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ORIGINAL PAPER

Cloning and Characterization of *MxVHA-c*, a Vacuolar H^+-ATPase Subunit C Gene Related to Fe Efficiency from *Malus xiaojinensis*

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Abstract The vacuolar H^+-ATPase plays a crucial role in secondary transport and in plant response to environmental stress. In this study, a vacuolar H^+-ATPase (*MxVHA-c*) gene, consisting of an ORF of 498 base pairs and 165 amino acid residues, has been cloned from the iron-efficient genotype of *Malus xiaojinensis*. Subsequently, this gene has been targeted to the tonoplast using transient expression analysis. Quantitative real-time (qRT) PCR results reveal that the *MxVHA-c* gene is expressed in both roots and leaves of Fe-deficient plants; however, it is sensitive to iron stress in roots. This suggests that *MxVHA-c* expression in roots may mediate iron-dependent responses. *MxVHA-c* expression is up-regulated following exogenous treatment with abscisic acid (ABA) and down-regulated following treatment with $CaCl_2$. Overexpression of the *MxVHA-c* gene in yeast strains has revealed that *MxVHA-c* transiently alleviated cadmium toxicity via the Cd^{2+}/H^+ antiport protein. H^+-ATPase activity is slightly increased in yeast overexpressing the *MxVHA-c* gene compared to that in yeast transformed with an empty vector. In addition, this transgenic yeast strain can grow in a liquid medium containing 40 μM ferrozine. These findings may provide useful information in elucidating molecular mechanisms that mediate resistance to iron deficiency.

Keywords H^+-ATPase activity · Iron deficiency · *Malus xiaojinensis* · Vacuolar H^+-ATPase

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Abbreviations

qRT-PCR	Quantitative real-time polymerase chain reaction
VHA	Vacuolar H^+-ATPase
YPD	Yeast growth medium
SD	Selection medium
RFU	Relative fluorescence unit
eGFP	Enhanced green fluorescent protein

Introduction

Iron (Fe) is an important nutrient element and is involved in many physiological and biochemical processes. Iron deficiency causes chlorosis and affects the production of crops (Briat et al. 1995; Mori 1999; Marschner et al. 1986). Plants can absorb iron from soil and also regulate iron balance in cells to satisfy the need of iron element. The vacuole is a primary storage site that mediates the distribution and transportation of nutrient in the plants. Following environmental damage in plants, vacuolar H^+-ATPase (V-H^+-ATPase) pumps H^+ into the vacuole to establish an electrochemical potential gradient between the vacuole and cytoplasm, which may promote ion transportation. Furthermore, V-H^+-ATPase can maintain ion balance under stress (Schumacher and Krebs 2010).

Malus xiaojinensis is an iron-efficient plant species that has been selected from more than 40 plant species and ecotypes in the genus *Malus* for investigation in this study. Understanding the transport of nutrients in fruit trees, from roots to scions, is very important (Han et al. 1994a; Xu et al. 2011). Thus, cloning and analysis of V-H^+-ATPase genes from plants, especially from woody stock plants, may aid in providing insights into our understanding of balance of ions, such as that of iron, at the cellular level.

V-H^+-ATPase is a functional complex and multi-subunit enzyme consisting of two sub-complexes as follows: a membrane-external V_1 subunit with eight sub-complexes (A, B, C, D, E, F, G and H) and a membrane-integral V_0 subunit with six sub-complexes (a, c, c′, c″, d and e) (Xiao et al. 2008; Ratajczak 2000; Dettmer et al. 2010). V-H^+-ATPase genes have been cloned from a broad range of plant species: monocots, dicots, trees as well as fruits such as wheat (Zhao et al. 2009), *Arabidopsis* (Kluge et al. 1999), tobacco (Rouquie et al. 1998), cotton (Wan and Wilkins 1994) and Fuji (Yao et al. 2009). V-H^+-ATPase subunits exhibit tissue specificity. *VHA-c1* is expressed ubiquitously, and *VHA-c3* expression is limited to roots caps. *VHA-E2* is a pollen-specific gene (Hirata et al. 2003; Padmanaban et al. 2004; Gaxiola et al. 2007). Disruption of each V-H^+-ATPase subunit (except for *VPH1* or *STV1*) results in an identical phenotype that is characterized by the inability of yeast cells to grow at a pH higher than 7 and sensitivity to calcium concentrations in the medium (Aviezer-Hagai et al. 2000; Drory et al. 2004). In *Arabidopsis*, *VHA-c* is likely to be involved in transporting protons, and the rotation of a ring of six c subunits is necessary for driving proton transport.

