Overexpression of Na+/H+ antiporter gene AtNHX1 from Arabidopsis thaliana improves the salt tolerance of kiwifruit (Actinidia deliciosa)

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Received 13 December 2009; received in revised form 21 July 2010; accepted 22 July 2010

Abstract

Salinity is the main limiting factor of plant growth and agricultural productivity. A lot of previous works showed that the introduction of Na+/H+ antiporter gene could improve the tolerance of plants to salt. In this study, a vacuolar Na+/H+ antiporter gene, AtNHX1 from Arabidopsis, was transferred into kiwifruit by Agrobacterium-mediated protocol. Polymerase chain reaction (PCR) and Southern blot analysis confirmed that AtNHX1 was successfully integrated into the kiwifruit genome. Reverse transcription (RT)-PCR analysis indicated that AtNHX1 expressed highly in transgenic plants. It was found that transgenic kiwifruit plants exhibited improved resistance to 200 mmol/l NaCl in comparison with wide-type plants. Under salt stress, these transgenic lines accumulated more Na+ than control, due to an increased Na+/H+ antiporter activity. In physiological analysis, the traits such as osmotic adjustment and antioxidation capability of transgenic lines under salt stress were obviously higher than that of wide-type plants. These results suggested that the overexpression of vacuolar Na+/H+ antiporter gene could increase the salt tolerance of kiwifruit.

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Keywords: Actinidia deliciosa; Agrobacterium tumefaciens; AtNHX1; Salt tolerance

1. Introduction

The genus Actinidia Lindl. is large, containing 50 to 70 species of climbing plants originating mainly in southern China (Ferguson, 2007), since ancient times people of that area have known of the very delicious flavor of kiwifruit. Kiwifruit has been used in Chinese herbal remedy and Chinese folk medicine for treatment cancer since prerecorded times, including stomach, lung and liver cancer (Motohashi et al., 2002). Over the past 30 years, kiwifruit has developed into an important horticultural fruit tree, firstly in New Zealand, and subsequently in many different parts of the world such as Chile, China and Italy (Ferguson and Huang, 2007; Nishiyama, 2007). In recent years, it attracts more preference for as much as its distinctive flavors, pleasant fragrance, healthful components and potential medical prospects.

Kiwifruit is one of the most nutrient-dense fruits and is a good source of vitamin C, low in fat and rich in dietary fibre and phytochemicals (Nishiyama et al., 2004). It is currently accepted that kiwifruit has a preventive effect against certain cancers and cardiovascular diseases such as hypertension, hyperlipidemia, arteriosclerosis and diabetes (Du et al., 2009; Jang et al., 2008), which are thought to be in correlation with its phytosubstances and diverse constituents’ ability in quenching active free radical oxygens, such as carotenoids and flavonoids (Kaur and Kapoor, 2001). The predominated flavonoid in kiwifruit is flavonol (Webby et al., 1994), and the main component in Actinidia deliciosa is rutin (Quercetin-3-rhamnoglucoside) (Greaves et al., 2001). In addition, the flower and fruit of kiwifruit contain many unique aroma compounds derived from different genetic pathways with potential for exploitation as nutraceuticals or medicine (Jordan et al., 2002).

Soil salinity is one of the major environmental factors limiting agricultural productivity in many regions of the world, because most crops are glycophytes and usually salt sensitive.
Salinity imposes two types of stress on plant tissues, which sequentially affect plant on many aspects, including mineral and water uptake, enzyme activities, photosynthesis and metabolism (Takahashi et al., 2009), one is the water deficit resulting from the relatively high solute concentrations of the soil, the other is ion-specific stresses resulting from altered K⁺/Na⁺ ratios, Na⁺ and Cl⁻ concentrations that are inimical to plants (Blumwald et al., 2000). Kiwifruit belong to glycophyte, and the soils they can grow are limited. The aim of our experiment is to alter the behavior and enhance the adaptability of kiwifruit to saline soil.

