



Transcript Regulation Related to Potassium Uptake Genes in *Nicotiana* Roots

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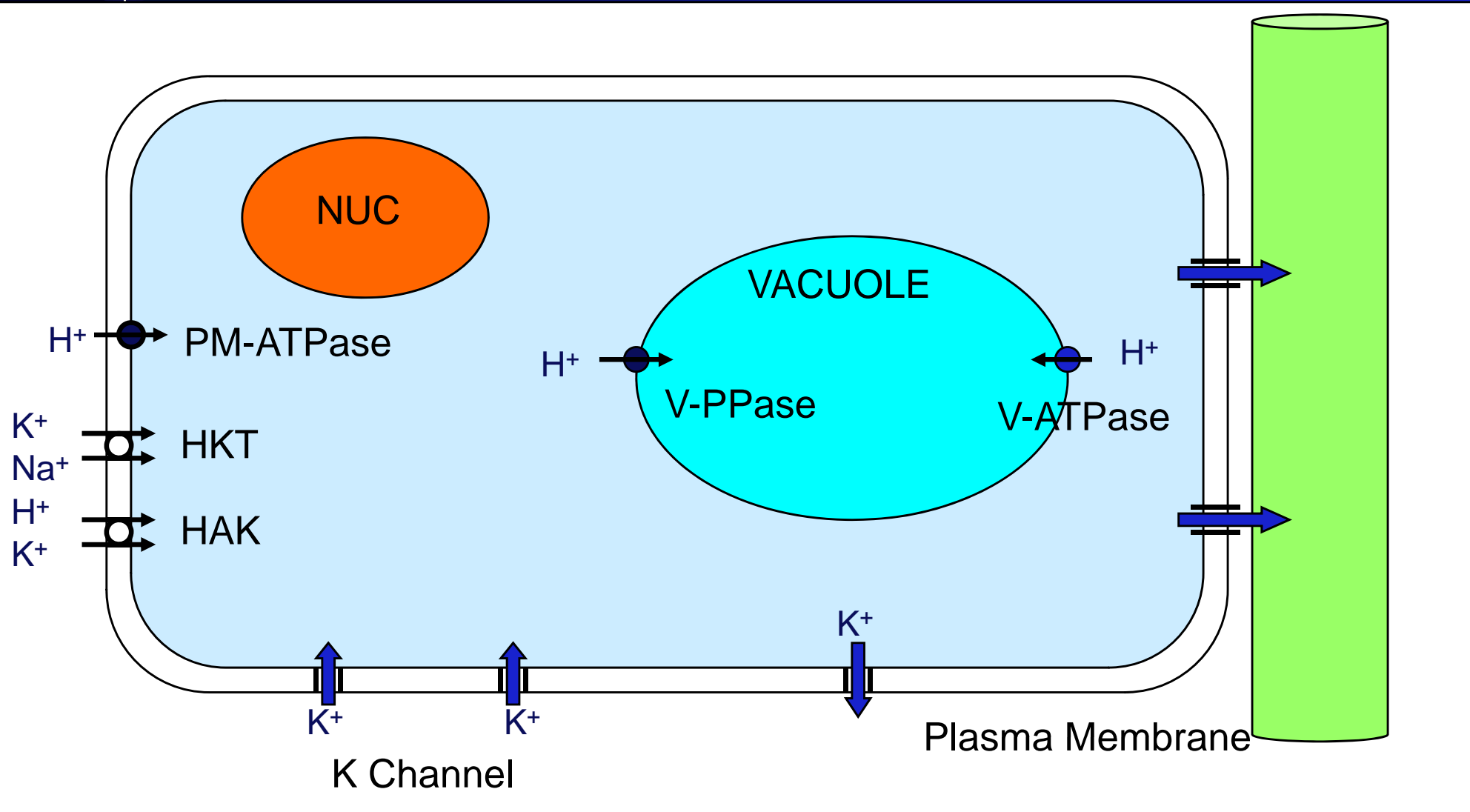
Introduction

- ◆ Except for affecting the disease resistance and stress responses in tobacco growing periods, the potassium nutrition has close relation with flavor, burn capacity and harmful materials such as tar. The potassium content in tobacco leaves is the important index for high quality tobacco leaf.

Introduction

- ◆ **Root absorption of K^+ has been described as biphasic, with a high-affinity system that sustains K^+ accumulation in plants when external K^+ is in the micro-molar range and a low-affinity transporter responsible for K^+ uptake in the presence of milli-molar K^+ concentrations.**

Multiple systems of K^+ transportation



Maathuis F. J. M. 1999)

Introduction

- ◆ **Translocation of K^+ may involve multiple transport systems including AKT/KAT channels, TRK/HKT transporters and KT/HAK/KUP transporters and other relative proteins.**

Purpose of this study

- ◆ The transcript of potassium uptake genes in *AtKup1*, *AtNHX1* and *Avp2* transgenic tobacco.
- ◆ The effects of K^+ starvation, Na^+ and NH_4^+ salt stress on transcript regulation of K^+ channel, K^+ transporter, plasma membrane H^+ -ATPase, vacuolar H^+ -ATPase and H^+ -PPase.

Materials

- ◆ Plant: *Arabidopsis thaliana* columbia
Nicotiana tabacum
- ◆ Vector and Strains:
 - ◆ pYH455 vector
 - ◆ *E. coli*: DH5α
 - ◆ *Agrobacterium tumefaciens*: LBA4404

Reading frame PCR primers

◆ *AtKUP1*

- ◆ R217: 5'-AAAGGATCCAACAATGAACCAATCACCATCTCTTATC-3'
- ◆ R219: 5'-AAAGAGCTCTTAGACGTAATAAACCAATTCCAAC-3'

◆ *AtNHX1*

- ◆ R221: 5'-AAAGGATCCAACATGTTGGATTCTCTAGTGTCGAAAC-3'
- ◆ R223: 5'-AAAGAGCTCTCAAGCCTTACTAAGATCAGGAGGG-3'

◆ *Avp2* :

- ◆ R221: 5'-AAAGGATCCAACATGTTGGATTCTCTAGTGTCGAAAC-3'
- ◆ R223: 5'-AAAGAGCTCTCAAGCCTTACTAAGATCAGGAGGG-3'

Screening PCR primers

◆ *AtKUP1*

- ◆ AtKup1-Z: 5'-CGCATAGAGTCGCCTTCATTTTCGCTCCA-3'
- ◆ AtKup1-Z: 5'-CGTCACGAAATGTCCGAAAACAGCTGGA-3'

