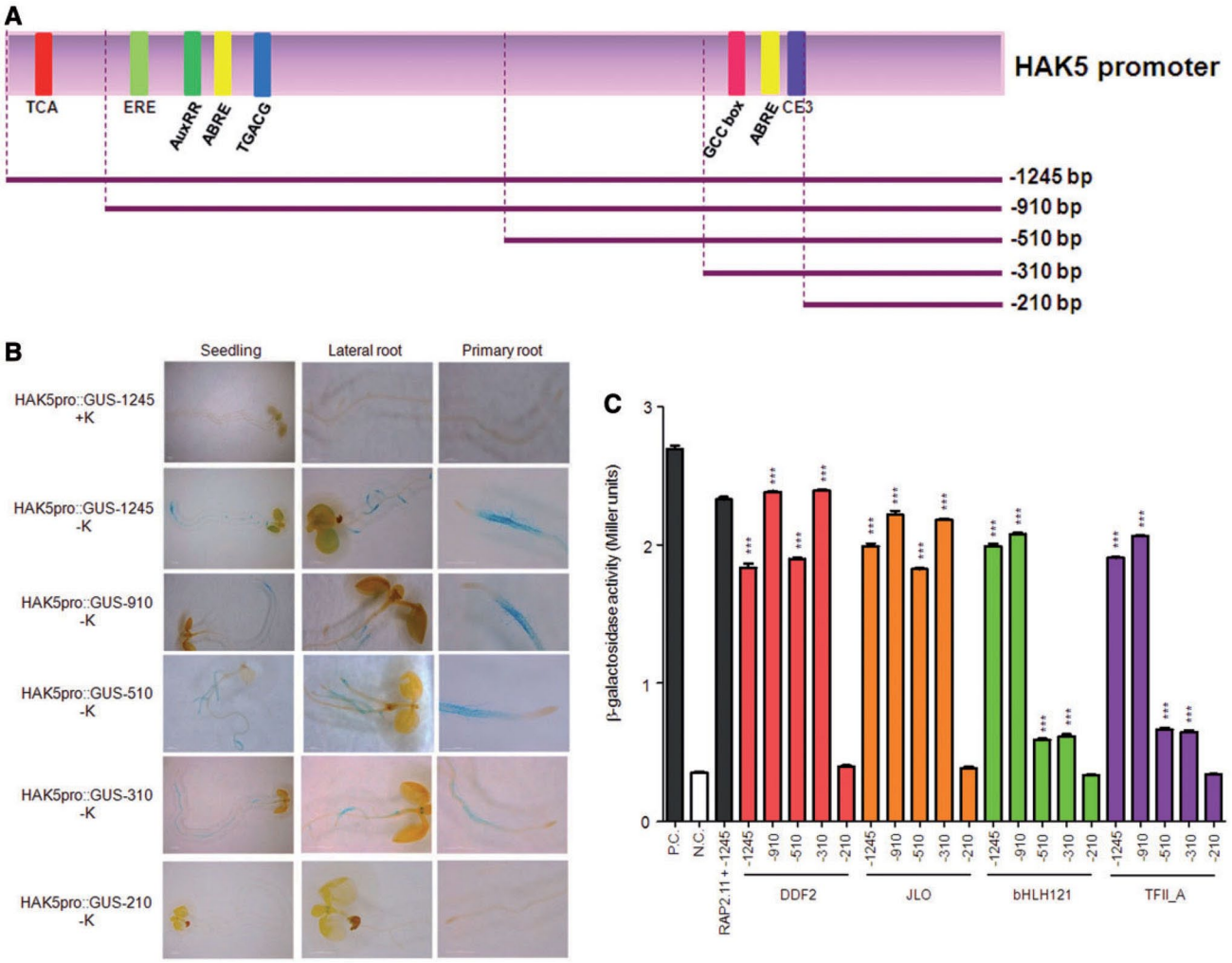


Figure 4. Histochemical GUS assays and β-galactosidase activity. (A) Diagram of the *HAK5* promoter deletion series. The −1,245 bp *HAK5* promoter is full length and includes the TCA element, ERE (ethylene-responsive element), TGACG motif, ABRE (ABA-responsive element), AuxRR-core, GCC-box and CE3 (coupling element 3). (B) Histochemical GUS assay of *HAK5* promoter::GUS transgenic lines under +K and −K conditions. The blue color indicates GUS expression in plants. HAK5pro::GUS-1245; −1,245 bp *HAK5* promoter, HAK5pro::GUS-910; −910 bp *HAK5* promoter, HAK5pro::GUS-510; −510 bp *HAK5* promoter, HAK5pro::GUS-310; −310 bp *HAK5* promoter, HAK5pro::GUS-210; −210 bp *HAK5* promoter. (C) Yeast one-hybrid analysis of four transcription factors and a *HAK5* promoter deletion series. ONPG assays were performed to quantify the binding between *HAK5* promoter segments and each transcription factor in yeast cells. This experiment was independently performed three times, and representative data are shown here. The error bars indicate the standard error and asterisks indicate values significantly different from the negative control (Empty vector) ($P < 0.05$).

