An efficient root transformation system for CRISPR/Cas9-based analyses of shoot–root communication in cucurbit crops

Running title: A root transformation system for grafted cucurbit crops

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Highlights

• Establishment of a rapid and efficient root transformation system for cucurbit crops
• The combination of this root transformation system with grafting provides a promising tool for studying shoot-root crosstalk
• CmoHKT1;1 contributes to salinity tolerance by limiting the long-distance transport of Na+
• CmoNHX4 confers salt tolerance to cucumber by lowering the Na+ content and raising the K+ content

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Abstract

Cucurbit crops are suitable models for studying long-distance signaling in horticultural plants. Although thousands of substances are graft transmissible in cucurbits, functional studies have been hampered by the lack of efficient genetic transformation systems. Here, we report a convenient and efficient root transformation method for several cucurbit crops that will facilitate studies of functional genes and shoot–root crosstalk. We obtained healthy plants with completely transformed roots and non-transgenic shoots within 6 weeks. Furthermore, we combined this root transformation method with grafting, which allowed for gene manipulation in the rootstock. We validated our system by exploring salt tolerance mechanisms using a cucumber (Cucumis sativus)/pumpkin (Cucurbita moschata Duch.) (scion/rootstock) graft in which the sodium transporter gene High-affinity K⁺ transporter1 (CmoHKT1;1) was edited in the pumpkin rootstock, and by overexpressing the pumpkin tonoplast Na⁺/H⁺ antiporter gene Sodium hydrogen exchanger4 (CmoNHX4) in cucumber roots.

Key words: Cucurbit crops; Root transformation system; Grafting; Salinity tolerance; Shoot-root crosstalk

Introduction

Cucurbit crops are economically important crops because of their dietary and medicinal value. Grafting of a high-yield scion onto a stress-resistant rootstock has been used to improve crop production for thousands of years, and grafting is also an ideal approach for exploring shoot–root crosstalk in plants. Grafting among the different genera of Cucurbitaceae is straightforward, and their phloem and xylem saps can be easily obtained; therefore, cucurbit crops are excellent models for studying long-distance signaling and graft-transmissible signals. However, the lack of an efficient transgene system in these crops has limited long-distance signaling studies. Although stable transformation systems have been established in watermelon (Citrullus lanatus) and cucumber (Cucumis sativus), they are lacking in other cucurbit species, such as pumpkin (Cucurbita moschata Duch.), melon (Cucumis melo), bottle gourd (Lagenaria siceraria), and luffa gourd (Luffa acutangula). Agrobacterium rhizogenes–mediated root transformation is a rapid and convenient alternative to conventional stable transformation procedures for gene manipulation in vivo. Moreover, the genetic modification of rootstock roots could offer a convenient way to
investigate crosstalk between rootstocks and scions. Nonetheless, an efficient *A. rhizogenes*–mediated root transformation system has not been established for most cucurbit crops.

Grafting is a practical approach to overcome soil-borne diseases and increase abiotic stress resistance in crops\(^\text{12}\). Pumpkin is the most widely used rootstock for cucurbit crops\(^\text{7,12,13}\). For instance, grafting onto pumpkin rootstock has been used to increase salt tolerance of a cucumber scion. In that case, the mechanism of the enhanced salt tolerance is related to the higher capacity of pumpkin roots to limit the long-distance transport of \(\text{Na}^+\) to shoots than that for cucumber roots\(^\text{13}\). X-ray analysis revealed that much more \(\text{Na}^+\) is sequestered in the cortex of pumpkin roots compared to cucumber roots, suggesting that the transport of \(\text{Na}^+\) to the stele is restricted; thus, its long-distance transport to shoots is limited\(^\text{14}\). The higher salt tolerance of pumpkin is also associated with a higher \(\text{K}^+\) uptake capacity relative to cucumber\(^\text{5}\). However, the molecular mechanism and the genes responsible for these differences between pumpkin and cucumber are largely unknown.

Here, we developed a convenient, efficient, and rapid root transformation system for pumpkin and several other cucurbit crops. Healthy plants with roots that had been completely transformed were obtained within 6 weeks. This root transformation system was combined with grafting to take advantage of gene manipulation in the rootstock. We demonstrated the feasibility of our method using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) genome editing technology to knock out the sodium transporter gene *CmoHKT1;1* in the pumpkin rootstock of cucumber/pumpkin grafted plants and by overexpressing the pumpkin tonoplast \(\text{Na}^+/\text{H}^+\) antiporter gene *CmoNHX4* in cucumber roots. Our results demonstrate that this robust root transformation system is efficient not only for functional analysis of genes in roots of cucurbit crops but also provides a promising approach for studying shoot–root communication.

