Phyton-International Journal of Experimental Botany
DOI:10.32604/phyton.2020.011882
Article

Phytosulfokine-α Promotes Root Growth by Repressing Expression of *Pectin Methylesterase Inhibitor* (*PMEI*) Genes in *Medicago truncatula*

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Received: 03 June 2020; Accepted: 14 June 2020

Abstract: Phytosulfokine-α (PSK-α), a sulfated pentapeptide with the sequence YIYTQ, is encoded by a small precursor gene family in Arabidopsis. PSK-α regulates multiple growth and developmental processes as a novel peptide hormone. Despite its importance, functions of PSK-α in *M. truncatula* growth remains unknown. In this study, we identified five genes to encode PSK-α precursors in *M. truncatula*. All of these precursors possess conserved PSK-α signature motif. Expression pattern analysis of these *MtPSK* genes revealed that each gene was expressed in a tissue-specific or ubiquitous pattern and three of them were remarkably expressed in root. Treatment of *M. truncatula* seedlings with synthetic PSK-α peptide significantly promoted root elongation. In addition, expression analysis of downstream genes by RNA-seq and qRT-PCR assays suggested that PSK-α signaling might regulate cell wall structure via PMEI-PME module to promote root cell growth. Taken together, our results shed light on the mechanism by which PSK-α promotes root growth in *M. truncatula*, providing a new resource for improvement of root growth in agriculture.

Keywords: Phytosulfokine-α; PSK-α; PMEI; root growth; *Medicago truncatula*

1 Introduction

Phytosulfokine (PSK), a disulfated pentapeptide, emerges as an important peptide hormone regulating multiple aspects of plant growth and development [1]. The first identified PSK is of the sequence Tyr(SO3H)-Ile-Tyr(SO3H)-Thr-Gln which was named PSK-α [2]. A C-terminal truncated form of PSK-α with extremely low activity was named PSK-β [3]. Our recent study identified a novel PSK with the sequence Tyr(SO3H)-Val-Tyr(SO3H)-Thr-Gln in soybean and referred to it as PSK-γ [4]. Among the three PSK analogs, PSK-α is a widespread peptide among plant species, including Arabidopsis [5–6], rice [7], carrot [8], cotton [9], soybean [4], etc. PSK-α is produced from precursor proteins which contain an N-terminal signal peptide sequence and the C-terminal PSK-α pentapeptide motif [1]. PSK-α matures through processing of precursors by post-translational sulfation in the Golgi apparatus [10] and subsequently by proteolytic cleavage in the apoplast [11]. PSK-α precursor gene family usually consists of multiple members. For instance, in Arabidopsis, five members were identified [5,12]; while in soybean, 14 members were reported [4]. These PSK-α precursor genes possess ubiquitous or organ-specific expression patterns, indicating differentiated functions among family members.

PSK-α signaling regulates various developmental events *in planta*. PSK-α was initially identified as an inducer for the division of cell cultures at low density [2]. Genetic evidences and peptide treatment assays revealed that PSK-α signaling promotes cell expansion and elongation to enhance plant organ growth in vivo [6,12–13]. Moreover, PSK-α has also been reported to positively regulate tracheary element differentiation [14], somatic embryogenesis [15–16], and leguminous root nodule formation [17]. Recently, Yu et al. reported that overexpression of PSK-α induces Arabidopsis male sterility by regulating cell wall development in anther, suggesting that this peptide also functions in reproductive development [6]. Nowadays, it is known that PSK-α is perceived by membrane-localized leucine-rich repeat (LRR) receptor kinases: PSKRs [5,8]. Binding of PSK-α stabilizes PSKR heterodimerization with a putative co-receptor, leading to allosteric activation of PSKR [18]. The intracellular kinase domain of PSKRs possesses overlapping serine/threonine kinase and guanylate cyclase activities [19–20], indicating dual signal outputs from the receptor. Recently, a plasma membrane-localized module composed of CNGC17, H^+-ATPase, and BAK1 was found to physically interact with PSKR to transduct PSK-α signaling in Arabidopsis [21]. However, despite increasing knowledge regarding the receptor, the signaling pathway, especially downstream key factors, remains unclear and needs further exploration.

