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Full Length Article

Cloning and Expression of Mitogen-activated Protein Kinase 4 (*MAPK4*) in Response to High Temperature in Lettuce (*Lactuca sativa* L.)

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Abstract

A cool temperature is preferred for lettuce cultivation, while high temperatures can cause premature bolting. To investigate the function of the lettuce *LsMAPK4* gene in bolting at high temperature, multiple bioinformatics tools were employed and real-time fluorescence quantitative PCR was applied to analyze expression patterns. The full-length *LsMAPK4* gene sequence was cloned from the lettuce strain GB-30. Sequence analysis showed that the full-length sequence was 1641 bp and the open reading frame was 1134 bp; the latter encoded 379 amino acids and had conserved mitogen-activated protein kinase domains. qRT-PCR analysis revealed that the expression of *LsMAPK4* in stems was significantly higher than in root and leaf. The expression of this gene in stem under high temperature treatment was significantly higher than that of control group. *LsMAPK4* may be intimately associated with high-temperature bolting in lettuce crop. © 2018 Friends Science Publishers

Keywords: Lettuce; *LsMAPK4*; Gene cloning; Expression analysis

Introduction

Lettuce (*Lactuca sativa* L.) originates from the Mediterranean coast (Han *et al*., 2013) and its leaves are consumed as food. Lettuce has a crispy texture and is rich in nutrients, including protein (Santamaria, 2006), fiber, iron, folic acid, and vitamin C (Kim *et al*., 2016). Therefore, it is popular with consumers. Lettuce grows best under cool temperature conditions in the range of 7-24°C. Growing crop above this range decreases quality and yield, which results in economically important physiological disorders such as tipburn, rib discoloration, premature bolting, and ribbiness (Jenni *et al*., 2013). A solution to this problem is urgently needed to improve annual production of lettuce. Currently, the relevant mechanisms of high-temperature lettuce bolting are unclear. Therefore, examining these molecular mechanisms is important to formulate scientific and reasonable measures to prevent high-temperature bolting.

Similar to ubiquitination, methylation, and acetylation, protein phosphorylation is a protein post-translational modification process that is widespread in biological organisms (Yao and Xu, 2017). This process acts as a molecular switch during regulation of enzyme activity and cellular signaling and is a crucial aspect of prokaryotic and eukaryotic metabolism (Bentem and Hirt, 2007). Protein phosphorylation is achieved by the transfer of phosphate moieties of ATP to specific sites on proteins, such as threonine, serine, and tyrosine residues, by protein kinases. In prokaryotes, histidine, glutamate, and aspartic acid residues have also been found to be phosphorylated (Engholm-Keller and Larsen, 2013). Plant protein kinases participate in the regulation of many processes, such as metabolism (Kempa *et al*., 2007; Polge and Thomas, 2007), cell cycle (Inze and Veylder, 2006), cytokinesis (Sasabe and Machida, 2006), stomatal closure and development (Mori *et al*., 2006; Wang *et al*., 2007), and stress and hormonal responses (Harper and Harmon, 2005; Nakagami *et al*., 2005; Belkhadir and Chory, 2006; Kempa *et al*., 2007; Takahashi *et al*., 2007; Wrzaczek *et al*., 2007).

Members of the mitogen-activated protein kinase (MAPK) family are integral protein kinases in cellular signaling, as they participate in many signal transduction pathways by phosphorylating transcription factors to regulate the expression of multiple genes. MAPK signal transduction pathways play important roles in mediating growth factors, hormonal responses, cellular proliferation and differentiation, extracellular environmental stress, and the regulation of intracellular stress responses (Meskiene *et al*., 2003). MAPK cascades include three integral protein kinases MAPKKK, MAPKK, and MAPK, which respond to external stimuli through phosphorylation and signal amplification to activate specific genes in the cell nucleus.

