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RESEARCH ARTICLE

Transcriptome analysis reveals dynamic changes in the gene expression of tobacco seedlings under low potassium stress

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Abstract

Potassium plays a key role in plant development and reproduction. In agricultural practice, potassium deficiency is common worldwide, and leads to crop growth inhibition and output reduction. In this study, we analysed the transcriptome of tobacco seedlings under low potassium stress. Tobacco seedlings with or without decreased potassium treatment were harvested after 0 (control), 6, 12, or 24 h and were submitted for microarray analysis. The results showed that up to 3790 genes were upregulated or downregulated more than 2-fold as a result of the decreased potassium treatment. Gene ontology analysis revealed significantly differentially expressed genes that were categorized as cation binding, transcription regulation, metabolic processes, transporter activity and enzyme regulation. Some potassium, nitrogen and phosphorus transporters; transcription factors; and plant signal molecules, such as CPKs were also significantly differentially expressed under potassium deficiency. Our results indicate that the expression profiles of a large number of genes involved in various plant physiological processes are significantly altered in response to potassium deficiency, which can result in physiological and morphological changes in tobacco plants.

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Introduction

Potassium is involved in many important physiological and biochemical processes in plants, including enzyme activation, assimilate transport, plant stoma regulation, electrical neutralization and osmoregulation (Clarkson and Hanson 1980). Plants take up potassium mainly through potassium absorption systems located in the cell membrane. This absorption is performed either by high-affinity potassium uptake transporters, including KUP/HAK/KT and HKT families, or by low-affinity potassium uptake ion channels, such as Shaker and KCO families (Véry and Sentenac 2003). High-affinity potassium transporters primarily function to absorb potassium under low potassium concentrations in the soil. In contrast, potassium channels play a key role when potassium concentration in soil is high. In worldwide agricultural practice, crops often encounter potassium shortages in soil which lead to crop growth inhibition and output reduction (Ashley *et al.* 2006). In response to a low potassium environment, plants have developed complex mechanisms to guarantee normal growth and development, including the regulation of expression levels of specific genes (Santa-María *et al.* 1997; Buschmann *et al.* 2000; Bañuelos *et al.* 2002; Cellier *et al.* 2004; Gierth *et al.* 2005; Fulgenzi *et al.* 2008) of signal transduction and of metabolic pathways (Armengaud *et al.* 2004; Ma *et al.* 2012).

Recently, several transcriptome analyses of crops under abiotic stress have been conducted, including conditions of low nitrogen (Krapp *et al.* 2011) and low phosphorus (Misson *et al.* 2005; Woo *et al.* 2012) in *Arabidopsis*. In rice, gene expression spectra have been also investigated under conditions of drought (Wang *et al.* 2011), low nitrogen (Lian *et al.* 2006), low phosphorus (Wasaki *et al.* 2003, 2006; Li *et al.* 2010) and low potassium (Ma *et al.* 2012). The results of these studies have provided valuable insight into the molecular mechanisms of plant adaptation to nutrient stress. However, the gene expression profile of tobacco seedlings under low potassium stress is largely unknown, and it is studied for the first time in this report. We hope our work will provide a frame of reference for research into the molecular mechanisms of tobacco potassium absorption.

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Keywords. tobacco; potassium deficiency; transcriptome; microarray.

Materials and methods

Plant growth conditions and decreased potassium treatment

Tobacco cultivar K326 (*Nicotiana tabaccum* L.) seeds were surface sterilized, planted in MS medium and placed in a light incubator for growth under the following conditions: photoperiod, 24 h light; temperature, 28°C. After four weeks, half of the tobacco plants were transferred to low potassium MS media (Xu *et al.* 2006) for potassium deficiency treatment, and the other half were transferred to normal MS media as a control. Seedlings from both the control and treatment groups were harvested at the indicated time for further assays. Three biological replicates at each time point were performed to ensure reliability of the experiment.

Sample preparation, RNA isolation and hybridization to genechips

Both the control and treatment seedlings were harvested at the indicated times (0, 6, 12 and 24 h), and immediately frozen in liquid nitrogen for RNA extraction. The total RNA of the plant samples were extracted using TRIzol reagent (Invitrogen, Carlsbad, USA). The quality of the RNA was assessed using a NanoDrop 2000 fluorospectrometer and formaldehyde denaturing gel electrophoresis. The total RNA was purified using an RNeasy Mini kit (Qiagen, Valencia, USA) and was used for cDNA and cRNA generation. The cRNA was then labelled, fragmented and used for hybridization on an Agilent tobacco gene chip according to the Agilent standard hybridization procedure at 65°C for 17 h. The standard wash protocol was applied and the arrays were scanned on a gene chip scanner (Agilent Microarray Scanner, Santa Clara, USA).