HAK5 promoter fully responded to −K conditions (Kim et al. 2012). Therefore, the 1,245 bp upstream region of *HAK5* is considered as a full length *HAK5* promoter in this study and includes an ethylene-responsive element (ERE) (Itzhaki and Woodson 1993), two ABA-responsive elements (ABREs) (Yamaguchi-Shinozaki and Shinozaki 1993), a GCC-box (Brown et al. 2003, Chakravarthy et al. 2003), a TCA element involved in salicylic acid responsiveness (Pastuglia et al. 1997), a TGACG motif involved in methyl jasmonate responsiveness (Rouster et al. 1997), a AuxRR-core involved in auxin responsiveness (Sakai 1996) and a coupling element 3 (CE3) involved in ABA responsiveness (Hattori et al. 1995).

In order to determine the conditional and spatial pattern of expression of *HAK5* in Arabidopsis plants, GUS (β-glucuronidase) activity was observed in a deletion series of *HAK5* promoter::GUS (HAK5pro::GUS) transgenic seedlings (−1,245, −910, −510, −310 and −210 bp) (Figure 4B). As shown in Figure 4B, GUS activity in the full-length HAK5pro::GUS (−1,245 bp) plants was detected in the elongation zone of primary and lateral roots under −K conditions but was not detected under +K conditions. GUS activity in HAK5pro::GUS −910, −510 and −310 plants was also detected in the elongation zone of primary and lateral roots in response to K deficiency. However, GUS activity in HAK5pro::GUS −210 plants was not detected under +K or −K conditions.

To identify specifically the preferential binding site on the *HAK5* promoter sequence for these four selected transcription factors, yeast one-hybrid analyses using the *HAK5* promoter deletion series and the four selected transcription factor genes were performed and the results were quantified by an ONPG (ortho-nitrophenyl-β-d-galactopyranoside) assay in yeast cells (Figure 4C). RAP2.11, an Arabidopsis AP2/ERF transcription factor that is known to bind to the *HAK5* promoter (Kim et al. 2012), was used as a positive control. RAP2.11 exhibited high β-galactosidase activity in the ONPG assay using the −1,245 bp *HAK5* promoter. Yeast cells expressing DDF2 and JLO showed high β-galactosidase activity using the −1,245, −910, −510 and −310 bp *HAK5* promoter sequences, but not with the −210 bp *HAK5* promoter (Figure 4C). Interestingly, bHLH121 and TFII_A showed high β-galactosidase activity in the −1,245 and −910 bp *HAK5* promoter, but showed relatively low β-galactosidase activity in the yeast cells containing the −510 and −310 bp *HAK5* promoters (Figure 4C). β-Galactosidase activity in the −210 bp *HAK5* promoter was similar to that of the negative control (Figure 4C). The combined results of the GUS and ONPG assays indicate that at least −310 bp of the *HAK5* promoter is required for mediating low K responsiveness.

Discussion

K plays an essential role in many cellular processes, including growth and reproduction. The mechanisms associated with its uptake and the cell's ability to maintain K homeostasis have been well studied (Ashley et al. 2006). In plants, many different types of ion channels and transporters play a role in K uptake and transport. The KT/KUP/HAK family, the largest family of K transporters, is comprised of 13 genes in Arabidopsis (Very and Sentenac 2003). The first eukaryotic KT/KUP/HAK transporter gene was cloned from *Schwanniomyces occidentalis* and was named *HAK1* (high-affinity K transporter 1) (Banuelos et al. 1995). Santa-Maria et al. (1997) identified HvHAK1 in barley and characterized its function as the main high-affinity K transporter responsible for K uptake in roots (Santa-Maria et al. 1997). Arabidopsis *HAK5*, an *HvHAK1* ortholog, was also identified as a high-affinity K transporter in roots (Gierth et al. 2005). Among the many families of K transporters, the KT/KUP/HAK family, especially Arabidopsis HAK5, has been actively studied.

In response to K deprivation, *HAK5* mRNA increases and then rapidly decreases after the resupply of K (Ahn et al. 2004). In order to study HAK5, and to take advantage of the tight regulation of *HAK5* expression by K availability, transgenic Arabidopsis plants were created using a luciferase reporter gene driven by the *HAK5* promoter (HAK5pro::LUC) (Kim et al. 2010). These transgenic lines have served as a useful resource to identify regulatory components associated with high-affinity K uptake and were used to identify transcription factors that regulate *HAK5* expression. Ichikawa et al. (2006) developed the FOX (Full-length cDNA Over-eXpressor) hunting system to enable the systematic exploration of gain-of-function mutants, and many publications have documented the useful applications of this system in both rice and Arabidopsis (Ichikawa et al. 2006). The Arabidopsis TF FOX library containing approximately 800 transcription factor cDNAs of Arabidopsis was created specifically to study overexpression of transcription factors. In the present study, our aim was to identify transcription factors that bind to the *HAK5* promoter sequence. However, candidate genes from general activation tagging methods, including an Arabidopsis whole-genome FOX library, have provided a wide range of genes but only a small number of transcription factors. Many candidates from those studies may only indirectly regulate the expression of *HAK5*. In order specifically to enrich our discovery of transcription factors interacting directly with the *HAK5* promoter, we created an activation tagging system using the promoter of *HAK5* fused to luciferase and then transformed these lines with the FOX library which exclusively contained transcription factors. This approach enabled the identification of many transcription factors that could potentially bind directly to the *HAK5* promoter. As shown in Figure 1A and Table 1, our screening strategy worked well for minimizing false positives using cut-off levels of luciferase activity and comparison with the endogenous *HAK5* level. The outcome of this screening approach confirms that this is an efficient strategy to enable the identification of candidate transcription factors that could function as direct upstream regulators of *HAK5* expression.