The physiological responses mediated by MAPK cascade pathways consist of two major types. The first type comprises signals in response to growth factors and hormones, which lead to cellular proliferation and differentiation. The second type comprises extracellular environmental stress signals that induce intracellular stress responses. *Arabidopsis* MAPK3, 4, and 6 are currently the most studied plant MAPKs. The results of previous studies have demonstrated that *AtMAPK3/6* not only participates in disease resistance and stress resistance in plants, but also play crucial roles in pollen and ovule development (Wang *et al*., 2008; Meng *et al*., 2013; Guan *et al*., 2014). In addition, *AtMAPK3/6* also participates in the regulation of responses to ethylene, abscisic acid, and other plant hormones (Gudesblat *et al*., 2007; Yoo and Sheen, 2008). *Arabidopsis MAPK4* participates in the cellular division of male gametophytes and many resistance and stress responses (Brodersen *et al*., 2006; Kosetsu *et al*., 2010; Zeng *et al*., 2011).

Previously differential proteomics studyin lettuce during high-temperature bolting revealed significant differences in the levels of MAPK4 protein (unpublished data). However, the effector mechanisms of the *MAPK4* gene in lettuce and its relationship with lettuce bolting are still unclear. Therefore, we cloned the *LsMAPK4* gene and carried out bioinformatics analysis, while real-time quantitative PCR was used to analyze the relative expression status of *LsMAPK4* gene under different temperatures and time points, in order to provide a basis for further studies on the *LsMAPK4* effector mechanisms involved in lettuce bolting.

Materials and Methods

Plant Material and Growth Conditions

The easy bolting leaf lettuce variety GB-30 was stored in our laboratory, sown in a sand/soil/peat (1:1:1 v/v) mixture, and grown in an experimental station in Beijing under standard greenhouse conditions (14 h light; 20±2°C during the day; 13±2°C at night; 10 h dark; and 50%-70% relative humidity). The seedlings were transplanted into 10 cm pots at the trefoil stage. Lettuce plants at sixth true leaf stage were moved to a growth chamber under temperatures of 20/13°C (day/night), a 14/10 h photoperiod, and 60% relative humidity for two days of acclimatization. After domestication and culture, 15 plants were selected for their roots, stems, and leaves, frozen in liquid nitrogen, and stored at -80°C. Next, the remaining plants were divided into two groups: the control group was kept under the standard greenhouse conditions, as described above; the other group was moved to another growth chamber and treated with high temperatures of 33 and 25°C during the day and night, respectively. Immediately, and then the 3rd, 6th, 12th, 24th, and 48th h and the 8th, 16th, 24th and 32nd days of treatment, the stems were taken as experimental materials and were stored at -80°C. In a preliminary experiment, the lettuce strain GB30 began to bolt on the 8th day of high temperature treatment. Therefore, sampling was performed at time points before and after bolting. At each time point, three stems were harvested and immediately frozen in liquid nitrogen. Three biological replicates were performed for each treatment. All samples were stored at 80°C prior to the extraction of RNA.

Methods

Total RNA extraction and cDNA first strand synthesis: A Spin Column Plant Total RNA Purification Kit (Songon Biotech, Shanghai, China) was used to extract the total RNA of lettuce, and then a TransScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) was used to reverse transcribe the RNA to cDNA. All cDNA strands obtained were stored at –20°C for use as a template for cloning the *LsMAPK4* gene and further used for qRT-PCR.