Na⁺ is not essential for plant growth, although it is required in some plants, particularly halophytes. Halophytes have achieved salt-tolerant capability in the long evolution process to adapt themselves to saline environment. In salinity-stressed plants, Na⁺/H⁺ antiporters play a key role in maintenance of the cytoplasmic K⁺/Na⁺ ratio, through pumping Na⁺ either out of cells (sodium extrusion) or into organelles, mainly vacuole (sodium compartmentation), in exchange for H⁺. From the first identification of the activity of Na⁺/H⁺ antiporter in barley root tips in 1976 (Ratner and Jacoby, 1976), many Na⁺/H⁺ antiporter genes have been characterized in plants, such as Arabidopsis thaliana (Apse et al., 1999), Oryza sativa (Fukuda et al., 1999; Gaxiola et al., 1999), Atriplex gmelini (Hamada et al., 2001), Mesembryanthemum crystallinum (Chauhan et al., 2000), Suaeda salsa (Ma et al., 2004), Beta vulgaris (Xia et al., 2002), Gossypium hirsutum (Wu et al., 2004) and Trifolium repens L. (Tang et al., 2009). The overexpression of Na⁺/H⁺ antiporter genes could increase tolerance under saline conditions in transgenic Arabidopsis (Apse et al., 1999), tomato (Zhang and Blumwald, 2001), Brassica (Zhang et al., 2001), rice (Chen et al., 2007; Ohta et al., 2002), wheat (Xue et al., 2004), buckwheat (Chen et al., 2008) and Petunia hybrida (Xue et al., 2009). These works demonstrated that Na⁺/H⁺ antiporter genes were crucial to plant salt-tolerance, and they could be used in crop improvement through genetic transformation.

Many crops have undesirable traits, such as salt sensitivity, thus improvement of their salt tolerance has been a tough task all the while in traditional breeding. The genetic improvement of tolerance by traditional cross-breeding has been hindered by the gynodioecious characteristics, genetic heterogeneity, and long life cycle of kiwifruit (Nakamura et al., 1999). Modern biotechnology creates a new approach for breeding with the development of tissue culture and transformation system. Several reports have been published in which gene function has been tested in transgenic kiwifruit (Actinidia delicosa) transformed by Agrobacterium. The expression of β-1, 3-endoglucanase gene from soybean in kiwifruit increased resistance of young leaves to Botrytis cinerea (Nakamura et al., 1999). The transgenic kiwifruit harbouring rice homeobox-containing gene OSH1 showed morphological changes including dissected leaf margins and dwarfism (Kusaba et al., 1999). Kobayashi et al. (2000) reported that expression of stilbene synthase gene from Vitis spp. led to production of resveratrol-glucoside in leaves of kiwifruit.

In the present work, AtNHX1 driven by a constitutive promoter was transferred into kiwifruit on the basis of a high-frequent regeneration system established by Tian et al. (2007). Several physiological parameters were examined to analyze the effect of salt stress on biomass production and the patterns of Na⁺ and K⁺ accumulation in transgenic plants. Our results confirmed that the biomass of AtNHX1 transgenic kiwifruit was improved under saline condition compared to wild-type plants.

2. Materials and methods

2.1. Plant materials and expression vector

The stems of kiwifruit genotype ‘Qin mei’ were collected from Hu county of Shaanxi province. Plant expression vector pHZX1 containing a selectable marker gene NPTII and Na⁺/H⁺ antiporter gene AtNHX1 was kindly provided by Dr. HX Zhang from the National Institute of Plant Physiology in Shanghai. AtNHX1 is controlled by cauliflower mosaic virus (CaMV) 35S promoter and 3′-UTR, polyadenylation signal and terminator region of nopaline synthetase gene (Nos).

2.2. Callus induction and plant regeneration

The stems were washed under running water for 6 h, followed by surface-sterilization with 75% ethanol for 3 min and 0.1% HgCl₂ for 8 min. After rinsing for 3 times with sterile distilled water, the stems were cut into 0.5 cm segments and inoculated on MS medium supplemented with 2.0 mg/l 6-BA, 1.0 mg/l NAA, 600 mg/l casein hydrolysate, 500 mg/l yeast extract for callus induction and regeneration under 2000 lx illumination, 16 h photoperiod and 25±2 °C. Calli and regenerated shoots were subcultured at regular intervals of 3 weeks. The regenerated shoots of 2.0 cm high were rooted on half MS medium (Murashige and Skoog, 1962) containing 1.0 mg/l IBA. All media contained 30 g/l sucrose and were solidified with 0.7% agar. The pH was adjusted to 5.8–6.2 prior to autoclaving at 121 °C for 25 min.