When plants undergo K limitation, phytohormones, ROS, AAA-ATPase and calcium ions play important roles for activating low K signaling and plant survival. Ethylene and ROS production were increased (Shin and Schachtman 2004, Jung et al. 2009), and AAA-ATPase-related genes and calcium-binding protein genes are induced in low K conditions (Hampton et al. 2004, Kim et al. 2012). Furthermore, many genes related to hormone metabolism and signaling were regulated by K availability (Rigas et al. 2001, Becker et al. 2003, Armengaud et al. 2004, Shin et al. 2007, Armengaud et al. 2009, Jung et al. 2009, Nam et al. 2012). These observations suggested that K signaling may be tightly linked to phytohormones. In the present study, we identified many different types of transcription factors as possible upstream components of *HAK5* in response to K deficiency (Table 1; Supplementary Table S1). Two ERFs (ERF104 and ERF110) and three AP2/ERF transcription factors were isolated. We also identified several auxin-related transcription factors, including two IAAs (IAA2 and IAA8) and one ARF (ARF11). JLO, an LBD (lateral organ boundary domain) transcription factor linked to auxin-dependent embryo development (Borghi et al. 2007), was also identified in this study. These results suggest that the ethylene- and auxin-related transcription factors found in this study may be involved in the regulation of *HAK5* expression in response to low K via an ethylene and/or auxin signaling pathway. Recently, it was shown that cytokinin also plays a regulatory role under low K conditions (Nam et al. 2012), and CRFs were also isolated as candidate regulators of HAK5 in this

study. In addition to phytohormones, salt stress resulted in induction of *HAK5* expression as well as four selected transcription factors (Table 2). Interestingly the induction of *HAK5* expression by low K was suppressed by salt stress (Nieves-Cordones et al. 2010) in Arabidopsis. Also, mild salt stress (50 mM NaCl) did not induce *HAK5* expression (Ahn et al. 2004). The expression levels of *HAK5* gradually increased following an increase in NaCl concentrations (Table 2). This suggests that plants utilize sodium instead of K when they are deprived of K, and plants recognize high sodium levels as K deprivation. Therefore, our results suggest that various phytohormone- and abiotic stress-regulated transcription factors may also be associated with the transcriptional regulation of *HAK5* in response to low K.

The Arabidopsis *HAK5* promoter contains many *cis*-acting elements (Figure 4A). In a previous study, an ERE and a GCC-box (GCCGGC) in the *HAK5* promoter were found to be important binding elements of RAP2.11 (Kim et al. 2012). One of the candidate transcription factors from the current study, DDF2, is homologous to DDF1 which binds to the DRE (drought-responsive element)-like motif (GCCGAC and ATCGAC) and is up-regulated in high salinity stress (Magome et al. 2008). The DRE motif is very similar to the GCC-box (GCCGCC or GCCGGC). Many hormone-related and stress-related transcription factors bind to the DRE motif or GCC-box. Since the *HAK5* promoter contains a GCC-box, and this motif is important for *HAK5* expression, DDF2 is also a promising candidate for a regulator of *HAK5* via binding on the GCC-box. The *ddf1* mutant was reported as a gibberellin-deficient mutant and exhibited a dwarf and late flowering phenotype (Magome et al. 2004). In a previous study, *DDF1* mRNA was induced in NaCl-treated Arabidopsis roots (Sakuma et al. 2002) and *DDF1*-overexpressing plants showed increased tolerance to high salinity stress (Magome et al. 2004). Similar to *DDF1*, our study revealed high levels of induction of *DDF2* mRNA by both NaCl treatment and K deprivation (Table 2). These data suggest that DDF2 is functionally involved in both K-deficient signaling and salt stress responses. The overexpression of *DDF2* enhances root growth under conditions of low K. We confirmed that DDF2 is capable of binding the *HAK5* promoter and at least −310 bp of the *HAK5* promoter was required in yeast cells (Figure 4C). Therefore, we suggest that a GCC-box located in the *HAK5* promoter sequence is an important binding motif for DDF2.