V-H^+-ATPase is not only a simple proton-pump but also has other functions. The Na^+/H^+ antiport protein in the tonoplast transports Na^+ into the vacuole based on the gradient by V-H^+-ATPase genes under salt stress (Zhang et al. 2009). V-H^+-ATPase genes are involved in glucose signaling and in regulating the vacuolar pH value, which is essential for *Arabidopsis* root gravity perception (Fasano et al. 2001). *VHA-c* has a major role in transporting ions. In addition, it is involved in many stress responses such as salt stress, low temperature, heat and so on. The enhancement in the expression of V-H^+-ATPase subunits has been demonstrated under salt- and abscisic acid (ABA)-induced stress (Kasai et al. 1994). Exogenous hormones can also induce the expression of the *VHA-c* gene (Zhang et al. 2006; Chinnusamy et al. 2006). *VHA-c* and H^+-PPase are mainly used to transport protons across the tonoplast. *VHA-c* enhances the SOD and POD activities to improve the salt tolerance in transgenic tobacco (Xu et al. 2010).

Although V-H^+-ATPase genes have been cloned from many species, there is little research analyzing these genes in woody plants, especially in apple. So we cloned a vacuolar H^+-ATPase subunit c from *M. xiaojinensis* and analyzed its expression patterns in roots and leaves in response to iron deficiency, ABA and $CaCl_2$. The sub-cellular location of *MxVHA-c* protein has been detected by transient expression analysis. Furthermore, the vacuolar H^+-ATPase activity and the cell growth of yeast with overexpressed *MxVHA-c* gene under ion stresses were investigated. Therefore, the function of *MxVHA-c* gene in ion absorption and bivalent ion transportation was confirmed in yeast.

Materials and Methods

Plant Material and Growth Conditions

M. xiaojinensis seedlings were grown in a container with Murashige and Skoog (MS) medium containing 0.5 mg L^{-1} Indole-3-butytric acid (IBA). After 30 days, when white roots were approximately 10 cm, the roots were rinsed in distilled water three times. The seedlings were then transferred to 1/2 Hoagland and Hoagland nutrient solution for 15 days, respectively. The composition of Hoagland nutrient solution was: 40 μM Fe(III)–EDTA, 0.5 mM KNO_3, 0.5 mM $NH_4H_2PO_4$, 1 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4{\cdot}7H_2O$, 0.5 mM $CaCl_2$, 0.3 mM $Mg(NO_3)_2{\cdot}6H_2O$, 23 μM H_3BO_3, 0.4 μM $ZnSO_4{\cdot}7H_2O$, 0.15 μM $CuSO_4{\cdot}5H_2O$, 0.05 μM $H_2MoO_4{\cdot}H_2O$ and 3 μM $MnCl_2$. The pH was adjusted to 6.3 with 0.1 N KOH. And then, the solutions were changed to iron-insufficient (4 μM Fe–EDTA) (Han et al. 1994b), ABA (50 mg L^{-1}) and $CaCl_2$ (50 mg L^{-1}) solutions. Roots and mature leaves were harvested being cultivated plants in iron-insufficient solutions for 0 h, 12 h, 1 day, 3 days, 6 days and 9 days, respectively. The same samples were harvested after being exposed to ABA and $CaCl_2$ for 0, 12 and 24 h, respectively.

Cloning of *MxVHA-c* cDNAs

Total RNA was isolated from the roots and mature leaves using CTAB methods with some modifications (Gasic et al. 2004). Approximately 1 μg of RNA was digested by DNase (TaKaRa Biotechnology Co. Ltd., Dalian, China) and reverse-transcribed using an oligo-dT primer and reverse transcriptase (TaKaRa Biotechnology Co. Ltd., Dalian, China) in a total volume of 20 μL. The initial complementary DNAs (cDNAs) were used to clone *MxVHA-c* cDNAs. Based on the gene sequence of *VHA* and the genome of the Golden Delicious (http://genomics.research.iasma.it/) (Velasco et al. 2010), the primers were designed using primer 5.0. The primers were 5′-AAAGAATTCATGTCTT CTTCAACCTTC-3′ with *Eco*RI site and 5′-AAAGTC GACCCCTCAGCTCTTGACTGACC-3′ with *Sal*I site. Amplification of the cDNA clone was performed at 94 °C for 5 min, 29 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and the final extension was performed at 72 °C for 10 min. The amplified PCR products were purified and subcloned into the Peasy-T1 vector (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. Three clones were sequenced. Alignment of

sequences was performed with DNAMAN software. Potential transmembrane segments were identified using TMHMM-2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/).