2.3. Transformation of the explants

Agrobacterium strain LBA4404 was inoculated into YEB liquid medium (5.0 g/l tryptone, 5.0 g/l sucrose, 1.0 g/l yeast extract, 0.5 g/l MgSO₄·7H₂O, 50 mg/l kanamycin and 100 mg/l streptomycin) and incubated at 28 °C with constant shaking (200 rpm) overnight. One millilitre bacterium suspension was harvested by centrifugation at 3000 rpm for 5–6 h and the bacteria were harvested by centrifugation at 3000 rpm for 10 min and resuspended in MS liquid medium, the OD₆₀₀ was adjusted to 0.2–0.8. Stem and leaf explants precultured for 0–4 days were immersed in Agrobacterium suspension for 5–25 min in the dark. Excess bacteria on the explant surface were removed with sterilized filter paper and the explants were co-cultured at 25 °C in the dark for 1–4 days. Acetosyringone (AS) was added at a different concentration (0–250 μmol/l) to the medium during the period of coculture. The infected explants were rinsed with sterile water more than 3 times, and subcultured on selection and regeneration medium supplemented with...
20 mg/l kanamycin, 500 mg/l cefotaxime at 3 weeks intervals. Approximately 8 weeks later, survived shoots were transferred onto half MS medium containing 1.0 mg/l IBA to induce roots. Transformation efficiency is usually expressed as the number of explant with kanamycin-resistant calli/initial number of explant inoculated.

2.4. PCR and Southern blotting analysis

Genomic DNA was extracted from leaf tissue of kanamycin-resistant plants using the cetyl trimethyl ammonium bromide (CTAB) method. The presence of AtNHX1 in putative transgenic plants was analyzed by PCR with a primer pair of 5′-CACTCACCTAAACCAGAAC-3′ and 5′-CAGACCACCAAATCACAAACC-3′. The amplification conditions used were 10 min predenaturation at 94 °C followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, elongation at 72 °C for 1 min, and a 10 min final extension step at 72 °C.

PCR positive plants were further confirmed by Southern blotting analysis. The 560-bp fragment amplified from AtNHX1 was purified and used as probe. Twenty microgram of genomic DNA was digested with EcoRI or with HindIII and EcoRI, respectively, were separated by electrophoresis in 1.0% agarose gel. After transferred to nylon membrane, genomic DNA was hybridized with AtNHX1 probe using DIG random labeling and detection system (Roche).

2.3. Expression of AtNHX1 in transgenic kiwifruit

Reverse transcription PCR (RT-PCR) was carried out to measure the expression level of AtNHX1 in transgenic lines. Total RNA was isolated according to the mini-prep procedure described previously (Wilkosz and Schläppi, 2000) and treated with RNase-free DNase I (Takara). One microgram of total RNA was added in 10 μl RT reaction systems with oligo dT as primer and cDNAs were synthesized with Reverse Aid First Strand cDNA Synthesis Kit (Fermantas). One microlitre of the RT reaction mixture was used as template in amplification of AtNHX1 in a 20 μl PCR system and the conditions were same as above-mentioned.

2.6. Determination of Na⁺, K⁺, proline and malondialdehyde contents in transgenic kiwifruit

Two transgenic lines (TL1 and TL2) and wild-type kiwifruit were cultured on MS medium for 2 weeks and then transferred to MS medium containing different concentrations of NaCl (0, 100 and 200 mmol/l respectively) for 3 days. Roots were rinsed with distilled water to remove NaCl on the surface, and then the leaves and roots of each treatment were harvested and dried at 60 °C overnight. Na⁺ and K⁺ concentrations were measured by a flame photometer (Thermo). Free proline content in leaves was determined according to the method given by Bates et al. (1973). The levels of lipid peroxidation in fresh leaves, stems and roots were measured in terms of malondialdehyde (MDA) content by thiobarbituric acid (TBA) reaction method (Kardeniz et al., 2005).

