

Abiotic Stress Resistance Analysis of *Lilium pumilum* Overexpressing the LpMT2 Gene

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Abstract

Plant metallothioneins (MT) are cysteine-rich proteins present in plants that can improve a plant's salt tolerance. Therefore, a greater understanding of the MT gene in lily (*Lilium pumilum*), *Liliaceae*, is an important factor in the development and cultivation of improved salt-tolerant varieties and enriching plant resources for saline soils. A type 2 MT gene (GenBank access number: MH319787, designated as LpMT2) was isolated from *L. pumilum* leaves. The response mechanism to stress was then investigated, which provided the basis for molecular breeding of *L. pumilum* for stress tolerance. The LpMT2 gene amino acid sequence is highly homologous to that of type 2 MT protein. Quantitative real-time PCR (qPCR) determined that different plant tissues expressed the LpMT2 gene differently and these expressions were dependent on the specific stress. Transgenic plants with LpMT2 gene exhibited significantly increased resistance to salt and oxidative stress compared with untransgenic plants. The LpMT2 transgenic plants had better growth, greater chlorophyll and proline content, less malondialdehyde (MDA) content and cell membrane permeability, greater superoxide dismutase (SOD) activity, less Na⁺ content, greater K⁺ content and Na⁺ efflux, and less K⁺ efflux. These results determined that the transformed LpMT2 gene in *L. pumilum* plays an important role in enhancing the plant's salt tolerance and antioxidant capacity.

Keywords: *Lilium pumilum*, lily, oxidant stress, metallothionein, salinity, salt tolerance

1. Introduction

1.1 Northeast China Salinity and Lily (*Lilium pumilum*)

Northeast China is one of the most severe saline areas in China and one of the three largest saline soils in the world with an area of 3.84 km² (Yao et al., 2006), accounting for 3.1% of the total area of the northeast China region (Y. H. Wang & S. X. Wang, 1994). Only a few salt tolerant plants can survive in this saline area (Jin et al., 2017). *L. pumilum* can grow well in saline soils, but there is limited research on its salt tolerant genes. The *Lilium* genus, in the family *Liliaceae*, was established by Linnaeus in 1753 (Takhtajian, 1986), and there are about 115 species identified worldwide (Fu, 2002). *L. pumilum* is a perennial herb of *Lilium* and distributed widely in north China. *L. pumilum* research mainly focuses on plant resources (Wu et al., 2006), hybrid breeding (Yang, 2016), the flowering biological characteristics (Fukai & Goi, 2001), tissue culture (Chen et al., 2013), and reproduction (Chojnowski, 1996). It is also reported that *Lilium* has resistance to drought and salt stress (Yue, 2012). However, research on the salt tolerance and cloning salt-tolerant genes in *Lilium* is limited. In Phase #1 of this research the objective was to screen a salt-tolerant Metallothionein (MT) gene from *L. pumilum* by qPCR under salt stress and investigate the gene function.

1.2 Metallothioneins

Metallothioneins (MTs) are low molecular weight proteins which are rich in cysteine and exists extensively in organisms (Leszczyszyn et al., 2003). Plant MT was first discovered in soybean (*Glycine max*) roots (Quan et al., 2006). MTs have many functions, including scavenging reactive oxygen species (ROS) (Akashi et al., 2004), reducing heavy metal ion toxicity (Ferraz et al., 2002), participating in metal ion transport (Fujimoto et al., 2013), remediating contaminated environments (Chen & Tang, 2013), stabilizing a plant's internal environment (Chang & Zhu, 2002), and participating in gene regulation (Robinson et al., 1993). MT gene molecular structures, physiological functions and evolutionary relationship were analyzed using the bioinformatics methods for four mushrooms (Qiang et al., 2012).

The MT gene is also related to a plant's resistance to salt and oxidative stress. Transgenic rice plants overexpressing *OsMT1a* demonstrated an enhanced tolerance to drought (Zhao et al., 2009). The *OsMT1e-P* transgenic tobacco plants improved the salt stress tolerance compared to the untransgenic tobacco plants (Kumar et al., 2012). The transgenic *Arabidopsis* plants overexpressing *cgMT1* from beefwood (*Casuarina glauca*) reduced the accumulation of H_2O_2 (Obertello et al., 2007). Transgenic tobacco (*Nicotiana tabacum*) plants overexpressing *GhMT3a* showed increased tolerance against abiotic stresses (salt, drought, and low-temperature stresses) compared to wild-type plants (Xue et al., 2009).

In addition, plant MT2 can directly eliminate reactive oxygen species (ROS), such as O_2^- and H_2O_2 (Hassinen et al., 2011). MT protein is thought to be involved in oxidative stress protection (Ahmad et al., 2016). MTs have been found to be responsible for oxidative stress and ROS scavenging (Naoki et al., 2006; Zhang et al., 2019). Salt stress induced ROS generation and ROS scavenging plays an important role in plant stress resistance. We hypothesized that the MT gene molecular pathway improved plant tolerance by inhibiting ROS production and reducing oxidative damage to plant cells.