The *bHLH121* transcription factor was also isolated as a potential regulator of *HAK5* expression, and its expression was induced by low K. The Arabidopsis genome contains >160 *bHLH* genes (Toledo-Ortiz et al. 2003), and most of the bHLH proteins exhibit transcriptional activity. The *bHLH* transcription factors which contain the highly conserved bHLH domain are the second largest class of plant transcription factors. bHLH proteins have been divided into 26 subgroups by phylogenetic analysis (Pires and Dolan 2009). Expression of an Arabidopsis *bHLH* transcription factor *GL3* (Glabra 3) was strongly up-regulated by nitrogen depletion in rosette leaves (Feyissa et al. 2009) and another *bHLH* transcription factor *PYE* (POPEYE) was up-regulated in iron-deficient roots (Long et al. 2010). Transgenic rice plants overexpressing the *bHLH* transcription factor, *PTF1*, showed greater tolerance to phosphate starvation (Yi et al. 2005). *ZmPTF1* in *Zea mays*, an *OsPTF1* ortholog, was rapidly up-regulated in phosphate-starved roots (Li et al. 2011). In our study, 11 *bHLH* transcription factors were identified, and *bHLH121* (At3g19860) was confirmed to function as a possible direct regulator of *HAK5* expression. The other 10 *bHLH* transcription factors (At1g35460, At1g32640, At2g18300, At2g20180, At2g41130, At3g21330, At3g47640, At3g61950, At4g02590 and At5g671110) isolated in this study also putatively bind the *HAK5* promoter and function as regulators of *HAK5* expression. The overexpression of four *bHLH* transcription factors (At5g67110, At3g19860, At2g20180 and At3g61950) was confirmed to activate endogenous *HAK5* gene expression (Table 1). These results support the premise that multiple *bHLH* transcription factors are involved in the signaling of K deficiency, as well as other nutrients.

In conclusion, at least 27 potential transcription factors were identified to be involved in the regulation of *HAK5* expression in response to K deficiency. Four of these transcription factors (DDF2, JLO, bHLH121 and TFII_A) whose expression was up-regulated by low K conditions were further characterized and confirmed to regulate *HAK5* expression in response to K deficiency and salt stress. In addition, promoter series deletion assays were utilized to confirm that these transcription factors were capable of specifically binding to the *HAK5* promoter. In this study, we found transcription factors which bound to the *HAK5* promoter and subsequently activated *HAK5* expression and consequently enhanced the growth of plants under K-limited conditions. Collectively, our findings increased our understanding of plant responses to K deficiency through the identification of novel contributing factors to nutrient signal transduction regulatory networks.

Materials and Methods

Plant materials and growth conditions

All Arabidopsis plants used in this study originated from the Col-0 background. Three previously generated Arabidopsis HAK5pro::LUC homozygous lines (HAK5pro::LUC-2, HAK5pro::LUC-8 and HAK5pro::LUC-12) that have 1,245 bp of *HAK5* promoter fused with luciferase (HAK5pro::LUC) were used for TF FOX transformation (Jung et al. 2009). The Arabidopsis TF FOX *Agrobacterium* library was transformed into the three HAK5pro::LUC homozygous lines by using the *Agrobacterium tumefaciens*-mediated floral dip method (Clough and Bent 1998). The various deletion series of the *HAK5* promoter fused to GUS (HAK5pro::GUS-1,245, -910, -510, -310 and -210) were used for GUS staining in the promoter deletion assay (Kim et al. 2012). All *Arabidopsis* seeds were surface sterilized in 70% (v/v) ethanol and 0.05% (v/v) Triton X-100 and washed five times in 100% ethanol. Sterilized seeds were planted on LSM [1.25 mM KNO_3, 2 mM $Ca(NO_3)_2$, 0.75 mM $MgSO_4$, 0.5

mM KH_2PO_4, 50 μM H_3BO_3, 10 μM MnCl, 2 μM $ZnSO_4$, 1.5 μM $CuSO_4$, 75 μM FeEDTA and 0.075 μM $(NH_4)_6Mo_7O_{24}$, pH 5.7] with 1% sucrose and 0.5% Seakem LE agarose (Lonza). The TF FOX transgenic T_1 seeds were grown on selective LSM containing 50 mg l^{-1} kanamycin and 50 mg l^{-1} carbenicillin. Dexamethasone (10 μM) was sprayed on the TF FOX transgenic plants. After cold treatment of the seeds at 4°C for 2 d, the plates were moved to a growth chamber at 22°C with a 16 h day-length at 150 μmol m^{-2} s^{-1}, unless otherwise indicated. Four-day-old seedlings were transferred onto potassium sufficient (+K) medium (1.75 mM KCl) and potassium deficient (–K) medium (10 μM KCl).

Construction of a binary vector for the Arabidopsis TF FOX library

To construct binary vectors for overexpression of each transcription factor, we used an entry clone library for Arabidopsis transcription factors that was previously produced (Mitsuda et al. 2010). Each open reading frame of a transcription factor was recombined individually into the destination plasmid, pBI35S-GW-GR, in-frame with the GR (glucocorticoid receptor) domain from the entry plasmid, pDONR207, using the GATEWAY cloning system (Invitrogen). To construct the pBI35S-GW-GR vector, the GUS gene of pBI121 was replaced with the GR fragment of a pMON721 derivative, provided by Dr. Aoyama (Kyoto University, Japan), and the 'reading frame B cassette' for the GATEWAY cloning system was inserted between the 35S promoter and the GR of the pBI121 derivative (Rusconi and Yamamoto 1987, Aoyama and Chua 1997). An aliquot of each destination clone was added and this mixed destination library comprised approximately 800 transcription factors. The library was subsequently introduced into *A. tumefaciens* (GV3101pMP90) by electroporation. Transformed *A. tumefaciens* were collected with LB medium from 20 selection plates containing approximately 1,000 colonies, resulting in an *Agrobacterium* library containing approximately 20,000 colonies.