2.7. Determination of flavonoids in transgenic kiwifruit

5% NaOH was employed to stain the flavonoids distributed in different tissues of plant (Tan et al., 2007). A modified spectrophotometric method was used to determine the flavonoid contents of transgenic kiwifruit. Dry samples were grinded to fine powder in a mortar and pestle, then immersed in methanol (0.1 g/20 ml), and treated with ultrasonic wave for 30 min. The samples were centrifuged at 12,000 rpm for 20 min. A 0.5 ml aliquot of the samples was transferred to a test tube and 3 ml of 30% ethanol and 0.3 ml of 5% sodium nitrite were added. The solution was mixed thoroughly and stood at room temperature for 5 min followed by addition of 0.3 ml of 10% aluminium chloride. After 6 min, 2 ml of 1 M sodium hydroxide was added to the test tube. The solution was then diluted with 30% ethanol to make the final volume up to 10 ml. The absorbance at 510 nm was checked and the flavonoid content was calculated with a standard calibration curve prepared with rutin (Subhasree et al., 2009).

3. Results

3.1. Effects of preculture, infection and coculture time on transformation frequency of kiwifruit

As shown in Fig. 1A, the transformation frequencies changed with explant type and preculture time. The result indicated that the stem was much more suitable for transformation by Agrobacterium tumefaciens than the leaf, presumably the leaf was vulnerable. Transformation frequency of leaf explants significantly increased if they were precultured for 1–2 days. On the contrary, preculture had no effect in stem transformation. However, the transformation frequencies decreased after 4 days preculture.

Time of Agrobacterium infection and coculture were crucial to transformation. The result (Fig. 1B and C) demonstrated that the appropriate infection time and coculture time for different explants were variable. The proper infection time was 2 min for leaf and 15 min for stem. Two days coculture with Agrobacterium was better for both stem and leaf. If the coculture time exceeded three days, the explants would be injured seriously by Agrobacterium. It is possible that leaves are more fragile to Agrobacterium than stems, which led to the differences between stem and leaf on infection and coculture time.

3.2. Effects of Agrobacterium density and AS on transformation of kiwifruit

The density of Agrobacterium influenced the transformation frequency obviously. Suitable densities of Agrobacterium not only facilitated the infection process of Agrobacterium, but also reduced its detrimental effect to explants. As shown in Fig. 1D, the fragile leaves required a low density of Agrobacterium (optimal OD_{600} = 0.2) compared to stems (optimal OD_{600} = 0.6).
Acetosyringone (AS) is regarded as one of the most effective signal molecules, with the function in activating expression of vir genes (Berthelot et al., 1998). It could significantly increase the transformation frequency in certain plant species (Ozawa, 2009), especially in monocotyledons which are insensitive to Agrobacterium. However, high concentration of AS is toxic to explants. To promote the transformation frequency, AS was added to the medium during the period of coculture. The effect of AS on transformation frequency was slight (Fig. 1E), so it was unnecessary to add AS in the process of kiwifruit transformation.

3.3. Regeneration of transgenic kiwifruit

The explants after 1–2 days coculture with Agrobacterium were selected on the regeneration medium containing kanamycin. 4 weeks later, most explants browned, only 17% of them
produced green calli (Fig. 2A). Subcultured green calli produced shoots in 4 weeks (Fig. 2B). The regenerated shoots produced roots on rooting medium in one month. Strong plantlets were exercised and transplanted to soil (Fig. 2E).

