Res. on Crops **15** (1) : 169-174 (2014) DOI : 10.5958/j.2348-7542.15.1.023
With three figures
Printed in India

Salinity stress accelerates abscission of tomato pedicel explants and expression of MAPK gene members

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(Received : November 2013)

ABSTRACT

The field experiment was conducted at the Horticulture Farm, Shenyang Agricultural University of China. The seeds of Liaoyuanduoli (*Solanum esculentum* Mill.), a popular tomato variety in north-east China bearing an indeterminate inflorescence, were planted in March 2012. The plants were grown in a greenhouse with natural light and normal management. We collected the flower organ to study abscission in blossom period. Abnormal abscission of flowers which usually relates to some types of stress can seriously impact crop yields. Our results show that salt stress may accelerate the tomato floral organ shedding and the abscission rate. Plant MAPK cascades are complicated networks and play vital roles in stress-induced signal transduction. By pre-treatment with 100 μM PD98059 to inactivate MAPK, we found that MAPK may be involved in the salt-induced tomato floral organ abscission and act as a positive regulator of salt tolerance. Here, three pairs of primers were designed to compare the expressions of *LeMAPKs* treated with different salt concentrations in tomato pedicel explants by using reverse transcriptase-polymerase chain reaction. Our findings indicated that *LeMAPKs* were extensively involved in salt stress response. The expression of *LeMAPK2* was obviously up-regulated, suggesting that *LeMAPK2* might be a salt-sensitive gene and play main defense function during tomato pedicel explant abscission induced by salt stress. And *LeMAPK3* might take effect in the late stage of flower abscission induced by salt stress. These results further suggest that MAPK cascades in plants had functions in signal transduction of salt stress and the expression patterns of MAPKs are complicated in tomato pedicel explants.

Key words : Abscission, accelerate, expression patterns, MAPK, salt stress

INTRODUCTION

Abscission is defined as the natural separation of organs from the parent plant which is initiated in response to environmental events such as disease or pathogens, or as a programmed shedding of organs that no longer provide indispensable function to the plant, such as the flower after aiding in pollination. Abscission has been a hotspot in the field of natural science research. It not only has tremendous commercial values to prevent abscission, but also is a tool to understand the growth and development of plants.

At the stage of flowering, some types of stress are more likely to cause abnormal abscission to the flowers. It may be nutritional stress, environmental stress, or a combination of the two. Tomato is a popular vegetable, and its production throughout the world is faced with a problem of floral organ shedding. Tomato plants lose blossoms for several reasons. The primary reasons are environmental, such as temperature and relative humidity or culture. Tomato plants can tolerate extreme temperatures for short periods, but several days or nights with temperatures beyond the ideal range will cause the flower to shed and focus on survival. The secondary reasons are water insufficiency, reduced or extended light exposure, excessive wind, insect damage, foliar disease, excessive pruning and heavy fruit set (Ozores-Hampton and McAvoy, 2010). Tomato yellow leaf curl virus (TYLCV), one major devastating virus of cultivated tomato, can cause flower abscission.

Salinity is the main environmental factor accounting for the decreased crop productivity throughout the world, especially in arid and semi-arid regions. Like many abiotic stresses, salt stress inhibits plant growth, as the increased salinity level can significantly decrease fresh-dry weight and

contents of photosynthetic pigments (chlorophyll a, b) in tomato plants (Manaa *et al.*, 2011). However, little attention has been paid to whether salt stress will induce tomato flower shedding.

Efficient perception of stresses and well-programmed signalling mechanisms are important for the growth and survival of a plant exposed to biotic or abiotic stress. Plants have developed some fine-tuned complex responsive pathways, among which one of the earliest signal pathways is mitogen-activated protein kinase (MAPK) cascade (Jonak *et al.*, 2002). The MAPK signalling cascade coordinates and amplifies the incoming information from other signalling pathways, conveys it to effectors and allows for various response patterns. Increasing evidence shows that MAPKs play an important role in the stress-related signal transduction in plants. MAPKs can be activated by stresses such as pathogens, cold, salinity, wounding, heat, ultraviolet (UV), osmotic shock and heavy metals (Pitzschke *et al.*, 2009; Li *et al.*, 2012). However, it is still largely unknown whether or not MAPKs participate in the signal transduction of abscission induced by salt stress in tomato.