Real-Time PCR

The cDNA reaction mixture was diluted with 20 μL distilled water for quantitative real-time (qRT) PCR, which was performed using the SYBR Premix Ex Taq (TaKaRa Biotechnology Co. Ltd., Dalian, China). Primers were designed by using Primer Premier 5 (PREMIER Biosoft International, Palo Alto, CA) to give an amplicon length of 100–150 bp. The primers were 5′-GGTAGTTATGGCGGGAGTGTGGGT-3′ and 5′-GGTAATAGGACTTAGCCAGCAGCCTTGGGGTT-3′. Gene specificity of primers was ensured using qRT-PCR and by analysis of the melting curves of the products. The *Malus domestica* 18S gene was used as a reference gene (GenBank: DQ341382), and the primers were 5′-ACACGGGGAGGTAGTGACAA-3′ and 5′-CCTCCAATGGATCCTCGTTA-3′. Each reaction was carried out in triplicate. The relative expression of

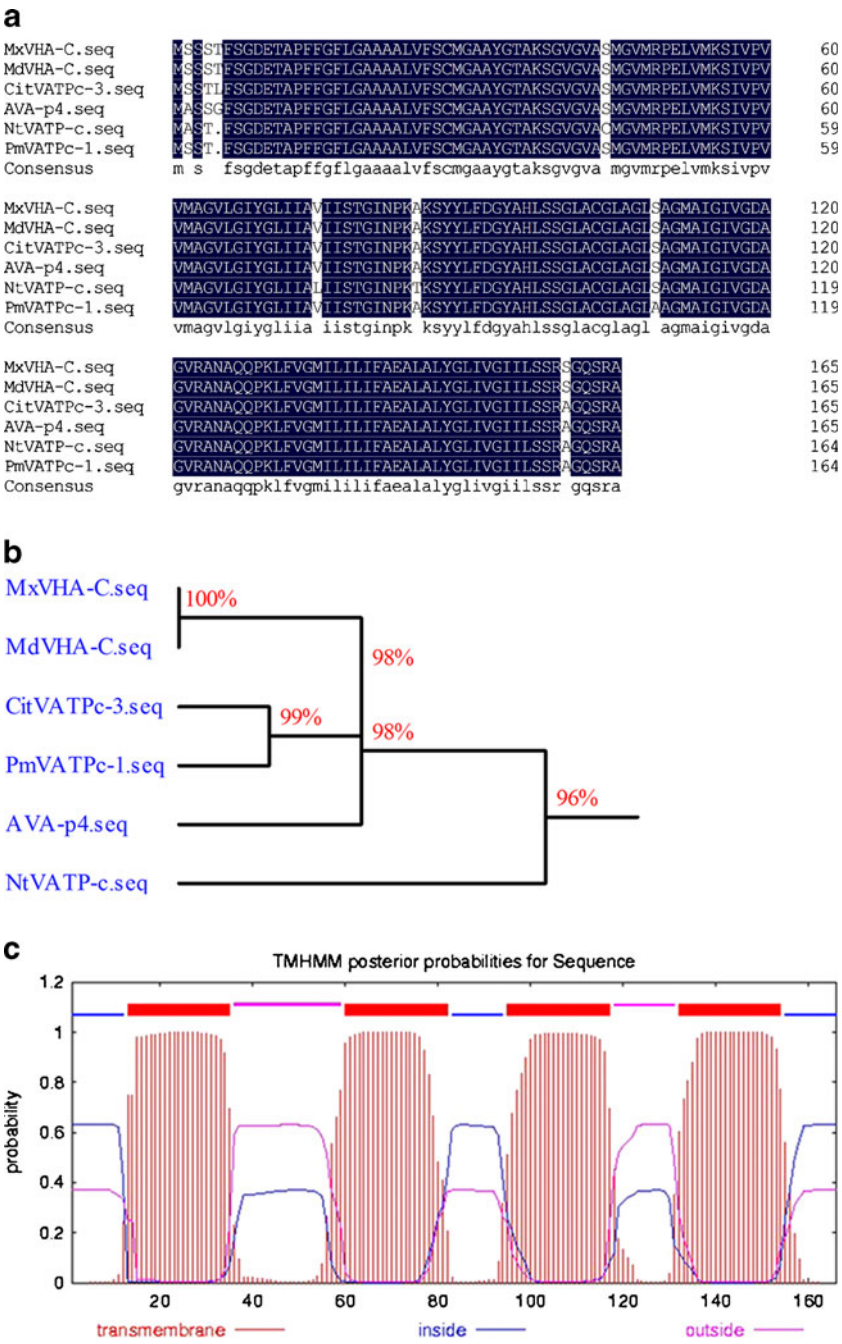

Fig. 1 Identification and sequence alignment of deduced amino acids of *MxVHA-c* with other related *VHA* genes. **a** Compared protein sequences are from *Arabidopsis thaliana* (*AVA-p4*), *Citrus unshiu* (*CitVATP-c*), *Nicotiana tabacum* (*NtVATP-c*), *Plantago major* (*PmVATPc-1*) and Golden Delicious (*MdVHA-c*). Identical domains are marked by *black color*. The different amino acids are labeled by *white color*. **b** Evolutionary tree analysis of *MxVHA-c* with *VHA* genes in others species. The identity is confirmed by percentage. **c** The transmembrane domains analysis of *MxVHA-c* protein by TMHMM-2.0

the target gene in the treatment was calculated using quantitative real-time PCR and the $2^{-\Delta\Delta C\ T}$ method (Livak and Schmittgen 2001; Qi et al. 2010; Tai et al. 2009).

Sub-cellular Localization of *MxVHA-c* Protein in Onion Epidermal Cells

The *MxVHA-c* gene and pEZS-NL plasmid (transient expression carrier) was digested using *Eco*RI and *Sal*I and ligated with T4 DNA ligase (TransGen Biotech, Beijing, China) to form the pEZS-NL+*MxVHA-c* plasmid. The recombinant plasmid was transformed into DH-5α for amplification. Recombined plasmid DNA was extracted. The recombinant plasmid DNA and *p*RTL2-*GFP* plasmid DNA (control) were integrated into onion epidermal cells using a particle bombardment device (Biolistic PDS-1000/He, Bio-Rad) according to the manufacturer's instructions. After 12 h, the fluorescence was observed using a florescence microscope (Nikon Eclipse TE2000-E).