Measurement of luciferase activity and selection of candidate lines

In order to isolate TF FOX transgenic plants with luciferase activity higher than control plants (HAK5pro::LUC-2, HAK5pro::LUC-8 and HAK5pro::LUC-12), antibiotic-resistant transgenic plants were transferred to LSM without kanamycin and grown for 6 d. These plants were sprayed with 100 μM luciferin. After incubating in the dark for 5 min, plants were analyzed for luminescence using a Luminescence Imaging System (HAMAMATSU). Fluorescence images were converted into pseudo-color images using AquaCosmos software version 1.3 (HAMAMATSU). Plants showing higher luciferase activity than control plants were selected for further analysis. The T_2 seeds of four selected lines (DDF2, line 2-1; JLO, line 2-5; TFII_A, line 8-6; and bHLH121, line 8-9) were germinated on LSM with 50 mg l^{-1} kanamycin and these plants were re-screened as described above.

Genomic DNA isolation and sequence analysis

One-month-old leaves of candidates showing higher luciferase activity than control plants were excised and immediately frozen in liquid nitrogen. Frozen leaves were pulverized using a TissueLyser II (Retsch), and total genomic DNA was isolated as previously described (An et al. 2003). In order to determine which TF FOX genes were inserted in the candidate lines, PCR was performed in a 50 μl total volume, using genomic DNA as a template, 10× ExTaq buffer, 0.4 mM deoxyribonucleotide triphosphate, 0.5 U of ExTaq polymerase (TAKARA) and 1 μM GR1 sense primer designed from the 35S promoter sequence (5'-GTCTTCAAAGCAAGTGGATTGATGTGATATC-3') and GR1 antisense primer designed from the GR gene sequence (5'-CGAAGTGTCTTGTGAGACTCC-3'). PCR products were eluted by the Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced. The full sequences were confirmed for integrity by comparing them with data available through the TAIR (The Arabidopsis Information Resource) database (http://www.arabidopsis.org).

Potassium deficiency and salt stress treatments, and total RNA isolation

For the K-deficient treatment, 5-day-old seedlings of Col-0 grown on LSM were transferred onto +K and –K medium. After 6 d, leaves and roots of these plants were harvested and immediately frozen in liquid nitrogen for total RNA extraction. For the salt stress treatment, 5-day-old seedlings of Col-0 grown on LSM were transferred to LSM containing either 0, 100 or 150 mM NaCl. After 3 d, these plants were harvested and immediately frozen in liquid nitrogen for total RNA extraction. Total RNA was isolated using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. Isolated total RNA was quantified using absorption of light at 260 and 280 nm ($A_{260}/_{280}$) and treated with DNase I (Invitrogen). The quality of DNase I-treated total RNA was determined by 1% agarose gel electrophoresis.

Quantitative RT–PCR analysis

A 5 μg aliquot of total RNA was reverse transcribed using the SuperScript III First-strand synthesis system for RT–PCR (Invitrogen) according to the manufacturer's instructions. QRT–PCR was conducted with the Stratagene Mx3000P system (Agilent Technologies) using THUNDERBIRD SYBR qPCR Mix (TOYOBO) and gene-specific primer sets. *HAK5* primers are sense (5'-CGAGACGGACAAAGAAGAGGAACC-3') and antisense (5'-CACGACCCTTCCCGACCTAATCT-3') (Ahn et al. 2004), *DDF2* primers are sense (5'-CCCTCAGCCAGTGAGTTTGACACG-3') and antisense (5'-TTTCTTCGTCCACGTACACACTCG-3'), *JLO* primers are sense (5'-ACCTCCCAACAATATCACCGTCGG-3') and antisense (5'-CGTGAATAAACTCACGCGCCAGTG-3'), *TFII_A* primers are sense (5'-ACTGAAGCTCTGGAGAGCCAAGTG-3') and antisense (5'-GAGCAGCTTGGAATCACATGCCAC-3'), *bHLH121* primers are sense (5'-CCAACGAAGTTGCAACACAACTCG-3') and antisense

(5'-CTGGAAGAACTGTGGTCTCGTACG-3'), and *Actin2* primers are ACT2F (5'-CTGGATCGGTGGTTCCATTC-3') and ACT2R (5'-CCTGGACCTGCCTCATCATAC-3'). *Actin2* (At3g18780) was used for the normalization of each transcript (Schneider et al. 2012). The fold change of transcripts was calculated using an efficiency-calibrated model (Yuan et al. 2006) and compared with *HAK5* transcripts and individual transcript levels under normal conditions. Statistical differences were evaluated by one-way analysis of variance (ANOVA) using delta Ct values (Yuan et al. 2006).

