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Overexpression of *SeNHX1* improves both salt tolerance and disease resistance in tobacco

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Recently, we found *NHX1*, the gene encoding a Na^+/H^+ exchanger, participated in plant disease defense. Although NHX1 has been confirmed to be involved in plant salt tolerance, whether the *NHX1* transgenic plants exhibit both salt tolerance and disease resistance has not been investigated. The T1 progenies of *Nicotiana tabacum* L. lines expressing *SeNHX1* (from *Salicornia europaea*) were generated for the present study. Compared with PBI-type control plants, *SeNHX1* transgenic tobaccos exhibited more biomass, longer root length, and higher K^+/Na^+ ratio at post germination or seedling stage under NaCl treatment, indicating enhanced salt tolerance. The vacuolar H^+ efflux in *SeNHX1* transgenic tobacco was increased after treatment of NaCl with different concentration. Meanwhile, the *SeNHX1* transgenic tobaccos showed smaller wilted spot area, less H_2O_2 accumulation in leaves after infection of *Phytophthora parasitica* var. *nicotianae*. Further investigation demonstrated a larger NAD(P)(H) pool in *SeNHX1* transgenic tobacco. These evidences revealed that overexpression of *SeNHX1* intensified the compartmentation of Na^+ into vacuole under salt stress and improved the ability of eliminating ROS after pathogen attack, which then enhanced salt tolerance and disease resistance simultaneously in tobacco. Our findings indicate *NHX1* has potential value in creating crops with both improved salt tolerance and disease resistance.

Plants have developed various biochemical and physiological processes to respond to environmental stresses because of their sessile nature. Sequestering excessive cytoplasmic Na^+ into the vacuole through Na^+/H^+ exchanger 1 (NHX1) is an important strategy in plants to deal with salt stress for maintaining ionic homeostasis.[1] Evidences in the *NHX1* transgenic plants and yeasts support the enhanced tolerance to salt stress by overexpressing Na^+/H^+ exchangers.[2-4] Recent studies suggest NHX1 affect the lumen pH[5] by regulating H^+ transportation, which is associated with cellular ROS metabolism.[6-8] In our previous study, we found NHX1 could regulate cellular ROS level, and participate in plant disease defense.[9] However, no evidence demonstrates that transformation of *NHX1* could improve both salt tolerance and disease defense in plant. In this study, the T1 progenies of *Nicotiana tabacum* L. (Wisconsin 38) lines expressing *SeNHX1* (from *Salicornia europaea*) were generated,[4] which were used to investigate the resistance to *Phytophthora parasitica* var *nicotianae* (*Ppn*) and the salt tolerance.

The analysis of salt tolerance was performed both at post germination and seedling stages between *SeNHX1* transgenic lines and PBI-type plants (pBI121 empty vector transformed tobaccos, as control). At post germination stage, *SeNHX1* transgenic tobacco exhibited longer main roots under 150 mM NaCl treatment, and higher fresh weight under 150 and 200 mM NaCl treatment (**Fig. 1A, B and C**). In addition, the 6-week-old seedlings of *SeNHX1* transgenic lines (TS1, TS11 and TS15) showed markedly higher biomass and K^+/Na^+ ratios than PBI-type plants after 6 weeks of 200 mM NaCl treatment (**Fig. 1D and E**).

The NMT[10-11] (Non-Invasive Micro-Test electrophysiological Technology) was further used to detect trans-tonoplast net H^+ fluxes in *SeNHX1* transgenic plants. The results showed the average net H^+ efflux (H^+ transportation out of vacuole) was 1.5 pmol m^{-2} s^{-1} in PBI-type plants, while it was two-fold higher in *SeNHX1* transgenic tobacco lines (4.2, 3.9 and 3.7 pmol m^{-2} s^{-1} in TS1, TS11 and TS15, respectively) without NaCl treatment (**Fig. 1F and G**). In addition, both of TS11 line and PBI-type tobacco plants exhibited enhanced net H^+ efflux in vacuoles along with increasing concentrations of NaCl instead of KCl (**Fig. 1H and I**). These results indicated SeNHX1 functions as a Na^+/H^+ exchanger in enhancement of H^+ efflux and Na^+ compartment in vacuole.