MT gene expression can be induced in plants, and the impact of metal ions on the MT gene expression in plants is most often studied. Understanding the impact of the MT gene in *Lilium pumilum* (*L. pumilum*) will make a significance impact in the development of excellent salt-tolerant varieties and enriching salt-tolerant plant resources. In Phase #2 of this research the objective was to clone a type 2 MT gene from the *L. pumilum* leaf under salt stress and determine if the gene will improve the plant's salt tolerance.

2. Methods and Materials

2.1 Phase #1: Cloning of Full-Length LpMT2 and the Analysis of Relative Expression

The complete transcription fragment was cloned from cDNA of *L. pumilum* by using sequence-specific primers. The sequence contains an open reading frame of 234 bp and encodes 77 amino acids. The result of BLAST search showed that amino acid sequence of LpMT2 had the highest homology of amino acid sequence with DcMT2 in *Dendrobium catenatum* (GenBank accession number: XP_020679854.1, similar degree 69%), the next were AcMT2 in *Actinidia chinensis* var. *chinensis* (GenBank number: PSR98280.1, similar degree 67%), PeMT2 in *Phalaenopsis equestris* (GenBank number: XP_020571140.1, similar degree 65%), PtMT2 in *Populus trichocarpa* (GenBank number: XP_002299873.1, similar degree 64%); GmMT2 in *Glycine max* (GenBank number: NP_001235506.1, similar degree 61%), AtMT2a in *Arabidopsis thaliana* (GenBank number: P25860.2, similar degree 55%), (Figure 1). Therefore, this protein was designated as LpMT2.

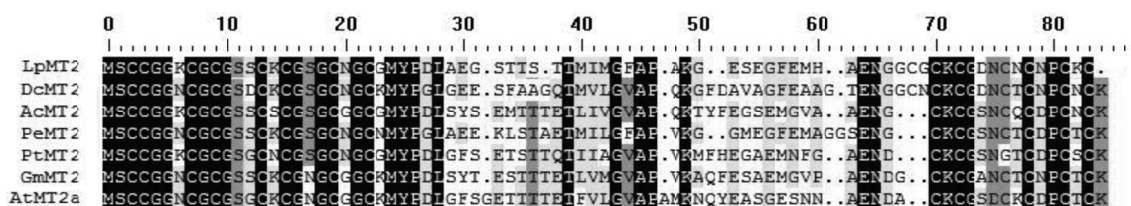


Figure 1. Comparison of the LpMT2 amino acid sequences with homologs from other species

GenBank accession numbers of the aligned sequences are as follows: *Dendrobium catenatum* DcMT2 (XP_020679854.1), *Actinidia chinensis* var. *chinensis* AcMT2 (PSR98280.1), *Phalaenopsis equestris* PeMT2 (XP_020571140.1), *Populus trichocarpa* PtMT2 (XP_002299873.1), *Glycine max* GmMT2 (NP_001235506.1), *Arabidopsis thaliana* AtMT2a (P25860.2).

2.2 Phase #2: Cloning of Full-Length *LpMT2* from *L. pumilum*

L. pumilum plants were grown on MS basal medium (Murashige & Skoog, 1962) in an illuminated incubator at 25°C under 2000 Lux irradiation with a 16 h light/8 h dark photoperiod. The total ribonucleic acid (RNA) was extracted from 8-week old leaves by RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). First-strand cDNA was synthesized by reverse transcribing 500 ng of total RNA with PrimeScript™ RT reagent Kit (TaKaRa Bio, Japan).

A transcript fragment was amplified by PCR from the cDNA with the forward primer (5'-ATGTCTTGCTGTGGTGGAAA-3') and reverse primer (5'-TTAGCACTTGCATGGGTTG-3') based on the transcriptome contract sequencing results of *L. pumilum*. Primers were designed using Primer Premier 5.0 software (Premier Biosoft, Canada). The PCR product was ligated into plasmid pMD18-T vector (Takara Bio, Japan) and sequenced. The sequences were identified using DNAMAN 6.0 software (Lynnon Biosoft, USA). The new gene was designated as *LpMT2*.