3.4. Molecular identification of transgenic plants

4 transgenic lines of kiwifruit were identified by PCR amplification of a 560 bp fragment of *AtNHX1* gene (TL1–TL4, TL5–TL8).
Three independent transgenic lines (TL1–TL3) were selected for Southern blotting analysis. When the DNA were double digested with HindIII and EcoRI, the expected 1.9 kbp band (corresponding to the fragment containing CaMV 35S promoter, the Na+/H+ antiporter cDNA and Nos terminator) was detected in all of the PCR positive plants (Fig. 3B, lanes TL1 to TL3), but not in the control plant (Fig. 3B, lane CK). To confirm the integration sites of the cDNA fragment in the transgenic plant genome, the genome DNA were single-digested with EcoRI because pHZX has a single restriction site of EcoRI. The transgenic plants showed one to two bands which represented junctions between T-DNA and adjacent plant DNA (Fig. 3D). These findings showed TL1 and TL2 possessed a single copy of AtNHX1, while TL3 has two copies. TL1 and TL2 lines were further analyzed on the transcription level by RT-PCR. As shown in Fig. 3C, AtNHX1 was expressed effectively in TL1 and TL2.

### 3.5. Determination of Na⁺, K⁺, proline and malondialdehyde contents of transgenic kiwifruits

Vacuolar Na⁺/H⁺ antiporters catalyze the exchange of Na⁺ for H⁺ across vacuolar membranes, and compartmentalize Na⁺ into vacuoles (Glenn et al., 1999). Sodium and potassium play important roles in salt stressed plants. To determine if over-expression of AtNHX1 could increase Na⁺ accumulation in kiwifruit, Na⁺ and K⁺ contents were examined in transgenic and wild-type plants. Physiological analysis was carried out in two transgenic lines TL1 and TL2 to evaluate the salt tolerance. Without salt stress, those contents were nearly the same in both transgenic and wild-type plants. Na⁺ contents in leaves and roots of the transgenic lines and wild-type kiwifruit both increased with the rising NaCl concentration (100–200 mmol/l). However, at the range of 100–200 mmol/l NaCl, Na⁺ contents in leaves (Fig. 4A) and roots (Fig. 4C) of transgenic lines were higher than that of the wild-type plants, especially at 200 mmol/l NaCl concentration. The result demonstrated that the transgenic lines accommodated more quickly to Na⁺ stress than wild-type plant. The reason might be efficient expression of Na⁺/H⁺ antiporter gene AtNHX1 in transgenic lines could sequester Na⁺ and compartmentalize it to vacuole to keep a high cytosolic K⁺/Na⁺ ratio.

The K⁺ contents in leaves and roots of transgenic lines and wild-type kiwifruit both decreased with the increase of NaCl concentration (Fig. 4B and D). Whereas, K⁺ contents in leaves and roots of transgenic lines were significantly higher than that...
of wild-type plants at a high concentration of NaCl (200 mmol/l). K⁺ is the most abundant cation in plants, involved in many metabolism processes, such as root and shoot growth, tropisms, cell expansion, enzyme activity, ion homeostasis, stomatal movements and osmotic regulation (Glenn et al., 1999). Hence the transgenic kiwifruit lines could maintain high K⁺/Na⁺ ratio in cytosol, so as to promote metabolism processes.

Proline can protect macromolecules from dehydration and be used as hydroxyl radical scavenger. When plants suffer from adversity, they usually produce abundant free proline to adjust osmotic-stress (Bates et al., 1973). After treated by NaCl, contents of free proline in leaves of transgenic lines were significantly higher than that of the wild-type plants (Fig. 5A). This result further confirmed the function of the Na⁺/H⁺ antiporter in opposing to salt stress.

Lipid peroxidation is related to the oxidative degradation of polyunsaturated fatty acids and involves free radicals. This membrane damage process results in deleterious effects and produces malondialdehyde (MDA) (Glenn et al., 1999). MDA level under stress conditions could mirror the antioxidative capacity of plant cell. In the present work, the contents of MDA in the leaves of transgenic lines and wild-type plants were measured to depict the membrane lipid peroxidation status of them stressed by different NaCl concentrations. After treatment with NaCl, the MDA contents increased in the transgenic lines and wild-type kiwifruit. However, in each concentration the MDA contents of transgenic lines were less than that of the wild-type plants after 3 d treatment with NaCl (Fig. 5B). These data indicated that overexpression of Na⁺/H⁺ antiporter gene *AtNHX1* was correlated with the alleviation of the detrimental effect of Na⁺.