◆ *AtNHX1*

- ◆ AtNHX1-Z: 5'-CGGTCTGATAAGTGCGTATG -3'
- ◆ AtNHX1-F: 5'-GTTCTGGTGCGGTAATAGGT -3'

◆ *Avp2* :

- ◆ AVP2-Z: 5'-GTGATTGGTATCGCCATCCTC-3'
- ◆ AVP2-F: 5'-AGGCTCGTGCTTATAGTCTGT-3'

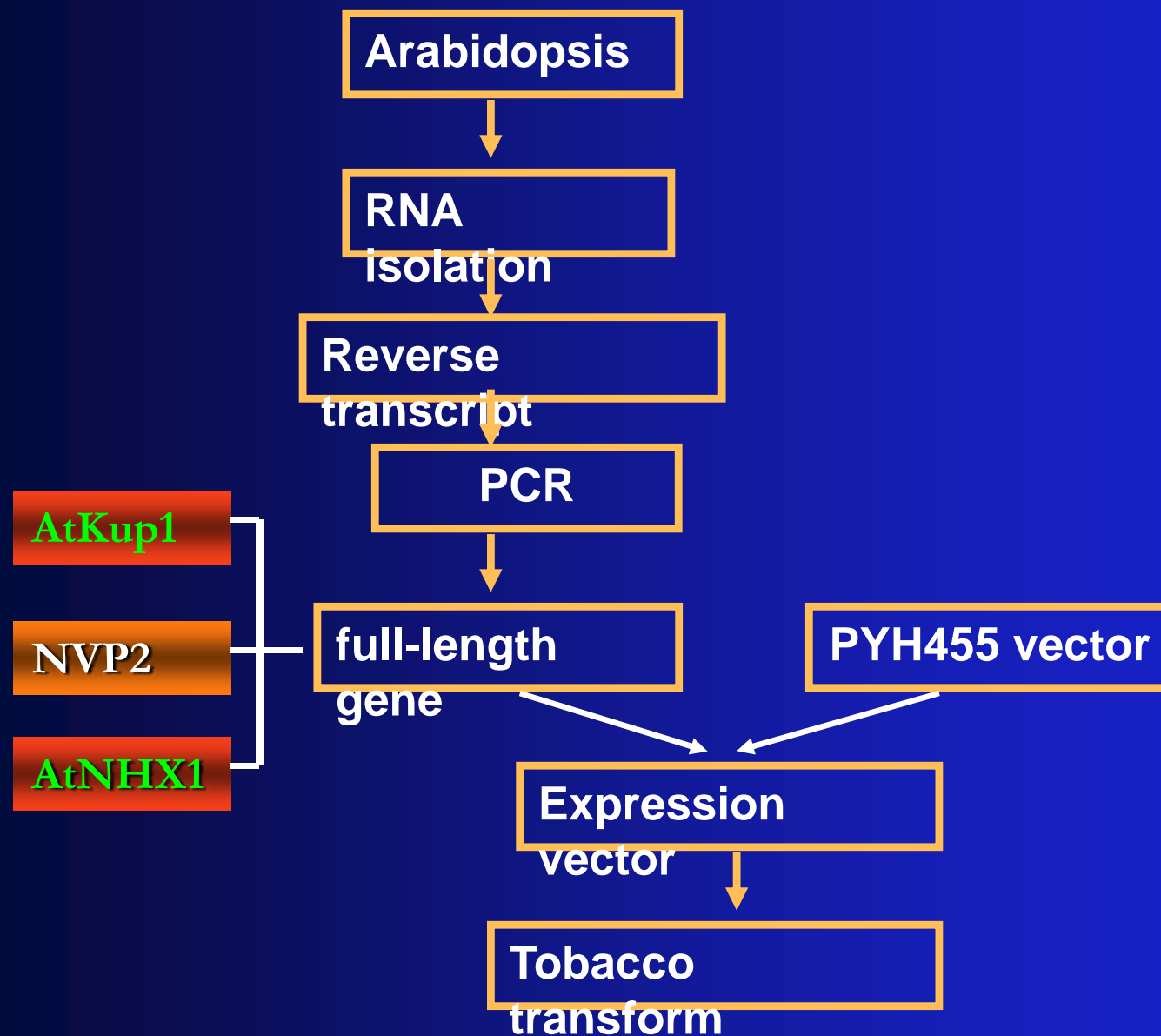
Real-time PCR primers

- ◆ AtNHX1S-F: 5'-CCTATTACCGCACCCAGAACG -3'
- ◆ AtNHX1S-R: 5'-GGTCGCGCATGAAGGAGTCATC -3'
- ◆ NVP-F: 5'-GCTGGAGGAATAGCTGAGAT-3'
- ◆ NVP-R: 5 -GTCATGGCAGAGAACCAGTA -3'
- ◆ NtHAK-S-F: 5'- CCTTATTGTGCCGTCATGCC- 3'
- ◆ NtHAK-S-R: 5'-CTGAGATTGCAGGAGTAAGG -3'
- ◆ NKT1 S-F: 5'- CAATCTTGGCCTCACTGCTT- 3'
- ◆ NKT1 S-R: 5'- TACCTCATCTGGCGATTCTGT- 3'
- ◆ NHA1-S-F: 5'-GCAAGAGCAGGGCATCCAAGA- 3'
- ◆ NHA1-S-R: 5'-CCACAGCCAAGGAACGAAGA- 3'
- ◆ Act-F: 5'-GATCTTGCTGGTCGTGATCT- 3'
- ◆ Act-R: 5'-ACTTCCGGACATCTGAACCT -3'.