The objective of this paper was to study the effects of salt stress on flower shedding in tomato. We determined the expression patterns of *LeMAPKs* (*Lycopersicon esculentum* mitogen-activated protein kinases) during tomato flower abscission induced by salt stresses. The results may help to understand the signal transduction mechanisms of *LeMAPKs* related to salt stresses and flower organ abscission.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

This study was conducted at the Horticulture Farm, Shenyang Agricultural University of China. The seeds of Liaoyuanduoli (*Solanum esculentum* Mill.), a popular tomato variety in north-east China bearing an indeterminate inflorescence, were planted in March 2012. The plants were grown in a greenhouse with natural light and normal management. When the fresh yellow flowers opened at an angle of ~ 90°, they were excised from the inflorescence and their pedicel explants were trimmed immediately into about 2 cm in length (Xu *et al.*, 2010).

NaCl Treatments and Abscission Rate Investigation

Forty explants were inserted from the proximal end into a petri dish containing a 0.9% agar medium. NaCl (0, 50, 100, 150 and 200 mM) was added to each of three dishes. The dishes were then placed in a container with a cling film cover (40 × 25 × 20 cm^3), and the explants were incubated at 25°C. The number of pedicels abscised was recorded after 8, 16 and 24 h, according to the method of Wang *et al.* (2005).

Inhibitor Treatments and Abscission Rate Investigation

Forty explants were inserted from the proximal end into a petri dish containing a 0.9% agar medium and 100 μM PD98059 (2'-Amino-3'-methoxyflavone, a specific inhibitor of MAPK pathway) for 12 h before the incubation with control and 150 mM NaCl (Liu *et al.*, 2009).

Total RNA Extraction

After incubation for the indicated time, the tissues (about 2 mm wide) containing the AZ (abscission zone) were briefly cut onto airlaid paper, weighed and then frozen in liquid nitrogen. Then the frozen AZ tissues were ground to fine powder in liquid nitrogen. Total RNA for reverse transcriptase-polymerase chain reaction (RT-PCR) was isolated from the AZ tissues using an RNAprep Pure Plant Kit (China Tiangen) according to the manufacturer's instructions. The kit contains an RNase-free Filtration Column CS for homogenizing and filtering viscous plant and an RNase-free Spin Column CR3 for purifying up to 100 ig of high-quality RNA using silica-membrane technology. All samples were treated with RNase-free DNase I to eliminate genomic DNA contamination. The integrity of the isolated RNA was detected by 1.2% agarose gel electrophoresis.

Primers Designed for Quantitative PCR Expression Analysis

Three tomato MAPK genes were aligned using DNAman, and exclusive primers for each gene were designed using Primer Express 3.0 (Applied BioSystems, USA). Each pair of primers was checked by the program BLAST

Table 1. Accession numbers and primer sequences used to analyze the expression of *LeMAPKs* by real-time RT-PCR

Category	SGN number	Primer sequences
LeMPK1	SGN-U576603	F : 5'-TGCACCTCCGGCTCAACAA-3' R : 5'-GGCAGTGCTCCTCAGATAAA-3'
LeMPK2	SGN-U576602	F : 5'-TCGTTTGCTGTTGGATTGTCTGTGT-3' R : 5'-TCATTTCTGGAACTAAAAATACAGA-3'
LeMPK3	SGN-U567335	F : 5'-CTAAATTTCTATCAATAATGGTTGATGC-3' R : 5'-GCGGAGGAATCACATTCCTT-3'
Actin	543519	F : 5'-AGGTGTTATGGTCGGAATGG-3' R : 5'-GAGAAAGCACAGCCTGGATAG-3'

in tomato genomic sequence available in databases Sol Genomics Network (SGN) and US National Center for Biotechnology Information (NCBI) to ensure the primers could amplify a unique structure without secondary structures and desired cDNA segment (Table 1).