Microarray data processing and analysis

Raw data were generated and normalized using Feature Extraction software 10.7 and Gene Spring Software 11.5.1 (Agilent technologies. Santa Clara, USA), respectively. When a gene was detected in all the three replicates and its detection *P* value was less than 0.05, we considered it to be expressed. A differentially expressed gene was determined by the following two criteria: (i) the gene expression level changed more than two-fold; and (ii) the *P* value of that fold change was less than 0.05. Hierarchical cluster analysis was conducted using Cluster3.0 software (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm). A Venn diagram was drawn to depict the genes that were upregulated or downregulated at one or more time points. The gene ontology (GO) web service (http://www.geneontology.org) was used to analyse the functional categories of the differentially expressed genes.

Real-time PCR analysis

To ensure the reliability and repeatability of our microarray results, the total RNA from control and treatment samples were extracted, and the genomic DNA was eliminated with DNase I (RNase-free) (Takara, Dalian, China). Gene-specific primers were designed using Primer 3 (http://frodo.wi.mit.edu/primer3/input.htm). Subsequently, cDNA generation and real-time PCR were performed according to Ma *et al*. (2012).

Results

Tobacco gene expression shows global changes under potassium deficiency

Tobacco seedlings from control and treatment groups were harvested at four time points (0, 6, 12 and 24 h). The total RNA of each was extracted, and both cDNA and cRNA were generated, and used for microarray experiments. The results showed 3790 genes with expression levels that were upregulated or downregulated more than 2-fold ($P < 0.05$) in the potassium-starved-tobacco seedlings compared to the control tobacco plants. Of these differentially expressed genes (DEGs), 2277, 1954 and 1835 were identified after 6, 12 and 24 h, respectively (table 1).

Our results showed that, at an early stage of potassium starvation treatment (6 h), a large number of DEGs were present (2277, table 1). However, as the duration of treatment increased from 6 to 12 and to 24 h, the number of DEGs decreased. Among these DEGs, more upregulated genes were identified than downregulated genes at all three time points. This observation indicates that potassium starvation may induce the expression of genes involved in the response and adaption to low potassium stress.

We performed a hierarchical cluster analysis and drew a Venn diagram (figure 1) to show the similarities and differences between the expression patterns of the DEGs at different durations of low potassium stress. These diagrams revealed that the DEGs at 12 and 24 h had similar expression patterns that were different from those at 6 h (figure 1a).

Table 1. Number of DEGs in tobacco during potassium deficiency.

Treatment	Total	Upregulated	Percentage of total DEGs (%)	Downregulated	Percentage of total DEGs (%)
6–0 h	2277	1270	33.51	1007	26.57
12–0 h	1954	1159	30.58	795	20.98
24–0 h	1835	1023	26.99	812	21.42