## Results

**An efficient root transformation system for pumpkin and several other cucurbit crops**

Constitutively expressed green fluorescent protein (GFP) was used as a reporter for transformation and to facilitate the selection of transgenic roots. The transformation was performed as shown in Supplementary Fig. S1a, with optimized *A. rhizogenes* strain, concentration of *Agrobacterium* solution, age of seedlings for injection, and co-cultivation time (Supplementary Fig. S1b-e). Briefly, pumpkin seedlings with cotyledons just unfolded were cut at the hypocotyls at angles...
approximately 2 to 3 cm below the cotyledons. These excised apical parts were used as explants for transformation (Fig. 1a). The cut end of the hypocotyl was immersed in 1 mL of A. rhizogenes strain K599 bacterial solution containing 35S:GFP for 30 min for infection (Fig. 1b). The infected explants were then cultivated in boxes containing sterilized vermiculite moistened with MS medium (Fig. 1c). After 4-d cultivation in the dark at 23°C, GFP fluorescence was examined. We observed that A. rhizogenes successfully infected the cut ends of the pumpkin hypocotyls (Fig. 1d) with an infection rate (ratio of hypocotyls with GFP fluorescence after cultivation) of approximately 95%. The explants were then transferred to trays containing sterilized vermiculite moistened with half-strength Hoagland solution. The trays were covered to maintain high humidity to promote the development of hairy roots. After 7 d, the lid was removed, the roots lacking GFP fluorescence were excised under UV light, and the plants were returned to the vermiculite for another week. When the length of GFP-fluorescent roots was more than 2 cm, the plants were cultured hydroponically to reveal the non-transgenic roots more easily and allow their removal without damaging the plants. Note that it was important to remove the non-transgenic roots at the early stages to promote the growth of transgenic roots. After the transgenic roots were fully developed, the growth of non-transgenic adventitious roots from the hypocotyl was greatly reduced. After approximately 2 weeks in hydroponic culture, healthy composite pumpkin plants with completely transgenic roots were obtained with a survival rate (ratio of healthy composite plants with completely transgenic roots) of 84.31% (Fig. 1e and j). The same root transformation method was applied to several other cucurbit crops, including cucumber, melon, bottle gourd, and luffa gourd, with survival rates of 73.33%, 68.9%, 73.9%, and 71.4%, respectively (Fig. 1f-j). Non-transformed plants were used as negative control under UV light, shown in Fig. S2. The morphology of transformed pumpkin roots was similar with the transgene-negative roots (Fig. S3).
Fig. 1 Root transformation system for cucurbit crops

a) Pumpkin seedling explants. b) Infection. c) Co-cultivation. d) GFP-fluorescence at hypocotyls cut end after co-cultivation. e) Composite pumpkin plants with entire roots emitting GFP fluorescence. f-i) Composite seedings of cucumber (f), melon (g), bottle gourd (h), and luffa gourd (i). j) Summary of survival rates of different cucurbit crops. From (d) to (i), bright-field images (left) and UV light images (right). From (a) to (c), scale = 1 cm; (d), scale = 1 mm; from (e) to (i), scale = 5 cm.

The root transformation system can be combined with grafting

Cucurbit crops are useful for studying grafting and graft-transmissible signals\(^6\), and grafting a rootstock with a scion from another species is a common way to characterize mobile signals\(^2\). We tested whether our root transformation system could be combined with grafting, reasoning that our system could provide a much needed and convenient way to genetically modify rootstocks in grafted plants. We used the cucumber/pumpkin (scion/rootstock) graft combination for our study. Pumpkin seedlings with just-unfolded cotyledons were used as the rootstock because this stage is ideal for our A. rhizogenic–mediated transformation method. The pumpkin seedlings were cut at the hypocotyls as described above. Grafting was performed by using the excised apical part with the hole insertion grafting method\(^{15,16}\). Freshly made grafts were used as explants for A. rhizogenic–mediated transformation (Fig. 2a). The transformation and cultivation steps were performed as described above for pumpkin. After that, the cucumber/pumpkin grafts were transferred to trays containing sterilized vermiculite moistened with half-strength Hoagland solution and covered with transparent plastic lids to maintain high humidity. One week of growth...
in high humidity conditions was essential as it promoted both the development of hairy roots and post-graft healing. For cucumber/pumpkin grafts, the infection rate 96.1% was very similar to that of pumpkin (Fig. 2b), but the survival rate of 78.56% was relatively lower (Fig. 2c). These results showed that this root transformation system could be combined with grafting without significantly reducing its infection and survival rates.