Root growth depends mainly on cell division at the tip and cell expansion/elongation at the elongation zone. Plant cell growth in volume is regulated by the interplay between intercellular turgor pressure and cell wall. One of the principal cell wall components involved in regulation of cell growth is pectin [22]. In dicots, homogalacturonan (HG) is the major pectic polymer in primary cell walls and undergoes modifications such as methylesterification [23]. The degree of methylesterification heavily influences the biomechanical properties of HG pectins, thereby influences the flexibility of cell wall. Pectin methylesterases (PMEs) catalyze the removal of methyl-groups from the HG backbone, facilitating enzymatic degradation of pectin and therefore increasing cell growth [24]. The activity of PMEs is antagonistically regulated by a family of proteinaceous inhibitors named pectin methylesterase inhibitors (PMEIs). It has been reported that overexpression of a PMEI encoding gene, *AtPMEI4*, delayed hypocotyl growth acceleration in Arabidopsis [25]. Conversely, a mutant of *AtPMEI4* with elevated PME activity in root cell walls showed a remarkably increase in root length [26]. A similar study in rice also found that overexpression of *OsPMEI28* inhibited culm elongation which resulted in a dwarfed phenotype [27]. These findings suggest that PMEI acts as a negative regulator in cell growth process by inhibiting pectin de-methylesterification catalyzed by PME. Our previous work in Arabidopsis has reported that PSK-α induced cell growth by regulating cell wall development; the underlying mechanism, however, is not clear. Given the importance of pectin methylesterification in cell growth, the relationship between PSK-α signaling and PME/PMEI activities deserves investigation.

In this study, we systematically searched *M. truncatula* genome and found five genes to encode PSK-α precursors. These *MtPSK* genes exhibited a tissue-specific or a ubiquitous pattern and three of them were remarkably expressed in root. Treatment of *M. truncatula* seedlings with synthetic PSK-α peptide significantly promoted root growth. Furthermore, expression analysis of downstream genes revealed that expression of *PMEI* genes were repressed by PSK-α signaling, suggesting that PSK-α might regulate pectin methylesterification status in cell wall to promote root cell growth.

2 Materials and Methods

2.1 Plant Material and Growth Conditions

M. truncatula cv. Jemalong A17 seeds were scarified in concentrated sulfuric acid for 6 min, rinsed with water, and stratified at 4°C for 2 days. After stratification, the seeds were sown in soil for germination and growth. To examine PSK-α effect on root growth, scarified A17 seeds were surface sterilized in 10% (v/v) sodium hypochlorite solution for 3 min and subsequently rinsed 3 times with sterile water. The seeds were then stratified and germinated on vertical FM medium for 7 days.

2.2 Peptide Treatment

The PSK-α peptide ($Y_{SO3}IY_{SO3}TQ$) and the randomly arranged pentapeptide (TYQYI) were chemically synthesized by Chinese Peptide Company (Hangzhou, China). Each peptide was dissolved in sterilized water at the concentration of 1 mM, which was used as a peptide stock solution. Stratified *M. truncatula* seeds were sown on FM-agar plates containing 1 μM of each peptide. After 7 days of growth on the vertical plates, seedlings were photographed to measure root length using ImageJ software (National Institute of Health, Bethesda, MD, USA).

2.3 RNA Extraction and qRT-PCR Analysis

Quantitative RT-PCR (qRT-PCR) was employed to determine the expression patterns of *MtPSK* genes and transcript levels of genes regulated by PSK-α. Total RNA was extracted from *M. truncatula* tissues using an RNAprep pure Plant kit (Tiangen, Beijing, China) following the manufacturer's instructions. Residual DNA was removed by on-column digestion with DNase I. Reverse transcription was performed at 50°C for 15 min with 1 μg total RNA to generate cDNA using the HiScript II Q-RT Supermix kit (Vazyme, Nanjing, China). qRT-PCR was performed with the cDNA and gene-specific primers (Tab. 1), and the Hieff qPCR SYBR Green Master Mix (Yeasen, Shanghai, China) and a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) were used. Gene expression relative to *MtActinB* was calculated by the 2-ΔΔCt method.