Isolation of the Vacuolar Membrane Vesicles and Enzyme Activity Analysis

The pEZS-NL+*MxVHA-c* and pEZS-NL plasmid DNAs were digested with *Eco*RI and *Xba*I to obtain *MxVHA-c*+*eGFP* and *eGFP* fragments. These two fragments were ligated to the pYES2.0 plasmid respectively which contained Ura selection maker driven by GAL promoter. Therefore, the recombinant yeast expression vector pYES2.0+*MxVHA-c*+*eGFP* plasmid was created, and pYES2.0+*eGFP* plasmid was used as a control. The recombinant plasmids were transformed into the wild-type yeast strain BJ2168 with pYES2 vector kit instructions (Invitrogen). Yeast growth (YPD) medium and selection (SD-Ura$^-$) medium were prepared. A single colony from the SD-Ura$^-$ plate was inoculated into 2 mL of SD-Ura$^-$ liquid medium and incubated at 30 °C with shaking at 200 rpm for 24 h. Vacuolar membrane vesicles were isolated using the supercompetent cell membrane vesicle preparation kit (GENMED SCIENTIFICS INC., USA). The ability of H^+ transport was measured by green fluorescence indicating H^+ transportation using an aspartame assay kit (GENMED SCIENTIFICS INC., USA).

The Function of *MxVHA-c* in Fe-Deficient Yeast

The *MxVHA-c* overexpressing yeast strains and the empty vector-expressing yeast strains were cultured overnight in SD-Ura$^-$ liquid medium. When the OD value reached 1, 40 μM ferrozine (to induce Fe deficiency) was added to the medium. After 12, 24 and 48 h, the OD values were determined.

The Tolerance of Cd^{2+}

The yeast strain expressing pYES2.0+*MxVHA-c*+*eGFP* and the strain transformed with the empty vector were grown in 20 mL SD-Ura$^-$ liquid medium until the OD reached 0.1, respectively. The cells were cultured with 10 μM $CdCl_2$ at 30 °C with shaking at 200 rpm (Gao et al. 2011). The OD values were measured at 0, 24, 48, 72 and 96 h since $CdCl_2$ addition.

Results

Cloning and Sequence Analyses of the *MxVHA-c* Gene from *Malus xiaojinensis*

A complete open reading frame of 498 base pairs was cloned from *M. xiaojinensis* using PCR. The predicted protein encoded by *MxVHA-c* gene consisted of 165 amino acids with a predicted molecular weight of 16.6 kDa.