Real-time PCR Analysis

The RNA samples were reversely transcribed into complementary DNAs (cDNAs). In brief, 3 µl of RNA was mixed with 0.6 µl of oligo d (T)15 µl, incubated at 70°C for 5 min, and then cooled immediately. Then with addition of 3 µl of an M-MLV 5 × reaction buffer, 0.4 µl of a cloned ribonuclease inhibitor, 3 µl of dNTP (2.5 mmol/l) and 0.6 µl of a Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase and RNase-free ddH_2O to the final volume of 15 µl, the system was mixed gently and incubated at 42°C for 1 h. The product cDNAs were stored at -20°C.

Quantitative RT-PCR was performed on an Applied Biosystems (ABI) 7500 Real Time PCR System and Software 7500 ver. 2.0.3 (ABI, USA) according to the manufacturer's instructions. The cDNA samples were used as a template and mixed with 200 nM of each primer and SYBR Green PCR Real Master Mix (Tiangen, Beijing, China) for real-time PCR, with the following procedure : 95°C for 3 min; 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. All experiments were run in triplicate with different cDNAs synthesized from biological triplicate. The fluorescence signal was collected during the elongation at 72°C in every cycle.

Statistical Analysis of Results

Date were analyzed for significant differences by one-way analysis of variance. Significant effects were determined using LSD multiple comparison prodeduce at the 5% level and at 1% level were considered significant and extremely significant, respectively.

RESULTS AND DISCUSSION

Effects of Salt Stress on Floral and Pedicel Explant Abscission

To evaluate the effects of salt stress on flower abscission, we examined tomato flower pedicels treated with different concentrations of NaCl for 24 h. Fig. 1 of statistical graph shows that NaCl treatment could accelerate the abscission of tomato flower pedicel explants. Increasing or decreasing the NaCl concentration resulted in a corresponding increase or decrease in the abscission rate. At 16 h, when abscission reached 25.9% in the control, 7.1 and 27.9% increases of abscission rate were observed at 50 and 150 mM NaCl, respectively. About 50% of abscission was obtained in the control after 24 h, but with the addition of 150 mM NaCl, the 50% abscission was accelerated by about 8 h. When a higher dose (200 mM) of NaCl was applied, the abscission increased to 57.1% at 16 h, but the pedicel explants were subjected to very serious osmotic stress. Therefore, 150 mM was considered as the most

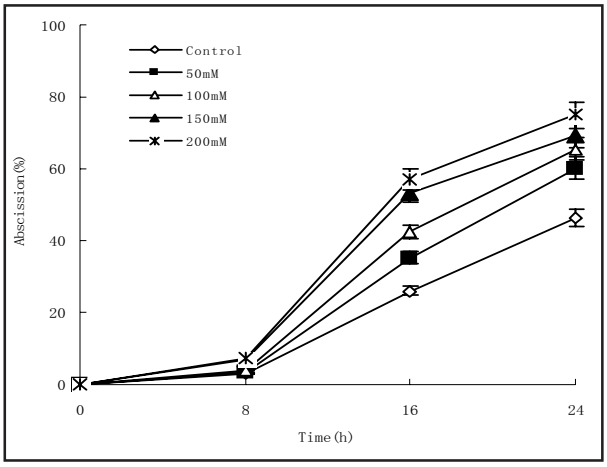

Fig. 1. Abscission timing of tomato flower pedicel explants imbibed in different NaCl concentrations. In control with no addition of NaCl. Vertical bars indicate ±SE (n=3).

effective dose in accelerating tomato pedicel abscission.

Effects of MAPK on Floral and Pedicel Explant Abscission of Tomato

Given that salt stress induced the acceleration of abscission and MAPKs were activated by salt stress, we then investigated whether there was a link between MAPKs and induction of shedding. The tomato flower pedicel explants were pre-treated with 100 μM PD98059 (a specific inhibitor of MAPK pathway) for 12 h before the treatment with 150 mM NaCl, and then the abcission rate was calculated.

As shown in Fig. 2, in pedicels pre-treated with PD98059 before incubation with 150 mM NaCl, abscission rate increased significantly in comparison to those treated with salt alone or PD98059. At 16 h, when the abscission rate reached 25.9% in the control, about 10% increase in abscission was observed after treatment with the inhibitor and the same uptrend was seen after treatment with NaCl+PD98059.