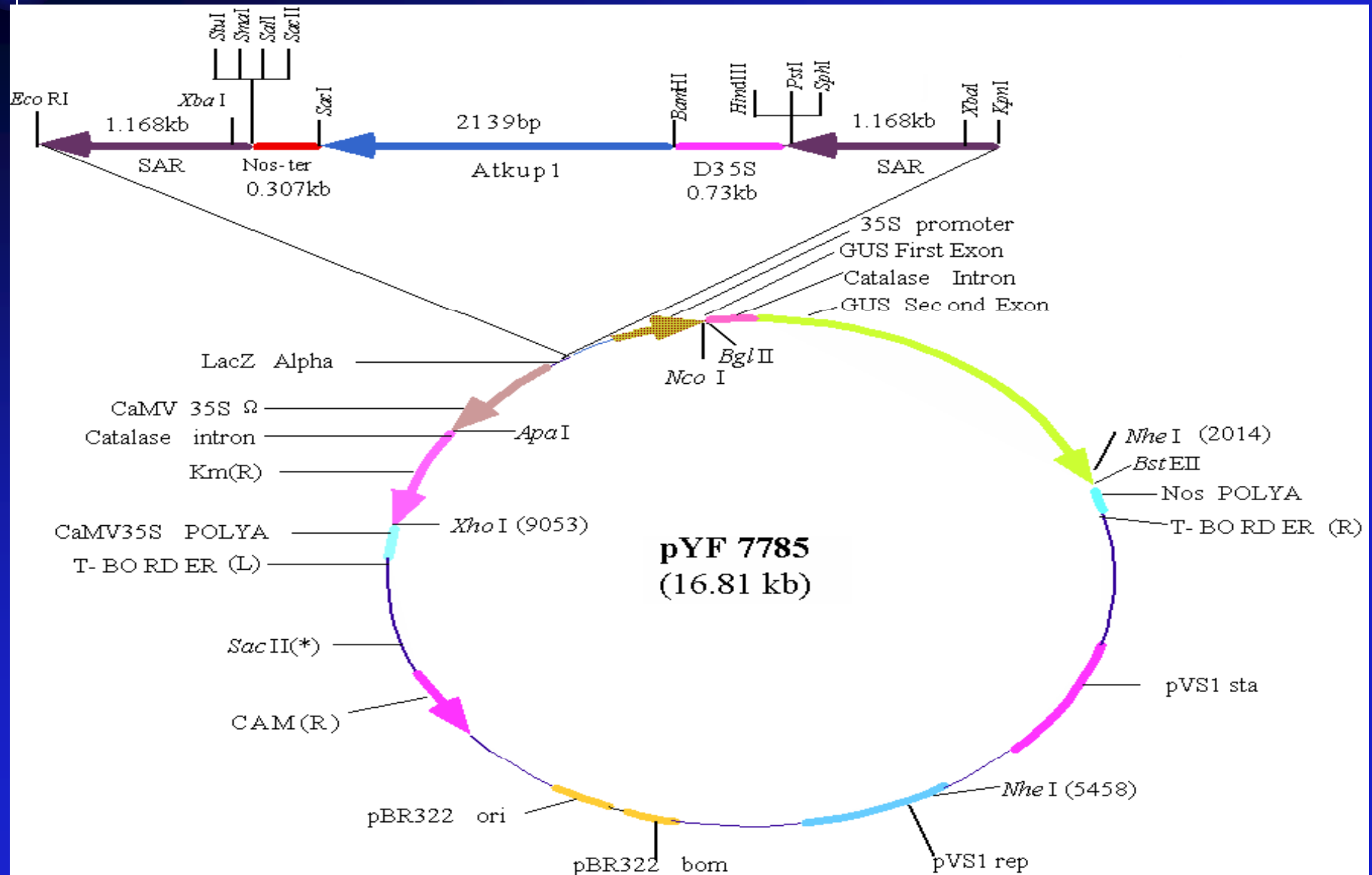


Methods and Results

Gene clone and transformation



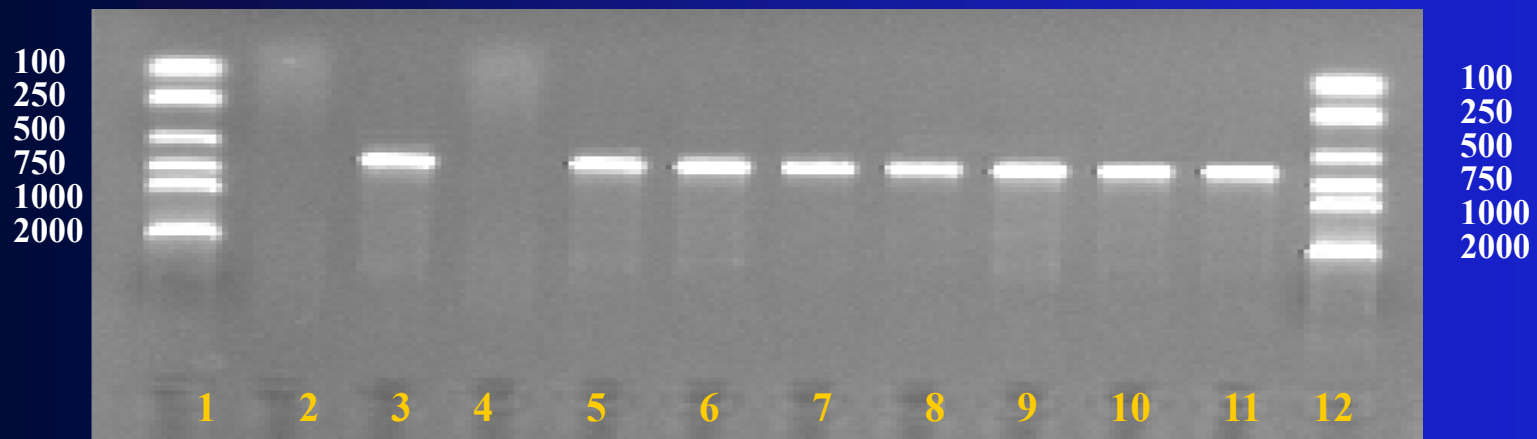
Expression vector



Trans-formants test



AtKup1 transformant PCR test



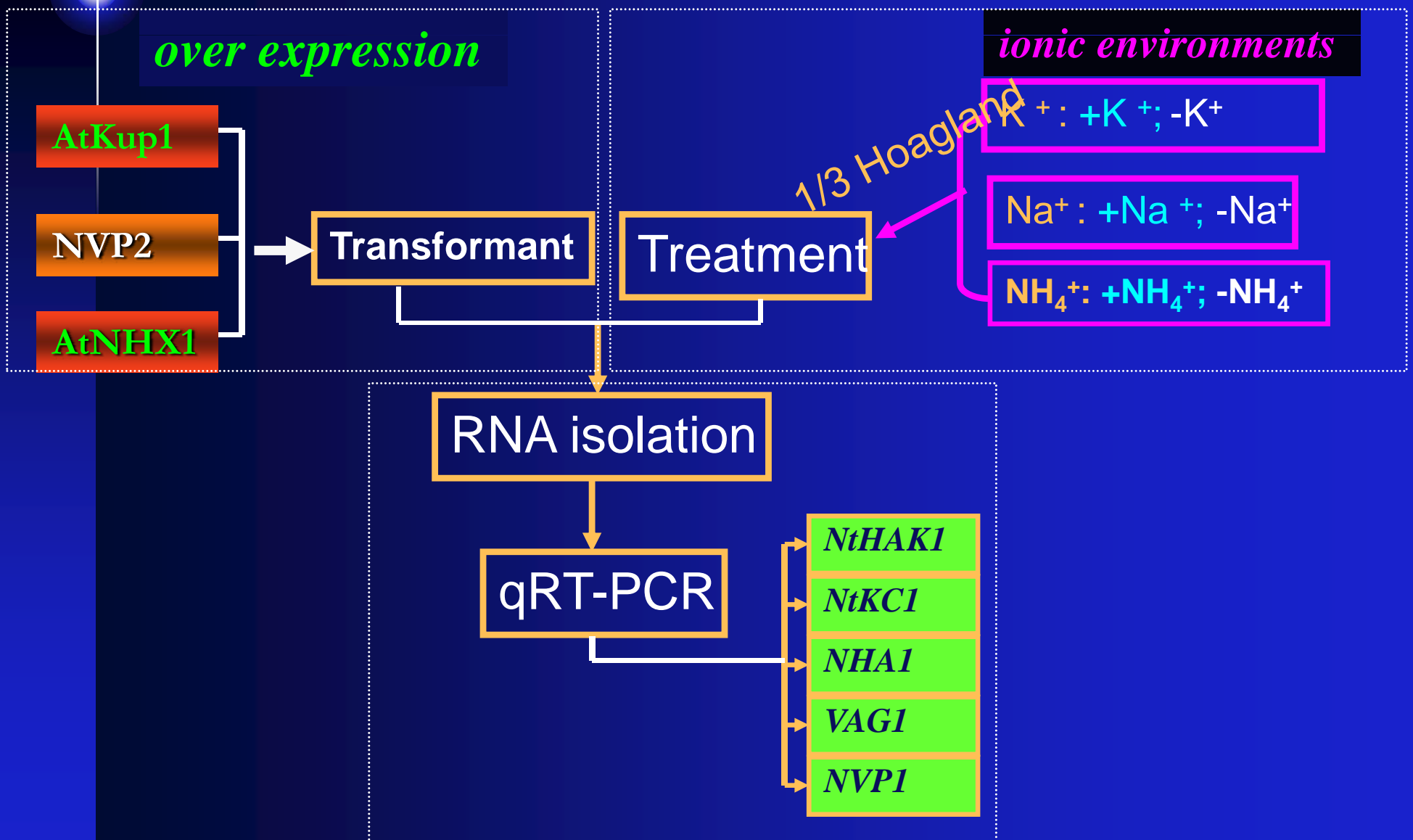
