

Supplementary Material

Table S1. Statistic analysis for the segregation ratio of target genes and selection-marker genes and co-transformed frequencies in T₀ and T₁ progeny tobacco plants.

Types	Number of kanamycin-resistance T ₀ progeny plants (total)			Number of T ₀ (G+P-) ^a	Number of T ₀ (G+P+) ^b	Co-transformation Rate (%)	Number of different T ₁ progeny (total)	Number of T ₁ progeny (G-P-) ^c	Number of T ₁ progeny (P+) ^d
	134	128	38						
<i>VpSH737</i> : <i>VpSH737-U4Cn</i> (1:1)						29.6	32	26	15
							28	20	13
							25	19	11
							31	25	13
							30	27	16
							28	18	14
							28	16	12
							32	22	17
		159	139	32		23.02	30	24	13
							22	17	9
<i>VpSH737</i> : <i>VpSH737-U4Cn-RcHak</i> (1:1)							26	20	10
							28	21	12
							25	19	9
							22	18	10
							23	16	11
							26	21	12

a Gus histochemical staining-positive with PCR amplification-negative;

b Gus histochemical staining-positive with PCR amplification-positive;

c Gus histochemical staining-negative with PCR amplification-negative;

d PCR amplification-positive.

Table S2. The statistical analysis of average thickness of both two transgenic plants leaf.

Group	Type	Simple sizes (N)	Leaf thickness (mm)	Levene's Test for Equality of Variances		T-test for Equality of Means
				F	Sig.	Sig. (2-tailed)
1	CN-HAK1	5	0.202±0.003**			0.000
	CN	5	0.161±0.002	2.165	0.179	0.000
	WT	5	0.143±0.003	0.490	0.504	0.000
2	CN-HAK1	5	0.196±0.002**			0.000
	CN	5	0.159±0.002	0.416	0.537	0.000
	WT	5	0.137±0.002	0.299	0.599	0.000
3	CN-HAK1	5	0.205±0.002**			0.000
	CN	5	0.161±0.002	0.096	0.764	0.000
	WT	5	0.140±0.001	2.353	0.164	0.000

(Data represent mean ±SE, **P<0.001; n=5)

Table S3. The statistical analysis of average sizes of necrotic spots in inoculated-leaf of both two tansgenic plants.

Group	Type	Sample sizes (N)	Size of necrotic spots (mm)	Levene's Test for Equality of Variances		T-test for Equality of Means
				F	Sig.	Sig.(2-tailed)
1	CN-HAK1	25	0.584±0.075**	0.711	0.406	0.000
	CN	25	1.084±0.057			0.000
2	CN-HAK1	25	0.568±0.060**	6.199	0.016	0.000
	CN	25	1.092±0.088			0.000
3	CN-HAK1	25	0.576±0.055**	4.903	0.032	0.000
	CN	25	1.132±0.083			0.000

(Data represent mean ± SE, **P<0.001; n=25)

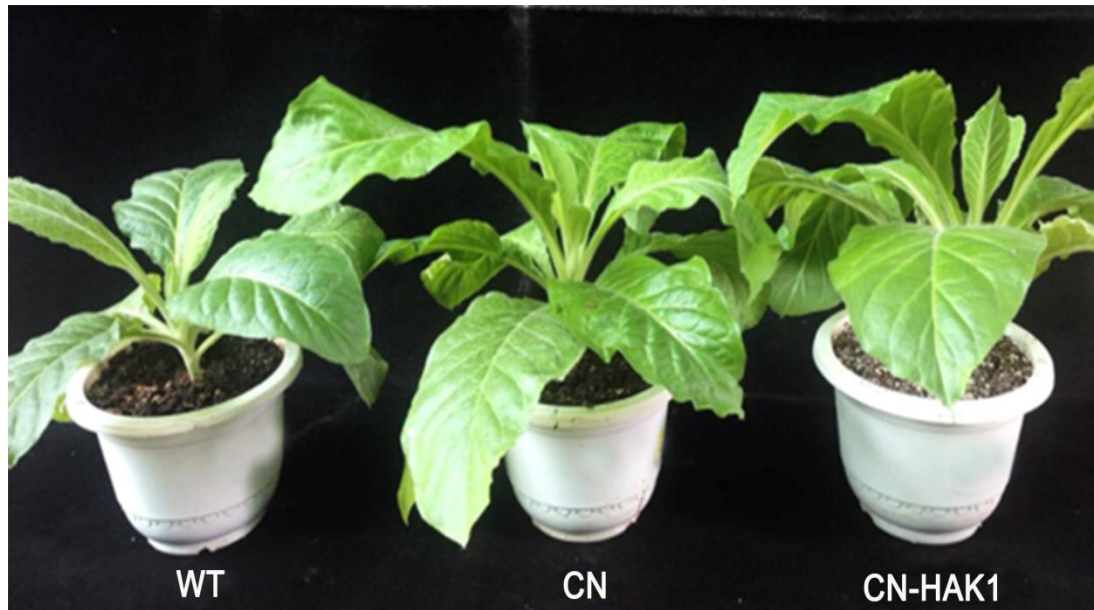


Fig. S1. The effect of TMV-infection on the growth of the two transgenic and wild-type tobacco plants. **a** Lateral view for wild-type and transgenic plants at 21 d after inoculation, **b** Top view for the upper leaf without TMV-inoculation **CN-HAK** trans-*CN-HAK1* plants, **CN** trans-*CN* plant, **WT** wild-type plant.