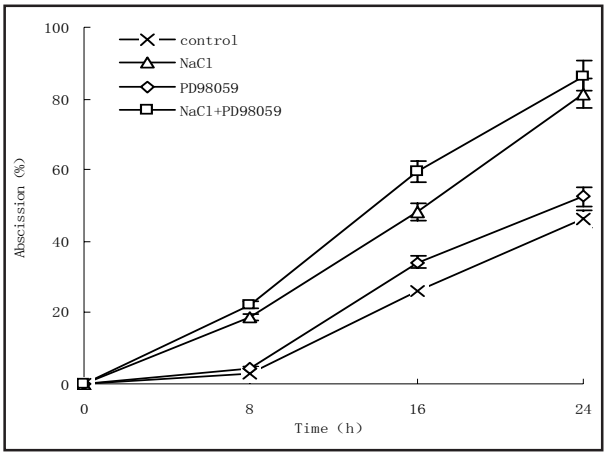

Fig. 2. Effects of MAPK on abscission timing of tomato flower pedical explants. Inhibitor : explants were pre-treated with 100 μM PD98059 for 12 h, before the incubation with control; Inhibitor+NaCl : pre-treated with 100 μM PD98059 for 12 h, before the incubation with 150 mM NaCl. Vertical bars indicate ±SE (n=3).

Expression Analysis of *LeMAPKs* in Tomato Blossom Drop Induced by NaCl Stress

In Fig.1, the NaCl stress accelerated the flower abscission. But is this acceleration related to changes in *LeMAPK* expression? To obtain more information about *LeMAPK* expression in tomato floral abscission, we analyzed the expressions of *LeMAPKs* under NaCl stress.

As shown in Fig. 3, the transcript levels of *LeMAPK1* increase, but the expression levels are significantly lower under salt stress than the control. Moreover, there is a swift rise and a peak at 6 h. The transcript levels of *LeMAPK2* are obviously up-regulated, which are higher under salt stress than in the control. Meanwhile, the degree increases at higher salt concentration. At 6 h, the *LeMAPK2* mRNA level starts to significantly rise and reaches 603.5

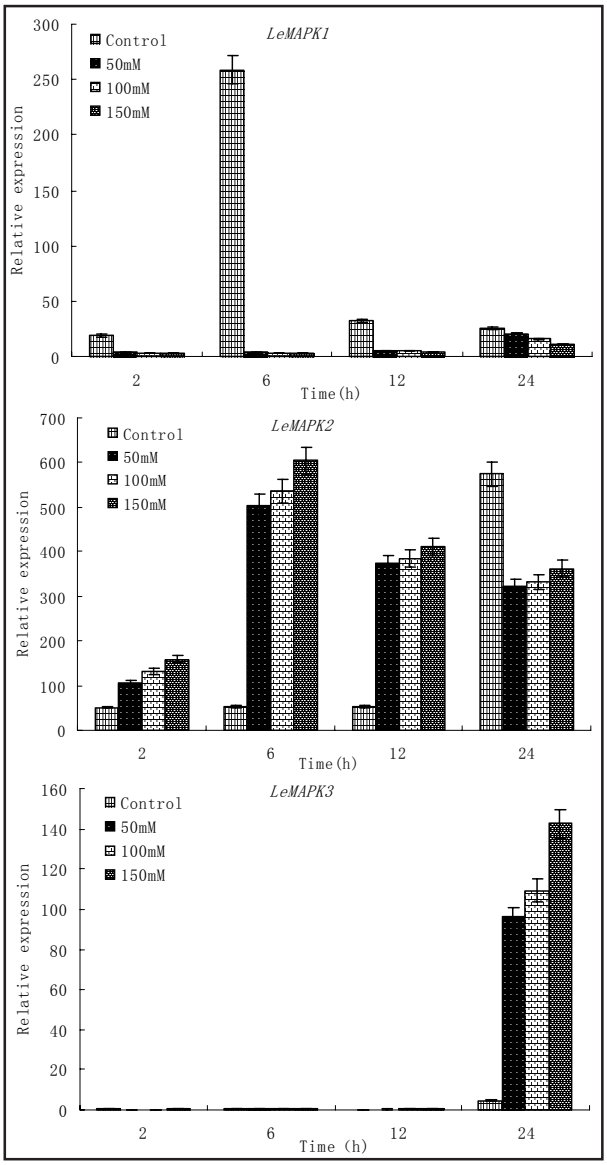

Fig. 3. Relative quantification of *LeMAPKs* during abscission of tomato pedicel explants induced by salt stress. Vertical bars indicate ±SE (n=3).

in 150 mM NaCl, and these changes occur 12 h earlier than in the control. The expression of *LeMAPK3* only slightly fluctuates from 2 to 12 h, and then tomato pedicel explant abscission rate starts to increase sharply both under salt stress and in the control; especially at 24 h, it is up to 30 times higher in 150 mM NaCl than in the control.