To investigate the homology and genetic relationship with other plants, the alignment of the deduced amino acid was performed. The *MxVHA-c* gene exhibited similarities with the *VHA* gene from *Arabidopsis thaliana* (GenBank ID: NP177693.1), *Citrus unshiu* (GenBank ID: BAA75516.1), *Nicotiana tabacum* (GenBank ID: CAA65063.1), *Plantago major* (GenBank ID: CAH58637.1) and Golden Delicious with the sequence identity up to 98 %. *VHA* gene families in different species may have similar structures. However, difference in a single amino acid may lead to species-specific distinctions (Fig. 1a, b). It was shown that this protein has four potential transmembrane domains (Fig. 1c, Table 1). Using the genome of domesticated apple as a comparison (Velasco et al. 2010), the results ascertained that *MxVHA-c* gene included three exons and two introns. This result was consistent with the structure of the *PgVHA-c1* gene (Tyagi et al. 2005).

Table 1 Structure analysis of *MxVHA-c* protein

Location	Amino acids
Inside	1–12
TM helix	13–35
Outside	36–59
TM helix	60–82
Inside	83–94
TM helix	95–117
Outside	118–131
TM helix	132–154
Inside	155–165

TM transmembrane

Expression Analysis of *MxVHA-c* Gene

The expression analysis of *MxVHA-c* gene during iron deficiency was investigated using qRT-PCR (Fig. 2). *MxVHA-c* expression was detected in roots and mature leaves. After iron deficiency for 12 h, *MxVHA-c* expression in roots increased by 4.0-fold. The transcript correlated with the length of iron deficiency. In leaves, the expression of *MxVHA-c* gene increased at the initial stages of iron deficiency, reached peak at 3 days and subsequently decreased. The transcript levels of the *MxVHA-c* gene were 1.2-fold and 1.8-fold up-regulated in leaves and roots under ABA stress. *MxVHA-c* expression was down-regulated in response to $CaCl_2$.

Sub-cellular Localization of the *MxVHA-c* Gene

The *MxVHA-c* gene encoded a membrane protein. The sub-cellular localization of *MxVHA-c* was visualized using a transiently expressed *eGFP* fusion protein. We detected *GFP*-tagged *MxVHA-c* protein around tonoplast on onion