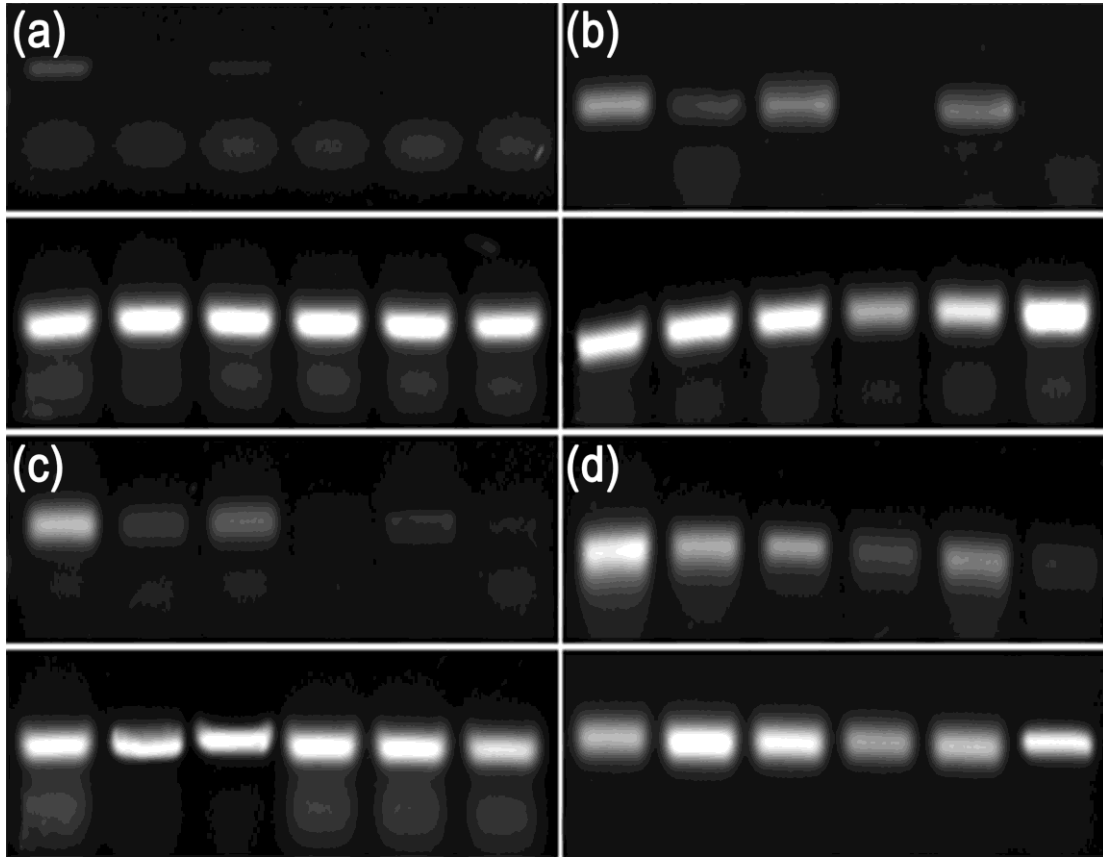


Fig. S2. The qRT-PCR analysis of TMV-CP gene in the inoculated and top uninoculated leaf of *CN-HAKI*-containing, *CN*-containing and wild-type tobacco plants. **a** The relative expression of TMV-CP in the inoculated and top uninoculated leaf of wild-type, *CN*-containing, and *CN-HAKI*-containing plants orderly at day 5 after TMV-inoculation. The pores 1, 3, 5 represents for the TMV-CP expression in the inoculated-leaf of wild-type, *CN*-containing, and *CN-HAKI*-containing tobaccos respectively, and the pores 2, 4, 6 represents for the TMV-CP expression in the top uninoculated-leaf of wild-type, *CN*-containing, and *CN-HAKI*-containing tobaccos respectively. **b** The expression levels of TMV-CP in transgenic and wild-type plants at day 12 after TMV-inoculation. **c** The expression levels of TMV-CP in transgenic and wild-type plants at day 18 after TMV-inoculation. **d** The expression levels of TMV-CP in transgenic and wild-type plants at day 25 after TMV-inoculation.