Cloning of *LsMAPK4* Gene

Primer Premier 5 software was used to design the primers needed for cloning the full-length *LsMAPK4* gene and Coding sequence (CDS), according to the sequence information obtained in the pre-transcriptome sequencing in the laboratory. The primer sequences are shown in Table 1. Primers were prepared by Sangon Biotech (Shanghai) Co., Ltd. cDNA from lettuce stems was used as a template for cloning the full-length *LsMAPK4* gene. The reaction conditions used were as follows: pre-denaturation at 98°C for 5 min; 35 cycles of denaturation at 98°C for 10 s, annealing at 56°C for 15 s, and extension at 72°C for 2 min; followed by a final extension of 72°C for 8 min. The PCR products were stored at 4°C. cDNA from lettuce stems was used as a template for cloning CDS according to the *LsMAPK4* gene. The reaction conditions used were as follows: pre-denaturation at 98°C for 5 min; 35 cycles of denaturation at 98°C for 10 s, annealing at 51°C for 15 s, and extension at 72°C for 1 min; followed by a final extension of 72°C for 8 min. The PCR products were stored at 4°C.

Agarose gel electrophoresis was carried out on the PCR products, and an EasyPure Quick Gel Extraction Kit (TransGen Biotech, Beijing, China) was used to recover the target band, which was then ligated to the pTOPO-Blunt vector (Aidlab Biotechnologies Co., Ltd, Beijing, China). The ligated vector was then transformed into *Escherichia coli* DH5α competent cells (Bao Biological Engineering Co., Ltd, Dalian, China), and the bacterial culture was sequenced by Sangon Biotech (Shanghai) Co., Ltd.

Sequences and Phylogenetic Analyses

ProtParam (Walker, 2005) (http://web.expasy.org/protparam) was used for online analysis of protein parameters. The conserved domains of

Table 1: Primer sequences

Name of primer	Sequence of primer (5′-3′)	purpose
LsMAPK4Q-F	TTTTGAGGAGGGTAAACTCTCGCTT	full-length
LsMAPK4Q-R	CGAACACAAAAAGCACTATACAAAA	full-length
LsMAPK4-F	ATGTCTGTGGTGGAGTCAAGCTC	CDS
LsMAPK4-R	GTGATTT GGATGGTCAG GATTG	CDS
qLsMAPK4-F	TTCTTACACATGGCGGTCGTTACG	qRT-PCR
qLsMAPK4-R	CAACAGGTCTGATCGGAGGAACATAC	qRT-PCR
Ls18S-F	GTGAGTGAAGAAGGGCAATG	qRT-PCR
Ls18S-R	CACTTTCAACCCGATTCACC	qRT-PCR

LsMAPK4 were obtained using the Conserved Domain Search Service. NCBI Protein BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for the online search of homologous sequences of the LsMAPK4 amino acid sequence. DNAMAN 7.0 software was used for the sequence alignment of MAPK4 amino acid sequences from lettuce, sunflower, parsley, papaya, and chili. The neighbor-joining algorithm of the MEGA 6.0 program (Tamura *et al*., 2003) was used to construct a phylogenetic tree with Poisson correction and pair-wise deletion parameters, and all other parameters were set to default. A total of 1000 bootstrap replicates were performed. The subcellular localization of the deduced polypeptides was predicted by Cell-PLoc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/) (Chou and Shen, 2008; Chou and Shen, 2010a, b).

Quantitative Real-time PCR

The fluorescence dye method was employed for real-time fluorescent quantitative expression analysis. According to the sequence information of gene *LsMAPK4*, fluorescent quantitative PCR primers were designed as follows: qLsMAPK4-F: 5′-TTCTTACACATGGCGGTCGTTA-CG-3′ and qLsMAPK4-R: 5′-CAACAGGTCTGATCGGAGGAACATAC-3′. The leaf lettuce 18S rRNA gene (GenBank accession number HM047292.1) was used as an internal reference gene.

The reaction system (10 μL) contained 2 × SYBR qPCR Mix (5 μL), cDNA template (1 μL), 10 μM forward primer (0.5 μL), 10 μM reverse primer (0.5 μL), and ddH_2O (3 μL). The reaction procedure was as follows: 3 min of pre-denaturation at 95°C, 10 s at 94°C, 30 s at 55°C, 20 s at 72°C, 40 cycles, and 5 min of extension at 72°C (Weng, 2017). The $2^{-\Delta\Delta CT}$ relative quantitation method was used to calculate the relative expression of the *LsMAPK4* gene (Livak and Schmittgen, 2001). Each experiment was repeated at least three times.