2.3 The qPCR Analyses of *LpMT2* Expression in Different Organs of *L. pumilum* and Under Different Stress

RNAs were extracted from the roots (three years old), bulbs (three years old), young leaves (eight week old), mature leaves (three years old), flowers (three years old) and seeds (harvested from three-year old plants) of *L. pumilum* using RNeasy Plant Mini Kit. First-strand cDNA was synthesized by reverse transcribing 500 ng of total RNA with using PrimeScript™ RT reagent Kit. The primers for qPCR were the same as primers for gene cloning primers. Subsequent qPCR analyses were conducted using SYBR green (TaKaRa Bio, Japan). Each amplification in a 96-well plate was performed in a 20 µL final volume containing 2.0 µL of 2× diluted cDNA template; 0.5 µL of each specific primer pair at 10 µM; 10 µL of 2× SYBR Premix Ex Taq and 8.0 µL of distilled deionized H₂O (dd H₂O). The expression level of the lily Actin (Gene Bank Accession Number: JX826390) gene was used as a reference. All analyses were conducted under the following conditions: denaturation for 10 min at 95 °C, 40 cycles of 30 s at 95 °C, 30 s at 55 °C and 72 °C at 1 min, using an IQ5 real-time PCR instrument (Bio-Rad, Hercules, CA, USA). Fluorescent detection was performed and the results of qPCR were analyzed by MxPro software (Agilent, USA).

Eight-week old untransgenic *L. pumilum* seedlings were transferred to fresh MS medium at 25 °C under 2000 Lux irradiation with a 16 h light/8 h dark photoperiod, supplemented with 250 mM NaCl, 20 mM NaHCO₃ and 11 mM H₂O₂. The leaves were harvested after 0, 6, 12, 24, 36, and 48 h. The *LpMT2* gene expression level in *L. pumilum* seedlings under different stresses were analyzed by qRT-PCR.

2.4 Construction of Plant Expression Vectors and *L. pumilum* Transformation

The coding region of the *LpMT2* gene was amplified from pMD18-T-*LpMT2* with the BamHI forward primer 5'-GGATCCATGTCTTGCTGTGGTGGAAA-3' and XhoI reverse primer 5'-CTCGAGTTAGCACTTGCATGGTTG-3'. The PCR product was ligated into plasmid pMD18-T vector. The plasmids were digested with BamHI and XhoI and then ligated into the BamHI and XhoI sites of pBI121 binary vector plasmids (We altered the vector). The plasmid DNA of pBI121-*LpMT2* was transformed into the *L. pumilum* bulbs by *Agrobacterium tumefaciens* (strain EHA105 Takara, Tokyo, Japan) mediated transformation (Ishida et al., 1996; Zhao et al., 2002). The bulbs were germinated on MS + 0.5 mg·mL⁻¹ BA + 0.05 mg·mL⁻¹ NAA plates containing 50 mg·L⁻¹ kanamycin (kana) to select kana-resistance plants, and the kana-resistance plants were transferred to fresh MS medium at 25 °C under 2000 Lux irradiation with a 16 h light/8 h dark photoperiod. Finally transgenic plants were identified using Northern blot (Jin et al., 2017). RNAs were extracted from eight-week old transgenic *L. pumilum* seedlings.

2.5 Stress Tolerance Analysis of the *L. pumilum*

Eight-week old *L. pumilum* untransgenic and transgenic seedlings at the same growth stage were selected for fresh MS medium + 200 mM, 250 mM, 300 mM NaCl, 9 mM, 11 mM, 13 mM H₂O₂, 20 mM, 50 mM, 80 mM NaHCO₃ stress treatment at 25 °C under 2000 Lux irradiation with a 16 h light/8 h dark photoperiod for 48 h to observe the extent of leaf injury. The leaf centers were determined by SPAD-502 Plus Chlorophyll Meter Model (Konica Minolta, Japan). The relative value of chlorophyll content in each leaf was measured. The free proline (Pro) content was determined by the ninhydrin method (Ma et al., 2007). Malondialdehyde (MDA) content was determined by the thiobarbituric acid method (Hodges et al., 1999). The degree of cell membrane damage was determined by conductance (Liu et al., 2008). Superoxide dismutase (SOD) was determined by its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) (Giannopolitis & Ries, 1977).

Eight-week old untransgenic and transgenic *L. pumilum* seedlings were treated without (control) or with each of following solutions: 20 mM NaHCO₃ and 250 mM NaCl, respectively for 48 h. Na⁺ and K⁺ ion content of leaves were measured by an atomic absorption spectrophotometer (AA800, Perkin Elmer, USA) (Barragan et al., 2012). Net flux of the roots' K⁺ and Na⁺ were measured using Noninvasive Micro-test Technology (Zhao et al., 2017).

The data were analyzed using a one-way analysis of variance using SPSS 20.0 (IBM, USA) with Duncan's one-way analysis of variance. The figures were drawn using SigmaPlot 12.5 (Systat Software Inc., USA).