Root growth in potassium-deficient plants

To analyze primary root length and lateral root numbers, 4-day-old seedlings of control (HAK5pro::LUC-2 and HAK5pro::LUC-8) and four FOX overexpression lines (DDF2, 2-1 line; JLO, 2-5 line; TFIIA, 8-6 line; and bHLH121, 8-9 line) grown on LSM (+K) were transferred to +K and −K medium. After 14 d, primary root length and lateral root numbers were recorded and statistical differences were determined by a one-way ANOVA and Tukey's multiple comparison test using GraphPad Prism 5 software. The experiment was performed three times and the presented data were from one representative experiment.

GUS assay of *HAK5* promoter::GUS

For the GUS assay, transgenic seeds of the *HAK5* promoter deletion series fused to GUS (Kim et al. 2012) were germinated on LSM. GUS staining was conducted using modification of a previously described method (Jefferson et al. 1987). Five-day-old plants were transferred to +K and −K medium and grown for an additional 7 d. The plants were then immersed in a GUS staining solution containing 1 mM X-Gluc (5-bromo-4-chloro-3-indoxyl-β-d-glucuronide cyclohexylammonium salt), 4 mM derricyanide [$K_3Fe(CN)_6$], 0.1% Triton X-100 and 20% methanol in 0.1 M phosphate buffer (pH 7.0) and incubated overnight at 37°C. The plants were immersed in 0.24 N HCl in 20% methanol and incubated at 57°C on a heat block for 15 min. After incubation, 7% NaOH in 60% ethanol was added and the plants were incubated at room temperature for an additional 15 min. GUS-stained plants were de-stained by sequential incubation in 40, 20, 10 and 5% ethanol at room temperature, and plants were subsequently mounted in 50% glycerol for photomicrography with a Leica MZ10F microscope.

Promoter binding analysis

For the promoter binding analysis, vector construction and a yeast one-hybrid assay were performed as previously described (Deplancke et al. 2006). The pMW#3, pGAD vector and the YM4271 yeast strain were provided by Dr. Walhout (University of Massachusetts Medical School, USA). A *HAK5* promoter deletion series (−1,245, −910, −510, −310 and −210 bp) was cloned into pMW#3 containing a Gateway cassette with the AttR4 and AttL1 recombination sites and the lacZ reporter gene. Four selected genes (DDF2, JLO, bHLH121 and TFII_A) were cloned into the pGAD vector containing an activation domain of Gal4p (AD). The *HAK5* promoter::lacZ constructs were linearized with the *Nco*I restriction enzyme. Linearized constructs were verified using 1% agarose gels in 0.5× TBE and transformed into YM4271 (MATa, *ura3-52, his3-200, his3-200, ade2-101, ade5, lys2-801, leu2-3, 112, trp1-901, tyr1-501, gal4-D, gal8D, ade5::hisG*) (Clontech) yeast cells using a high-efficiency yeast transformation method (Amberg et al. 2006).

Transformed yeast cells were grown on SD/−Ura medium and incubated for at least 3 d at 28°C. Yeast colonies were confirmed by PCR analysis for identification of transformed vectors, and an X-β-gal filter assay was performed for identification of self-activation. After confirming no self-activation, the AD–transcription factor constructs were transformed into yeast cells. For quantifying the binding between the *HAK5* promoter and transcription factors, ONPG assays were performed as described previously (Gietz et al. 1997). The confirmed yeast cells were incubated overnight at 28°C, collected in a 1.5 ml tube and resuspended in 500 µl of Z buffer (100 mM $Na_2HPO_4{\cdot}7H_2O$, 30 mM $Na_2PO_4{\cdot}H_2O$, 10 mM KCl, 1 mM $MgSO_4{\cdot}7H_2O$, pH 7.0). A 50 µl aliquot of 0.1% SDS was added and the 1.5 ml tubes were vigorously vortexed. A 50 µl aliquot of chloroform and 100 µl of ONPG (4 mg ml^{-1}) were added and the 1.5 ml tubes were subsequently incubated at 37°C for 2 h. The reactions were stopped by the addition of 500 µl of 1 M Na_2CO_3, and β-galactosidase activity was measured at OD_{420} using a spectrophotometer (Beckmann). The units of β-galactosidase activity were calculated as described previously (Gietz et al. 1997). The *HAK5* promoter full-length DNA- and RAP2.11-transformed yeast cells were used as a positive control (Kim et al. 2012) and pMW#3 empty DNA and pDEST-AD empty DNA-transformed yeast cells were used as a negative control. At least two experiments were independently performed and the presented data are a representative set. Statistical differences were determined by one-way ANOVA and a Tukey's multiple comparison test using GraphPad Prism 5 software.

Supplementary materials follow the **References**.