**Fig. 2 Root transformation system for cucumber/pumpkin grafts.**

a Cucumber/pumpkin grafts used as explants. b GFP-florescence at hypocotyls cut end after co-cultivation. c Cucumber/pumpkin grafts with entire roots emitting GFP florescence. Csa, cucumber; Cmo, pumpkin; *, graft junction; for (a) scale = 1 cm and (b), scale = 1 mm; for (c), scale = 5 cm.

*CmoHKT1;1* contributes to salinity tolerance of cucumber/pumpkin grafts by limiting long-distance Na⁺ transport

Grafting onto pumpkin has been reported to increase the salt tolerance of cucumber because the pumpkin rootstock limits the transport of Na⁺ from the roots to the shoots and thus prevents the accumulation of toxic Na⁺ in the cucumber scion. The sodium transporter gene *CmoHKT1;1* plays a role in the long-distance transport of Na⁺ from roots to shoots. However, the function of *CmoHKT1;1* has not been tested in pumpkin due to lack of an efficient and stable transformation system. In this study, we generated mutations in *CmoHKT1;1* in the pumpkin roots from cucumber/pumpkin grafts using CRISPR/Cas9 technology. The pBSE403G-sgRNA*CmoHKT1;1* vector was constructed by placing a *CmoHKT1;1*-targeting sgRNA in pBSE403G, which was constructed by inserting 35S:GFP into pBSE401. 35S:GFP was used to verify transformation. Cucumber/pumpkin grafts were transformed using *A. rhizogenes* containing pBSE403G and pBSE403G-sgRNA*CmoHKT1;1* vectors as described above. Four weeks after transformation, we used Hi-TOM sequencing to assess the gene-editing efficiency in the transgenic roots that were positive
for GFP fluorescence\textsuperscript{19}. The gene-editing efficiency of \textit{CmoHKT1;1} reached 80.14\% (Fig. 3a). The role of \textit{CmoHKT1;1} was further evaluated with a 75 mM salt treatment, as described previously\textsuperscript{17}. We determined that the \textit{CmoHKT1;1\textsuperscript{CR}} plants (i.e., plants transformed with pBSE403G-sgRNA\textsuperscript{CmoHKT1;1}) were more sensitive to salt stress than the control plants (i.e., plants transformed with the empty pBSE403G vector) (Fig. 3b). Moreover, when subjected to salt stress, the \textit{CmoHKT1;1\textsuperscript{CR}} plants accumulated more Na\textsuperscript{+} in the shoots and less Na\textsuperscript{+} in the roots than the control plants (Fig.3c and d), suggesting disturbed Na\textsuperscript{+} transport from roots to shoots in \textit{CmoHKT1;1\textsuperscript{CR}} plants. Meanwhile, when subjected to salt stress, the Na\textsuperscript{+} content was higher in the xylem sap of the \textit{CmoHKT1;1\textsuperscript{CR}} plants relative to the control plants (Fig.3e and f). These data support the conclusion that \textit{CmoHKT1;1} contributes to salinity tolerance by limiting the long-distance transport of Na\textsuperscript{+} in cucumber/pumpkin grafts.

\textbf{Fig. 3.} Mutation of \textit{CmoHKT1;1} in rootstock disturbed Na\textsuperscript{+} transport from rootstock to scion in cucumber/pumpkin grafts.

\textbf{a} Editing efficiency of \textit{CmoHKT1;1}. \textbf{b} Cucumber/pumpkin grafts transformed with \textit{CmoHKT1;1\textsuperscript{CR}} or an empty vector subjected to NaCl treatments, scale = 5 cm. \textbf{c-d} Na\textsuperscript{+} content in shoots and roots. \textbf{e} Collection of xylem sap, scale = 1 cm. \textbf{f} Na\textsuperscript{+} content in xylem sap. CK, 0 mM NaCl; NaCl, 75 mM NaCl. The values are mean ± standard error of three biological replicates for each treatment. Different letters indicate statistically significant differences, \(P<0.01\).