3. Results

3.1 *LpMT2* Gene Expression in *L. pumilum*

LpMT2 gene had the greatest expression in flowers, followed by young leaves, seeds, mature leaves, bulbs and roots (Figure 2a). It was confirmed that the expression of *LpMT2* gene was greater in the aboveground plant parts (flowers, leaves, and seeds). Under 250 mM NaCl stress, the *LpMT2* expression reached the greatest level at 36 h, about 18 times greater than the gene expression in the control (Figure 2b). The *LpMT2* expression for 250 mM NaCl tended to stabilize from 12 to 24 h and then dramatically increased at 36 h. When exposed to 20 mM NaHCO₃ stress, the gene expression was relatively low (less than 2) until 48 h, when it was about 14 times greater than the control (Figure 2c). The *LpMT2* expression due to 11 mM H₂O₂ stress increased gradually with treatment time, reaching the greatest level at 48 h, which was about 4.5 times greater than the control (Figure 2d).

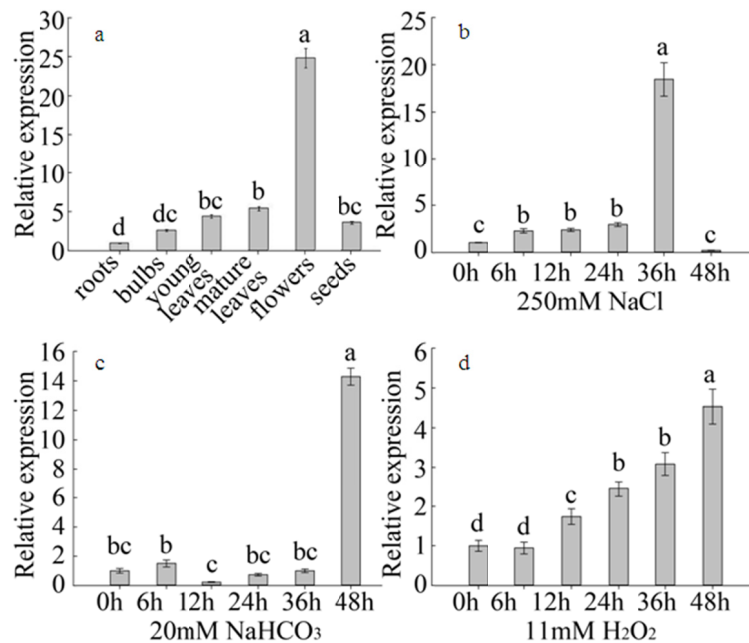


Figure 2. Relative expression of *LpMT2*. (a) Relative expression of *LpMT2* in different organs of *L. pumilum*. (b) Relative expression of *LpMT2* gene under NaCl treatment. (c) Relative expression of *LpMT2* gene under NaHCO₃ treatment. (d) Relative expression of *LpMT2* gene under H₂O₂ treatment

3.2 Identification of Transgenic *L. pumilum* Lines Using Northern Blot

Three transgenic *L. pumilum* lines (#1, #2 and #3) were selected for Northern blot analysis. The overexpression of the *LpMT2* gene in transgenic *L. pumilum* was due to the CaMV35S promoter. The transgenic lines had a greater *LpMT2* gene expression than the untransgenic plants due to the stronger hybridization signals (Figure 3).

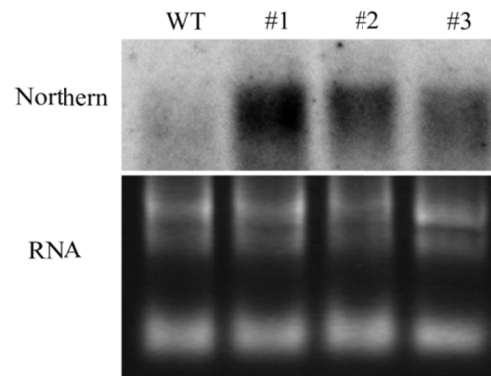


Figure 3. Northern blot analysis of *LpMT2* gene expression in transgenic plants

Untransgenic plant genotype was negative control (CK), and transgenic plants were #1, #2, #3. Total RNA (lower panel of the picture) was isolated from leaves and hybridized with DIG-labelled cDNA probe for *LpMT2*. 5 μ g RNA was used into each lane. Full-length blot and gel are presented in Supplementary Figure 1.

3.3 Analysis of Plant Phenotype, Physiological Index, Icon Content, and Flux Under Various Stress Treatments

After 48 h, under different stress concentrations, the leaves showed injury signs at low concentrations with increasing injury as the stress levels increased. Phenotype observation determined that the transgenic lines were more stress tolerant than the untransgenic plants (Figure 4). As the stress concentrations increased, the plant chlorophyll content gradually decreased. The chlorophyll content of the untransgenic plants was generally less than the transgenic plants under the same stress conditions. These results indicate that the *LpMT2* gene enhanced the plant's photosynthesis to varying levels (Figure 5a). The proline content in the plants increased significantly after stress treatments and the increase was greater for the transgenic plants. It was determined that transgenic plants could produce more proline under stress, and, therefore, had a greater resistance to stress (Figure 5b). With the increase of stress concentration, the content of MDA increased first and then decreased for both the transgenic and untransgenic plants (Figure 5c). When under stress, the increase MDA content was greater for the untransgenic plants than the transgenic plants. The MDA content in untransgenic plants was 140%, 48%, and 26% greater than the transgenic plants under 250 mM NaCl, 80 mM NaHCO₃, and 11 mM H₂O₂, respectively.