Unfavourable environmental conditions can cause tomato plants to drop their blooms, which seriously impact yields. It is well known that salt stress can cause metabolic modifications, especially reactive oxygen species (ROS), hormonal changes and photosynthesis. Therefore, at a whole-plant level, the effects of salinity are reflected by growth decline and yield reduction. The more actual response to salinity varies with growing conditions, crop variety and the sensitivity of crops at different growth stages. In *Populus euphratica* and citrus, leaf drop is a visible symptom of salt damage. In cotton, salt stress leads flower buds to massive sudden abscission when the salt concentration is up to 1% (Ye *et al.*, 2007). In wild-type *Arabidopsis*, three days of salt-shock treatment results in the abortion of flower buds and the smaller number of seeds per siliqua (Sulpice *et al.*, 2003). Nearly 90% of the ovules aborted when roots were incubated for 12 h in a hydroponic medium containing 200 mM NaCl (Sun *et al.*, 2004). In the present study, with the increase of salt concentration, salt stress accelerated the tomato floral organ shedding and the abscission rate. After treatment with 150 mM NaCl for 16 h, the abscission rate reached 53.2% (Fig. 1). These results suggest that salt stress may cause different levels of abscission to leaves, flowers and other organs.

In plants, MAPK pathways are involved in responses to a diversity of environmental stimuli as well as in the regulation of development, growth and Programmed Cell Death (PCD). PD98059 at 100 ìM can effectively inhibit MAPK activity in plants (Pitzschke *et al.,* 2009). Although in-gel protein kinase assay was not performed, we detected the ratio of abscission. In this work, the ratio of abscission reached 34.2% after the pre-treatment with PD98059, while it was only 25.9% at 16 h in the control (Fig. 2). The results imply that MAPK in tomato may act as a positive regulator of salt tolerance.

The number of members in the MAPK gene family has not been fully determined in most of the plants. In the completed *Arabidopsis* genome sequence, 20 genes encoding possible MAPKs were identified, but only three or four MAPKs were known to be stress-responsive in a single plant species. In tomato, only three MAPK genes have been identified to be stress-related. These genes may simultaneously or separately participate in different stress reactions. *LeMAPK1* and *LeMAPK2* are activated upon stress responses caused by the systemin, oligosaccharide elicitors, UV-B radiation, and the fungal toxin fusicoccin (Higgins *et al.*, 2007). *LeMAPK2* and *LeMAPK3* are activated in a Pto-specific manner upon expression of AvrPto and AvrPtoB (Pedley and Martin, 2004). In the present study, three new pairs of primers were designed to compare expressions of *LeMAPKs* after salt treatment by using RT-PCR (Table 1).

The roles of MAPK pathway in tolerance to salt stress in some plants have been studied. For instance, salt stress will activate the *Arabidopsis* MAPKs-MAPK6 and MAPK4. Overexpression of maize MAPK gene, *ZmSIMK1* in *Arabidopsis* increases tolerance to salt stress. These results suggest that *ZmSIMK1* may play an important role in tolerance to salt stress in plants. *CsNMAPK* is involved in positive regulation of ROS scavengence and osmotic adjustment under salt stress (Xu *et al.*, 2011).

Our findings indicate that tomato MAPK genes are extensively involved in salt stress response. The mRNA level of *LeMAPKs* changes to different extents in response to salt stress. As shown in Fig. 3, the transcript levels of *LeMAPK1* increase similarly, but the expression level is significantly lower under salt stress than in the control. The transcript levels of *LeMAPK2* are obviously up-regulated and the degree increases with the elevated salt concentration. *LeMAPK3* level increases largely at 24 h and we speculate that it may take effect at the late stage of the salt-induced flower abscission. Our results further suggest that MAPK cascades in plants may function in the signal transduction under salt stress. Meanwhile, these results suggest that *LeMAPK2* may be a salt-sensitivity gene and play main defense function during tomato pedicel explant abscission induced by salt stresses.