Statistical Analysis

Microsoft Office Excel 2016 was used for data processing, SPSS 20.0 software was used for the analysis of variance and determination of significance of the data. OriginPro 9.0 software was used to plot the graphs.

Results

Cloning of the *LsMAPK4* Gene in Leaf Lettuce

The complete sequence of the MAPK gene was cloned from lettuce leaves by RT-PCR. The gene had the highest homology with *Helianthus annuus* mitogen-activated protein kinase (MAPK4, XP_021969289). Concordance at the nucleotide and amino acid levels was 88% and 95%, respectively. The gene was named *LsMAPK4* and cDNA was 1641 bp in length (Fig. 1A). The coding region was 1134 bp in length (Fig. 1B) and encoded a protein of 378 amino acids (Fig. 2).

Sequence Analysis of *LsMAPK4*

The deduced molecular weight of LsMAPK4 was 43.46 kDa and the theoretical isoelectric point (pI) was 6.32. The leucine and arginine content was 10.1% and 7.4%, respectively, there were 49 (Asp + Glu) negative residues and 44 (Arg + Lys) positive residues, which were unstable (with an instability index of 42.96). LsMAPK4 is a labile protein with a mean hydrophilicity of -0.388, predicted to be a hydrophilic protein. The estimated half-life is more than 20 h in yeast, *in vivo* and more than 10 h in *Escherichia coli*, *in vivo*. Moreover, the protein did not contain a signal peptide or transmembrane domain, and was not a secreted protein. The results also indicated that *LsMAPK4* mainly localized in cytoplasm Golgi apparatus.

Analysis of Conserved Domains in Lettuce LsMAPK4 and Multiple Sequence Alignment

LsMAPK4 was found to contain a plant TEY subtype MAPK domain (Fig. 3). The kinase activity sites of this protein include an ATP-binding site and a substrate binding site. In addition, the protein also contains a kinase interaction motif (KIM) docking site and an activating loop (A loop), also known as a regulatory T-loop.

LsMAPK4 has a high degree of similarity to the MAPK4 proteins of other species (Fig. 4). The C terminal is more highly conserved than the N terminal, as the latter contains multiple variable sequences. LsMAPK4 contains 11 conserved protein kinase domains, which are located at the VII and VIIII domains in the catalytic core, including a “TEY” threonine and tyrosine phosphorylation site.