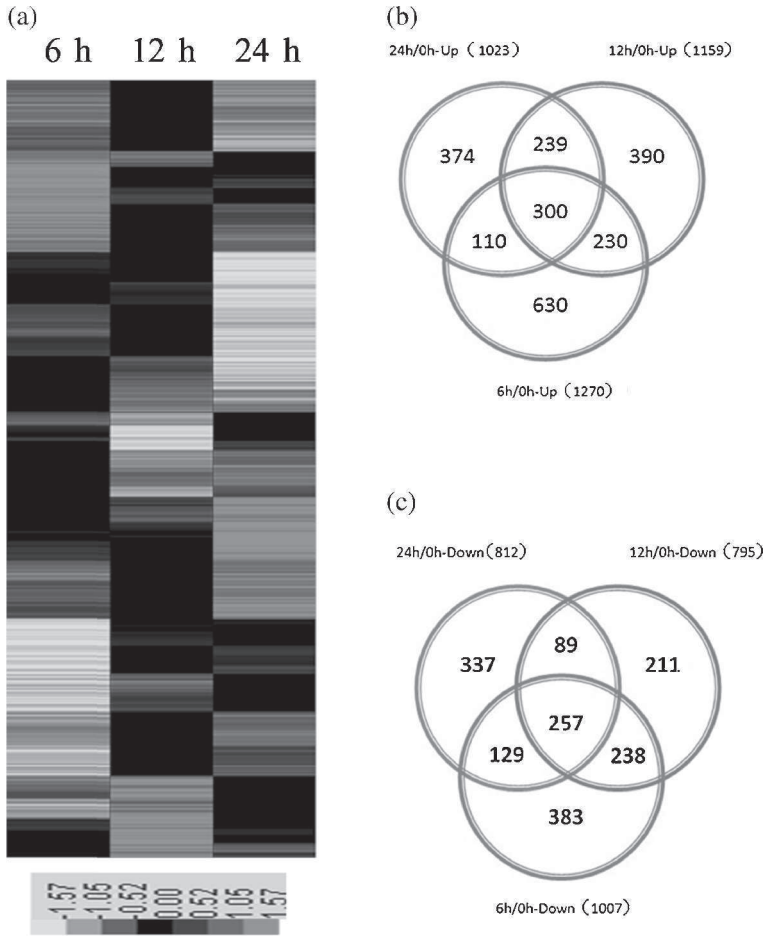


Figure 1. Hierarchical cluster analysis and Venn diagrams of tobacco genes showing transcriptional changes in response to potassium deficiency. (a) Hierarchical cluster analysis of genes showing transcriptional changes at the indicated times (6, 12 and 24 h) during potassium deficiency treatment. (b, c) Venn diagrams of upregulated (b) and downregulated (c) genes at different durations of low potassium treatment.

At 6 h, some DEGs were upregulated and others were downregulated. This result suggests that DEGs observed after 6 h of treatment could be related to a short-term response to potassium deficiency in tobacco. In addition, some DEGs showed transcriptional changes at all three time points, indicating that these genes may function at all stages of low potassium stress (figure 1b). To ensure the validity of the generated microarray data, we analysed 12 randomly selected genes using qPCR analysis. The results showed that the expression patterns of most of the genes were similar to those predicted by the tobacco microarray data (figure 2).

GO analysis of DEGs in response to low potassium stress

We performed GO analysis using the GO web service (http://www.geneontology.org) to assess the possible functions of DEGs in response to potassium deficiency. The results showed that 3790 DEGs were classified into 18 functional categories including response to stimulus, transporter activity, enzyme regulator activity, metabolic processes, transcription regulator activity and binding (figure 3). Most of the 18 categories possessed more upregulated than downregulated genes. This observation was especially true in the GO categories of antioxidant activity, structural molecule activity, anatomical structure formation and enzyme regulator activity, which did not contain downregulated genes.

Transmembrane transport

Transmembrane transport is a vital mechanism of nutrient transport in plant cells and is the foundation of the exchange of substances across a membrane. We identified 82 upregulated genes and 48 downregulated genes in the category of transmembrane transported activity whose expression levels changed more than 2-fold ($P < 0.05$). The 82 upregulated genes can be divided into nine subcategories based on the substance they transport: metal ion (30%), water (12%), amino acid and protein (10%), phosphate (9%), carbohydrate

