Effects of top excision on the potassium accumulation and expression of potassium channel genes in tobacco

Xiao Yan Dai1,2, Yi Rong Su2,* Wen Xue Wei2, Jin Shui Wu2 and Ye Kuan Fan1

1 College of Resource and Environment, Huazhong Agriculture University, Wuhan 430070, PR China
2 The Key Laboratory of Subtropical Agro-ecology, Institute of Subtropical Agriculture, CAS, Changsha 410125, PR China

Received 10 September 2008; Revised 18 October 2008; Accepted 21 October 2008

Abstract

The effects of the removal of the shoot apex of tobacco on the relative transcript levels of potassium channel genes, determined by real-time PCR, and on the relationship between the expression of genes encoding potassium channels and potassium concentration, were studied. The results from the study indicated that comparatively more assimilates of photosynthesis were allocated to the apex in control plants than in both decapitated and IAA-treated decapitated plants. By contrast, dry matter in the upper leaves, roots, and stems in both decapitated and IAA-treated plants was significantly increased relative to control plants. The potassium level in whole plants decreased post-decapitation compared with control plants, and so did the potassium concentration in middle and upper leaves, stem, and roots. Expression of \(NKT1\), \(NtKC1\), \(NTORK1\), and \(NKT2\) was inhibited by decapitation in tobacco leaves with a gradual reduction after decapitation, but was induced in roots. The relative expression of \(NKT1\), \(NTORK1\), and \(NKT2\) in tobacco leaves was higher than that in roots, whereas the expression of \(NtKC1\) was higher in roots. The levels of inhibition and induction of \(NKT1\), \(NtKC1\), \(NTORK1\), and \(NKT2\) in leaves and roots, respectively, associated with decapitation were reduced by the application of IAA on the cut surface of the decapitated stem. Further results showed that the level of endogenous auxin IAA in decapitated plants, which dropped in leaves and increased in roots by 140.7% at 14 d compared with the control plant, might be attributed to the change in the expression of potassium channel genes. The results suggest that there is a reciprocal relationship among endogenous auxin IAA, expression of potassium channel genes and potassium accumulation. They further imply that the endogenous IAA probably plays a role in regulating the expression of potassium channel genes, and that variations in expression of these genes affected the accumulation and distribution of potassium in tobacco.

Key words: Decapitation, gene expression, potassium channel, potassium accumulation, tobacco (\textit{Nicotiana tabacum} L.).

Introduction

The plant apex is not only an important source of auxins, but also a centre of growth and metabolism in higher plants. It is standard practice in tobacco production that the apices of plants are excised when the first flower on the inflorescence blossoms. This is beneficial for getting high yield of quality leaves, but it results in a fall in leaf \(K^+\) concentration (Jiang et al., 2001; Yang et al., 2007). \(K^+\) is one of the most abundant cations in cells of higher plants and plays vital roles in plant growth and development. The \(K^+\) concentration in leaves is an important index for tobacco quality (Leggett et al., 1977). Yang’s results indicate that the sink–source relationship in tobacco plants is changed by top excision and the concentration of endogenous hormone IAA decreases from shoots to roots which could activate plasmalemma potassium channels and regulate \(K^+\) uptake from soil, and affect \(K^+\) transport and distribution (Blatt and Thiel, 1993; Claussen et al., 1997; Jiang et al., 2001; Yang et al., 2007).

Epstein and colleagues provided the first evidence of the operation of at least two (high- and low-affinity) \(K^+\) uptake systems in plants (Epstein et al., 1963; Kochian and Lucas, 1988). The two transport systems were proposed to play roles...
in uptake that correspond with external K⁺ concentrations. The high-affinity system plays a role in low external K⁺ concentration (1–200 μmol l⁻¹), when K⁺ uptake follows a Michaelis–Menten dynamics equation primarily through carrier absorption, with H⁺, Na⁺ co-transport (Maathuis and Schroeder, 1996). The low-affinity system plays a role in high external K⁺ concentration (1–10 mmol l⁻¹), when K⁺ uptake is mainly regulated by potassium channels. Generally, the exchange K⁺ concentration in tobacco planting soils is within 1–10 mmol l⁻¹. Although potassium channels are not the only means for plant K⁺ absorption, they may play the leading role under normal tobacco planting soil conditions.