AtNHX1 transformant PCR test

Trans-formants test



Avp2 transformant PCR test

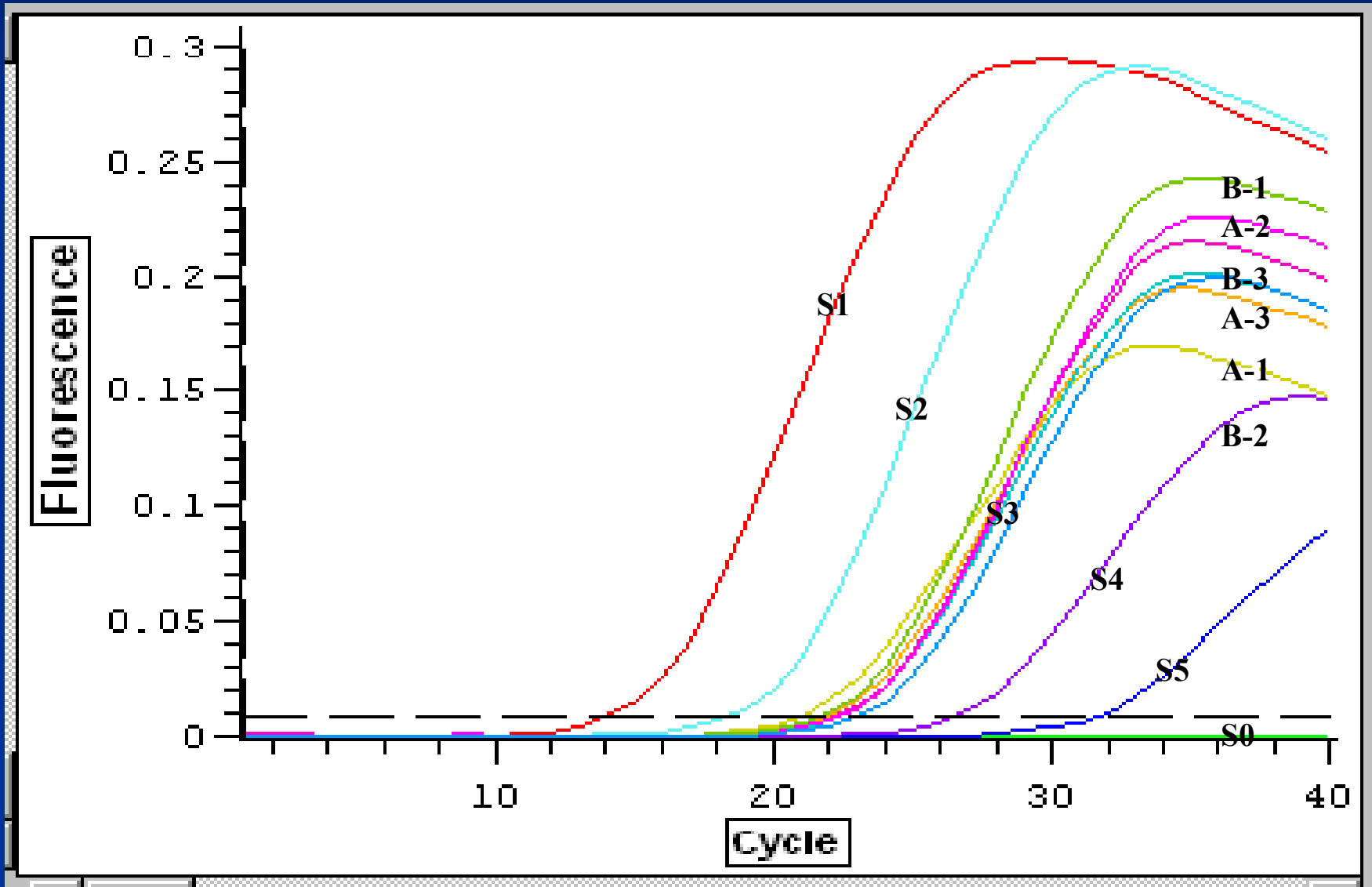
Transcript analysis



Transcript analysis

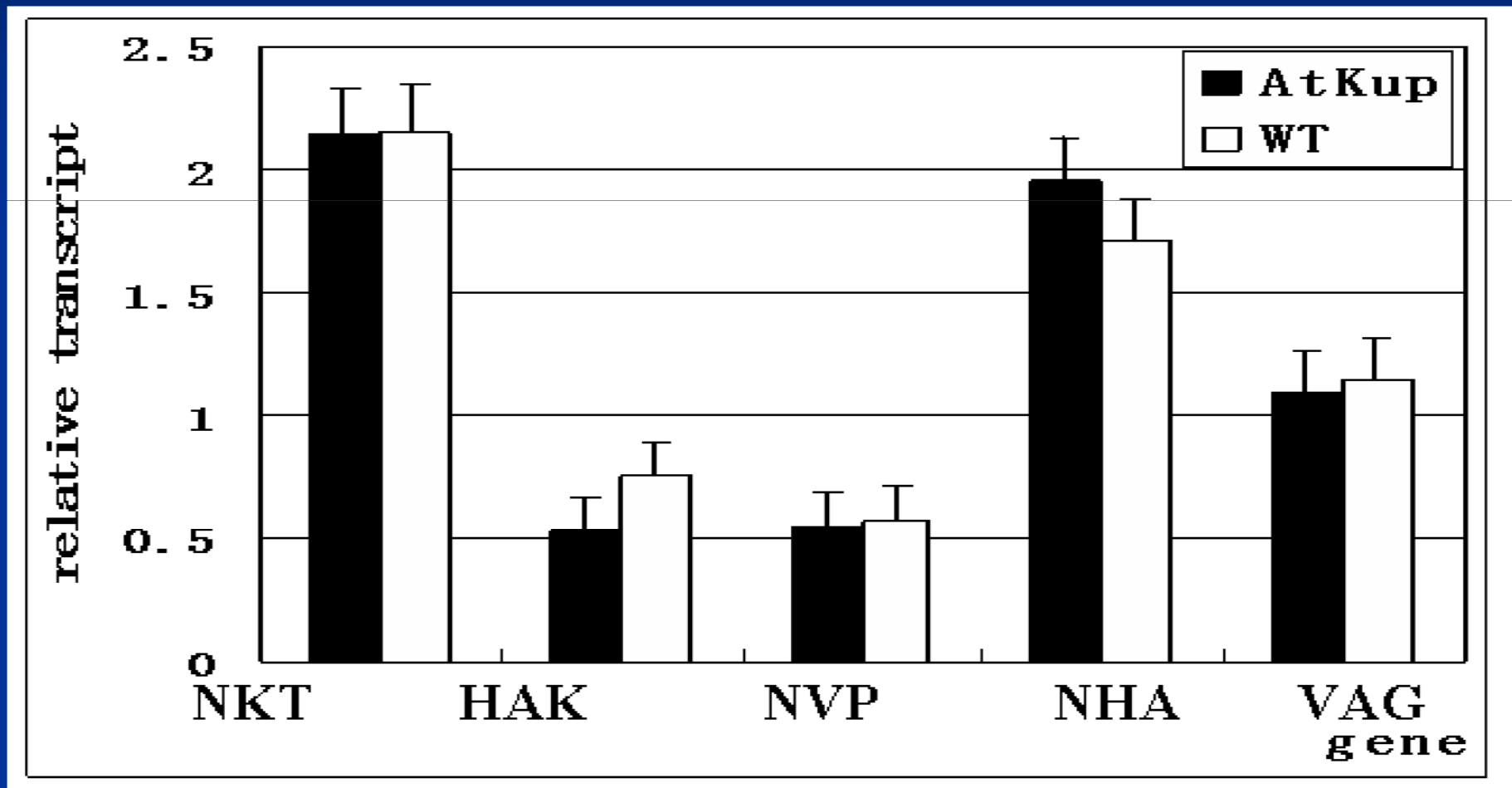
- **Methods: qRT-PCR**
- **Fluorescence staining: SYBGreen I**
- **Standards template: 10^2 , 10^3 , 10^4 , 10^5 and 10^6 copies/ml**
- **Housekeeping gene: *β -actin***
- **Relative transcript: a ratio of the copy number of target *gene* to that of *β -actin*.**