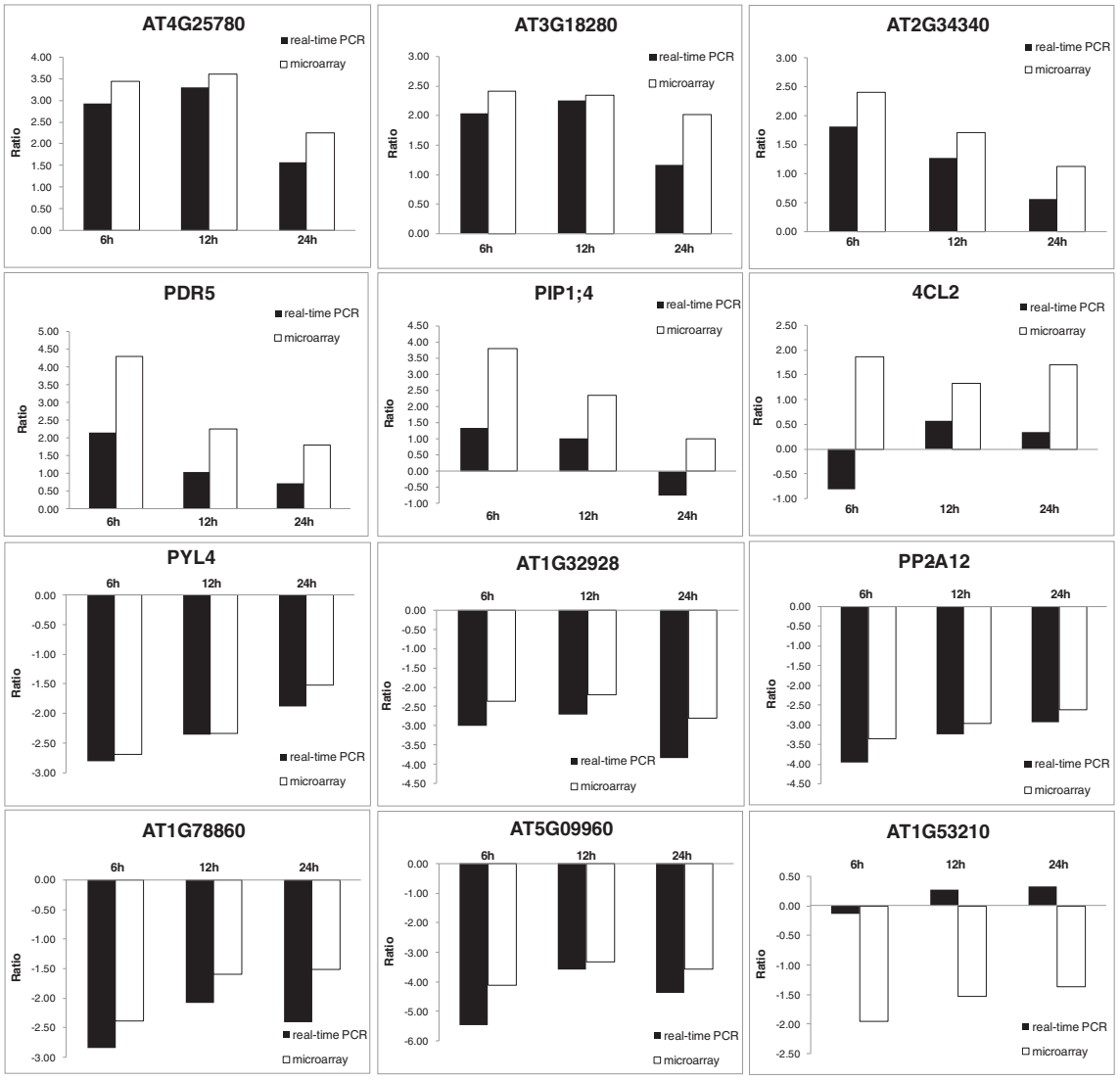


Figure 2. Confirmation of transcriptomic profiles of selected genes by qPCR. Ratio refers to expression levels of selected genes at the time indicated compared with that of 0 h.

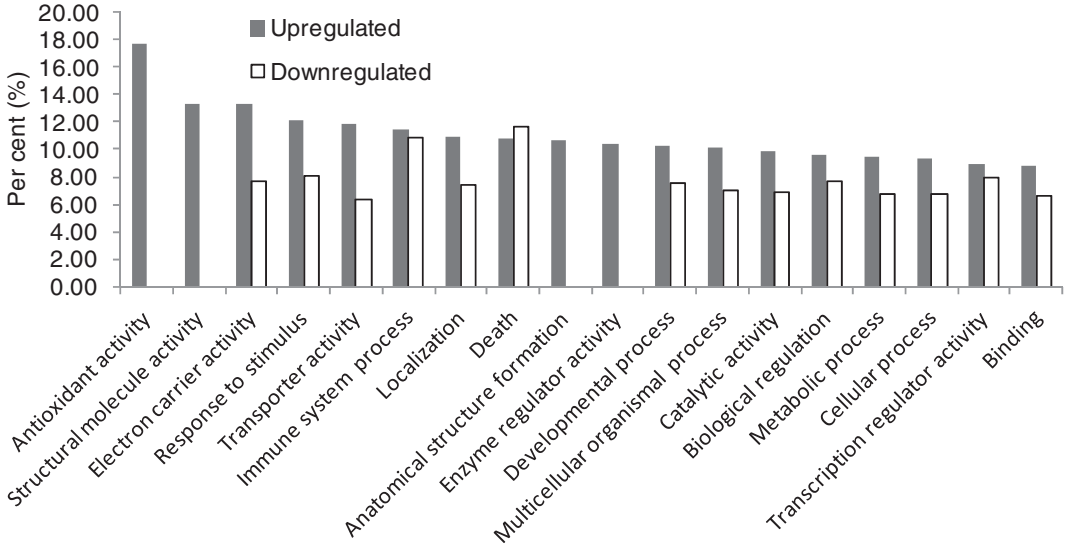


Figure 3. GO analysis of DEGs. Detailed DEG classifications were analysed using the GO web service.

(5%), sulphate (5%), nitrate (4%) and others. The 48 downregulated genes can be divided into 10 subcategories: metal ion (25%), amino acid and protein (13%), carbohydrate (11%), water (4%), sulphate (4%), anion (4%), phosphate (2%) and others.

As the largest subcategory of transmembrane transporter activity was metal ion and our study is related to the adaption of tobacco plants to potassium deficiency, we further analysed this category. The results showed that several transporter genes involved in the transport of macroelements, including nitrate (3), ammonium (2), phosphate (5), potassium (4), calcium (5), magnesium (2) and sulphate (4) were significantly expressed (table 2). In addition, several genes for transporting microelements, such as copper, iron, zinc, sodium and chloride were also significantly expressed. Most of these DEGs were upregulated. Only a few were downregulated, such as PHT1;7, KUP6, KUP11, COPT5, ZIP4 and CLC-C.