Table 1: qRT-PCR primers used in this study

Gene	Orientation	Sequence (5'–3')
MtPSK1	Forward	AACTAGTCACCCTCTTCACC
	Reverse	GAAACAATATCCTCGTGTAGT
MtPSK2	Forward	ATTGCAGCCTTGTTCTTCATG
	Reverse	ACTGCATCAACATCCAGTTTC
MtPSK3	Forward	CCCATTTGCCTGGCAGTGTTC
	Reverse	CCTTGTCATCTTCCAAAACCTC
MtPSK4	Forward	CTACTTTCTTCCTCAATACTC
	Reverse	CCATTCCTAACATCTTCAAAG
MtPSK5	Forward	AGCTTCTCCTCGCCTCATTG
	Reverse	CTCTAATCCCATTAGTTGTTC
Medtr4g124855	Forward	CATGCGCACAAGCAAGATACC
	Reverse	GGACCCTGCTACCATATTGG
Medtr0458s0010	Forward	GGCTACATTTGCTGTAGTTGG
	Reverse	ACAATGGGTCCCTGATAACCC
MtActinB	Forward	AACTGGAATGGTCAAGGCTGG
	Reverse	TATAGGATACTTCAACGTGAGG

2.4 RNA Sequencing (RNA-seq)

Total RNA (approx. 10 μg) from peptide-treated A17 roots were extracted as described above. Samples of three independent treatments were pooled together. The RNA-seq libraries were constructed according to Illumina standard instruction and were sequenced with an Illumina HiSeq 2500 system by Shanghai

Biotechnology Corporation (Shanghai, China). The generated paired-end reads were mapped to the *M. truncatula* genome. Gene transcription levels were normalized as fragments per kilobase of exon per million mapped reads (FPKM). Differentially expressed genes were identified as those with a fold change and *P*-value of differential expression above the threshold (fold change >2.0, $p < 0.05$).

3 Results

3.1 PSK Precursor Proteins in M. truncatula

Using Arabidopsis *PSK* sequences as queries, we searched the genome database of *M. truncatula* (Mt4.0v1, https://phytozome.jgi.doe.gov/pz/portal.html) and identified five putative *MtPSK* genes encoding precursor proteins. These precursors have a length from 76 to 93 amino acids, a typical length of PSK precursors [1], and were designated MtPSK1 (Medtr1g017700), MtPSK2 (Medtr5g015140), MtPSK3 (Medtr1g106030), MtPSK4 (Medtr2g084855), and MtPSK5 (Medtr1g061120) (Fig. 1A). Sequence alignment showed that, similar with Arabidopsis PSK precursor proteins, the MtPSK family members are relatively conserved with respect to the PSK-α signature motif in the C-terminal half. Specifically, the YIYTQ pentapeptide sequence is highly conserved among members; and the aspartate residue at the −1 position of the peptide sequence and the cysteine, di-basic amino acids, and histidine are also well conserved (Fig. 1A). These conserved amino acids are essential for sulfonylation and proteolytic cleavage to form bioactive PSK-α peptide. Phylogenetic tree analysis demonstrated that the MtPSK members are close orthologs of the Arabidopsis PSK members (Fig. 2), indicating functional conservation of PSK in the evolution.

Figure 1: Sequence alignment of PSK precursor proteins in *Arabidopsis thaliana* and *Medicago truncatula*. The amino acid sequences of these precursor proteins were aligned using BioEdit software. Stars indicate identical amino acids and dots indicate the conserved di-basic amino acids essential for mature PSK peptides formation. The PSK-α pentapeptide motifs near the C-terminus of the precursor proteins are underlined

3.2 Expression Patterns of MtPSK Genes

To investigate the expression patterns of *MtPSK* genes in various tissues of *M. truncatula*, qRT-PCR was performed. The results showed that each gene was expressed in a tissue-specific or ubiquitous pattern. Of the five *MtPSK* genes, *MtPSK1* was dominantly expressed in root, root nodule, and pod; *MtPSK2* was mainly detected in root, root nodule, and seed; while *MtPSK3* showed a ubiquitous expression pattern with the highest expression in stem and pod. By contrast, *MtPSK4* exhibited a seed-specific expression pattern,