\textbf{Pumpkin \textit{CmoNHX4} confers salinity tolerance to cucumber roots}

We also tested whether our method could be useful for cell biological analyses in roots of cucurbit crops. The higher salt tolerance of pumpkin relative to cucumber is associated with the higher K\textsuperscript{+} uptake capacity of pumpkin\textsuperscript{5}. The tonoplast Na\textsuperscript{+}/H\textsuperscript{+} antiporter \textit{AtNHX1} mediates potassium uptake\textsuperscript{20}. In this study, we observed that the expression of \textit{CmoNHX4} —a homolog of \textit{AtNHX1}— was induced by a NaCl treatment and was the most highly expressed member of the \textit{CmoNHX}
gene family in pumpkin roots treated with NaCl (Fig. 4a and Fig. S4). Thus, we tested whether overexpression of CmoNHX4 in cucumber could improve salt tolerance. Vectors were constructed by inserting the CmoNHX4 coding sequence with or without a GFP tag downstream of the 35S-promoter (Fig. S5a,b). 35S:DsRed2 was used to monitor transformation \(^{21}\). The vectors were transformed as mentioned above. The 35S:CmoNHX4-GFP vector was used for the subcellular localization of CmoNHX4-GFP. The expression of CmoNHX4-GFP in cucumber roots was low and inconsistent (Fig. S6a,b), which was similar to 35S:AtNHX1-GFP expression in transgenic roots of Arabidopsis \(^{22}\). According to report \(^{22}\), the GFP signal within the vacuolar lumen and endosomes in cells with bright GFP expression may be a result of 35S promoter-driven-overexpression (Fig. S6a,b). However, the CmoNHX4-GFP signal was found on the vacuolar membrane in cells with lower GFP, as evidenced by the nuclei stained with DAPI detected outside the membrane boundary (Fig. 4b). In pumpkin roots, the expression pattern of 35S:CmoNHX4-GFP was similar to cucumber roots, with the exception that the fluorescence intensity in pumpkin roots was lower (Fig. S6c-e). The intensity of GFP signal was diminished after plasmolysis of root cells induced by sucrose, with vacuolar membrane located GFP signal hardly detectable and only GFP signal within the vacuolar lumen or endosomal bodies observed outside the nuclei (Fig. S6f). Then, the tonoplast membrane marker 35S:AtTIP1-RFP was transiently co-expressed with 35S:CmoNHX4-GFP in Nicotiana leaves \(^{23}\). The colocalization of CmoNHX4-GFP with AtTIP1-RFP provided additional evidence that CmoNHX4-GFP was localized on the vacuolar membrane (Fig. S6g). 35S:CmoNHX4 vector (without GFP tag) were used to overexpress CmoNHX4, and the resulted cucumber seedlings with transformed roots were used for phenotypic analysis and CoroNa green staining. Realtime-PCR analysis showed that CmoNHX4 expression increased significantly in roots transformed with 35S:CmoNHX4 vector compared to the empty vector (Fig. S5c). The overexpression of CmoNHX4 in cucumber roots significantly improved the salt tolerance of cucumber (Fig. 4c). We measured the Na\(^+\) and K\(^+\) content in the control and CmoNHX4-overexpressing roots and determined that CmoNHX4 overexpression decreased the Na\(^+\) content and increased the K\(^+\) content of cucumber roots (Fig. 4d and e). We used CoroNa staining \(^{24}\) to assess the accumulation of Na\(^+\) in the vacuoles of cucumber roots and observed that the CmoNHX4-overexpressing roots accumulated less Na\(^+\) in their vacuoles than the control roots.
These data indicate that \textit{CmoNHX4} conferred salinity tolerance in cucumber plants by increasing the K$^+$ content and decreasing the Na$^+$ content of cucumber roots.

![Fig. 4](https://example.com/fig4)

**Fig. 4.** Overexpression of pumpkin \textit{CmoNHX4} enhanced cucumber salt tolerance.

- a \textit{CmoNHX} expression, numbers indicating FPKM values.
- b Subcellular localization of \textit{CmoNHX4-GFP}.
- c Phenotype of \textit{CmoNHX4-OE} plants treated with NaCl.
- d-e Na$^+$ and K$^+$ content in roots.
- f Na$^+$ in vacuoles with CoroNa staining. The values are mean $\pm$ standard error of three biological replicates for each treatment. Different letters indicate statistically significant differences, $P<0.01$.