Transcript analysis



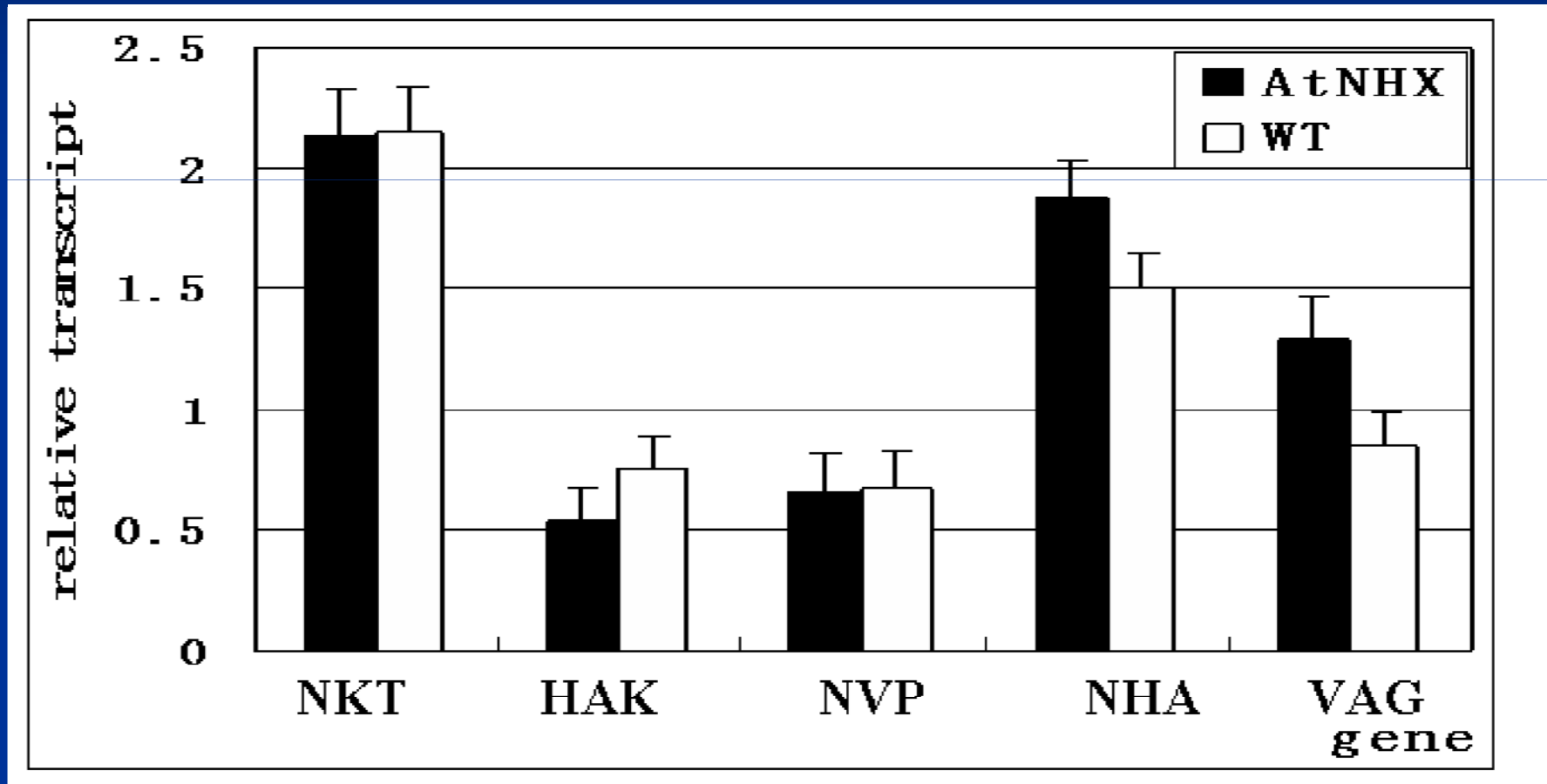
two treatments and triplicate

Transcript analysis in AtKup1 transformant



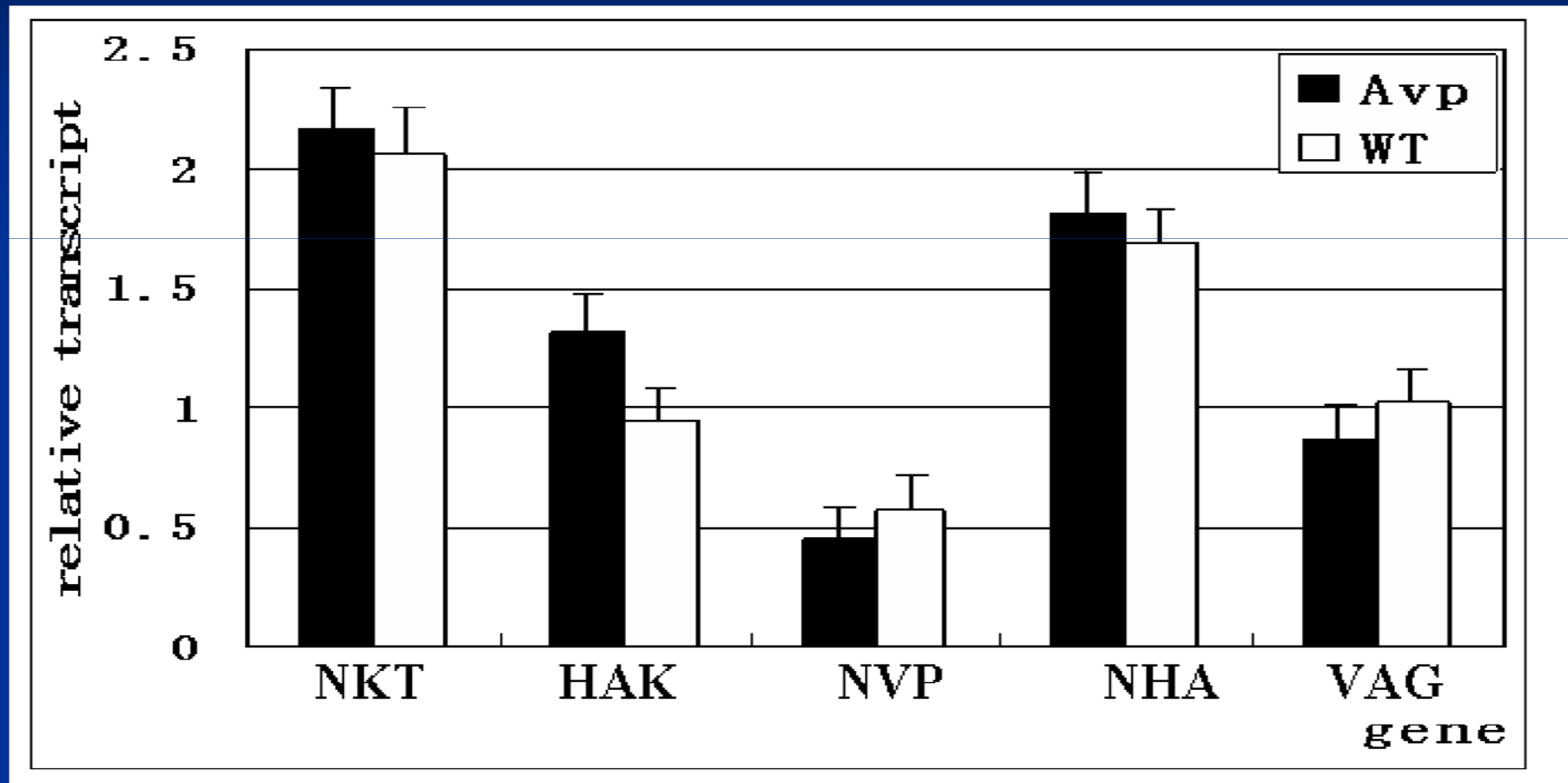
***NtHAK1* gene transcript was reduced and that of *NHA1* was increased in AtKup1 transformants**