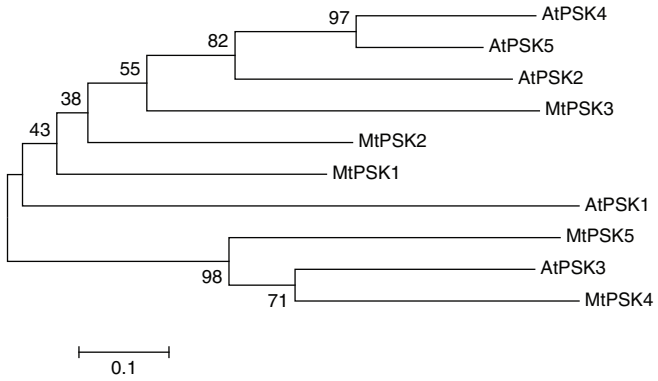

Figure 2: Phylogenetic tree of AtPSK and MtPSK family members. The tree was constructed using MEGA6 software with the neighbor-joining method. The bootstrap values from 1000 replications were included

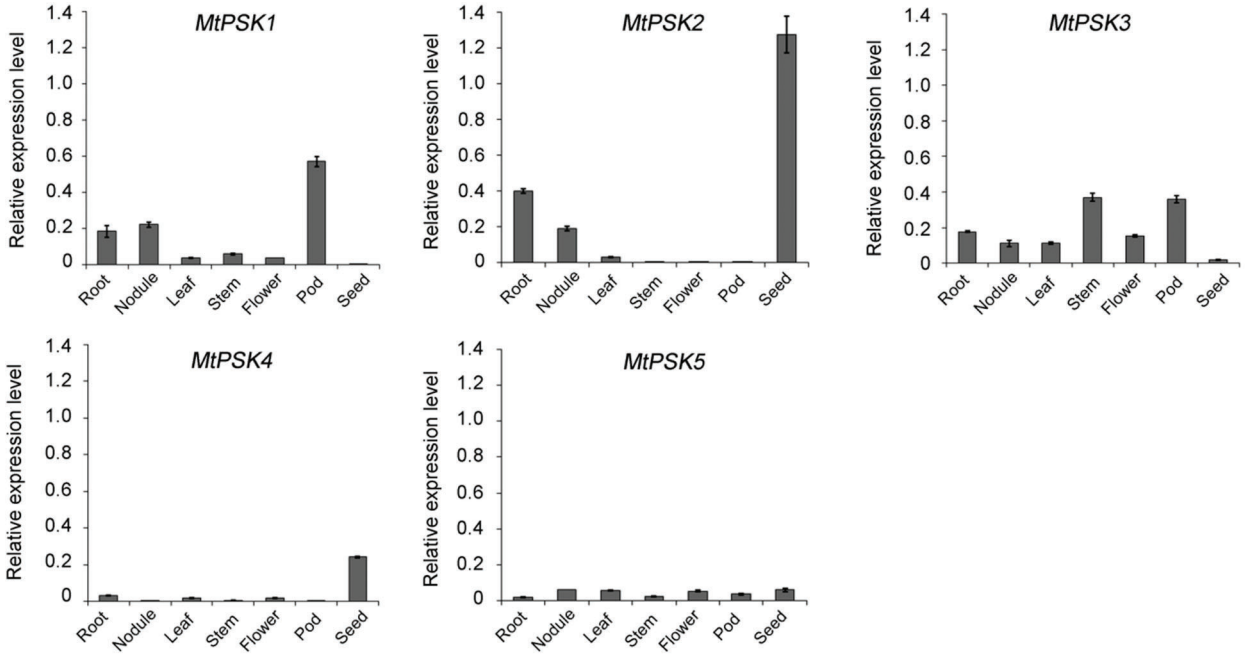

Figure 3: qRT-PCR analyses of the expression pattern of *MtPSK* family genes in various tissues. Values are the mean ± SD of three independent biological replicates normalized against the reference gene *MtActinB*

while *MtPSK5*, the last one, had a ubiquitous but relatively low expression pattern (Fig. 3). In terms of root, three out of the five *MtPSK* genes, *MtPSK1*, *−2*, and *−3*, were significant expressed, suggesting roles of PSK-α signaling in *M. truncatula* root growth and development.