In conclusion, we developed a robust root transformation system for cucurbit crops. We obtained composite plants with transgenic roots using apical parts of seedlings as explants. A hydroponic culture procedure allowed us to readily identify non-transgenic roots and excise them without damaging the plants. By combining our transformation system with grafting, we provide a promising approach for studying shoot–root communication. Using this system, we further explored the mechanism of higher salt tolerance in pumpkin compared to cucumber by revealing the roles of \textit{CmoHKT1;1} and \textit{CmoNHX4}.

**Discussion**

Stable genetic transformation mediated by \textit{Agrobacterium tumefaciens} is time-consuming, labor-intensive, and a major constraint in the functional genomics of cucurbit crops. Additionally, the recalcitrant nature of some cucurbits reduces their transformation efficiencies and requires an alternative technique to address these issues\textsuperscript{1,25}. The hairy root transformation has been
successfully performed in hundreds of plant species\textsuperscript{26,27,28}, including legumes like soybean\textsuperscript{11} and peanuts\textsuperscript{29}, vegetables like potato\textsuperscript{30} and tomato\textsuperscript{31} and even fruits such as avocado\textsuperscript{32}, grapevine\textsuperscript{33} and prunus\textsuperscript{34}. The hairy root transformation system has been used in biological research for decades and has proven to be a viable tool for studying gene function.

In the current study, we successfully developed a hairy root transgenic system that might be deployed to investigate the gene function in cucurbit crops. This approach highlights the basic need for genetic verification systems, particularly in pumpkin, melon, bottle gourd, and luffa gourd, which currently lack highly efficient stable transgenic systems. This root transformation system along with stably transformed roots, could be used for gene function studies in the roots of cucurbit crops using overexpression and knockout techniques in the fields of root metabolites, nutrient acquisition, root development, and root resistance to soil-borne disease and abiotic stress. It could also be used for cell biological analyses in roots, such as subcellular localization and cell staining. Moreover, this root transformation system was used in combination with grafting to explore root-shoot communication in grafted cucurbit plants. However, there were few limitations, such as the inability to obtain homozygous mutations edited by CRISPR/Cas9, and the produced mutations could not be transmitted to the next generation. Nevertheless, the convenience, rapidity, and high efficiency make this system a promising tool for preliminarily validating candidate genes before conducting stable transformation. Our method of studying scion-rootstock communication by integrating the root transformation system and grafting technique could be used to other crops such as flowers and fruit trees.

Materials and methods

Plant material and growth conditions

Pumpkin (\textit{Cucurbita maxima} × \textit{C. moschata}, Fenglejinjia), cucumber (\textit{Cucumis sativus} L, Jinchun No. 4), melon (\textit{Cucumis melo}, Yilishabai), bottle gourd (\textit{Lagenaria siceraria}, Jingxinzhen No.1), and luffa gourd (\textit{Luffa acutangular}, Zaojia) were used in this study. The seedlings were grown in a climate chamber with a light intensity of 350 μmol·m\textsuperscript{−2}·s\textsuperscript{−1}, a photoperiod of 16 h light/8 h dark, and day/night temperatures of 28°C/18°C.

Vector construction

The pBSE403G vector was constructed by inserting 35S:GFP:Terminator into the EcoRI site of pBSE401\textsuperscript{18}. The sgRNA targeting \textit{CmoHKT1;1} was designed using Geneious software as
described\(^8\). CmoHKT1;1-targeting sgRNA was cloned into pBSE403G as described previously\(^{18}\).

For overexpression of CmoNHX4, the intermediate vector pBSE401R was first built by inserting 35S:DsRed2:Terminator\(^{21}\) into the EcoRI site of the pBSE401 vector. Then, CmoNHX4 or CmoNHX4-GFP was cloned into pBSE401R between the XbaI/SacI sites and downstream of the 35S promoter. The pBSE401R-CmoNHX4 was used for phenotypic analysis and CoroNa green staining. pBSE401R-CmoNHX4-GFP was used to study subcellular localization of CmoNHX4. The maps of the two vectors are provided in supplementary data Fig.S4.