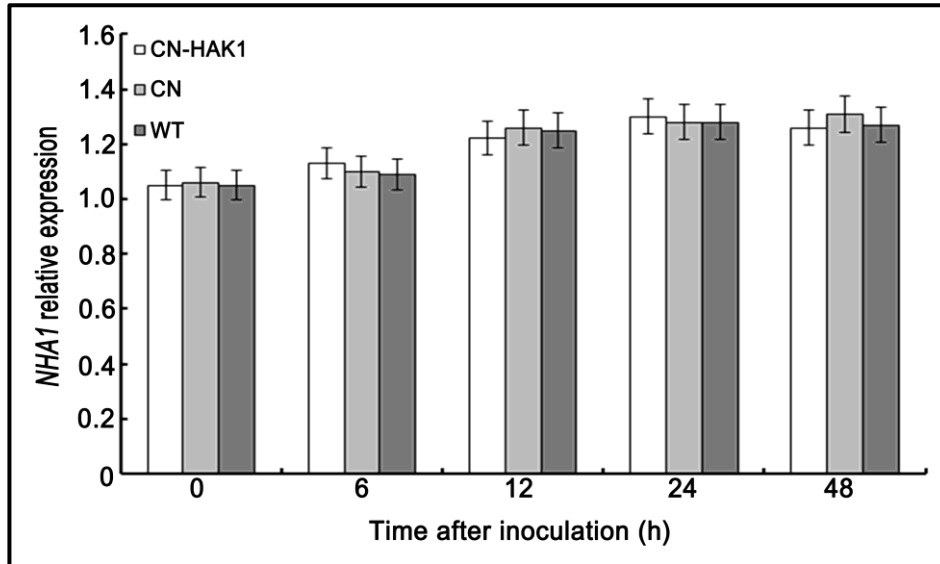


Fig. S3. The relative expression of NHA1 gene. The gene has been reported to plays an important role in K⁺-absorbing in response to abiotic stress (Lu et al., 2005), but there was seemly no significant difference in NHA1 expression level in response to biotic stress (TMV-infection) in this study.

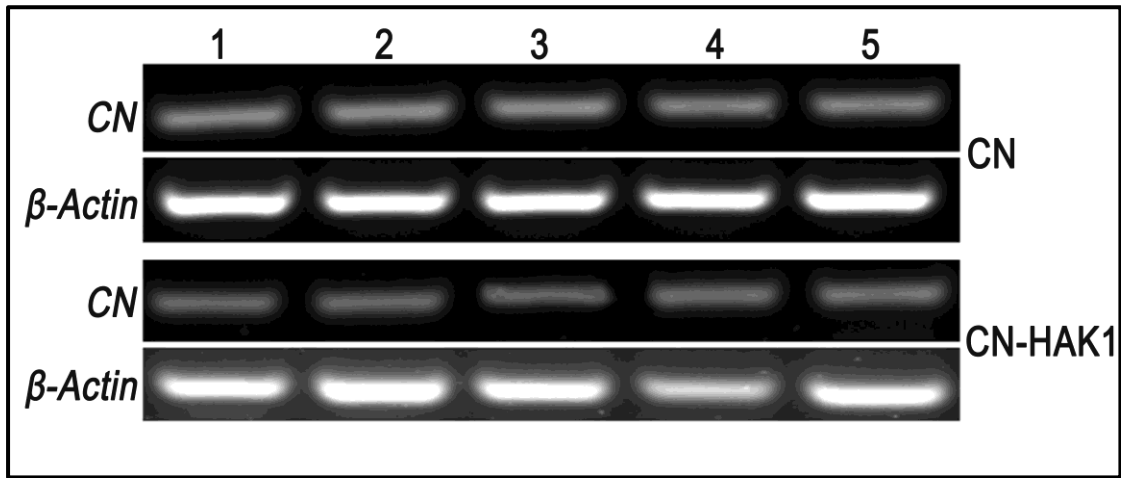


Fig. S4. The relative expression of *CN* gene in *CN*- and *CN-HAK1*-containing tobacco plants. **CN-HAK1**: trans-*CN-HAK1* tobaccos, **CN**: trans- *CN* tobaccos.

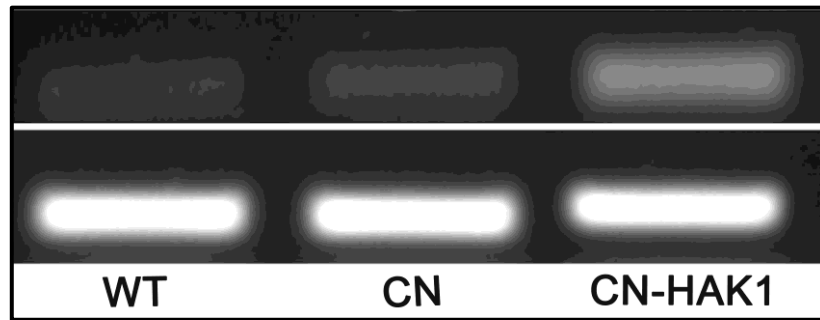


Fig. S5. The qRT-PCR analysis for *HAK1* gene under water-planting. **CN-HAK1**: trans-*CN- HAK1* tobaccos, **CN**: trans- *CN* tobaccos, **WT** wild-type tobaccos.