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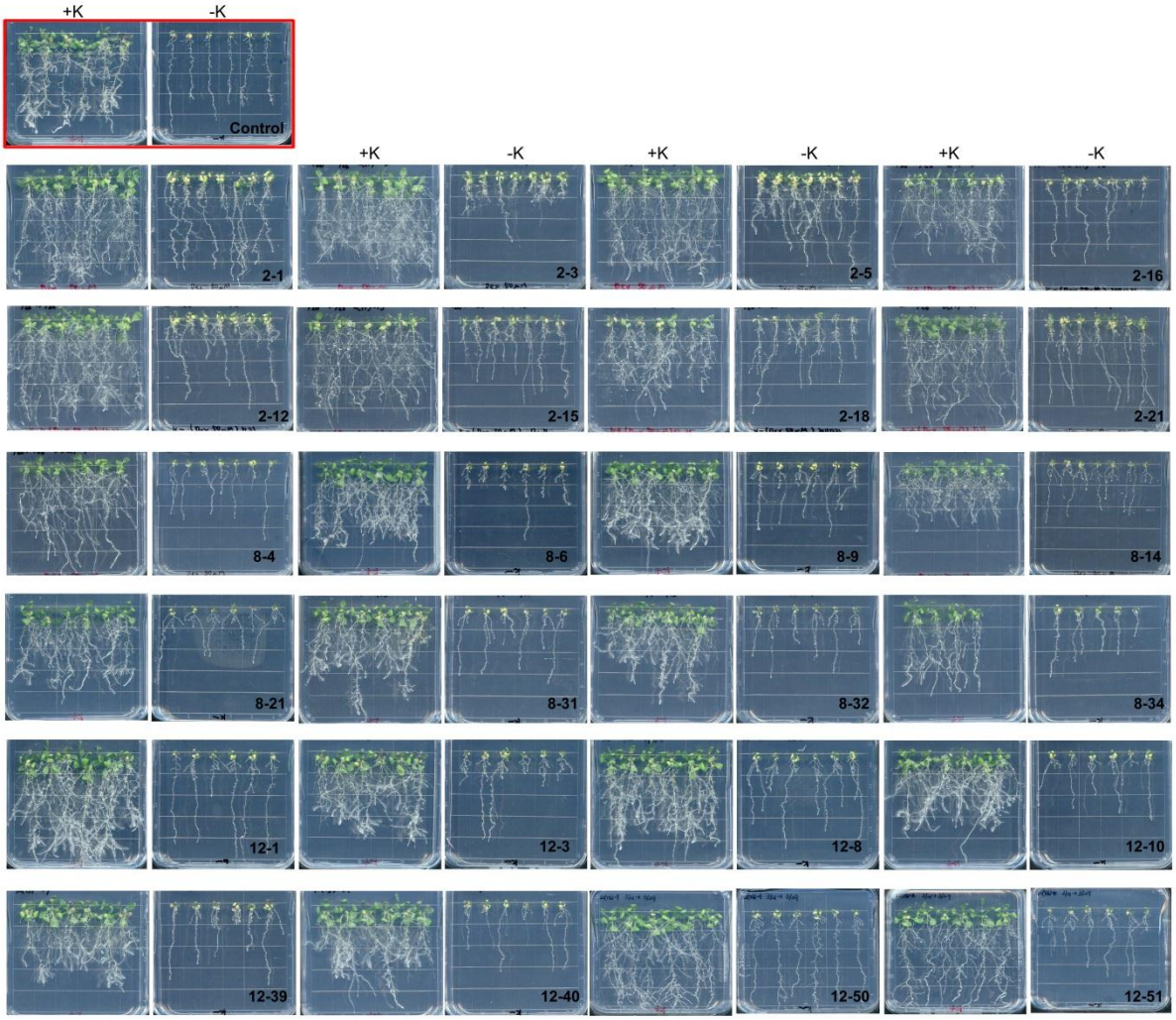
Supplementary Fig.1



Supplementary Fig. 1 Growth pattern of selected transgenic lines in +K and -K medium. T2 transgenic seeds were germinated on +K medium with kanamycin. Five-day-old antibiotic resistant seedlings were transferred to +K and -K medium without antibiotics and were subsequently grown for an additional 14 days. The plates were scanned after two weeks. The control for the experiment was HAK5pro::LUC-2 plants.

Supplementary Table 1. List of all TF FOX lines that showed higher luciferase activity than control plants (HAK5pro::LUC-2, HAK5pro::LUC-8, and HAK5pro::LUC-12). The full-length sequences were confirmed by the TAIR (The Arabidopsis Information Resource) database (http://www.arabidopsis.org).