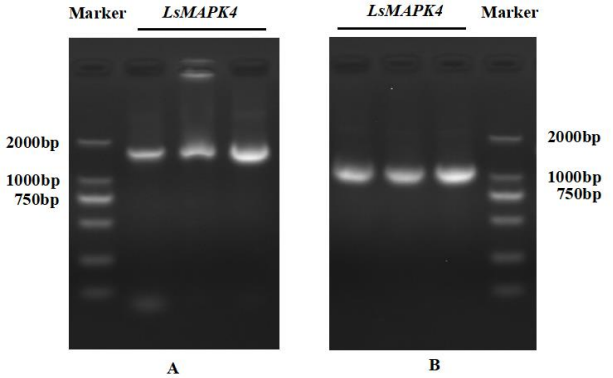

Fig. 1: The amplification of *LsMAPK4* gene in lettuce

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  1 M  S  V  V  E  S  S  S  A  T  T  A  D  Q  S  N  V  K  G  V
  1 ATGTCTGTGGTGGAGTCAAGCTCTGCTACTACAGCGGATCAGAGTAACGTTAAGGGGGTT
 21 L  T  H  G  G  R  Y  V  Q  Y  N  V  Y  G  N  L  F  E  V  S
 61 CTTACACATGGCGGTCGTTACGTTCAGTATAATGTGTACGGTAATCTTTTCGAAGTTTCC
 41 R  K  Y  V  P  P  I  R  P  V  G  R  G  A  Y  G  I  V  C  A
121 CGGAAGTATGTTCCTCCGATCAGACCTGTTGGTAGAGGCGCTTATGGAATCGTTTGTGCT
 61 A  T  N  A  E  T  R  E  E  V  A  I  K  K  I  G  N  A  F  D
181 GCAACGAATGCGGAGACACGTGAAGAAGTTGCCATAAAGAAAATTGGGAATGCTTTTGAC
 81 N  R  I  D  A  K  R  T  L  R  E  I  K  L  L  R  H  M  E  H
241 AACAGAATAGATGCGAAAAGGACTCTAAGAGAAATTAAGCTTCTTCGTCACATGGAACAT
101 E  N  V  I  A  I  K  D  I  I  R  P  P  Q  K  E  N  F  N  D
301 GAAAATGTTATTGCAATCAAAGACATCATACGGCCTCCACAGAAGGAAAACTTCAATGAT
121 V  Y  I  V  Y  E  L  M  D  T  D  L  H  Q  I  I  R  S  N  Q
361 GTTTACATTGTTTATGAGTTGATGGACACGGATCTTCATCAAATAATACGCTCTAATCAA
141 P  L  A  D  D  H  C  R  Y  F  L  Y  Q  I  L  R  G  L  K  Y
421 CCTCTGGCTGATGATCATTGTCGGTATTTTCTTTACCAAATTCTAAGAGGACTGAAATAC
161 V  H  S  A  H  V  L  H  R  D  L  K  P  S  N  L  L  L  N  A
481 GTTCATTCGGCACACGTGTTGCATCGTGATCTAAAACCAAGCAACTTACTTCTGAATGCA
181 N  C  D  L  K  I  G  D  F  G  L  A  R  T  T  S  E  T  D  F
541 AATTGTGACCTAAAAATTGGGGATTTTGGGCTTGCAAGAACCACTTCAGAAACGGATTTC
201 M  T  E  Y  V  V  T  R  W  Y  R  A  P  E  L  L  L  N  C  S
601 ATGACCGAATATGTTGTGACTCGTTGGTATCGCGCCCCTGAATTGCTCTTAAATTGTTCC
221 E  Y  T  A  A  I  D  I  W  S  V  G  C  I  L  G  E  I  L  T
661 GAGTACACGGCCGCCATTGACATCTGGTCCGTCGGCTGCATCCTTGGTGAGATCCTCACT
241 R  Q  P  L  F  P  G  K  D  Y  V  H  Q  L  R  L  I  T  E  L
721 CGACAGCCCTTGTTTCCAGGCAAAGATTATGTTCATCAGCTCAGACTTATCACAGAGCTC
261 I  G  S  P  D  D  A  S  L  G  F  L  R  S  D  N  A  R  R  Y
781 ATTGGTTCACCTGATGATGCAAGTCTTGGCTTTCTAAGAAGCGATAATGCAAGAAGATAT
281 V  R  Q  L  P  Q  Y  P  R  Q  Q  F  S  A  R  F  P  N  K  S
841 GTGAGACAGCTTCCTCAGTATCCAAGACAACAATTCTCTGCCAGATTCCCAAATAAATCC
301 P  G  A  L  D  L  L  E  K  M  L  V  F  D  P  N  R  R  I  T
901 CCTGGAGCTTTAGATCTGCTTGAAAAGATGCTTGTATTTGACCCAAACAGGCGTATTACA
321 V  D  E  A  L  C  H  P  Y  L  A  P  L  H  E  I  N  D  E  P
961 GTTGATGAGGCGTTATGTCACCCGTATTTGGCACCTCTTCATGAAATCAACGATGAGCCG
341 V  C  P  H  P  F  S  F  D  F  E  Q  P  S  C  T  E  E  H  I
1021 GTGTGCCCTCATCCTTTTAGCTTCGACTTTGAGCAGCCTTCATGCACTGAAGAACACATC
361 K  E  L  I  W  R  E  S  V  K  F  N  P  D  H  P  N  H  *
1081 AAAGAGCTTATTTGGAGGGAGTCTGTCAAATTCAATCCTGACCATCCAAATCACTGA
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Fig. 2: Nucleotide and amino acid sequence of *LsMAPK4*