Besides, we found that transformation of *SeNHX1* improved the resistance of tobacco to *Ppn*. The PBI-type plants exhibited larger wilted spots than *SeNHX1* transgenic tobaccos at 60 hpi (hour post inoculation) after *Ppn* inoculation (**Fig. 2A and B**). The disease classification[9] indicated more severe infection occurred in PBI-type plants compared with transgenic lines (**Fig. 2C**). The quantitative analysis of H_2O_2 contents showed PBI-type tobaccos accumulated more H_2O_2 than *SeNHX1* transgenic plants at 60 hpi (**Fig. 2D**). In addition, *SeNHX1* transgenic tobaccos exhibited higher

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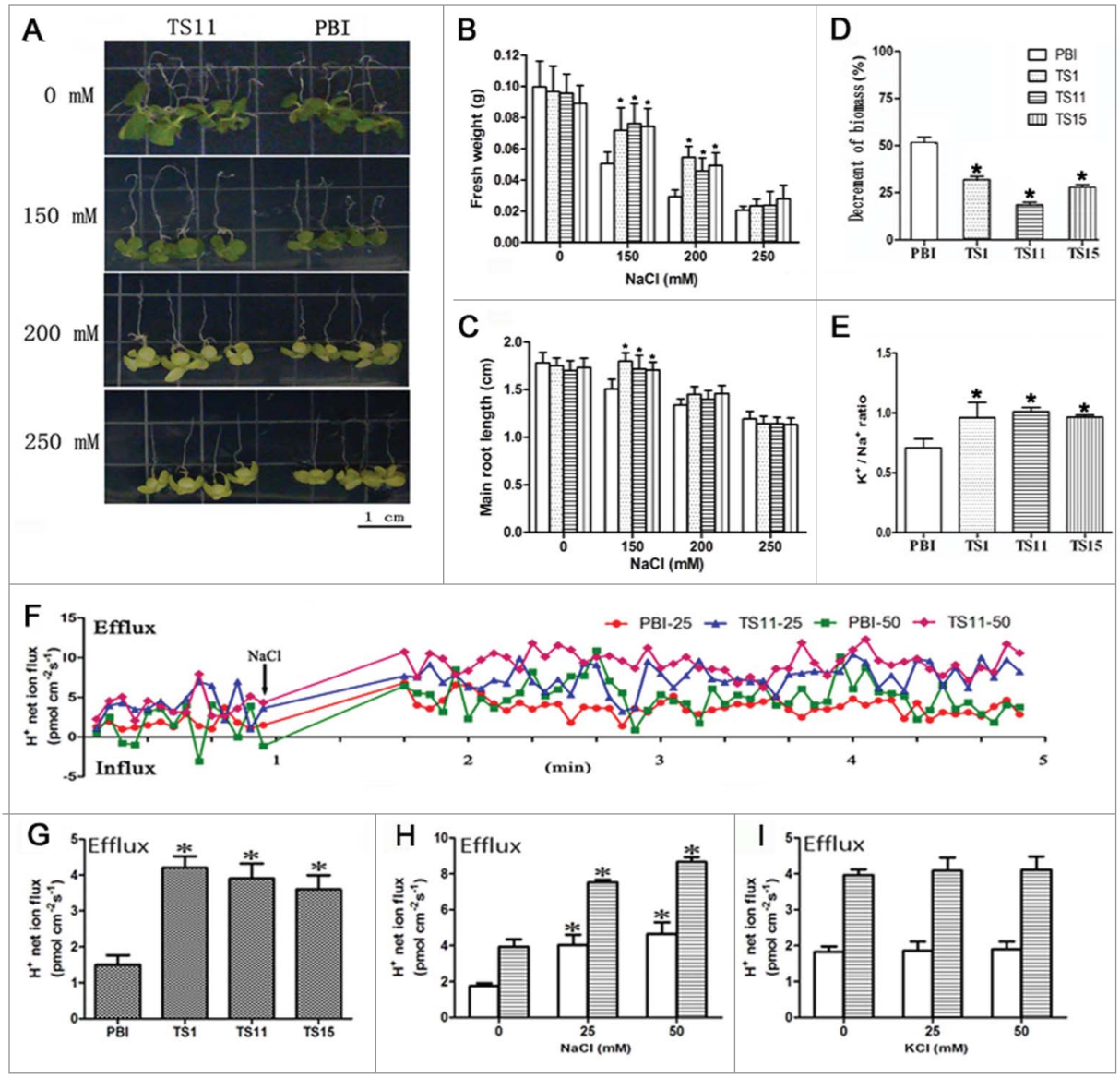