Potassium channels are important in K⁺ uptake, and are characteristically large molecules (Mäser et al., 2001; Maathuis and Schroeder, 1996). There are three types of potassium channels in plants including hyperpolarization-activated inward-rectifying channels, leak-like inward-rectifying channels, and depolarization-activated outward-rectifying channels. Direct evidence has been obtained that potassium channels participate in several processes at the cell and whole plant levels, such as cell elongation, stomatal movements, and regulation of gas exchanges, and in the transduction of various signals (Hosy et al., 2003; Dolan and Davies, 2004; Iwashikina et al., 2005). Besides these roles, potassium channels are involved in K⁺ uptake from the soil solution and contribute to K⁺ translocation towards the shoots (Gaymard et al., 1998; Hirsch et al., 1998; Gierth et al., 2005; Johansson et al., 2006), and play an important role both in loading in source leaves and unloading in sink organs (Marten et al., 1999; Lacombe et al., 2000; Pilot et al., 2001; Xicluna et al., 2007). Potassium channels, which include the inward-rectifiers NKT1, NKT2, NtKCl, and the outward-rectifier NTORK1, were cloned from BY2 cells of tobacco (Sano et al., 2007). In line with their affiliation to the known subfamilies of plant Shaker-like K⁺ channels, NKT1, NKT2, and NtKCl belong to the Arabidopsis thaliana AKT1, AKT2/3, and AtKCl subfamilies, respectively, while NTORK1 is homologous to members of the A. thaliana SKOR/GORK subfamily (Kasukabe et al., 2006; Sano et al., 2007).

Until now, it was not clear whether the activities of potassium channels could be regulated directly by decapitation in tobacco plants and no attempts had been made to understand the molecular mechanisms of potassium regulation in decapitated tobacco plants. Similarly, the relationship between potassium channel activities and potassium accumulation and distribution remains unclear. In this paper, studies aimed at understanding the possible mechanisms of regulation of the K⁺ content in tobacco by decapitation, and by topical application of exogenous auxin to the decapitated plants are reported. The effects of decapitation and of the replacement of the apex by an exogenous source of auxin (IAA) on the levels of expression, as measured by real-time PCR, of potassium channel genes in tobacco plants were investigated. The relationship between the expression of potassium channel genes and potassium accumulation and distribution in tobacco plants was also investigated.

### Materials and methods

#### Plant growth

Tobacco seeds (Nicotiana tabacum L. Yun 87) were germinated in a mixture of 60% (w/w) peat, 20% (w/w) perlite, and 20% (w/w) vermiculite, and grown in a seedbed in a naturally illuminated greenhouse until the normal transplanting stage (about 3 months). The plants were then transferred to the pots (31×33 cm, one plant per pot) containing 16 kg growth medium (70% perlite, 15% soil, and 15% sawdust). The above material was well mixed with 50 g of rape seed cake, 49.5 g of diammonium phosphates, 49.5 g of potassium soleplates, and 15.8 g of ammonium nitrate. Throughout the experiments the K⁺ concentration of the substrate mixture was maintained between 2–4 mmol l⁻¹. At 3 weeks and 5 weeks after transplanting, Hoagland’s nutrient solution containing 1 mM KH₂PO₄, 5 mM KNO₃, 5 mM Ca(NO₃)₂, 2 mM MgSO₄, 4.6×10⁻² mM H₂BO₃, 7.65×10⁻⁴ mM ZnSO₄·7H₂O, 3.2×10⁻⁴ mM CuSO₄, 1.6×10⁻⁵ mM (NH₄)₆MoO₂₄·9×10⁻³ mM MnCl₂·4H₂O, and 3.7×10⁻² mM FeEDTA, was provided (200 ml each time per plant) (Jiang et al., 2001). The pots were adequately watered for the growth of tobacco during the period of culture and treatments.

#### Treatments and harvest procedures

Thirty-six tobacco plants were used in the experiment. Decapitation and IAA application treatments were performed when the first flower inflorescence was blossoming. Plant leaves were numbered in ascending order, starting with the lowest expanded leaf, which was designated as leaf no.1. Smaller leaves, which had already senesced, were removed. The youngest unfolded leaf was no. 27. Plants, at similar stages of development, were treated as follows: (i) intact plants, no treatment (control); (ii) apices were excised above the young unfolded leaf, no. 24 from the base (decapitated); and (iii) apices were excised in the same way above leaf no. 24 and immediately a paste of 30 mg kg⁻¹ indole acetic acid (IAA) in lanolin was applied to the decapitated stem stump (decapitated+IAA). The control plants had 27 leaves including the top and three young unfolded leaves. In both decapitated and IAA-treated plants, lateral bud outgrowths were excised when they reached about 0.5 cm in length. The harvest times were 1 h, 5 h, 24 h, and 14 d and they were, respectively, at 09:00 h on 23 May, 13:00 h on 23 May, 08:00 h on 24 May, and 08:00 h on 6 June. The time of decapitation treatment was at 08:00 h on 23 May. About 10 min later, the plant auxin IAA was used on the cut face of tobacco stems. There were three separately harvested plants for every treatment at each sampling time, and the three separately harvested plants were regarded as three separate materials for the following analysis.