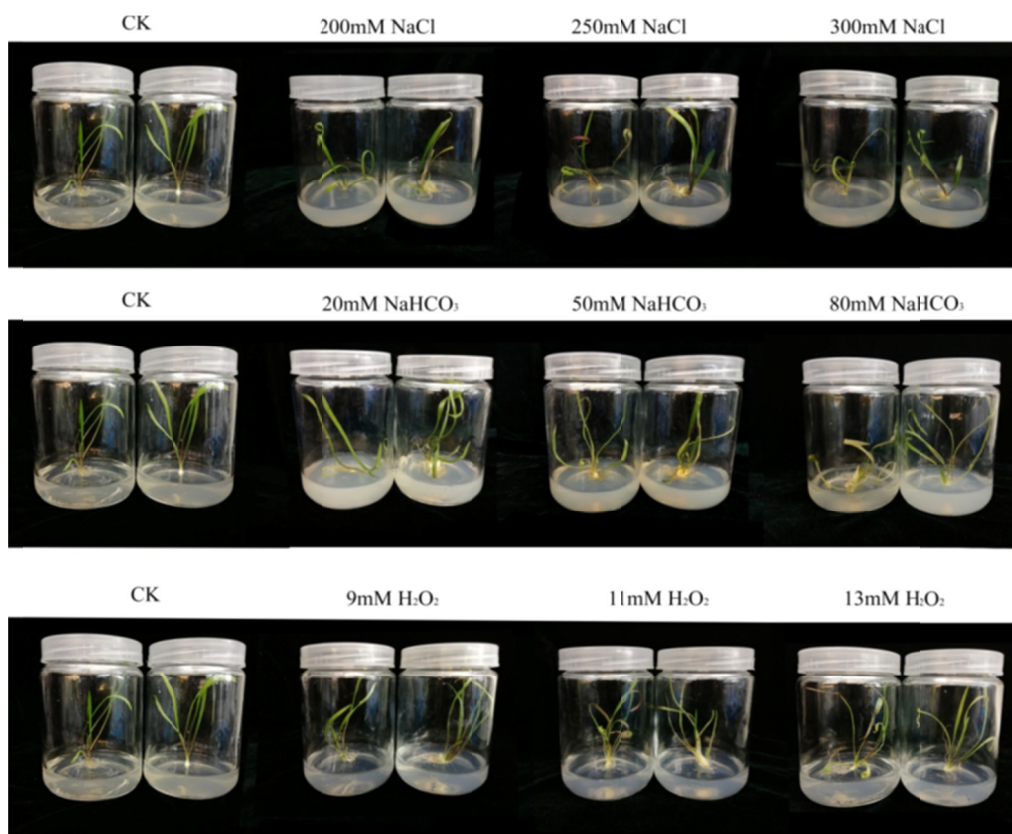


Figure 4. Phenotype of untransgenic and transgenic plants under different stress

Eight-week old *L. pumilum* untransgenic and transgenic seedlings at the same growth stage were selected for fresh MS medium + 200 mM, 250 mM, 300 mM NaCl, 9 mM, 11 mM, 13 mM H₂O₂, 20 mM, 50 mM, 80 mM NaHCO₃ stress treatment at 25 °C under 2000 Lux irradiation with a 16 h light/8 h dark photoperiod for 48 h. The plant on the left is the untransgenic plant and the transgenic one is on the right.

The change in electrical conductivity (EC) may reflect the degree of plasma membrane damage and the strength of the plant's resistance to stress. The change in EC of transgenic plants was less than the untransgenic plants when under stress. The plant's EC increased as the stress concentration increased, indicating that the degree of cell membrane damage is more serious in untransgenic plants (Figure 5d).

The SOD activity for untransgenic plants was significantly less than that the transgenic lines under stress. The SOD activity first increased and then decreased with increasing stress (Figure 5e). Under 250 mM NaCl, 50mM NaHCO₃, and 11 mM H₂O₂ stress treatments, the SOD activity in transgenic plants was 60%, 10% and 60% greater than the untransgenic plants, respectively.