3.3 PSK-α Promotes M. truncatula Root Growth

To test the possible effect of PSK-α on root growth, we treated *M. truncatula* seedlings on plate with 1 μM synthetic sulfated PSK-α peptide. Seedlings treated with 1 μM randomly arranged pentapeptide were taken as a control. At 7 days of age on vertical plates, PSK-α treatment caused an approx. 22% increase in root length compared to seedlings treated with random peptide (Figs. 4A, 4B). These results demonstrate that PSK-α signaling has a promotive effect on *M. truncatula* root elongation.

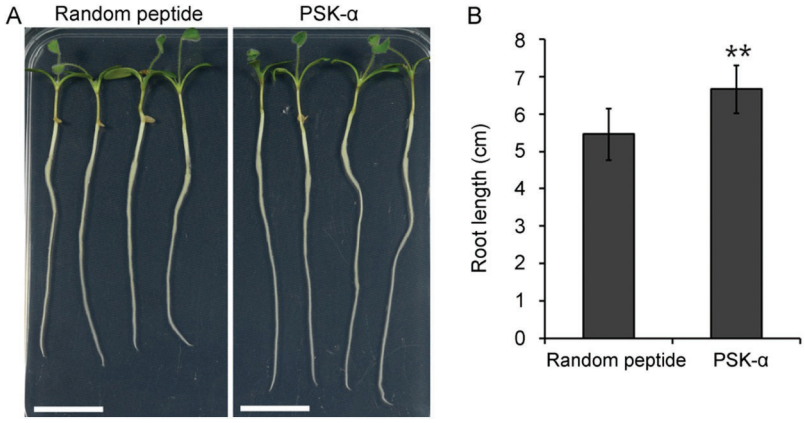

Figure 4: Application of PSK-α peptide promotes root growth in *M. truncatula*. A) Seven-day-old *M. truncatula* (A17) seedlings grown on vertical FM plates containing 1 μM PSK-α peptide or 1 μM randomly arranged pentapeptide. Bars = 2 cm. B) Measurement of root length of the *M. truncatula* seedlings shown in A. Values are the mean ± SD from three independent biological replicates with a total of 30 seedlings per treatment. **, $p < 0.01$, student's *t*-test

3.4 PSK-α Represses Expression of PMEI Genes in M. truncatula Roots

To investigate the mechanism by which PSK-α promotes root growth, we performed RNA-sequencing (RNA-seq) assay to identify downstream genes that differentially expressed in *M. truncatula* roots treated with PSK-α or random peptide. Among the 27,066 transcripts detected by RNA-seq, differentially expressed genes were identified as those with a threshold fold change > 2 and *p*-value < 0.05. According to this criterion, 61 genes were significantly up-regulated (Tab. S1) and 55 genes were down-regulated (Tab. S2) in PSK-α-treated roots relative to the control. Among these genes, two *PMEI* genes *Medtr4g124855* and *Medtr0458s0010* which are putative cell expansion inhibitors were significantly down-regulated (Tab. S2). Repression of these two genes' expression by PSK-α signaling was further validated by qRT-PCR analysis (Fig. 5). These results demonstrate that PSK-α promotes *M. truncatula* root growth by repressing *PMEI* expression.

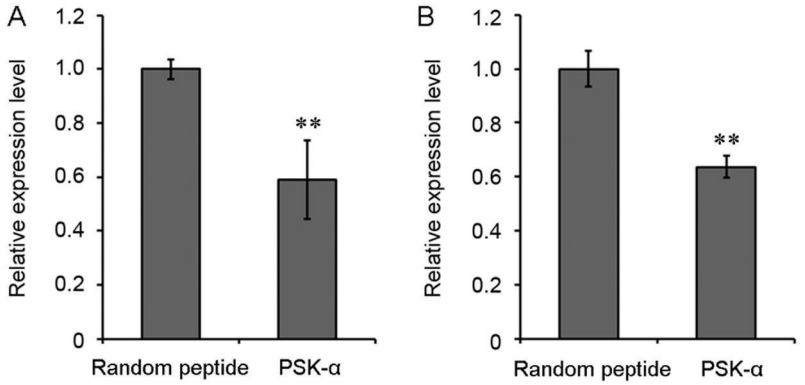

Figure 5: Treatment of PSK-α peptide represses expression of two *Pectin Methylesterase Inhibitor* (*PMEI*) genes in *M. truncatula* roots. Ten-day-old A17 seedlings were treated with 1 μM PSK-α peptide or 1 μM randomly arranged pentapeptide for 12 h, and qRT-PCR was used to examine the expression levels of two *PMEI* genes, *Medtr4g124855* (A) and *Medtr0458s0010* (B), in the treated roots. Values are the mean ± SD of three independent biological replicates normalized against the reference gene *MtActinB*. **, $p < 0.01$, student's *t*-test