The expression changes of the stress-inducible MAPK genes in this study may confer stress tolerance in tomato. The MAPK signalling

network related to abiotic and biotic stresses is very complex. However, further study is needed to dissect its regulatory mechanisms. Studies on the stress-regulation of MAPK genes will help to understand the functions of these genes in stress responses and the induction of tolerance in tomato. Such knowledge will be useful to improve the tomato tolerance to environmental stresses and ensure high and stable yield of tomato.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support for this work from the National Natural Science Foundation of China (30871714 and 31171966).

REFERENCES

Higgins, R., Lockwood, T., Holley, S., Yalamanchili, R. and Stratmann, J. W. (2007). Changes in the extracellular pH are neither required nor sufficient for activation of mitogen-activated protein kinases (MAPKs) in response to systemin and fusicoccin in tomato. *Planta* **225** : 1535-46.

Jonak, C., Okresz, L., Bogre, L. and Hirt, H. (2002). Complexity, crosstalk and integration of plant MAP kinase signalling. *Curr. Opin. Plant Biol.* **5** : 415-24.

Li, F. F., Xia, J., Liu, S. S. and Wang, X. W. (2012). p38 MAPK is a component of the signal transduction pathway triggering cold stress response in the MED cryptic species of *Bemisia tabaci*. *J. Integrative Agric.* **11** : 303-11.

Liu, Y. K., Liu, Q. Z., Xing, X. and Li, D. Q. (2009). Effects of MAPKK inhibitor PD98059 on the gravitropism of primary roots of maize. *Plant Growth Regul.* **59** : 191-98.

Manaa, A., Ahmed, B. H., Smiti, S. and Faurobert, M. (2011). Salt-stress induced physiological and proteomic changes in tomato (*Solanum lycopersicum*) seedlings. *OMICS : A Journal of Integrative Biology* **15** : 801-09.

Ozores-Hampton, M. and McAvoy, G. (2010). What causes blossom drop in tomatoes? *The Tomato Magazine* **8** : 4-5.

Pedley K. F. and Martin, G. B. (2004). Identification of MAPKs and their possible MAPK kinase activators involved in the Pto-mediated defense response of tomato. *J. Biol. Chem.* **279** : 49229-35.

Pitzschke, A., Schikora, A. and Hirt, H. (2009). MAPK cascade signalling networks in plant defence. *Curr. Opin. Plant Biol.* **12** : 421-26.

Sulpice, R., Tsukaya, H., Nonaka, H., Mustardy, L., Chen, T. H. H. and Murata, N. (2003). Enhanced formation of flowers in salt-stressed *Arabidopsis* after genetic engineering of the synthesis of glycine betaine. *The Plant J.* **36** : 165-76.

Sun, K. L., Hunt, K. and Hauser, B. V. (2004). Ovule abortion in *Arabidopsis* triggered by stress. *Plant Physiol.* **135** : 2358-67.

Wang, Y. C., Li, T. L., Meng, H. Y. and Sun, X. Y. (2005). Optimal and spatial analysis of hormones, degrading enzymes and isozyme profiles in tomato pedicel explants during ethylene-induced abscission. *Plant Growth Regul.* **46** : 97-107.

Xu, H. N., Sun, X. D., Wang, X. F., Shi ,Q. H., Yang, X. Y. and Yang, F. J .(2011). Involvement of a cucumber MAPK gene (CsNMAPK) in positive regulation of ROS scavengence and osmotic adjustment under salt stress. *Sci. Hort.* **127** : 488-93.

Xu, T., Li, T. L. and Qi, M. F. (2010). Calcium effects on mediating polygalacturonan activity by mRNA expression and protein accumulation during tomato pedicel explant abscission. *Plant Growth Regul.* **60** : 255-63.

Ye, W. W. (2007). Abscission and its mechanism of three different salt-tolerant types of upland cotton under the stress of salinity. *Cotton Sci.* **19** : 59.