Line No.	Locus No.	Gene description
2-1	At1g63030	DDF2, Dwarf and delayed flowering 2
2-2	At5g47140	GATA27, GATA transcription factor 27
2-4	At3g04570	AHL19, AT-hook motif nuclear-localized protein 19
	At5g67110	ALC, Alcatraz
2-5	At4g00220	JLO, Jagged lateral organs
2-6	At5g18240	MYR1, MYB-related protein 1
2-7	At5g67110	ALC, Alcatraz
2-8	At3g04570	AHL19, AT-hook motif nuclear-localized protein 19
	At5g67110	ALC, Alcatraz
2-9	At2g33550	Homeodomain-like protein
2-13	At2g37120	S1FA-like DNA-binding protein
2-14	At1g17310	MADS-box transcription factor
2-16	At5g10030	OBF4, OCS element binding factor 4
2-17	At3g61790	RING/U-box and TRAF-like domain protein
2-18	At3g21330	Basic helix-loop-helix (bHLH) DNA-binding protein
2-20	At1g08290	WIP3, WIP domain protein 3
2-22	At1g23380	KNAT6, KNOTTED1-like homoebox gene 6
2-23	At2g33550	Homeodomain-like protein
2-24	At1g44810	DNA-binding storekeeper protein-related transcriptional regulator
2-27	At2g45410	LBD19, LOB domain-containing protein 19
2-30	At5g24800	bZIP9, basic leucine zipper 9
2-33	At5g51590	AT hook motif DNA-binding protein
2-35	At1g17310	MADS-box transcription factor
2-38	At1g22985	CRF7, Cytokinin response factor 7
2-41	At3g21175	GATA24, GATA transcription factor 24
2-42	At5g53290	CRF3, Cytokinin response factor 3
2-45	At1g30210	TCP24, Teosinte branched 1, cycloidea, and PCF family 24
2-50	At3g47640	PYE, Popeye
2-51	At2g24500	FZF, C2H2 zinc finger protein
2-54	At1g48000	MYB112, Myb domain protein 112
2-57	At1g01520	Altered seed germination 4
8-1	At1g64380	ERF/AP2 transcription factor
8-4	At3g23030	IAA2, Indole-3-acetic acid inducible 2
8-5	At1g18570	MYB51, Myb domain protein 51
8-6	At4g24440	TFII_A, Transcription initiation factor IIA gamma chain
8-7	At1g65330	AGL37, Agamous-like 37
8-9	At3g19860	bHLH121, Basic helix-loop-helix 121
8-14	At2g28710	C2H2-type zinc finger protein
8-18	At2g24790	COL3, Constans-like 3
8-20	At5g23280	TCP family transcription factor
8-21	At1g75410	BLH3, BEL1-like homeodomain 3
8-22	At5g50080	ERF110, Ethylene response factor 110
8-23	At4g37750	ANT, Aintegumenta
8-26	At2g20180	PIF1, PHY-interacting factor 1
8-27	At2g27100	SE, Serrate
8-28	At5g02470	Core cell cycle gene
8-31	At2g46530	ARF11, Auxin response factor 11
8-32	At3g61950	Basic helix-loop-helix (bHLH) DNA-binding protein
8-33	At3g10330	Cyclin-like protein
8-34	At2g31380	STH, Salt tolerance homologue

8-35	At5g10510	AIL6, Aintegumenta-like 6
8-39	At5g09240	ssDNA-binding transcriptional regulator
8-42	At2g22670	IAA8, Indole-3-acetic acid inducible 8
8-43	At5g61600	ERF104, Ethylene response factor 104
8-44	At3g57990	Unknown protein
8-45	At3g61790	RING/U-box and TRAF-like domain protein
8-46	At4g11140	CRF1, Cytokinin response factor 1
8-49	At1g74930	ERF/AP2 transcription factor
8-50	At1g22985	CRF7, Cytokinin response factor 7
8-51	At2g02070	IDD5, Indeterminate(ID)-domain 5
8-53	At5g60970	TCP5, Teosinte branched 1, cycloidea, and PCF transcription factor 5
8-54	At4g37730	bZIP7, basic leucine zipper 7
12-2	At3g24860	Homeodomain-like protein
12-4	At5g61600	ERF104, Ethylene response factor 104
12-6	At4g23750	CRF2, Cytokinin response factor 2
12-7	At1g35460	Basic helix-loop-helix (bHLH) DNA-binding protein
12-11	At3g60530	GATA4, GATA transcription factor 4
12-15	At3g04570	AHL19, AT-hook motif nuclear-localized protein 19
12-16	At4g02590	UNE12, Unfertilized embryo sac 12
12-23	At3g60390	HAT3, Homeobox-leucine zipper protein 3
12-27	At3g22760	SOL1, CXC domain containing TSO1-like protein 1
12-32	At2g18300	Basic helix-loop-helix (bHLH) DNA-binding protein
12-33	At1g31320	LBD4, LOB domain-containing protein 4
	At2g28710	C2H2-type zinc finger protein
12-39	At5g09790	Trithorax-related protein 5
12-40	At1g32640	MYC2, Myc-related transcriptional activator
12-41	At1g36060	ERF/AP2 transcription factor
	At5g67420	ASL39, Asymmetric leaves 2-like 39
12-42	At4g39100	SHL1, Short life
12-43	At3g54320	Wrinkled 1, AP2/ERWEBP transcription factor
12-44	At1g14510	AL7, Alfin-like 7
12-46	At3g42790	AL3, Alfin-like 3
12-48	At4g37730	bZIP7, basic leucine zipper 7
12-49	At4g37730	bZIP7, basic leucine zipper 7
12-50	At2g41130	Basic helix-loop-helix (bHLH) DNA-binding protein
12-51	At1g13600	bZIP58, basic leucine zipper 58
12-52	At5g42820	U2AF35B, U2 auxillary factor small subunit
12-53	At1g30210	TCP24, Teosinte branched 1, cycloidea, and PCF family 24
12-54	At5g05610	AL1, Alfin-like 1
12-55	At5g65590	Dof-type zinc finger DNA-binding protein
12-56	At5g02470	DPA transcription factor