4 Discussion

To date, PSK-α peptide and its precursor genes have been extensively reported in a variety of plant species. However, little is known about PSK-α in leguminous plants, especially in the model legume species, *M. truncatula*. In this study, we have systematically searched *M. truncatula* genome and found five genes to encode PSK-α precursors. Like in Arabidopsis, MtPSK family members are conserved at the PSK-α signature motif in the C-terminal half, including the YIYTQ pentapeptide and several key amino acids essential for peptide maturation, indicating conservation of PSK family during plant evolution.

Expression pattern analyses of the five *MtPSK* genes revealed that each gene was expressed in a tissue-specific or ubiquitous pattern. On the other hand, with respect to each organ, one or more genes were significantly expressed, suggesting that PSK-α functions throughout the whole *M. truncatula* plant. Our previous studies have shown that PSK-α and its analog PSK-γ positively regulate root nodule and seed development, respectively, in legume species. In detail, PSK-α promotes nodule formation by interfering with JA signaling in *Lotus japonicas*, another model legume species [17]; while expression of PSK-γ induces cell expansion to increase seed growth and yield in soybean [4]. Nonetheless, PSK-α's function in other organs of leguminous plants has not yet been elucidated. In the present study, three *MtPSK* genes (*MtPSK1*, *−2*, and *−3*) were found to be significant expressed in *M. truncatula* root, and application of PSK-α peptide markedly promoted root elongation, indicating an important role of PSK-α signaling in leguminous root growth.

Except for root, PSK-α has also been reported to promote the growth of leaf and hypocotyl [6,13]. However, the underlying mechanism is largely unclear. Here, through RNA-seq and qRT-PCR assays, we found that expression of *PMEI* genes were down-regulated by PSK-α signaling in *M. truncatula* root. PMEIs are inhibitors of PMEs, the latter catalyze the de-methylesterification of pectin to increase cell wall extensibility and promote cell growth [24,28]. On the basis of these results, we propose a model to illustrate the mechanism of PSK-α signaling in promoting root growth. As shown in Fig. 6, the PSK-α peptide represses expression of *PMEI* genes, reducing their inhibitory effect against PME. The elevated PME activity induces cell wall loosening by modulating degree of methylesterification in pectin, leading to cell growth in volume and eventually promoting root growth.

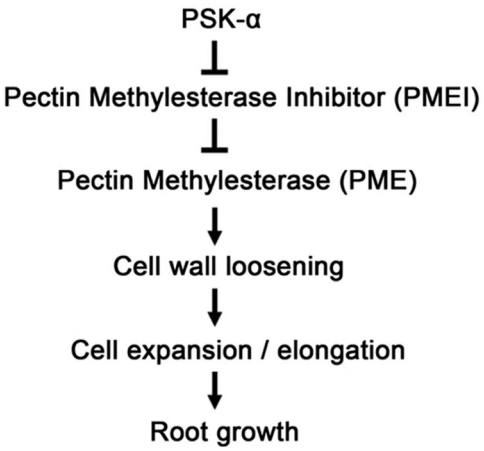

Figure 6: Proposed model illustrating the mechanism of PSK-α signaling in promoting root growth. The PSK-α signaling represses expression of *Pectin Methylesterase Inhibitor* (*PMEI*) genes, therefore attenuating the inhibitory effect against Pectin Methylesterase (PME). The elevated PME activity induces cell wall loosening by modulating pectin structure, leading to cell expansion and elongation and eventually root growth

5 Conclusions

This research identified five PSK-α-encoding precursor genes in *M. truncatula* genome. These *MtPSK* genes exhibited a tissue-specific or ubiquitous pattern and three of them were remarkably expressed in root. Treatment with PSK-α peptide promoted *M. truncatula* root growth by repressing expression of *PMEI* genes, suggesting that PSK-α might regulate pectin methylesterification status to induce root growth.