Figure 1. Analysis of vacuole net H^+ fluxes and NaCl tolerance of *SeNHX1* transgenic tobaccos. **A to C**, Phenotype changes (**A**), and fresh weights (**B**), and root lengths (**C**) of 6-day-old seedlings after 0 (MS), 150, 200 or 250 mM NaCl treatment for 12 d. Data are means ± SE ($n = 3$ plates, 4 seedlings in each plate). **D-E**, Effect of 200 mM NaCl on biomass (**D**) and K^+/Na^+ ratio (**E**) of PBI-type and 3 transgenic lines (TS1, TS11 and TS15) subjected to 200 mM NaCl for 6 weeks. Data are means ± SE ($n = 6$ seedlings). **F**, Time-course dynamic curves of vacuolar net H^+ fluxes under different concentrations of NaCl shock. PBI-25/50 represents vacuoles of PBI-type plants under 25 mM or 50 mM NaCl shock, respectively. TS11-25/50 represents vacuoles of TS11 plants under 25 mM or 50 mM NaCl shock, respectively. **G to I**, Mean net H^+ fluxes in vacuoles for 12 min test under normal condition (**G**), and supplied with 0, 25 and 50 mM NaCl (**H**) or KCl (**I**). The value obtained from NMT indicates net ion flux, the positive values of ion flux in figures represent cation efflux or anion influx and *vice versa*. Data are means ± SE ($n = 6$ vacuoles from 6 independent transgenic lines). PBI means tobacco plants transformed with pBI121 empty vector. TS1, TS11, and TS15 represent T1 progenies of *SeNHX1* transgenic tobacco lines. Asterisks on bars indicate significant differences from PBI-type plants (difference from untreated plants in H and I) under the same treatment at $P \leq 0.05$.

contents of NAD(P)(H) components and a larger NAD(P)(H) pool (**Fig. 2E and F**), contributing to the enhancement of ROS elimination ability.[12]

Several molecules including transcriptional factors, kinases and hormones are involved in crosstalk between abiotic and biotic stress responses. In this study, we reported that *SeNHX1*, a gene encoding Na^+/H^+ exchanger 1, participated in both salt stress responses and *Ppn* resistance in tobacco. Our previous study revealed that involvement in *Ppn* resistance was not only the characteristic of *SeNHX1*, but also of *AtNHX1* from *Arabidopsis thaliana* and *NbNHX1* from *Nicotiana benthamiana*.[9] Therefore, the regulation of vacuolar Na^+ and H^+ flux by NHX1 can not only compartment Na^+ in vacuole in response to salt stress, but also affect lumen pH