Transcription factors

Transcription factors (TFs) are proteins that bind to a specific DNA sequence to activate or inhibit gene transcription. In plant genome, a large number of transcription factors play

Table 2. Genes encoding ion transporters show changes in expression subjected to low potassium stress.

		Fold change			
	Symbol	6 h	12 h	24 h	Description
Ammonium	AMT1;1			4.26	Ammonium transporter 1;1
	AMT1;2	2.64	3.02	−2.48	Ammonium transporter 1;2
	VHA-E3		2.25	2.06	V-type proton ATPase subunit E3
Calcium	ACA2			0.49	Ca2+-transporting ATPase
	ACA8	−4.14	−3.75		Calcium-transporting ATPase 8
	ACA9			2.53	Ca2+-transporting ATPase
	ECA1		5.07	2.51	Ca2+-transporting ATPase
	ECA2			−2.02	Ca2+-transporting ATPase
Potassium	KUP3			3.82	Potassium transporter 4
	KUP11	−2.34			Potassium transporter 11
	KUP6	−2.18			Potassium transporter 6
	SKOR		2.01		Potassium channel SKOR
Phosphate	PHO1		2.49	3.43	Phosphate transporter PHO1
	PHT1;8	2.59			Putative inorganic phosphate transporter 1-8
	PHT1;9		2.81		Putative inorganic phosphate transporter 1-9
	PHT4;5		2.83		Putative anion transporter 6
	PHT1;7			−26.71	Putative inorganic phosphate transporter 1-7
Sulphate	SULTR1;3		−2.69	4.41	Sulphate transporter 1.3
	SULTR2;1	3.97	4.92	6.91	Sulphate transporter 2.1
	SULTR3;4	3.19			Putative sulphate transporter 3.4
	SULTR4;2		2.11		Putative sulphate transporter 4.2
Magnesium	MGT2	2.43			Magnesium transporter MRS2-1
	MGT3	2.20			Magnesium transporter MRS2-5
Sodium	NHX4		2.60	2.30	Sodium/hydrogen exchanger 3
Iron	IRT3		3.46		Fe(2+) transport protein 3
	VIT1	2.29			Vacuolar iron transporter 1
Copper	COPT5	−2.06			Copper transporter 5
Zinc	ZIP11	4.02	3.01		Zinc transporter 11
	ZIP5	16.07	5.96	3.85	Zinc transporter 5
	ZTP29			2.36	ZIP metal ion transporter
Nitrate	NRT1.7	6.21			Nitrate transporter 1.7
	NRT2.4	8.25			Nitrate transporter 2.4
	WR3		2.14		Nitrate transmembrane transporter
Chloride	CLC-C	2.65	2.72	3.61	Chloride channel protein CLC-c
Others	CAX11	−2.94	−2.08		Cation exchanger 11
	CAX3			−2.31	Vacuolar cation/proton exchanger 3
	CNGC1	−2.03	−2.58		Cyclic nucleotide-gated ion channel 1
	CNGC17		2.17		Cyclic nucleotide gated channel
	ATCHX13	2.48	4.52	3.08	Cation/H(+) symporter 13
	NRAMP3	3.69	3.83	4.23	Metal transporter Nramp3
	NRAMP1	2.11			Metal transporter Nramp1
	NRAMP2	2.88	2.54	2.20	Metal transporter Nramp2
	NRAMP4	2.11		2.36	Metal transporter Nramp4
	NRAMP6	3.44	2.84	3.06	Metal transporter Nramp6

Positive numbers in fold change are upregulated and negative numbers are downregulated.

Table 3. Genes encoding transcription factors show changes in expression subjected to low potassium stress.