Transcript analysis in AtNHX1 transformant



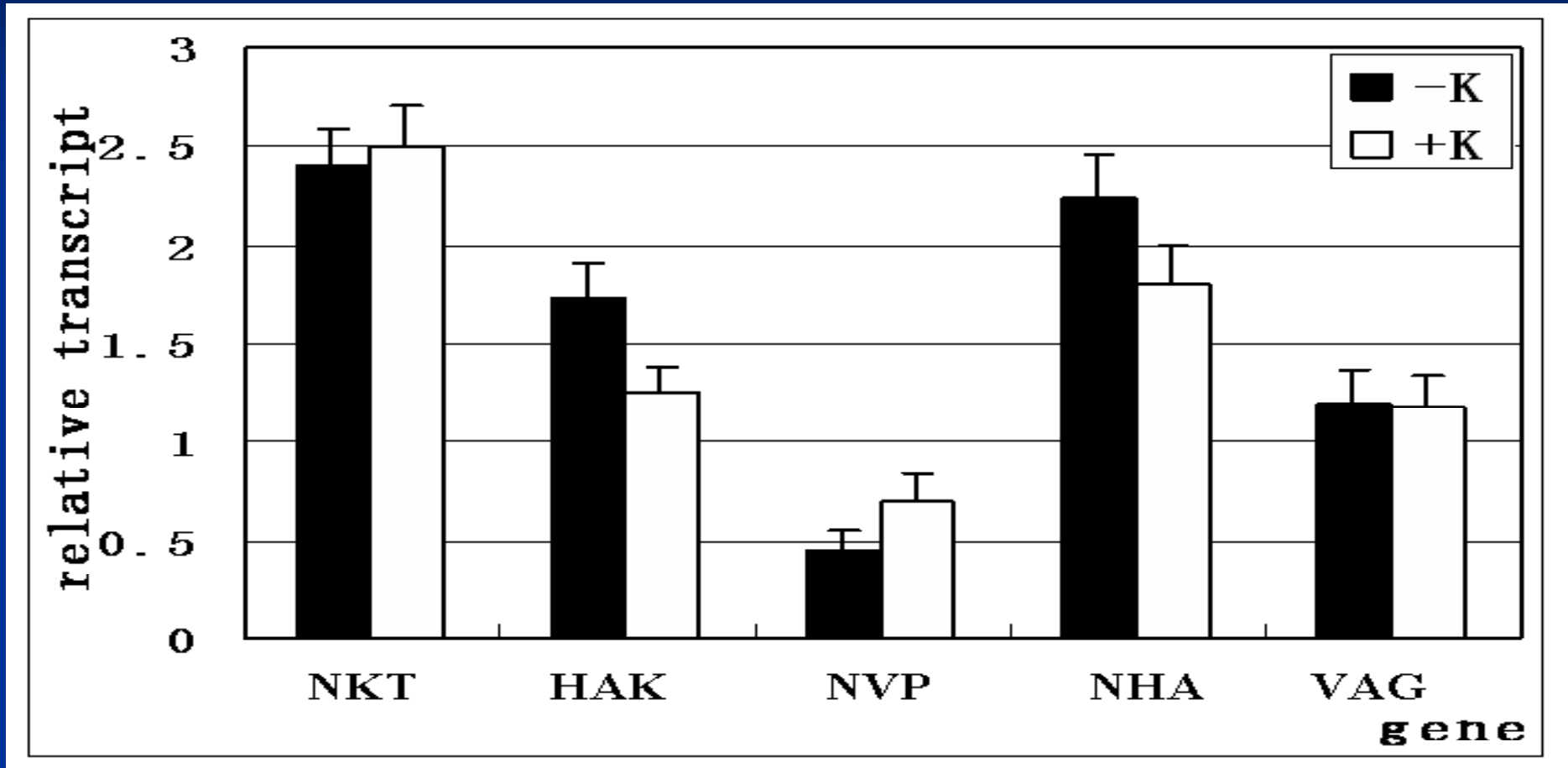
***NtHAK1* gene is down-regulation, and transcript of *NVP1* and *NHA1* were up-regulated**

Transcript analysis in Avp2 transformant



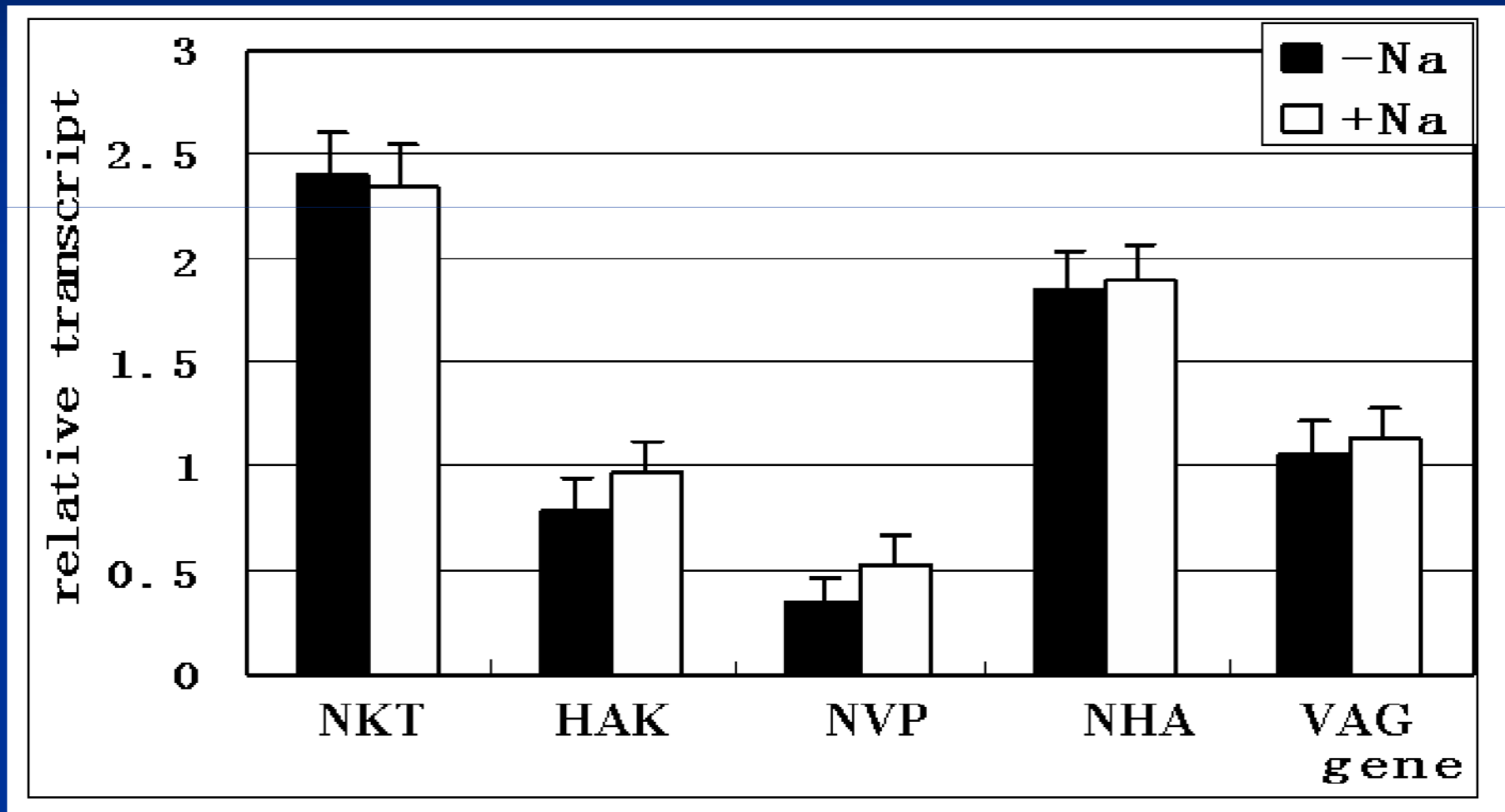
Transcript of *NtHAK1* was up-regulated, That was down-regulated of *VAG1* and *NVP1* in *AVP2* transformants

