

***AtHKT1* gene regulating K⁺ state in whole plant improves salt tolerance in transgenic tobacco plants**

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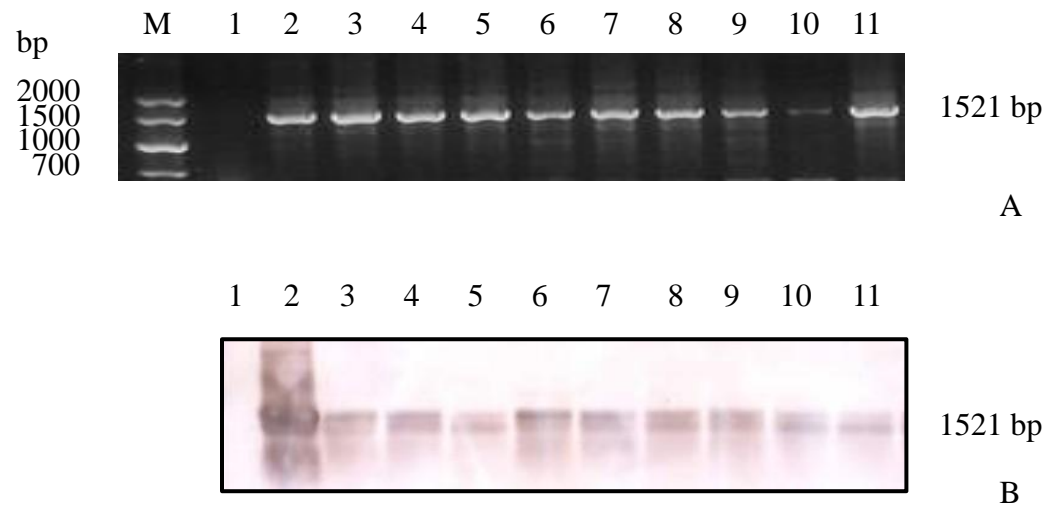


Figure S1. Verification of transgenic plants using (A) the PCR assay and (B) PCR-Southern of *AtHKT1* gene. M, DNA Marker V; 1, untransformed tomato plant as a negative control; 2, plasmid *AtHKT1*-GFP as a positive control; 3 to 11, transgenic tobacco lines. Hybridized with a *AtHKT1* probe labeled with digoxigenin. The grouping of gels/blots cropped from same part of the same gel.

Table S1. Genes primers, PCR and qPCR amplification program

| Type | Genes | Primers | Amplification program | Notes |
|----------------|---------------|---|--|---|
| PCR and RT-PCR | <i>AtHKT1</i> | Forward primer: 5'-CGGGATCCATGGACAGAGTGG TGGCAAAAATAG-3' Reverse primer: 5'-CGCCCGGGTTAGGAAGACGA GGGGTAAAGTATCC-3' | 1 min at 94 °C; 35 cycles of 94 °C for 50 s, 53 °C for 50 s, 72 °C for 120 s, and 10 min final extension at 72 °C. | Cloning of <i>AtHKT1</i> gene |
| | <i>AtHKT1</i> | Forward primer: 5'-CGGGATCCATGGACAGAGTGG TGGCAAAAATAG-3' Reverse primer: 5'-CGCCCGGGTTAGGAAGACGA GGGGTAAAGTATCC-3' | 1 min at 94 °C; 35 cycles of 94 °C for 50 s, 55 °C for 60 s, 72 °C for 90 s, and 10 min final extension at 72 °C. | Transgenic plants PCR and RT-PCR detection |
| | <i>GFP</i> | Forward primer: 5'-ATGAGTAAAGGAGAACTTTTC -3' Reverse primer: 5'-TTATTATTTGTATAGTTCATCC ATG-3' | 3 min at 94°C; 35 cycles of 45 s at 94°C, 45 s at 59°C, and 60 s at 72°C, a final stage was for 5 min at 72°C. | Transgenic plants PCR and RT-PCR detection |
| qPCR | <i>AtHKT1</i> | Forward primer: 5' - ATC TGG CTC CTA ATC CCT CAA - 3' Reverse primer: 5' - CCG TCA CTC CAA GAA GAA CAC - 3' | 30 s at 95°C; 40 cycles of 5 s at 95°C, and 34 s at 60°C | Gene expression assays with qPCR |
| | <i>efla</i> | Forward primer: 5'-CAA GGATGA CCC AGC CAA G -3' Reverse primer: 5'-TTCCTT ACC TGAACGCCT GT-3' | 30 s at 95°C; 40 cycles of 5 s at 95°C, and 34 s at 60°C | Gene expression assays with qPCR, internal control gene |