	Symbol	Fold change 6 h	Fold change 12 h	Fold change 24 h	Description
MYB	MYB106	2.16			MYB domain protein 106
	MYB11	2.28			MYB domain protein 11
	MYB117	−2.95	−2.99	−2.28	MYB domain protein 117
	MYB12	3.12	2.88		Transcription factor MYB12
	AtMYB103	−2.01		−2.37	MYB domain protein 103
	MYB14	−2.24			MYB domain protein 14
	MYB15			3.63	MYB domain protein 15
	MYB19		2.13	3.55	MYB domain protein 19
	MYB26		4.87		MYB domain protein 26
	MYB305		2.70		MYB domain protein 305
	RVE1	2.13	5.43	−13.58	MYB family transcription factor
	MYB36		2.71	4.01	MYB domain protein 36
	MYB43		2.41	2.10	MYB domain protein 43
	MYB47		5.12		MYB domain protein 47
	MYB5			−3.79	Transcription repressor MYB5
	MYB56		2.60	3.10	MYB domain protein 56
	MYB6	−2.62	−2.54	−2.14	Transcription repressor MYB6
	MYB61	2.50	2.44	2.17	MYB proto-oncogene protein
	MYB82		2.41		Transcription factor MYB82
	MYB9			2.84	MYB domain protein 9
	MYB93		3.14	3.62	MYB domain protein 93
WRKY	WRKY11	−2.87	−3.61	−3.91	Putative WRKY transcription factor
	WRKY18	−11.92		−3.64	WRKY transcription factor 18
	WRKY20	2.00			Putative WRKY transcription factor
	WRKY22		−3.65		WRKY transcription factor 22
	WRKY26	3.00			WRKY DNA-binding protein 26
	WRKY3	2.30			WRKY DNA-binding protein 3
	TTR1	2.53		2.29	Putative WRKY transcription factor 16
	WRKY31		−4.58	−4.49	WRKY DNA-binding protein 31
	WRKY32			−2.41	WRKY DNA-binding protein 32
	WRKY33	−9.51	−10.37	−5.64	Putative WRKY transcription factor
	WRKY40	−10.71	−9.59		Putative WRKY transcription factor
	WRKY41	3.04	2.16	2.24	Putative WRKY transcription factor
	WRKY47			2.15	Putative WRKY transcription factor
	WRKY53	−25.90		−4.24	Putative WRKY transcription factor
	WRKY57	−2.87	−4.57	−3.02	Putative WRKY transcription factor
	WRKY67		−2.60	−2.85	Putative WRKY transcription factor
ERF	ERF-1	−4.51	−6.31	6.78	Ethylene-responsive transcription factor 1A
	ERF104	−5.53	−4.15	−2.88	Ethylene-responsive transcription factor ERF104
	ERF110	14.36			Ethylene-responsive transcription factor ERF110
	ERF13		3.11	4.67	Ethylene-responsive transcription factor 13
	ERF3	4.11	2.40	2.12	Ethylene-responsive transcription factor 3
	ERF4	−8.24	−6.56		Ethylene-responsive transcription factor 4
	ERF5	−22.52		−4.19	Ethylene-responsive transcription factor 5
	ERF9	−5.39	−6.21		Ethylene-responsive transcription factor 9
bZIP	bZIP48	3.09			Basic leucine-zipper 48
	bZIP53	−5.96			Basic region/leucine zipper motif 53 protein
	bZIP9			2.18	Basic leucine zipper 9
NAC	NAC007			2.08	NAC domain-containing protein 7
	NAC035			−2.20	NAC domain containing protein 35
	NAC058		4.54	3.81	NAC domain containing protein 58
	NAC073	3.76		2.35	NAC domain containing protein 73
	NAC075	2.65	2.29	2.59	NAC domain containing protein 75
	NAC080	2.37		−2.39	NAC domain containing protein 80
	NAC088	−2.91	−2.17		NAC domain containing protein 88
	NAC100	−4.58	−2.71		NAC domain containing protein 100
	NAC105	−13.01	−16.60	−18.48	NAC domain containing protein 105

Positive numbers in fold change are upregulated and negative numbers are downregulated.

Table 4. Genes encoding calcium signal transduction proteins showing expression changes under potassium stress.

Symbol	Fold change 6 h	Fold change 12 h	Fold change 24 h	Description
ANNAT1	2.39			Annexin D1
ANNAT4	3.15			Annexin D4
ANNAT7		2.13		Annexin D7
ANNAT8		3.11	2.20	Annexin D8
CAM5	−19.43	−16.80	−13.08	Calmodulin 5
CAM9	−5.59	−6.48	−2.07	Calmodulin-like protein 9
SOS3		2.08		Calcineurin B-like protein 4
CBL7	−2.01		−2.67	Calcineurin B-like protein 7
CML24			−3.45	Calcium-binding protein CML24
CML37	−64.72	−38.08	−26.28	Calcium-binding protein CML37
CNX1	2.88			Calnexin 1
CPK1	−2.03	−2.33	−3.87	Calcium dependent protein kinase 1
CPK7	−4.06			Calmodulin-domain protein kinase 7
CPK15		−2.34	−2.68	Calcium-dependent protein kinase 15
CPK18		3.74	6.53	Calcium-dependent protein kinase 18
CPK20	−7.56			Calcium-dependent protein kinase 20
CPK23	−5.09			Calcium-dependent protein kinase 23
CRT1b	3.27	2.09		Calreticulin-2
CRT3			−2.10	Calreticulin-3

Positive numbers in fold change are upregulated and negative numbers are downregulated.

key roles in the biotic or abiotic stress response (Eulgem *et al.* 2000; Dong *et al.* 2003; Fujita *et al.* 2006; Sakuma *et al.* 2006; Alves *et al.* 2013; Li *et al*. 2014). In our study, the expression levels of 108 upregulated TF genes and 93 downregulated TF genes changed more than 2-fold ($P < 0.05$) under low potassium stress (table 3). These DEGs were classified into MYB (21), WRKY (16), ERF (8), bZIP (3) and NAC (9) transcription factor families. The expression levels of *RVE1* (MYB family, 5.43) and NAC105 (NAC family, −18.48) were the highest of all the upregulated or downregulated genes encoding TFs, respectively. These results indicate that potassium deficiency induces global changes in the expression of genes involved in many physiological processes to adapt to the stress caused by this deficiency.