Transgenic lines (TL1 and TL2) and wild-type plants were transferred to soil and grown for two weeks, then watered with nutrient solution containing 0–200 mmol/l NaCl. After 20 days, obvious salt stress effect on the growth of the transgenic plants and control could be observed. The leaves of the wild-type plants gradually turned yellow and withered after two weeks when the concentration of NaCl reach 200 mmol/l, whereas transgenic lines still kept green and grown (Fig. 2C, D). The inhibition of NaCl on vegetative growth was shown in Table 1. It demonstrated that the growth status of the transgenic lines obviously excelled the control plants. The transgenic lines were less-inhibited by salt stress compared to wild-type plants further confirmed that the overexpression of the Na⁺/H⁺ antiporter gene could indeed improve salt tolerance.

### 3.6. Determination of flavonoids in different tissues of transgenic kiwifruit

Flavonoids take part in prohibiting cell aging and antagonizing ROS, especially when plants are stressed by salt, drought, pathogen, ultraviolet and other adverse factors (Glenn et al., 1999). They are important secondary metabolites and nutrition components of kiwifruit. In the present work, antioxidative capacity of *AtNHX1* transgenic kiwifruit was analyzed in terms of flavonoids contents. To estimate the ability of reducing ROS of the transgenic kiwifruit, the flavonoids contents in leaves, stems and roots of transgenic lines were measured after treatment with 200 mmol/l NaCl for three days. As shown in Fig. 6, in different tissues of kiwifruit, the flavonoids contents of leaf were the highest and stem contained more flavonoids than root in general. The contents of flavonoids in transgenic lines were significantly higher than that of the control, especially in

| NaCl concentration (mmol/L) | WT   | TL1   | TL2   |
|-----------------------------|------|------|------|
|                             | 0    | 150  | 0    | 150  | 0    | 150  |
| Height (cm)                 | 2.60±0.14 | 1.03±0.04  | 2.55±0.07  | 2.05±0.07  | 2.67±0.10  | 1.83±0.07 |
| Fresh weight (g)            | 2.06±0.02  | 0.64±0.03  | 2.12±0.05  | 1.64±0.02  | 1.97±0.04  | 1.44±0.02 |

Fig. 5. Proline (A) and MDA (B) contents in leaves of transgenic lines and wild-type kiwifruit stressed by NaCl. Error bars (n=3)±S.D. are shown.

Table 1

Effects of NaCl on the vegetative growth of transgenic lines and wild-type kiwifruit.
leaves of transgenic lines. The flavonoid contents in whole plants of transgenic lines TL1 and TL2 increased 70.6% and 40.0% respectively compared to the wild-type plants (Table 2). The contents of flavonoids in leaves of transgenic lines TL1 and TL2 increased 68% and 44% respectively compared to that of wild-type plants (Table 2). All these results coincided with the change of MDA content and confirmed that the transgenic lines had higher ROS elimination activity.

4. Discussion

Agrobacterium-mediated protocol is an effectual approach for transformation of plants, especially dicot, many scientifically and agronomically important species are routinely transformed using Agrobacterium. In this paper, we studied the factors affecting the transformation of Actinidia deliciosa mediated by Agrobacterium. We found that Actinidia deliciosa showed high sensitivity to Agrobacterium without the facilitation of AS, and the young stem was much more suitable explant in comparison with young leaf, due to its better endurance to Agrobacterium, although they had a difference in regeneration capability. The damage of Agrobacterium to leaf was alleviated by lowering the density of Agrobacterium (OD600 = 0.2) and shortening the infection time (2 min). This phenomenon was also observed in Agrobacterium-mediated transformation of rice (Ozawa, 2009). Coculture of explants with Agrobacterium in the presence of AS, a vir gene inducer, has become a routine exercise in the transformation of recalcitrant crops such as rice, maize, barley and wheat (Bartels and Sunkar, 2005). However, influence of AS is trivial on promotion of Actinidia deliciosa transformation. The results showed that AtNHX1-positive transgenic plants could be regenerated effectively from stem co-cultured for 2 days with Agrobacterium (OD600 = 0.2) and after infection for 15 min.