Transcript analysis in K⁺ starvation



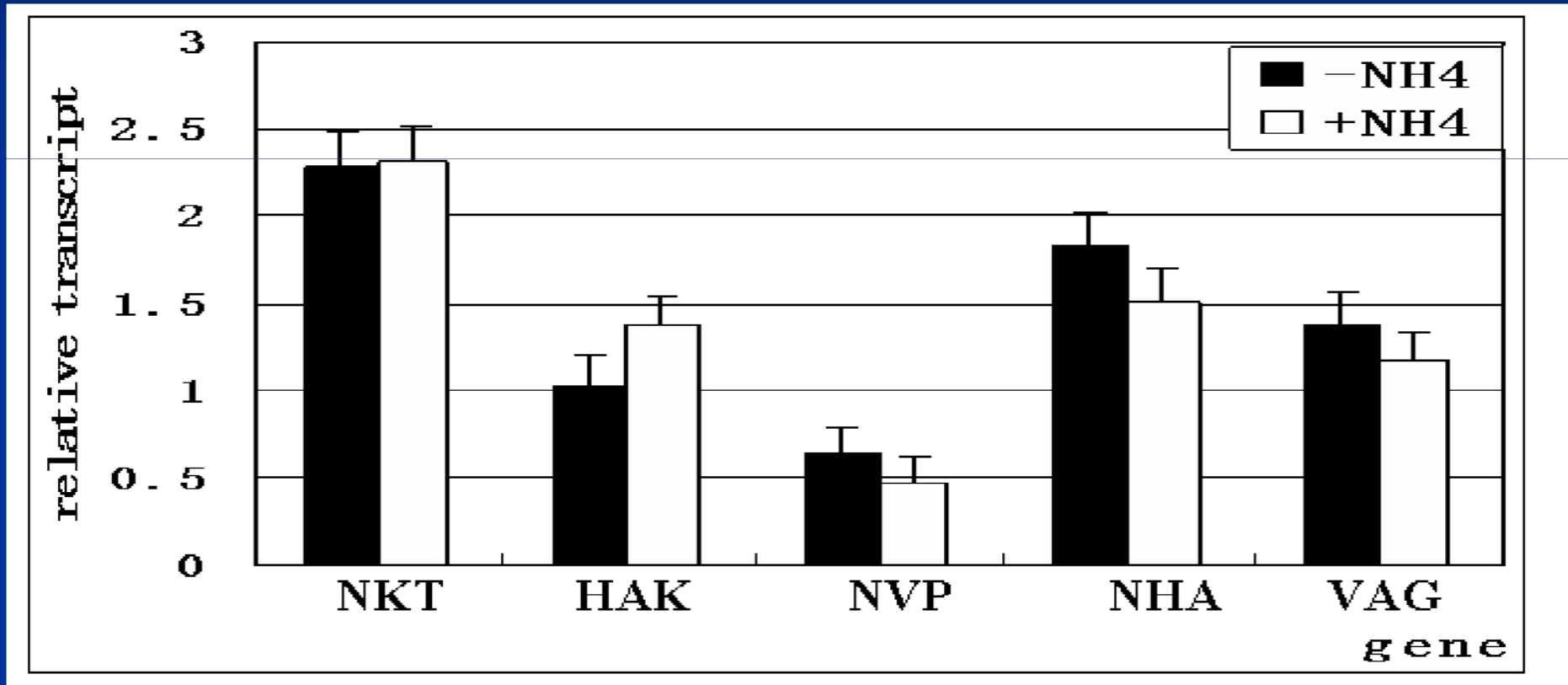
Transcripts of *NtHAK1* and *NHA1* were stimulated and that of *NVP1* decreased under an external K⁺ starvation solution.

Transcript analysis in high Na⁺ stress



Transcript of *NtHAK1* and *NVP1* were up-regulated by excessive Na⁺ stress

Transcript analysis in high NH_4^+ stress



high ammonia caused a decline in *VGA*, *NHA* and *NVP* transcript levels, whereas *NtHAK1* exhibited increased expression in 5 mM NH_4^+ treated roots

Summary

Factor		Tobacco internal K ⁺ uptake genes				
		NrHAK1	NKT	NVP1	NHA1	VAG
Gene	<i>AtKup1</i>	down	--	--	up	--
	<i>AtNHX1</i>	down	--	up	up	up
	<i>AVP2</i>	up	up	down	--	down
Ionic	K ⁺	up	--	down	--	--
	Ca ²⁺	down	down	--	down	down
	Na ⁺	up	--	up	--	--
	NH ₄ ⁺	up	--	down	down	down

Summary

- The results demonstrated that the transcript of *NtHAK1* was reduced under all treatments and *NHA1* was increased in the roots of *AtKup1* transformants.
- The *NtHAK1* transcript was down-regulated, but the *NHA1*, *VAG1* encoding proton pump and *NVP1* were significantly up-regulated in the roots of *AtNHX1* transformants,
- *VAG1* encoding H⁺-ATPase and *NVP1* encoding vacuolar H⁺-PPase were down-regulated. The *NtHAK1* and *NKT1* transcripts were slightly increased in *AVP2* over-expressed tobacco.

Summary

- The study demonstrated that *NtHAK1* and *NHA1* transcripts were significantly stimulated and *NVP1* expression decreased under an external K^+ starvation solution. The results also confirmed that the K^+ transporter gene *NtHAK1* was induced by excessive Na^+ stress, but inhibited when the tobacco plants were in a 5 mmol/L NH_4^+ solution. *NKT1* transcript levels exhibited no response to potassium starvation and sodium or ammonium stress treatments.



Thanks