Calcium signal transduction proteins

Three primary types of calcium signal transduction proteins exist in plants, CaM, CPK and CBL, and these play key roles in plant development. Previous studies have shown that these proteins are strongly related to plant responses to biotic or abiotic stresses (Reddy and Reddy 2003; Xu *et al.* 2006; Luan 2009; Weinl and Kudla 2009; Ma *et al.* 2012). Our microarray analysis revealed 18 genes encoding calcium signal transduction proteins that were significantly expressed to adapt to potassium deficiency. These DEGs were classified into *Annexin* (4), *CaM* (2), *CPK* (6), *CBL* (2), *CML* (1) and *CRT* (2) families. All of the *Annexin* genes were upregulated, and all of the *CaM* genes and most of the *CPK* genes were downregulated (table 4). The expression level of *CPK 18* was upregulated more than 6-fold, and that of *CML37* was downregulated more than 26-fold. These results indicate that potassium deficiency induced changes in tobacco plant calcium signal transduction, likely to signal physiological processes necessary for adaptation to the stress caused by potassium deficiency.

Discussion

Potassium is one of the main cation involved in the plant development. Though there are millions of tons of potassium mineral deposited in the earth's crust, crops often encounter potassium deficiency in agricultural practice (Ashley *et al*. 2006). To adapt to this abiotic stress, plants develop complex mechanisms to survive, including enhanced ROS and Ca^{2+} signalling, potassium channel regulation and increased expression of potassium transporter genes (Wang and Wu 2013). In our study, potassium deficient conditions induced novel transcriptional changes in a large number of genes involved in mineral element transport, TF regulation, nitrogen metabolism, cell wall formation, carbohydrate metabolism, signal transduction, ageing and photosynthesis.

Mineral element transporters

Potassium absorption and uptake are mainly accomplished by potassium transporters and channels situated in the plasma membrane (Véry and Sentenac 2003; Ashley *et al*. 2006; Ward *et al*. 2009). Previous studies have shown that the expression of many potassium transporters, such as *AtHAK5*, *HvHAK1* and *OsHAK1* is induced by potassium deficiency (Santa-María *et al.* 1997; Bañuelos *et al.* 2002; Ahn *et al.* 2004; Gierth *et al.* 2005; Fulgenzi *et al.* 2008). In addition, rice root transcriptome analysis under low

potassium stress showed that four rice potassium transporters, *OsHKT2;1*, *OsHAK1*, *OsHAK7* and *OsHAK11* were upregulated throughout stress conditions (Ma *et al.* 2012). Of potassium channels, *OsAKT1*, *OsAKT2/3* and *OsKCO1* were also upregulated in response to potassium deficiency (Ma *et al.* 2012). In our microarray data, potassium transporter, *KUP3* and potassium channel, *SKOR* were upregulated under low potassium stress. This result is consistent with previous studies and indicates that a common regulatory mechanism exists across plant species whereby the transcription of genes encoding potassium transporters increases. This approach could be an efficient strategy for plants to increase potassium uptake in potassium deficient surroundings. Thus, we speculate that tobacco *KUP3* and *SKOR* are two potassium transport components involved in potassium absorption under low potassium stress.

Apart from potassium transporter genes, the expression levels of some nitrate and phosphate transporter genes under potassium deficient conditions also changed significantly ($P < 0.05$) in our study. Two nitrate transporter genes, *NRT1;7* and *NRT2;4* were upregulated after 6 h of stress. Similarly, four phosphate transporter genes, *PHO1*, *PHT1;8*, *PHT1;9* and *PHT4;5* were upregulated in response to potassium deficiency; however, PHT1;7 was downregulated (>26-fold). In rice, after 5 d of potassium starvation, two phosphate transporter genes (*PHO1* and *PHT1;4*) were upregulated, while two nitrate transporter genes (Os02g 0112600 and Os02g 0689900) were downregulated (Ma *et al*. 2012). In *Arabidopsis*, potassium deficiency reduced the expression levels of three nitrate transporter genes (*AtNRT2;1*, *AtNRT2;3* and *AtNRT2;6*) (Armengaud *et al*. 2004). Interestingly, previous studies have shown that nitrogen and phosphate deficient conditions also increase the transcriptional levels of potassium transporter genes including *HAK5*, *KUP10* and *KUP11* in *Arabidopsis* (Shin *et al.* 2005; Misson *et al.* 2005).