Leaf no. 23 was used for gene expression analysis. Leaf no. 24 was nipped for endogenous hormone analysis. Roots were washed free of potting mixture with distilled water. For expression and endogenous hormone analysis, radicles about 0–10 cm in length were nipped into tubes (10 ml) which had been frozen in liquid nitrogen beforehand. All
samples, which were used for gene expression and endogenous hormone analyses, were immediately frozen in liquid nitrogen after harvest and stored at −70 °C until RNA isolation or hormone analysis. At harvest, plants that were used for potassium analysis were separated into roots, stem, top (apex and enclosed leaves), and lower (from leaf 1 to leaf 10), middle (from leaf 11 to leaf 18), and upper stratum leaves (from leaf 19 to leaf 22). All plant parts for potassium analysis were weighed (fresh wt.), dried (60 °C) and weighed again (dry wt.).

**Measurement of potassium concentration and endogenous hormone IAA**

Potassium of different plant organs was digested by H2SO4–H2O2, after 50× dilution, and then the K in the digested solution was measured by AAS analysis. The K concentration in different organs was determined using a GBC932 analyser (GBC, Australia). The methods for extraction and purification of IAA were modified from those described by Bollmark et al. (1988) and He (1993). A small quantity of tobacco tissues (0.5 g) was ground in an ice-cooled mortar in 10 ml 80% (v/v) methanol extraction medium containing 1 mM butylated hydroxytolence as an antioxidant. The extract was incubated for 4 h at 4 °C and then centrifuged at 4000 g for 15 min at 2–8 °C. The supernatant was passed through Chromosep C18 columns (C18 Sep-Park Cartridge, Waters Corp., Millford, MA), prewashed with 10 ml 100% (w/v) and 5 ml 80% (v/v) methanol, respectively. The hormone fractions eluted with methanol, respectively. The hormone fractions eluted with 10 ml 100% (v/v) methanol and 10 ml ether from the columns, then dried under N2, and dissolved in 2 ml phosphate buffer saline (PBS) containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) for analysis by ELISA..

The mouse monoclonal antigens and antibodies against IAA and IgG horseradish peroxides used in ELISA were produced at the Phytohormones Research Institute (China Agricultural University; see He, 1993). Colour development in each well was detected using an ELISA Reader (model EL310, Bio-TEK, Winooski, VT) at an optical density of A490. The IAA content was calculated following Weiler et al. (1981).

**RNA isolation and analyses**

Total RNA samples from leaves and roots of tobacco at different times after treatments were extracted with TRizol and treated with deoxyribonuclease I (Invitrogen, Carlsbad, CA, USA) for 30 min at 37 °C. The reaction was terminated by heating for 10 min at 70 °C. RNA was quantified and its quality assessed by measuring its OD at 260 nm and 280 nm. To verify the RNA integrity, 10 μg of total RNA from each sample was fractionated in a 2.2 M formaldehyde–1.5% agarose gel, stained with ethidium bromide, and visualized under UV light. The presence of intact 28S and 18S rRNA bands was used as a criterion of RNA integrity. The intensity of the rRNA bands was used to confirm the quantification by spectrophotometry and the equalized loading of RNA in each lane of the agarose gels used for blotting.

**cDNA preparation and quantitative real-time PCR**

First strand cDNA synthesis was performed in a standard reaction using 0.8 μg of total RNA in combination with oligo(dT)-18 as a primer (Fermentas, UK) in a 20 μl volume using M-MLV reverse transcriptase. All the real-time PCR reactions were performed using a Rotor-Gene RG-3000 Detection System (Corbett Research). The SYBR Premix Ex Taq™ PCR Reagent kit was used for the PCRs (Takara Bio Inc., Otsu, Japan). The thermal cycling conditions comprised an initial step at 95 °C for 15 s, followed by 40 cycles at 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 15 s. In all the experiments, appropriate negative controls containing no template DNA or RNA were subjected to the same procedures to exclude or detect any possible contamination or carryover. Each sample analysis was repeated at least three times. In real-time PCR analysis, quantification is based on the threshold cycle. The PCR cycle at which the increase of SYBR green fluorescence becomes statistically significant is called the threshold value (Ct). The Primer Premier 5.0 programme was used for the design of gene-specific primers. The primers used are summarized in Table 1. Gene-specific primers were synthesized by the Invitrogen Corporation (Carlsbad, CA, USA) to generate PCR products of lengths between 100 bp and