*Agrobacterium rhizogenes*–mediated root transformation system

Seeds were soaked in water at 55\(^\circ\)C for 5 h and then incubated in a Petri dish at 28\(^\circ\)C in the dark until germination. *A. rhizogenes* strain K599 from cultures with OD\(_{600}\) values between 0.8 and 1.0 were collected and re-suspended in MS medium containing 200 \(\mu\)M AS for bacterial infection. The subsequent steps are described in detail in the supplementary data. The hole insertion grafting method was used for grafting\(^{15,16}\). Briefly, we removed the apical meristem of the rootstock, leaving the cotyledons, and made a hole on the top portion with a grafting needle. Then, the cucumber scion was cut at angles on both sides of the hypocotyls and inserted into the hole made in the rootstock.

DNA isolation and Hi-TOM analysis

About 2 weeks after co-cultivation, healthy composite plants with completely transgenic roots were used to analyze the efficiency of gene editing. The GFP-positive transgenic roots were used for DNA extraction using the CTAB protocol. The fragment containing the target site was PCR amplified and analyzed by Hi-TOM sequencing\(^{19}\).

Hydroponic cultivation and salt treatment

Hydroponic cultures were done by growing plants in half-strength Hoagland solution in plastic boxes (380 mm \(\times\) 245 mm \(\times\) 100 mm) as described\(^{17}\). Foam boards were used to anchor and support the plants. The Hoagland solution was renewed once a week. For the salt treatment, healthy plants with completely transgenic GFP or DsRed2 fluorescent roots and consistent growth were selected and grown in 5 L 1/2-strength Hoagland solution containing 0 or 75 mm NaCl for 5 days. Three biological replicates per treatment were used for the experiment.

Measurements of Na\(^+\) and K\(^+\) content
Three plants per treatment were harvested. The roots and shoots were separated and put into Kraft paper bags, which were placed at 105°C for 15 min and then at 80°C until dry. The dried samples were ground into powder and weighed. Then, 0.1 g of powder was digested in 5 mL H$_2$SO$_4$ at 300°C for 1 h, and a drop of H$_2$O$_2$ was added every half hour until the solution became colorless and transparent. The digested solution was diluted and used to determine the concentrations of Na$^+$ and K$^+$ using an atomic absorption spectrophotometer (6300C, Shimadzu). For determination of Na$^+$ concentration in xylem sap, 20 μL xylem sap was diluted in 5 mL ddH$_2$O and directly measured without digestion. Three biological replicates per treatment were used for the experiment.

**Gene expression analysis by qRT-PCR**

Cucumber roots transformed with an empty vector and pBSE401R-CmoNHX4 vector were harvested and immediately frozen in liquid nitrogen for further gene expression analysis. The gene expression was measured for CmoNHX4 according to the described method$^{35}$. Primer3plus software was used to generate specific sequences of primers as mentioned in supplementary Table S1.

**Xylem sap collection**

Cross sections of the salt-treated and control plants were cut 1 cm above the grafting union. Then, the wound surface was wiped with filter paper two to three times, and the xylem fluid, which flowed out due to root pressure, was carefully removed with a pipette and put into a 1.5-mL centrifuge tube for subsequent ion determination.

**Light microscopy and CoroNa staining**

The Na$^+$ content in the vacuoles of pumpkin roots was measured with the green fluorescent Na$^+$ dye CoroNa as described previously$^{34}$. Transgenic roots positive for 35S:DsRed2 were used for analysis. The DMSO-dissolved dye was diluted to 20 μM with the measuring buffer (10 mM KCl, 5 mM Ca$^{2+}$-MES, pH 6.1). About 1 cm of excised root tip segments was immersed and incubated in the dark for 2.5 h for staining. The stained roots were washed with measuring buffer three times and then observed with a confocal laser scanning microscope (SP8; Leica Microsystems). Co-localization of CmoNHX4-GFP and AtTIP-RFP was conducted in tobacco leaves as described$^{17}$. The nuclei was stained with 10 µg/ml DAPI solution for 10 minutes.

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Author contributions
L. Y. and Z. B. conceived and designed the experiments. S.G., H. S., H. C., J. S., Z. C., L. Z., W., and R. Y. performed the experiments and analyzed the data. L. Y. and Z. B. wrote the paper.

Data availability
All the data generated in this study are included in this publishing article and its supplementary information.

Conflict of interest
The authors declare that they have no competing interests.

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