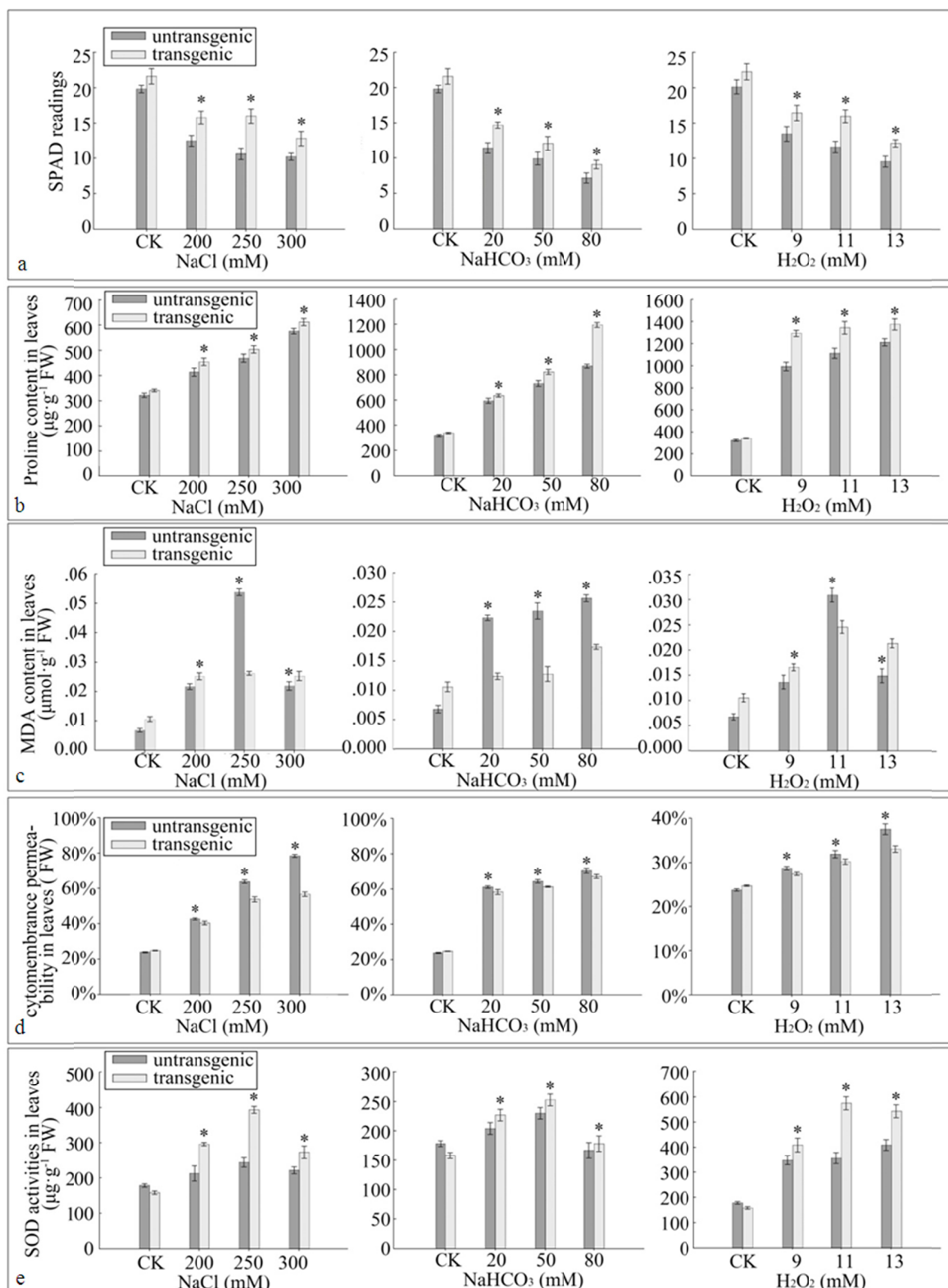


Figure 5. Physiological index of untransgenic and transgenic plants under stress conditions. (a) SPAD readings (SPAD is a unit of relative value of chlorophyll content measured using SPAD-502 Plus Chlorophyll Meter Model). (b) Proline content. (c) MDA content. (d) Cytomembrance permeability. (e) SOD activity. Asterisks indicate significant differences between untransgenic and transgenic plants ($P < 0.05$)

Compared with the control, the Na^+ content and salt concentration significantly increased after saline stress. The increasing trend of transgenic plants was less than that the untransgenic plants (Figure 6a). The K^+ content of the transgenic plants decreased significantly and was less than the untransgenic plants (Figure 6b). NMT flux data revealed that Na^+ efflux in the roots' apical regions significantly increased in all plants under saline conditions. Under 250 mM NaCl and 20 mM NaHCO_3 treatments, the Na^+ efflux rate in transgenic plants was 15% and 20% greater than the untransgenic plants (Figure 6c). NaCl and NaHCO_3 treatment changed the K^+ flux from influx to efflux. The K^+ efflux rate in untransgenic plants was 46% and 26% greater than the untransgenic plants (Figure 6d).