This site is required for MAPK kinase activity and is a classical characteristic of MAPKs. It is concluded that the cDNA sequence cloned in this study is the full-length cDNA sequence of the lettuce MAPK gene (*LsMAPK4*).

The secondary structure of the LsMAPK4 protein was predicted by SOPMA, within the NPS server, and found to be composed of alpha helices, random curls, extended chains, and beta corners, with contents of 43.12%, 35.45%, 15.61% and 5.82% respectively (Fig. 5). The three-dimensional structure of LsMAPK4 was predicted by applying SWISS-MODEL software (Fig. 6).

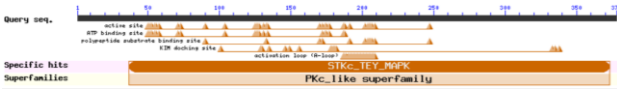

Fig. 3: Conserved domain of the LsMAPK4 protein

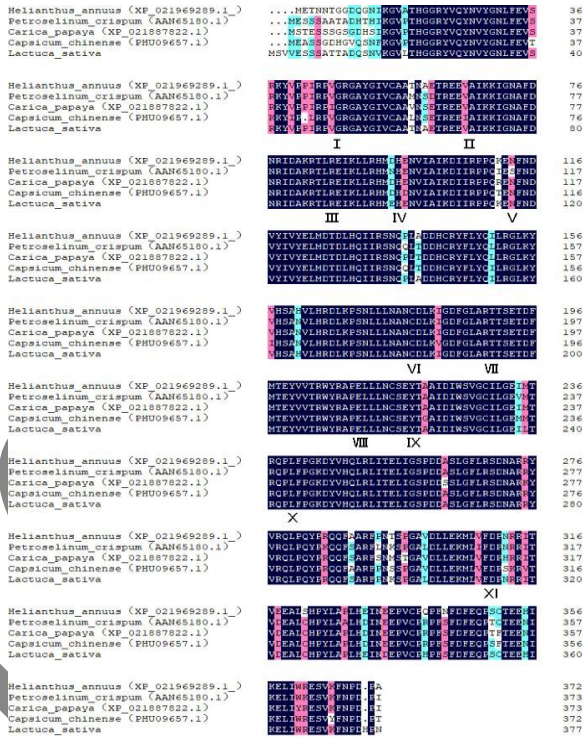

Fig. 4: Alignment and analysis of the amino acid sequence of LsMAPK4

Roman numerals I to XI represent eleven conserved catalytic domains. TEY" represents the
threonine and tyrosine phosphorylation site

Phylogenetic Analysis of LsMAPK4 Protein in Leaf Lettuce

The LsMAPK4 amino acid sequence obtained was aligned with MAPK4 sequences. LsMAPK4 was found to have a high degree of homology with the MAPK4 protein from 9 types of plants, including *Helianthus annuus*, *Petroselinum crispum*, *Carica papaya*, and *Capsicum chinense*. Lettuce and *Helianthus annuus* are both crops from the family *Asteraceae*, with a closer degree of homology, followed by *Capsicum chinense* and *Arabidopsis* (Fig. 7).

Analysis of *LsMAPK4* Gene Expression

The relative expression of the *LsMAPK4* gene in the roots and leaves are similar. In the stem, the expression was significantly higher than in the roots and leaves, suggesting that the gene may function in the stem (Fig. 8).