### Table 1. Primers used in qPCR experiments

<table>
<thead>
<tr>
<th>Target gene</th>
<th>GenBank accession no.</th>
<th>Sequence of primers</th>
<th>PCR products/bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKT1</td>
<td>AB196790</td>
<td>5’-TTTGCTGTGTGATGGTACTCCAG-3’</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-TACCCGCCCCCTAGATTAGTG-3’</td>
<td>131</td>
</tr>
<tr>
<td>NIKC1</td>
<td>AB196791</td>
<td>5’-CATATTGTCAATGGGCGAGATG-3’</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-TCCTCGGTACATCGGTTTCTG-3’</td>
<td>175</td>
</tr>
<tr>
<td>NTORK1</td>
<td>AB196792</td>
<td>5’-AGTGAAACAAACTTGGAGATCTCC-3’</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-GAGAAGACTAAATGCTGACATGG-3’</td>
<td>199</td>
</tr>
<tr>
<td>NKT2</td>
<td>AB196789</td>
<td>5’-GCTCAAGATCGTGGAGTCGAG-3’</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-CTGAGATGCAAGTTTGGCATG-3’</td>
<td>175</td>
</tr>
<tr>
<td>ACTIN</td>
<td>AB158612</td>
<td>5’-AACAGTTTGTGGAGTTCTTCG-3’</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-CATGAGATAAAAAGGCGGGATG-3’</td>
<td>199</td>
</tr>
</tbody>
</table>
250 bp. The $2^{-\Delta\Delta C_{t}}$ method was used to calculate the quantitative PCR data (Kenneth and Thomas, 2001). The expression of potassium channel genes was relative to Nicotiana tabacum actin (AB158612; Yasuda et al., 2005). In order to detect the suitability of actin as an internal gene, the difference of the actin expression in leaves and roots in different treatments and times was done and no significant difference was found (data not shown).

**Statistical treatments**

$K^{+}$ concentration was determined from three replicates of each treatment at different sampling times. All further analyses were made with three individual samples for each organ. All the values reported in this paper were the means of three replicates. All data obtained was subjected to one-way analysis of variance (ANOVA) and the mean differences were compared by the least significant difference (LSD) test. Comparisons with $P$-values <0.05 were considered significantly different. Where appropriate, data are presented as ± standard error (SE) of the mean.

**Results**

**Plant growth**

The results on the dry weights of organs in different treatments are shown in Table 2. The dry matter of different organs obtained for the three treatments had increased significantly at 14 d compared with that at 1 h after decapitation. There were no significant differences in dry matter of different organs among the three treatments from 1 h to 24 h, however, the dry weights in stems and roots of decapitated and IAA-treated plants were significantly higher than those of the control plants at 14 d ($P < 0.05$). This suggested that there were more assimilates of photosynthesis being deposited in stems and roots in the treated plants, and removing the shoot apex resulted in the over-proportional growth of stems and roots. In both decapitated and IAA-treated plants, the dry matter in the upper leaves increased significantly compared with non-decapitated plants ($P < 0.05$), and there was a slight rise in the middle leaves. However, in the control plants, there was a substantial dry matter gain in the top and newly formed leaves, which accounted for 15% of the total dry weigh of the plants. This showed that the presence and growth of shoot apices strongly inhibited the growth of the upper leaves.

**Response of $K^{+}$ content and concentration in tobacco to different treatments**

The potassium content in whole plants showed an increasing trend from 1 h to 14 d, which represented a rise of about 42.7%, 27.5%, and 31.5% in control, decapitated, and IAA-treated plants at 14 d, compared to at 1 h, respectively (Fig. 1A). Furthermore the rate of potassium accumulation and the amount of accumulated potassium in control plants were higher than those of decapitated and IAA-treated plants. But there was no significant difference found among the three treatments at every sampling time. The absorbed $K^{+}$ in non-decapitated plants was mostly deposited in the shoot apices and young leaves which were the main $K^{+}$ accumulating sink organs, accounting for 14.5% of the whole plant at 14 d (Fig. 1B). The $K^{+}$ distribution in the middle and upper leaves of non-decapitated plants accounted for 38.3% of the whole plant, but in decapitated plants it accounted for 47.9%. Compared with control plants, the absorbed potassium in decapitated and IAA-treated plants mostly accumulated in the middle and upper leaves because of the removal of the shoot apices (Fig. 1B). This implies that there is strong competition for this mineral nutrient between the apex and leaves, and that though the apices in control plants contributed to $K^{+}$ accumulation in the whole plant, they reduced the deposition of the mineral in leaves. Thus, excising the shoot apex of tobacco plants could greatly reduce unnecessary in vivo consumption of potassium, and replacement of the apex by an exogenous source of IAA can contribute to the accumulation of potassium in the upper and middle leaves (Fig. 1B).