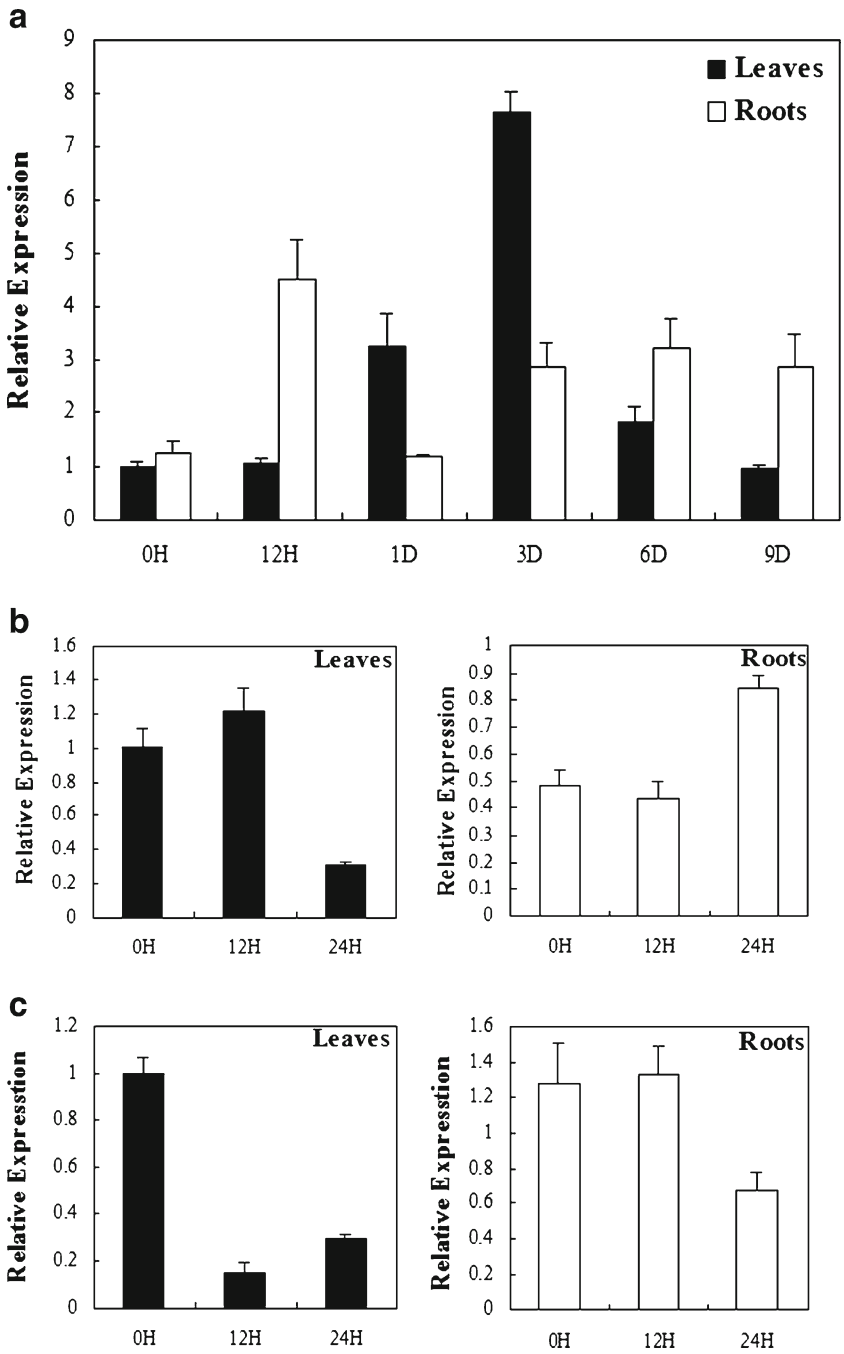

Fig. 2 Quantitative real-time PCR analysis of *MxVHA-c* expression in leaves and roots. With 18 s as control, the relative expression of this gene was calculated by $2^{-\Delta\Delta C\ T}$ method. Values are means of three biological replicates. Standard error is also labeled. **a** Iron deficiency stress. **b** 50 mg L^{-1} IAA. **c** 50 mg L^{-1} $CaCl_2$

epidermal cells (Fig. 3d), while the pRTL2-*GFP* was located around all the cells (Fig. 3b). There was no *GFP* fluorescence in other sites of cells.

Vacuolar H^+-ATPase Activity Analysis

H^+ transport ability reflected vacuolar H^+-ATPase activity. The relative fluorescence unit (RFU) in transgenic yeast was lower than that in yeast with pYES2.0+*eGFP* (Fig. 4). This result illustrates that *MxVHA-c* gene enhances H^+ transport from the cytoplasm into vacuole, indicating that the *MxVHA-c* gene encoded a vacuolar H^+-ATPase of *M. xiaojinensis*.

The Growth of Transgenic Yeast in the Iron-Deficient Medium

MxVHA-c overexpression yeast strains exhibited normal growth in iron-deficient medium. The OD value of transgenic yeast was more than 1.5 times (1.6±0.02) greater than that of the control (1.096±0.002) at 12 h. After 12 h, the differences between the two values were less dramatic. These results indicate that the kinetics of *MxVHA-c* mediating Fe^{2+} transport is time-dependent with a peak at 12 h (Fig. 5).

Cd^{2+} Tolerance in the Transgenic Yeast

To determine whether the transgenic yeast can enhance the tolerance of heavy metals, we measured the cell growth value. During cultivation, 10 μM $CdCl_2$ inhibited the growth of wild-type yeast that was transformed with the empty vector compared to that of yeast transformed with the *MxVHA-c* gene. The OD value of yeast with empty vector decreased, whereas the OD value of yeast transformed with *MxVHA-c* gene increased within 24 h. After 24 h, the growth decreased (Fig. 6). This observation suggested that cadmium toxicity was transiently alleviated in the transgenic yeast strain. However, transgenic yeast cannot maintain normal growth after 2 days.

Discussion

Plants must make appropriate physiological and biochemical changes in order to deal with environmental stresses (Fu et al. 2010). The H^+-ATPase gene plays a crucial role in these processes. In this study, we reported a V-H^+-ATPase subunit c gene consisting of 498 base pairs fragment in the *M. xiaojinensis*. This gene has a high homology with VHA genes in other species. The proton-pumping activity may be regulated by complex formation with the V_0 subcomponent, which is located within the membrane. One of V-H^+-ATPase's functions in plants is to create the electrochemical gradient across the tonoplast, which maintains a stable environment despite stress. Together with other V-H^+-ATPase genes, *VHA* genes may influence the pH homeostasis in the cytoplasm (Nishi and Forgac 2002). The V-H^+-ATPase subunit c is essential for transporting protons and regulating V-H^+-ATPase activity (Sze et al. 2002). The vacuole is an important storage organ for nutritional elements, including Fe. The *MxVHA-c* gene expression was up-regulated in Fe-deficient plant roots and leaves. The yeast strain transformed with the *MxVHA-c* gene displayed an improved survival rate under 40 μM ferrozine, relative to the strain transformed with the empty vector. This result indicates that *MxVHA-c* regulates ion transport and