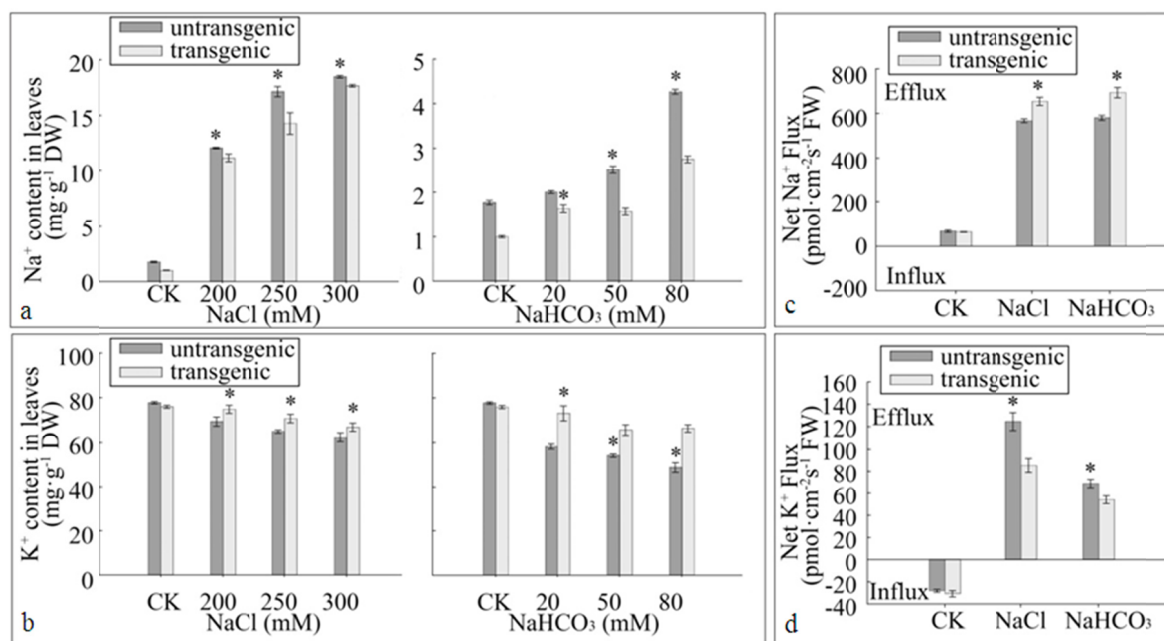


Figure 6. Na^+ and K^+ content and Fluxes of leaves in untransgenic and transgenic plants under NaCl and NaHCO_3 stress. (a) Na^+ content. (b) K^+ content. (c) Net Na^+ Fluxes. (d) Net K^+ Fluxes. Positive values represent efflux. Asterisks indicate significant differences between untransgenic and transgenic plants ($P < 0.05$)

4. Discussion

Depending on the position and arrangement of the cysteine residues, type 2 MTs sequence has three characteristics, the first and second Cys, which appear in the form of Cys-Cys contiguous, and most of them exist in the 3rd and 4th position of the N-terminal, a highly conserved sequence MSCCGGNCGCG exists in the N-terminal, and three Cys-X-Cys elements are arranged in the structure domain of C-terminal (Cobbett & Goldsbrough, 2002). The cysteine arrangement in the LpMT2 gene has the characteristics of type 2 MTs, and LpMT2 belongs to the type 2 metallothionein family.

Different MTs often exhibit different expression patterns. The specific expression of MhMT2 gene in *Malus hupehensis* was analyzed by Wang et al. (2012). The results showed that the MhMT2 gene expression was the greatest in leaves, followed by roots, and least in stems. Our results showed that LpMT2 transcripts were preferentially expressed in flowers, followed by mature leaves, young leaves and seeds, but also expressed to a lesser extent in the underground plant tissues.

One of the basic characteristics of MT genes is inducibility (Jin et al., 2006). MT genes are expressed in response to salinity, ROS, heavy metals, environmental stress, hormones, damage, and virus infection in some plants (Nishiuchi et al., 2007). The MT stress resistance is primarily focused on metal resistance. Although there are studies related to salt stress, there are few related to oxidative stress. Liu et al. (2011) cloned the metallothionein gene of *Limonium sinense* and analyzed its expression pattern under high salt exposure. When treated with ABA, H_2O_2 , mechanical damage, and NaCl, the MhMT2 gene expression was up-regulated in *Malus hupehensis* leaves, among which H_2O_2 treatment was the most obvious. In our research, *L. pumilum* was treated with different salts (NaCl and NaHCO_3) and oxidative (H_2O_2) stressors and treatment exposure times. LpMT2 gene expression in

leaves increased gradually with the increase time exposed to NaHCO_3 and H_2O_2 stress. While under the NaCl stress, the LpMT2 gene expression in leaves increased and then decreased. These results indicate that the induction of LpMT2 gene expression was related to the salt and oxidative stresses.