There was no significant difference in the $K^{+}$ concentration of all plant organs among the decapitated plants, IAA-treated plants, and non-decapitated plants within 24 h after decapitation (data not shown). By contrast, at 14 d after decapitation, the $K^{+}$ concentration in decapitated and IAA-treated plants rose in the lower leaves and dropped in middle leaves, upper leaves, stems, and roots compared with that of control plants (Table 3). However, the $K^{+}$ concentration of roots and lower leaves of IAA-treated plants was higher than those of decapitated plants and there was no significant difference in the middle and upper leaves because of their relatively greater increase in dry matter.

**Effects of decapitation on the expression of potassium channel genes in tobacco**

Inward-rectifier potassium channel genes: The mRNA transcript levels of $NKT1$ in tobacco leaves and roots are

Table 2. Dry weights in different organs of tobacco 14 d after treatments (g DW per organ)

Values are mean ± SE (n=3). Means in the same column followed by different letters are significantly different at $P < 0.05$ as determined by the ANOVA test.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Top+new leaves</th>
<th>Upper leaves</th>
<th>Middle leaves</th>
<th>Lower leaves</th>
<th>Stem</th>
<th>Roots</th>
<th>Whole plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>No decapitation</td>
<td>28.31±4.55</td>
<td>17.94±0.89 b</td>
<td>60.67±7.20 a</td>
<td>30.65±8.97 a</td>
<td>48.42±3.94 b</td>
<td>36.17±3.84 b</td>
<td>222.17±19.13 a</td>
</tr>
</tbody>
</table>
shown in Fig. 1. Kasukabe et al. (2006) compared the deduced amino acid sequence with other reported K+ channels and found that NKT1 could be classified into the inward-rectifying K+ channels subfamily, exhibiting 61% identity with the A. thaliana AKT1, which contributes to K+ uptake from the soil solution even at low external K+.

The expression of NKT1 in leaves of non-decapitated control plants reached a maximum at 5 h and then dropped (Fig. 2A). Expression in leaves of decapitated tobacco was clearly inhibited, showing 9.5-fold lower levels of expression relative to the non-decapitated plants at 5 h, and showed a decrease of 1.4-fold at 1 h, 1.5-fold at 24 h, and 1.1-fold at 14 d, respectively (Fig. 2A). The observed differences of NKT1 expression in leaves between non-decapitated plants and decapitated plants were significant at 1 h and 5 h after treatment, but not at the other sampling times (P <0.05).

A similar trend was observed in the expression of NKT1 in roots of non-decapitated versus decapitated plants. In marked contrast to that in leaves, the expression in roots was elevated in decapitated tobacco showing an increase of 118.5% at 1 h, 100.0% at 5 h, 50.0% at 24 h, and 100.0% at 14 d compared with non-decapitated plants (Fig. 2B). This suggested that excision of the apex could induce the expression of NKT1 in roots. But no significant difference was found in the NKT1 expression in roots between the decapitated plants and control. The expression of NKT1 in leaves was higher than in roots in both decapitated and non-decapitated plants (Fig. 2A, B). This suggested that NKT1 might not be the key gene for K+ uptake from soil, in contrast to AKT1 in A. thaliana which is expressed mainly in the plasma membrane of root periphery cells and has low expression in leaves (Bassett et al., 1995; Lagarde et al., 1996; Broadly et al., 2001).

NtKC1 forms an inwardly-rectifying potassium channel corresponding to the AtKC1 gene of A. thaliana which is expressed predominantly in root hairs and root endodermis and contributes to K+ influx from the soil solution together with AKT1 and AKT2 (Dreyer et al., 1997; Reintanz et al., 2002; Sano et al., 2007). The expression of NtKC1 in leaves of non-decapitated plants appeared to be under circadian control, but that of decapitated plants decreased gradually with a 2.5-fold reduction relative to that of non-decapitated plant and the difference between them was significant (P <0.05) at 24 h (Fig. 3A). However, the difference in the expression in leaves was not significant between the control and decapitated plants at 1 h, 5 h, and 14 d. The patterns of expression of NtKC1 and NKT1 in roots of non-decapitated plants were similar. The expression of NtKC1 in roots of decapitated plants was highest at 1 h and remained constant from 5 h. This corresponded to increases of 80.9% at 5 h, 21.5% at 24 h, and 4.4% at 14 d, compared with non-decapitated plants (Fig. 3B). The difference was significant in the NtKC1 expression of roots at 5 h (P <0.05). Different from NKT1, the expression of NtKC1 in leaves was lower than that in roots (Fig. 3A, B). This observation is consistent with NtKC1 playing an important role in the regulation of K+ uptake from soil among potassium channels.