Funding Statement: This work is supported by the National Natural Science Foundation of China (31500197).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Sauter, M. (2015). Phytosulfokine peptide signalling. *Journal of Experimental Botany, 66(17),* 5161–5169. DOI 10.1093/jxb/erv071.
2. Matsubayashi, Y., Sakagami, Y. (1996). Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of Asparagus officinalis L. *Proceedings of the National Academy of Sciences of the United States of America, 93(15),* 7623–7627. DOI 10.1073/pnas.93.15.7623.
3. Matsubayashi, Y., Hanai, H., Hara, O., Sakagami, Y. (1996). Active fragments and analogs of the plant growth factor, phytosulfokine: structure-activity relationships. *Biochemical and Biophysical Research Communications, 225(1),* 209–214. DOI 10.1006/bbrc.1996.1155.
4. Yu, L., Liu, Y., Zeng, S., Yan, J., Wang, E. et al. (2019). Expression of a novel PSK-encoding gene from soybean improves seed growth and yield in transgenic plants. *Planta, 249(4),* 1239–1250. DOI 10.1007/s00425-019-03101-w.
5. Matsubayashi, Y., Ogawa, M., Kihara, H., Niwa, M., Sakagami, Y. (2006). Disruption and overexpression of Arabidopsis phytosulfokine receptor gene affects cellular longevity and potential for growth. *Plant Physiology, 142(1),* 45–53. DOI 10.1104/pp.106.081109.
6. Yu, L., Liu, Y., Li, Q., Tang, G., Luo, L. (2016). Overexpression of phytosulfokine-alpha induces male sterility and cell growth by regulating cell wall development in Arabidopsis. *Plant Cell Reports, 35(12),* 2503–2512. DOI 10.1007/s00299-016-2050-7.
7. Yang, H., Matsubayashi, Y., Nakamura, K., Sakagami, Y. (1999). Oryza sativa PSK gene encodes a precursor of phytosulfokine-alpha, a sulfated peptide growth factor found in plants. *Proceedings of the National Academy of Sciences of the United States of America, 96(23),* 13560–13565. DOI 10.1073/pnas.96.23.13560.
8. Matsubayashi, Y., Ogawa, M., Morita, A., Sakagami, Y. (2002). An LRR receptor kinase involved in perception of a peptide plant hormone, phytosulfokine. *Science, 296(5572),* 1470–1472. DOI 10.1126/science.1069607.
9. Han, J., Tan, J., Tu, L., Zhang, X. (2014). A peptide hormone gene, GhPSK promotes fibre elongation and contributes to longer and finer cotton fibre. *Plant Biotechnology Journal, 12(7),* 861–871. DOI 10.1111/pbi.12187.
10. Komori, R., Amano, Y., Ogawa-Ohnishi, M., Matsubayashi, Y. (2009). Identification of tyrosylprotein sulfotransferase in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America, 106(35),* 15067–15072. DOI 10.1073/pnas.0902801106.
11. Srivastava, R., Liu, J. X., Howell, S. H. (2008). Proteolytic processing of a precursor protein for a growth-promoting peptide by a subtilisin serine protease in Arabidopsis. *Plant Journal, 56(2),* 219–227. DOI 10.1111/j.1365-313X.2008.03598.x.
12. Kutschmar, A., Rzewuski, G., Stuhrwohldt, N., Beemster, G. T., Inze, D. et al. (2009). PSK-alpha promotes root growth in Arabidopsis. *New Phytologist, 181(4),* 820–831. DOI 10.1111/j.1469-8137.2008.02710.x.
13. Stuhrwohldt, N., Dahlke, R. I., Steffens, B., Johnson, A., Sauter, M. (2011). Phytosulfokine-alpha controls hypocotyl length and cell expansion in Arabidopsis thaliana through phytosulfokine receptor 1. *PLoS One, 6 (6),* e21054. DOI 10.1371/journal.pone.0021054.
14. Matsubayashi, Y., Takagi, L., Omura, N., Morita, A., Sakagami, Y. (1999). The endogenous sulfated pentapeptide phytosulfokine-alpha stimulates tracheary element differentiation of isolated mesophyll cells of zinnia. *Plant Physiology, 120(4),* 1043–1048. DOI 10.1104/pp.120.4.1043.