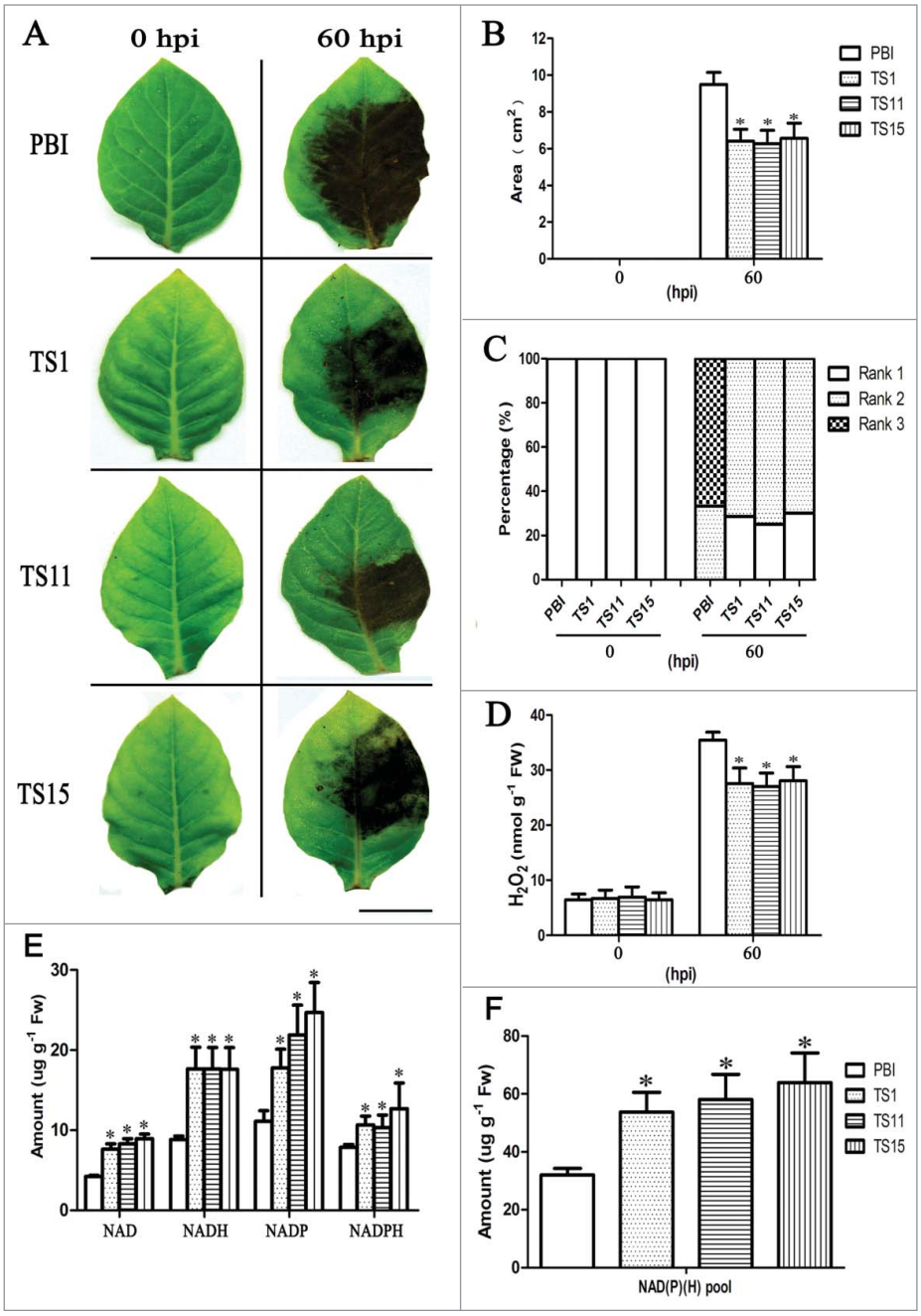

Figure 2. Comparison of NAD(P)(H) pool and the disease development between PBI-type and *SeNHX1* transgenic tobaccos. (**A**) Symptoms on tobacco leaves of PBI-type and 3 *SeNHX1* transgenic lines (TS1, TS11, and TS15) at 0 and 60 hpi. Left side of leaf with wounding-only treatment was served as control (Scale bar = 2 cm). (**B**) Area of wilt spots after *Ppn* inoculation. (**C**) Evaluation on disease courses. The rank of disease symptom in leaves was classified into 3 levels from Rank 1 to 3 according to area of wilt spots.[9] The higher rank means more serious infection. (**D**) H_2O_2 contents in leaf tissue at 0 and 60 hpi. Data are means ± SE (n = 30 leaves). (**E**) The content of NAD(P)(H) components. (**F**) NAD(P)(H) pool as NAD(P) + NAD(P)H. Data are means ± SE (n = 6 seedlings). PBI means tobacco plants transformed with pBI121 empty vector. TS1, TS11, and TS15 represent T1 progenies of *SeNHX1* transgenic tobacco lines, respectively. Asterisks on bars indicate significant differences from PBI-type plants under the same treatment at $P \leq 0.05$.

and the cellular ROS level, thus participate in plant disease defense.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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