**Fig. 1.** K accumulation and distribution in tobacco plants. K accumulation in whole plants was calculated as the product of K content by biomass of all organs of the tobacco plants (A). K distribution in different organs of tobacco plants after 14 d post-treatment (B). Key to symbols: (white bars), no decapitation; (hashed bars) decapitation; (black bars) decapitation+IAA. The bars denote satandard errors of the mean, n=3.

**Table 3.** K+ concentration in different organs of tobacco in 14 d after treatments (%)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Top + new leaves</th>
<th>Upper leaves</th>
<th>Middle leaves</th>
<th>Lower leaves</th>
<th>Stem</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>No decapitation</td>
<td>4.04±0.17</td>
<td>4.30±0.28 a</td>
<td>3.77±0.12 a</td>
<td>5.70±0.50 a</td>
<td>3.00±0.06 a</td>
<td>1.59±0.14 a</td>
</tr>
<tr>
<td>Decapitation</td>
<td>4.10±0.18 a</td>
<td>3.74±0.35 a</td>
<td>5.95±0.33 a</td>
<td>2.44±0.03 b</td>
<td>1.44±0.12 a</td>
<td></td>
</tr>
<tr>
<td>Decapitation +IAA</td>
<td>4.05±0.16 a</td>
<td>3.71±0.09 a</td>
<td>6.00±0.40 a</td>
<td>2.44±0.14 b</td>
<td>1.49±0.11 a</td>
<td></td>
</tr>
</tbody>
</table>
non-decapitated and decapitated plants, and it was inhibited in decapitated plants showing 2.4-fold lower levels of expression relative to non-decapitated plants and the difference was significant at 24 h \((P<0.05)\) (Fig. 4A). The expression of \(\text{NTORK1}\) in roots was regulated by decapitation, with mRNA accumulation increasing from 1 h, with increases of 77.8% at 5 h, 39.1% at 24 h, and 23.1% at 14 d, compared with its expression in non-decapitated plants (Fig. 4B). The differences in the \(\text{NTORK1}\) expression in roots between the control and decapitated plants were not significant during the sampling period, except at 5 h.

\(\text{NTORK1}\) activity is modulated by external K+ due to K+-dependent gating and the depolarization activity of \(\text{NTORK1}\) in guard cells is weakened when the external \([\text{K}^+]\) is very low, and thus the expression of the gene in leaves is inhibited (Ache \textit{et al.}, 2000; Sano \textit{et al.}, 2007). The expression of \(\text{NTORK1}\) in tobacco leaves was also higher than that in roots, as for \(\text{NKT1}\).

Interestingly, a reverse of the variation of expression was observed between \(\text{NKT1}\) and \(\text{NtKC1}\), \(\text{NTORK1}\) in roots of decapitated plants (Figs 2B, 3B, 4B). This indicated that the effects of decapitation on the expressions of inward-rectifying and outward-rectifying potassium channel genes were different.

**Leak-like inward-rectifier potassium channel gene: NKT2**

belongs to the \(\text{A. thaliana AKT2/3}\) subfamily which is expressed in the phloem vasculature both in leaves and roots (Sano \textit{et al.}, 2007; Dennison \textit{et al.}, 2001). It mainly participates in the aerial part of K+ transport in the plant and regulates the phloem cell membrane voltage, sharing cell K+ osmotic potential in leaves. There was no significant difference of expression of \(\text{NKT2}\) in non-decapitated plants leaves from 1 h to 24 h, but the expression dropped significantly at 14 d (Fig. 5A). The expression of \(\text{NKT2}\) in leaves of decapitated plants dropped after 5 h. \(\text{NKT2}\) expression in tobacco leaves was significantly inhibited in decapitated plants being 52.94% lower than that in the leaves of non-decapitated plants at 24 h \((P<0.05)\) (Fig. 5A). But there were no significant differences at other
times in the NKT2 expression in leaves. The expression of NKT2 in roots was lower than that in leaves for both decapitated and control plants (Fig. 5A, B). In non-decapitated plants it dropped gradually from 1 h to 24 h and rose at 14 d. Decapitated plants showed a reverse pattern of expression with a rapid increase, and at 14 d it decreased to the same level as at 5 h (Fig. 5B). The difference of expression of NKT2 in roots of decapitated and non-decapitated plants was significant at 5 h and 24 h (P < 0.05). In contrast with that in leaves, the expression of NKT2 in roots of decapitated plants was induced, showing 1.4-fold and 4.6-fold higher levels than in non-decapitated plants at 5 h and 24 h, respectively. Figure 5 also shows that the expression of NKT2 in leaves and roots of control and decapitated plants had reverse trends. This fact led to the hypothesis that NKT2 may play a role both in K⁺ loading in source leaves and unloading in sink organs, because it mediates potassium influx and potassium efflux and it displays two distinct gating modes (Dreyer et al., 2001).