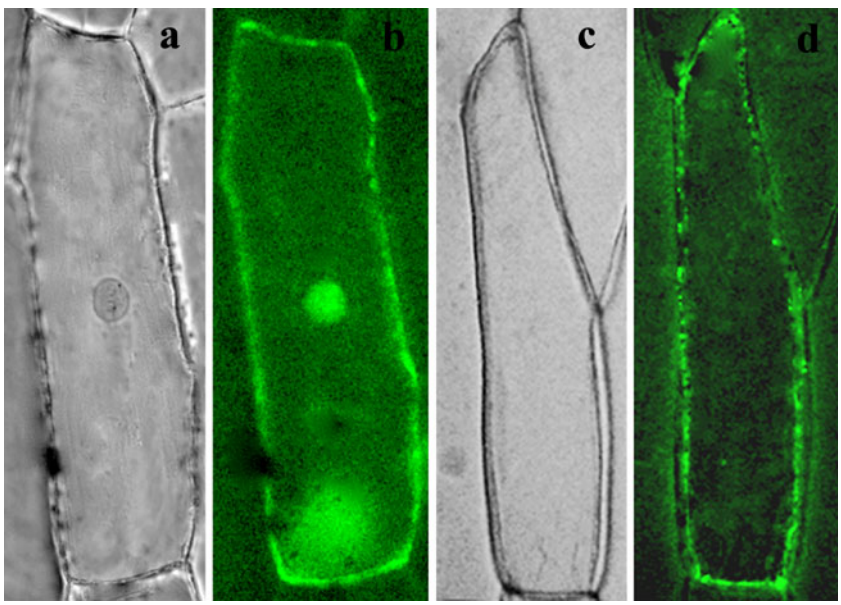

Fig. 3 Sub-cellular localization of *MxVHA-c* protein in onion epidermal cells using *eGFP* by common florescence microscope. Cells were observed after 12 h of infection. Images **a** and **c** are pictured in the dark field, while **b** and **d** are bright field images. Panels **a** and **b** represent fusion protein (*GFP*) in the pRTL2-*GFP*, while **c** and **d** represent *MxVHA-c* fused to *GFP* in the pEZS-NL

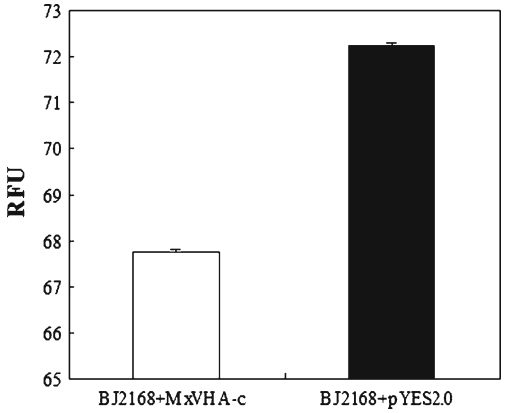

Fig. 4 Vacuolar H^+-ATPase activity analysis in transformed yeast strains. Vacuolar membrane vesicles were isolated from wild-type yeast (BJ2168) transformed with *MxVHA-c*+pYES2.0+*eGFP*, as control, transformed with pYES2.0+*eGFP*

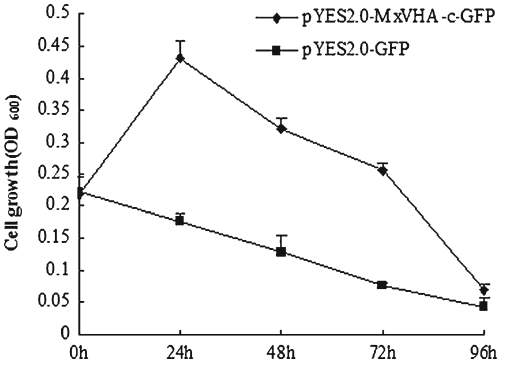

Fig. 6 Yeast strains which overexpressed *MxVHA-c* gene can transiently alleviate cadmium toxicity. Pre-cultured cells were cultured in the SD-Ura^- medium with 10 μM $CdCl_2$ at 30 °C with shaking at 200 rpm which started from an OD of 0.1. After 24, 48, 72 and 96 h of culture, cell growth can be got by OD_{600}. Three replicates can be done every time. The average value and standard error are also printed

balance in transformed cells. Iron balance in the cells requires the coordination of many genes. *AtNRAMP3* and *AtNRAMP4*, which encode proteins located on the surface of the tonoplast, are expressed earlier than *IRT1* (iron-transport protein 1). These proteins transport Fe^{2+} to the cytoplasm to meet the demands of plants (Lanquar et al. 2005). The *VIT1* (vacuolar membrane transport) gene regulates iron balance between vacuole and cytoplasm, and vit1 mutants cannot grow in alkaline soil (Kim et al. 2006). In our study, *MxVHA-c* gene was expressed in Fe-deficient plant roots and leaves. In addition, *MxVHA-c* mediates H^+ balance in the vacuole. From above analysis, we can deduce that *MxVHA-c* gene may also interact with other genes to regulate iron homeostasis in *M. xiaojinensis*.