Fig. 5: The model of the secondary structure of the LsMAPK4 protein
(h: α-Helix; e: Extended strand; t: β corner; c: Random coil)

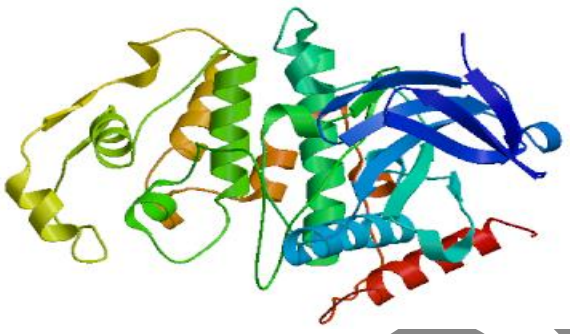

Fig. 6: The tertiary structure prediction of LsMAPK4

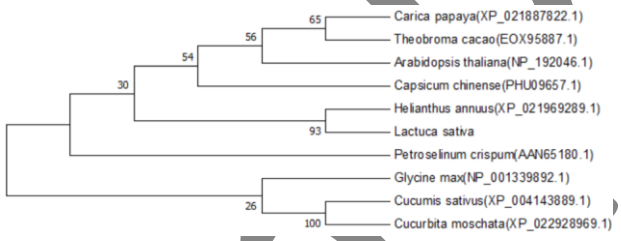

Fig. 7: Phylogenetic analysis of LsMAPK4

The expression of the *LsMAPK4* gene in lettuce leaves changed significantly under high temperature, and its expression was up-regulated in the high temperature group during the early stage of treatment (48 h). However, from the 8th day of treatment, the expression of *LsMAPK4* gene was down-regulated under high temperature conditions (Fig. 9).

Discussion

Since the 1990s, more MAPK family members have been found in plants than in animals and yeasts (Mizoguchi *et al.*, 1997). MAPK is the most downstream member of the MAPK signal cascade and has many substrates for phosphorylation, including protein kinases, transcription factors, and cytoskeletal binding proteins, (Rodriguez *et al.*, 2010). MAPK phosphorylates substrates to regulate the expression of specific genes, which ultimately initiates the corresponding physiological responses in organisms (Tena *et al.*, 2001; Zhang *et al.*, 2006). Plant MAPK signaling pathways play a crucial role in plant growth and development and various responses to stress and hormones (Zhang and Klessig, 2001; Nakagami *et al.*, 2005; Yoo and Sheen, 2008; Pitzschke *et al.*, 2009; Meng and Zhang, 2013). One study showed that in alfalfa plants, MMK3 is associated with cell division and is localized to phragmoplast (Bogre *et al.*, 1997). A study revealed that AtMAPK4 in *Arabidopsis* is associated with cell division and is necessary for formation of the cell plate (Kosetsu *et al.*, 2010). In addition, the MAPK3/6 protein of *Arabidopsis* has been shown to regulate pollen development by phosphorylating WRKY transcription factors (Guan *et al.*, 2014).