Under certain stress levels, a plant's cytochrome system will be destroyed, leading to a decrease in chlorophyll content (Li et al., 2016). The chlorophyll content of untransgenic plants' leaves had a greater decreasing trend than the transgenic *L. pumilum* plants under salt stress. This is an indication that LpMT2 has a significant impact on plant chlorophyll content. Proline, as an effective plant osmotic regulator, can scavenge reactive oxygen species (ROS), reduce lipid peroxidation, and prevent toxic amino acids accumulation (Li et al., 2011). When plants are subjected to environmental stress, the accumulation of proline increases significantly, which can enhance plant resistance to stress (Zhang et al., 2006). When under stress, the proline content was greater in the transgenic plants compared to the untransgenic plants. Various adverse environments often first influence the cell membrane (Xu et al., 2007). Relative conductivity reflects the cell membrane integrity, while MDA is an indicator of membrane lipid peroxidation, which together reflects the degree of cellular damage (Verslues et al., 2006). The increase in MDA content in the early stage of stress indicates that the membrane lipid peroxidation occurred. As the treatment concentration increased, the MDA content decreased, which may be due to the excessive lipid peroxidation consumption due to respiration. The cell membrane destruction in transgenic plants was less than the untransgenic plants. SOD plays a very important role in protecting cells from oxidative damage (Giannopolitis & Ries, 1977). SOD activity decreased more in transgenic plants than that in the untransgenic plants as a result of stress. This is an indication that the LpMT2 gene transfer reduced membrane peroxidation and protected the plants. When comparing the physiological indexes between the transgenic and untransgenic *L. pumilum*, the transgenic resistant plants were significantly greater than the untransgenic plants.

Salt stress destroys the plant's intracellular nutritional balance, primarily due to the excessive Na^+ accumulation, which causes ion poisoning and other elemental deficits (Flowers et al., 2010). K^+ is an essential nutrient for plant growth, but because of the antagonism between Na^+ and K^+ , it is necessary to maintain a high K^+ content in order to improve the salt tolerance of plants (Adams et al., 1992). Under salt stress, due to the accumulation of Na^+ , K^+ uptake is inhibited and there is a decrease in plant K^+ . The Na^+ concentration in the transgenic plants was less than the untransgenic plants, while the K^+ concentration was greater under saline stress. These results demonstrate that transgenic plants can control Na^+ uptake, maintain K^+ content in leaves, and ensure normal growth under stress.

Salt stress weakens Na^+ absorption and transport, and accelerates the Na^+ poisoning (Guo et al., 2005). The transgenic plant Na^+ efflux rate under salt stress was significantly greater than the untransgenic plants, while K^+ efflux rate was the opposite. This is an indication that the transgenic plants can adapt to a saline environment by increasing the Na^+ efflux rate and reducing the K^+ efflux rate, therefore, the transgenic plants had greater stress resistance.

The biological MT protein has been studied for over 60 years, but its exact function is still unclear. In recent years the function of the MT gene and protein relationship to abiotic stress has been predominantly understood, but the molecular mechanism of how the MT gene regulates a plant response to stress is not clear, therefore, the investigation of plant MT continues to be important.

The over expression of LpMT2 gene improves the salt tolerance of *L. pumilum*, but the impact of high salt stress on plant growth and development is very complex. Halophytes can grow in a saline environment as the result of a combination of various salt-tolerant mechanisms. In addition, the function of MT in abiotic stress has been primarily understood in recent years, but the molecular mechanism of how the MT regulates a plant's response to stress is not yet clear. Further research on the physiological metabolism and cell structure of transgenic *L. pumilum* will be helpful to reveal the role of MT accumulation in plants and its relationship to salt tolerance in plants.

5. Conclusions

In this research we isolated a LpMT2 gene from *L. pumilum*, proved that the gene expression was different in different plant organs or under different stresses, and characterized its function in *L. pumilum*. This study indicates that the LpMT2 gene enhanced the transgenic plants' tolerance to salts (NaCl and NaHCO_3) and oxidative (H_2O_2) stress. The following conclusions can be drawn from this research: 1) A 234bp long MT2 gene encoding 77 amino acids was cloned by PCR using cDNA of *L. pumilum* leaves as a template. QRT-PCR results showed that the LpMT2 gene has the greatest expression in flowers and the expression was significantly induced under salt and oxidative stress. 2) The plant expression vector pBI121-LpMT2 was constructed and transferred into the bulbs of *L. pumilum* by *Agrobacterium*-mediated successfully. 3) The untransgenic and transgenic *L. pumilum* were treated with different concentrations of NaCl , NaHCO_3 , and H_2O_2 for 48 h. Compared with untransgenic plants, transgenic

plants had better growth, greater chlorophyll and proline content, less MDA content, smaller cell membrane permeability, greater SOD activity, less Na⁺ content, greater K⁺ content, greater Na⁺ efflux and less K⁺ efflux.

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