In Fe-deficient plants, roots initially induce signals in response to Fe deficiency. Our results are consistent with this finding. QRT-PCR indicates that *MxVHA-c* gene is expressed in roots and leaves. *MxVHA-c* expression peaks at 12 h in the roots. The increasing of H^+ in the vacuole causes acidification, enhancing Fe^{2+} removal. The signals that are involved in this process have not been well characterized. Iron content in the surrounding soil of the roots induces changes in local signals in the plants (Curie and Briat 2003; Yang et al. 2010). V-H^+-ATPase activity and endogenous ABA content increase under salt stress. In addition to this, exogenous ABA enhances V-H^+-ATPase activity (Zhao et al. 2009). The transcriptional levels will be up-regulated under ABA and $CaCl_2$ treatments (Tyagi et al. 2005). Exogenous ABA enhanced the expression of *MxVHA-c* gene. Ca^{2+} signaling has different effects on V-H^+-ATPase genes in different plants (Tyagi et al. 2005). $CaCl_2$ suppressed *MxVHA-c* expression in *M. xiaojinensis*. Because Ca^{2+} and ABA may influence *MxVHA-c* gene expression, the properties of *MxVHA-c* gene in signal pathway require further investigation.

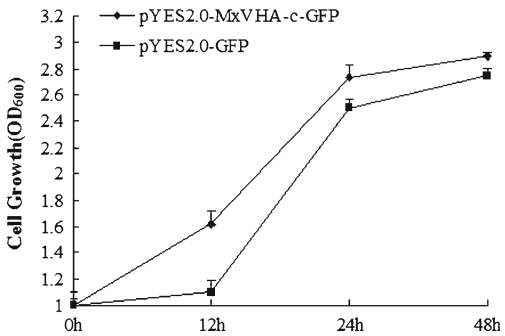

Fig. 5 Yeast strains which overexpressed *MxVHA-c* gene can grow in the iron-deficient medium. Cells were cultured overnight in the SD-Ura^- medium at 30 °C with shaking at 200 rpm. Until the OD value was up to 1, then 40 μM ferrozine was added in the medium; after that the OD value was determined after 12, 24 and 48 h. Three replicates can be done every time. The average value and standard error are also printed

Yeast is a useful model organism that has been extensively studied, and it has a mechanism of iron absorption that is similar to that of plants (Zhang et al. 2009). Yeast stores many ions in the vacuole. Overexpressing of *MxVHA-c* gene in yeast induces elevation of V-H^+-ATPase activity compared to yeast transformed with an empty vector. Yeast transformed with *MxVHA-c* gene reveals lower levels of RFU. This result indicates that H^+ transportation from the cytoplasm into vacuole influences ion distribution between cytoplasm and vacuole. This phenomenon is in accordance with previous studies. Transferability of Cd^{2+} in plants is regulated by pH. In oat roots, Cd^{2+} transportation from cytoplasm to vacuole across the tonoplast is demonstrated through Cd^{2+}/H^+ antiport protein (Prasad 1995). And also the absorption rate of Cd^{2+} in the Fe-deficient plant roots is seven times greater than that in the Fe-sufficient plant roots (Cohen et al. 1998; Kabała et al. 2008). The increasing of Cd^{2+} absorption may cause metal ion poisoning. The *ThVHA-c1* can greatly improve $CdCl_2$ tolerance in transgenic yeast (Gao et al. 2011). The transgenic yeast had a relatively high

survival rate under 10 μM $CdCl_2$. This phenomenon illustrated that overexpression of *MxVHA-c* gene inhibited cadmium toxicity. Therefore, we hypothesize that Cd^{2+}/H^+ antiport protein is present in the tonoplast to transport excess Cd^{2+} from cytoplasm into vacuole in *M. xiaojinensis*. However, the transporting activity of the Cd^{2+}/H^+ antiport protein is limited. Similar to the cellular response to salt stress, this process may also be regulated by SOS signals (Zhao et al. 2009).

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