In this study, a newly identified gene in lettuce, containing a mitogen-activated protein kinases domain was cloned and named *LsMAPK4*. Phylogenetic tree analysis suggested that *LsMAPK4* clustered with the *Helianthus annuus* MAPK4 protein, which is highly conserved in many plant species and its subcellular localization was predicted to be in the Golgi bodies. From the results of expression analysis of *LsMAPK4* in various organs, we hypothesize that this gene may elicit its effects in lettuce stem. Quantitative PCR revealed that temperature also affects the expression of *LsMAPK4* while causing bolting in lettuce (Fig. 9). *LsMAPK4* expression in the group that was exposed to high temperature for 48 h was higher than that in the control environment. However, from the 8th day after treatment, the expression level in the high-temperature treatment group was lower than that in the control group. The results of our previous study indicated that after 8 days of high-temperature treatment, lettuce flower buds start to differentiate, and bolting begins. Thus, we hypothesize that the *LsMAPK4* gene may elicit its function during high-temperature bolting in lettuce. In addition, this occurs through increased expression levels of *LsMAPK4* in the first 8 days of high-temperature treatment, which leads to the phosphorylation of other substrates and results in the regulation of cell division and hormonal levels to promote lettuce bolting. On the 8th day of high-temperature treatment, when lettuce starts to bolt, the expression level of the *LsMAPK4* gene starts to become relatively stable, having achieved its function. Studies have shown that the expression of MAPK in oat aleurone cells (Huttly and Phillips, 1995) and peas (Marcote and Carbonell, 2000) is regulated by gibberellin (GA). Later, a study found that gibberellins play an important role in the bolting of lettuce (Fukuda *et al.*, 2012). However, the mechanism behind gibberellin regulating high temperature bolting in lettuce has not been fully revealed. A study in 2016 showed that MAPK11 might be related to the gibberellin signaling pathway in cotton (Wang *et al.*, 2016). We have reason to speculate that MAPK may affect lettuce bolting by regulating the gibberellin signaling pathway.

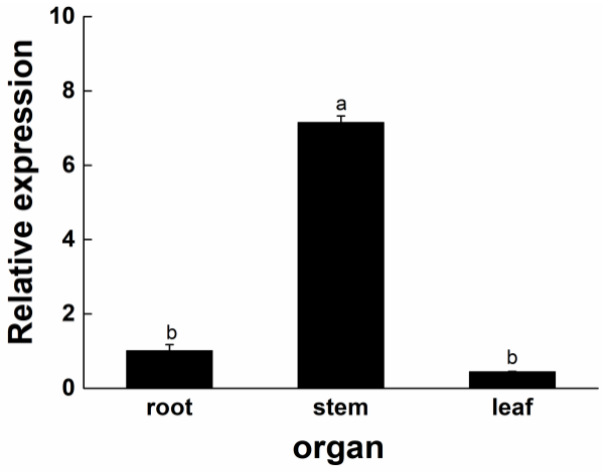

Fig. 8: Relative expression of *LsMAPK4* gene in different organs

Identical superscript letters indicate that the difference is not significant, whereas different superscript letters imply a significant difference. $P < 0.05$

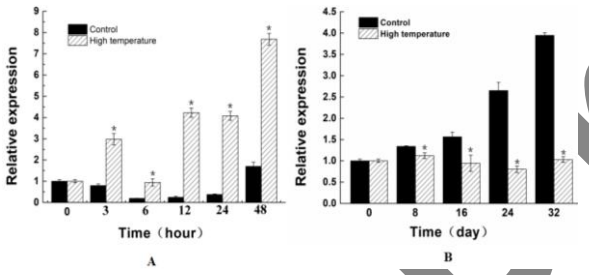

Fig. 9: Relative expression analysis of *LsMAPK4* under 20/13°C (Control) and 33/25°C (High temperature) of lettuce

* $P < 0.05$

Previous studies have shown that MAPK4 participates in cellular division in male gametophytes and plant hormone signaling pathways (Brodersen *et al*., 2006; Kosetsu *et al*., 2010; Zeng *et al*., 2011). In this study, we analyzed the protein structure of LsMAPK4 and its mode of gene expression under high-temperature treatment to provide a foundation to examine the biological functions of this gene during high-temperature bolting in lettuce and the molecular mechanisms behind high-temperature bolting.

Conclusion

In this study, the full-length *LsMAPK4* gene sequence was cloned from lettuce strain GB-30. This gene encoded 379 amino acids, which harbored the conserved domains of mitogen-activated protein kinases. qRT-PCR analysis showed that the expression of *LsMAPK4* in stems was significantly higher than in the roots and leaves. Under high temperature treatment, the expression of this gene was significantly higher than that of the control group. These results suggest that *LsMAPK4* might be a bolting-related gene in lettuce.

Acknowledgments

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