High-salt stress is one of the major adverse environmental conditions that affect plant growth, development and crop yield. To avoid salt damage, plants have evolved different mechanisms to limit Na⁺ uptake or compartmentalize Na⁺ into vacuoles, and Na⁺/H⁺ antiporters play a key role in the maintenance of osmotic balance (Bartels and Sunkar, 2005). Recently, great progress of improving plant salt tolerance has been made through adopting the strategies of reestablishing ion homeostasis, such as manipulating plant vacuolar Na⁺/H⁺ antiporter to produce transgenic plants. Overexpression of plant vacuolar Na⁺/H⁺ antiporter gene, AtNHX1 from Arabidopsis, in several dicotyledonous and monocotyledonous species, including tomato, Brassica, rice, wheat, and other crops, enable transgenic plants to grow in high concentration of salt,
demonstrating the feasibility of producing salt-tolerant crop plants via introducing plant vacuolar Na⁺/H⁺ antiporter into aimed plants (Yamaguchi and Blumwald, 2005). We obtained similar results in kiwifruit, further verifying the importance of AtNHX1 in salt tolerance.

Intracellular high concentration K⁺ and low concentration Na⁺ are important for the activities of many cytosolic enzymes and for maintaining membrane potential and an appropriate osmoticum for cell volume regulation. Under salt stress, the rate of transporter can be affected by excessive Na⁺ in cytosol through its competition for K⁺ binding sites of K⁺ transporters such as AKT1 (Zhu, 2003). In this study, it was also found that Na⁺ and K⁺ accumulation in leaves of the transgenic kiwifruit were higher than that of wild-type plants under severe saline conditions (200 mmol/l NaCl), and maintained a higher K⁺/Na⁺ ratio. The transgenic plant cells obtained the enhanced ability to efficiently sequester excessive Na⁺ into vacuole and decrease the Na⁺ concentration in cytosol. It thus not only averts ion toxic effect on cytosolic enzymes and plasma membrane but also maintains higher K⁺ concentration through alleviating the inhibition of K⁺ uptake. These results indicated that AtNHX1 overexpression increased the plant salt tolerance by elevating Na⁺ compartmentalization and keeping the K⁺/Na⁺ balance mainly in the leaves.

A remarkable improvement in biomass production at the vegetative growth stage was observed between the transgenic lines and wild-type kiwifruit under salt stress. In the present work, the fresh shoot weight of wild-type plants was 31% of the non-saline condition with the treatment of 150 mmol/l NaCl for 20 days, in comparison with that of the transgenic line TL1, 77% of the non-saline control, which was two times higher than the wild-type plants. The growth reduction of kiwifruit in saline conditions is mainly contributed to the Na⁺-specific toxicity and nutrient imbalance. Improved growth of the transgenic plants might be the result of the transporter compartmentalizing Na⁺ into the vacuole and maintaining high level K⁺ in cytosol.

The concept of using physiological criteria to improve the growth and productivity in saline environment has been successfully assessed in breeding programs (Mansour et al., 2003). In the present work, several physiologic parameters of transgenic lines were analyzed. Under salt stress, the transgenic lines showed better osmotic adjustment, higher antioxidant capacity and lower membrane damage and more vigorous growth than wild-type plants. Apparently, no toxicity and extra detrimental metabolic burden were observed for the overexpression of AtNHX1 in kiwifruit using the constitutive CaMV 35S promoter. These findings demonstrate that AtNHX1 can be useful for practical applications with cultivated crops. However, further field tests at various sites are necessary for confirmation of the commercial viability of these AtNHX1 kiwifruit lines.

Acknowledgements

We thank Hongxia Zhang for providing pHZX1 plasmid and Yafu Zhou for help with showing flavonoids in tissue sections. This work was supported by the National Natural Science Foundation of China (Grant number: 30870194), the Research Project of Provincial Key Laboratory of Shaanxi (Grant number: 08JZ70), Development Project of Science and Technology Research of Shaanxi Province (the Program for Tackling Key Problems) (Grant number: 2010 K16-04-01) and the Postgraduate Innovation Research Project of Northwest University (09YZZ58, 09YSY38).

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