Together, the findings indicated that excision of the apex had important but diverse effects on the expression of NKT1, NtKC1, NTORK1, and NKT2 in tobacco plants. The observed differences in the variation of expression of the four potassium channel genes may be related to their specific roles in K⁺ uptake and transport.

Effects of application of IAA on the expression of potassium channel genes in tobacco

The expression of NtKC1, NTORK1, and NKT2 in leaves of IAA-treated plants showed similar trends, with maximal expressions at 5 h (Figs 3A, 4A, 5A). For both NtKC1 and NTORK1 the levels of expression were similar between 1 h and 24 h, but the expression of NKT2 at 24 h was significantly higher than that at 1 h. The expression of NKT1 in leaves of IAA-treated plants showed a steady state at all times tested. The expression of NKT1, NtKC1, NTORK1, and NKT2 in leaves of IAA-treated-plants was lower than in decapitated plants at 1 h, and higher than that of decapitated
plants at 5 h and 24 h, and the same as that of decapitated plants at 14 d.

In roots of IAA-treated plants, the maximal expression of \( NKT1 \) occurred at 1 h and then quickly decreased (Fig. 2B). This contrasted with the expressions of \( NiKC1 \) and \( NTORK1 \), which were at their lowest level at 1 h and then increased (Figs 3B, 4B). The expression of \( NKT2 \) in roots of IAA-treated plants was nearly constant throughout. These results suggested that IAA may play an important role in the regulation of expression of potassium channel genes.

These results (Figs 2, 3, 4, 5) also indicated that the expression of potassium channel genes was inhibited by decapitation in the leaves from 1 h, but not in roots, except \( NiKC1 \). The expression of \( NKT1 \) and \( NTORK1 \) in leaves and of \( NKT1 \), \( NiKC1 \), \( NTORK1 \), and \( NKT2 \) in roots was affected by decapitation and by IAA application throughout the duration of the experiment from 1 h to 14 d. By contrast, the expressions of \( NiKC1 \) and \( NKT2 \) in leaves were under short-term control. As shown in Fig. 2A and Fig. 5B the expression of \( NTK1 \) in leaves and of \( NKT2 \) in roots of IAA-treated plants, respectively, remained constant at all times. This indicates that \( NKT1 \) and \( NKT2 \) were steadily down-regulated by auxin.

**Discussion**

*The relationship between the expression of K*⁺* channel genes and potassium nutrition in tobacco*

Excision of the apex is usually reported to cause a drop of potassium concentration in tobacco. The same results were observed at each of the sampling times except at 1 h after excision (Fig. 1A). Changes in the distribution of the mineral have generally been used to explain these results. Jiang et al. (2001) and Yang et al. (2007), using sink–source relationships, explained that there were higher net flows of K⁺ in the phloem than in the xylem and K⁺ uptake activity in roots was restrained after decapitation. In this study, the expression of potassium channel genes in leaves and roots of tobacco changed after decapitation. However, no significant positive or negative correlation was found between the expression of potassium channel genes and potassium accumulation. A possible explanation might be that the accumulation of potassium is not regulated by the expression of a single gene but rather may depend on a balance of expression of several potassium channel genes. Indeed, K⁺ uptake and flux in plants are mediated by many families of transporters and channels (Chérel, 2004). Physiological analyses indicate that a large set of transport systems, differing in their affinity for K⁺, selectivity and energetic coupling, are involved in K⁺ uptake from the soil, and its translocation and compartmentalization (Lebaudy et al., 2007). For example, the members of the Shaker-like plant K⁺ channel family share a similar universal structure, but they are highly diverse in their specific function. Also different tissues and organs of a plant show expression of different K⁺ channel genes (Poreé et al., 2005). Thus the potassium channel genes \( NKT1 \), \( NiKC1 \), \( NTORK1 \), and \( NKT2 \) tested here, as well as other related but unknown potassium transport genes in tobacco may jointly coordinate K⁺ uptake and distribution.