In addition, sulphate, magnesium, sodium, iron and zinc transporter genes were also upregulated during low potassium stress according to our microarray data. The cross-talk among these mineral elements suggests that plant absorption of these nutrients is interrelated. Deficiency in one element could cause changes in the absorption and translocation profiles of others. Thus, further research is needed to explore this phenomenon.

Transcription factors

One important function of TFs is to respond to environmental stimuli (Li *et al.* 2014). Under unfavourable conditions, TFs such as WRKY, MYB, ERF, bZIP and NAC are induced and cause gene expression that protects plants from damage caused by stress (Chen *et al.* 2012). Previous studies have shown that many TFs are induced under abiotic stresses including drought, salt, temperature, metal and light (Soo 2006; Rushton *et al.* 2010; Mizoi *et al.* 2012; Nakashima *et al.* 2012; Li *et al.* 2014). However, only a few TFs respond to mineral starvation. Under phosphate (Pi) deficient conditions, transcription of *CrPSR1*, *PAP1*, *PAP2*, *MYB108*, *At1g 13300*, *At1g 68670*, *AtPHR2*, *BnPHR1* and *At3g 25790* is upregulated, while that of *MYB111* is downregulated (Todd *et al.* 2004; Nilsson *et al.* 2010; Ren *et al.* 2012). Overexpression of *OsPHR2* leads to enhanced Pi uptake and accumulation (Zhou *et al.* 2008). In addition, AtMYB2 and OsMYB2P-1 can positively regulate Pi absorption and translocation in response to Pi deprivation (Dai *et al.* 2012; Baek *et al.* 2013). When *Arabidopsis* encounters nitrogen starvation, *AtNSR1* is upregulated (Todd *et al.* 2004). In *Cyanidioschyzon merolae, CmMYB1* plays a pivotal role in response to nitrogen depletion (Liu *et al.* 2013). Consistent with these findings, we identified 57 TFs whose transcription levels changed significantly in our microarray data. Among them, 16 TFs from MYB, six from WRKY, five from NAC, three from ERF and two from bZIP families were upregulated (table 3). These results indicate that plant TFs extensively participate in the regulation of potassium absorption and translocation in response to potassium depletion. However, further research is needed to elucidate the mechanistic details.

Calcium sensors

Some calcium-binding proteins are important potassium responsive components in *Arabidopsis* (Armengaud *et al*. 2004). Under low potassium stress, CBL1/9 together with CIPK23 can activate potassium uptake in roots by modulating the activity of the potassium channel, AKT1 (Li *et al*. 2006; Xu *et al*. 2006). CBL3 interacting with CIPK9 can regulate potassium homeostasis in response to potassium deficiency (Liu *et al.* 2013). By forming a calcium sensor/protein kinase complex, CBL4/CIPK6 can enhance AKT2 activity (Held *et al.* 2011). In addition, CBL10 can directly regulate AKT1 activity and enhance potassium uptake (Ren *et al.* 2013). In rice, under low potassium stress, the expression levels of the calcium sensor protein genes *OsCBL5*, *OsCML1*, *OsCML18*, *OsCML20* and *OsCML31* are upregulated while those of *OsCPK9*, *OsCML5*, *OsCML11* and *OsCCaMK1* are downregulated (Ma *et al.* 2012). In addition, drought and salt can induce the expression of genes from Annexin (Huh *et al.* 2010) and CML families (Xu *et al.* 2011). Consistent with previous studies, 18 genes from CBL, CPK, CML, Annexin and CRT families were differentially expressed in our microarray data (table 4). These results suggest that calcium sensor proteins also play vital roles in potassium-responsive signal transduction and that a plant may recruit downstream genes in response to abiotic stresses through similar signal transduction networks.

Conclusion

Our research identified 3790 genes that exhibited significant changes in their transcription levels in response to low potassium stress. These DEGs were mainly in GO categories

of binding, transcription regulation, metabolic processes, transports and enzyme regulation. Many genes in the TF, calcium sensor and ion transporter families were upregulated at all three time points, such as MYB 61, WRKY 41, ERF3, NAC 075, SULTR2;1, ZIP5, CLC-C, ATCHX13, NRAMP3, NRAMP2 and NRAMP6. However, many genes classified into these categories like MYB117, MYB6, WRKY11, WRKY33, WRKY57, ERF-1, ERF104, NAC105, CAM5, CAM9, CML37 and CPK1 were significantly downregulated. For potassium transporters, SKOR and KUP3 were upregulated at 12 and 24 h, while KUP6 and KUP11 were downregulated at 6 h, respectively. Nevertheless, detailed studies are required to clarify the mechanisms behind these phenomena.

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