15. Hanai, H., Matsuno, T., Yamamoto, M., Matsubayashi, Y., Kobayashi, T. et al. (2000). A secreted peptide growth factor, phytosulfokine, acting as a stimulatory factor of carrot somatic embryo formation. *Plant and Cell Physiology, 41(1),* 27–32. DOI 10.1093/pcp/41.1.27.
16. Igasaki, T., Akashi, N., Ujino-Ihara, T., Matsubayashi, Y., Sakagami Y. et al. (2003). Phytosulfokine stimulates somatic embryogenesis in Cryptomeria japonica. *Plant and Cell Physiology, 44(12),* 1412–1416. DOI 10.1093/pcp/pcg161.
17. Wang, C., Yu, H., Zhang, Z., Yu, L., Xu, X. et al. (2015). Phytosulfokine is involved in positive regulation of Lotus japonicus nodulation. *Molecular Plant-Microbe Interactions, 28(8),* 847–855. DOI 10.1094/MPMI-02-15-0032-R.
18. Wang, J., Li, H., Han, Z., Zhang, H., Wang, T. et al. (2015). Allosteric receptor activation by the plant peptide hormone phytosulfokine. *Nature, 525(7568),* 265–268. DOI 10.1038/nature14858.
19. Hartmann, J., Fischer, C., Dietrich, P., Sauter, M. (2014). Kinase activity and calmodulin binding are essential for growth signaling by the phytosulfokine receptor PSKR1. *Plant Journal, 78(2),* 192–202. DOI 10.1111/tpj.12460.
20. Kwezi, L., Ruzvidzo, O., Wheeler, J. I., Govender, K., Iacuone, S. et al. (2011). The phytosulfokine (PSK) receptor is capable of guanylate cyclase activity and enabling cyclic GMP-dependent signaling in plants. *Journal of Biological Chemistry, 286(25),* 22580–22588. DOI 10.1074/jbc.M110.168823.
21. Ladwig, F., Dahlke, R. I., Stührwohldt, N., Hartmann, J., Harter, K. et al. (2015). Phytosulfokine regulates growth in Arabidopsis through a response module at the plasma membrane that includes CYCLIC NUCLEOTIDE-GATED CHANNEL17, H+-ATPase, and BAK1. *Plant Cell, 27(6),* 1718–1729. DOI 10.1105/tpc.15.00306.
22. Palin, R., Geitmann, A. (2012). The role of pectin in plant morphogenesis. *Biosystems, 109(3),* 397–402. DOI 10.1016/j.biosystems.2012.04.006.
23. Wolf, S., Mouille, G., Pelloux, J. (2009). Homogalacturonan methyl-esterification and plant development. *Molecular Plant, 2(5),* 851–860. DOI 10.1093/mp/ssp066.
24. Wormit, A., Usadel, B. (2018). The multifaceted role of pectin methylesterase inhibitors (PMEIs). *International Journal of Molecular Sciences, 19(10),* 2878. DOI 10.3390/ijms19102878.
25. Pelletier, S., Van Orden, J., Wolf, S., Vissenberg, K., Delacourt, J. et al. (2010). A role for pectin de-methylesterification in a developmentally regulated growth acceleration in dark-grown Arabidopsis hypocotyls. *New Phytologist, 188(3),* 726–739. DOI 10.1111/j.1469-8137.2010.03409.x.
26. Senechal, F., Mareck, A., Marcelo, P., Lerouge, P., Pelloux, J. (2015). Arabidopsis PME17 activity can be controlled by pectin methylesterase inhibitor4. *Plant Signaling & Behavior, 10(2),* e983351. DOI 10.4161/15592324.2014.983351.
27. Nguyen, H. P., Jeong, H. Y., Jeon, S. H., Kim, D., Lee, C. (2017). Rice pectin methylesterase inhibitor28 (OsPMEI28) encodes a functional PMEI and its overexpression results in a dwarf phenotype through increased pectin methylesterification levels. *Journal of Plant Physiology, 208,* 17–25. DOI 10.1016/j.jplph.2016.11.006.
28. Levesque-Tremblay, G., Pelloux, J., Braybrook, S. A., Muller, K. (2015). Tuning of pectin methylesterification: consequences for cell wall biomechanics and development. *Planta, 242(4),* 791–811. DOI 10.1007/s00425-015-2358-5.