Replacement of auxin from an exogenous source after apex excision can partially compensate for apical dominance and stimulate K⁺ uptake activity (Rodrigo and Garcia-Martinez, 1998; Jiang et al., 2001; Morris et al., 2005). However, the K⁺ content in whole IAA-treated plants was lower than that in non-decapitated and decapitated plants at 14 d (Fig. 1A). This observation may be explained by the concentration and types of hormones used in this study. In line with this possibility, Jiang et al. (2001) and Yang et al. (2007) found that the net increase in K⁺ content in leaves and whole plants after NAA application was higher than that in non-treated decapitated plants. The choice of experimental material used may be another possible factor in explaining the results. Although ideally the same leaf and root materials should be used both to detect the expression of potassium channels and to determine the potassium content simultaneously, this was not possible in our experiments because the unfolded leaves in the plants were too small. In our previous experiments with tobacco, the potassium concentration in leaves significantly increased by 14.5–23.6% following the application of an identical amount of IAA (30 mg kg⁻¹) on the cut surface of the stem of decapitated plants (Zou and Su, 2008). Thus, in spite of some potential drawbacks, the application of IAA remains a valuable technique to increase the potassium concentration of tobacco in field experiments.

**Mechanism of regulation of K*⁺* channel genes expression by decapitation in tobacco**

To gain insight into the functions of plant growth regulators on increasing the potassium concentration in tobacco, a study to characterize the expression of potassium channel genes in IAA-treated plants was initiated. It was found that all of the four potassium channel genes tested were induced or repressed in leaves or roots of the treated tobacco plants relative to non-treated plants. Also the expression of \( NKT1 \) in leaves and of \( NKT2 \) in roots of IAA-treated plants showed a steady state at all the times tested. The results suggested that IAA may play an important role in regulating the expression of potassium channel genes. Figure 6 presents the concentrations of the endogenous hormone IAA in leaves and roots of tobacco under different treatments. Compared with non-decapitated plants, the IAA concentration decreased markedly in leaves of decapitated plants and increased in roots by 140.7% at 14 d. Our study demonstrated the positive effect of IAA application after decapitation on the regulation of IAA concentration in the leaves and roots of tobacco (Fig. 6A, B). IAA concentration in leaves and roots of IAA-treated plants was close to the levels found in non-decapitated plants. Auxin is produced in shoot apices and young leaves and it can be transported basipetally into roots to regulate plant growth (Lomax et al., 1995). Decapitation removed the dominant source of auxin and the distribution balance in the tobacco plants was...
upset. Studies on the relationship between plant hormones and potassium channels showed that auxin can rapidly induce the expression of the maize \textit{ZMK1} gene in the plasmalemma, and that auxin modulation of K\(^+\) channels plays a role in the signalling cascade leading to auxin-induced cell elongation (Claussen \textit{et al.}, 1997; Philippar \textit{et al.}, 1999; Thiel and Weise, 1999). Also, plant hormonal treatments (2,4-D, ABA, BA) differentially affected the mRNA levels of \textit{AKT1}, \textit{AKT2}, \textit{AtKC1}, and \textit{SKOR} in sprouts and roots of \textit{A. thaliana} (Pilot \textit{et al.}, 2003). Physiological studies have also provided evidence that K\(^+\) secretion into the xylem sap is under hormonal control (Kochian and Lucas, 1993). However, other studies indicate that IAA inhibited net K\(^+\) uptake and reduced the activity of K\(^+\) channels as measured by patch-clamp (Paul \textit{et al.}, 2005). Therefore, a reciprocal relationship is conjectured between decapitation, the endogenous IAA levels and distribution, and the expression of potassium channel genes, according to which the removal of the shoot apex altered IAA distribution. This, in turn, affected the expression of potassium channel genes and, as a consequence, the allocation of potassium in the plants changed.

Although the control plants in this study did not undergo decapitation, their leaves showed a large rise in the expression of \textit{NKT1} at 5 h, and the expression of \textit{NtKC1} and \textit{NTORK1} dropped and then rose within 24 h, while that of \textit{NKT2} kept a steady state. One possible reason for the large change in the expression of \textit{NKT1} may be the changes in light conditions affecting the shoot apex. Light is known to provide an easy-to-manipulate environmental switch for the state of shoot meristem activity and can help to address fundamental questions in meristem function. For example, Lopez-Juez \textit{et al.} (2008) showed that, in dark-growth shoot apices, genes coding for components of regulated proteolysis, signalling, and specific groups of transcription factors are expressed upon light exposure. Both light and the circadian clock have been shown to regulate K permeability of the motor cell membranes (Lowen and Satter, 1989; Kim \textit{et al.}, 1992; Suh \textit{et al.}, 2000). Putative potassium channel genes in \textit{Samanea saman} were also shown to be under circadian regulation (Moshelion \textit{et al.}, 2002).

**Acknowledgements**

The authors are grateful that the project was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KZCX2-XB2-08-01, KSCX2-YW-N-48-1) and National Key Basic Research and Development Program (2006CB403208). Thanks to Dr Pedro Rocha (Institute of Subtropical Agriculture, the Chinese Academy of Sciences) for valuable suggestions to improving the manuscript.

**References**


