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Commentary

Phytosulfokine-δ: A Small Peptide, but a Big Player in Symbiosis Gene Regulation

Gurparsad Singh Suri [1] and Manish Tiwari [2,*]

[1] California Baptist University, Riverside, CA 92504, USA
[2] Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53705, USA
* Correspondence: mtiwari5@wisc.edu

Abstract: Nitrogen availability is one of the critical determinants of agricultural yield. Biological nitrogen fixation, such as legume–rhizobia symbiotic association, might function as a solution to fix nitrogen. Using phytosulfokine (PSK)-α sequences as a query, Yu et al., 2022 performed a comprehensive genome-wide search of legume species to identify PSK-δ, a divergent pentapeptide differing in single amino acid. Furthermore, PSK-δ exhibited nodule-specific expression with lower expression in the root, substantiating the nodule-specific temporal expression and suggesting its role in nodule development and nitrogen fixation. Additionally, in planta functional characterization in *Medicago truncatula* using overexpression and *Tnt1*-insertion mutant analysis indicated the role of PSK-δ in symbiotic nodulation. Interestingly, a similar phenotype of *MtPSKδ* mutant (*mtpskδ*) with that of wild-type control led to the hypothesis of its functional redundancy with PSK-α in nodule organogenesis. Further investigation regarding its position in the Nod-factor signaling pathway revealed the downstream function of PSK-δ in association with *MtENOD11* in regulating nodule formation.

Keywords: *Glycine max*; *Lotus japonicus*; *Medicago truncatula*; phytosulfokine; root nodule symbiosis; small-signaling peptide; *Tnt1* mutant

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Nitrogen is involved in building DNA and proteins; however, plants can procure minimal soil inorganic nitrogen [1]. Therefore, nitrogen availability is a key factor determining agricultural yield. Biological nitrogen fixation (BNF), such as the legume–rhizobia symbiotic association, appeared as a solution to fix nitrogen [2,3]. Legume–rhizobia interaction starts with the secretion of flavonoids by the legumes, which acts as a signal for rhizobia; in turn, rhizobia secrete lipo-chitooligosaccharides (LCOs) or Nod-factors in response to plant flavonoids [4]. Upon LCOs perception by Nod-factor receptors, a phosphorylation signaling cascade is activated, also known as the common symbiotic pathway (CSP), leading to calcium spiking [5]. Several essential proteins, such as DMI1, DMI2 and DMI3, participate in the CSP to induce calcium spiking. The calcium spiking is interpreted by calcium calmodulin-dependent protein kinase to activate the transcription of important genes such as *NIN*, *NSP2* and *ENOD11*, etc. [6]. The activation of these genes is accompanied by rhizobial entry into the host. Simultaneously the cortical cells divide to facilitate the formation of a niche for bacteria known as root nodules. Phytohormones auxin and cytokinin have been well characterized in inducing nodule organogenesis [7].

Small-signaling peptides recently appeared as signaling peptide species regulating different plant developmental processes, including arbuscular mycorrhizae association and root nodulation [8]. Phytosulfokine-α (PSK-α) is a signaling peptide (disulfated pentapeptide) perceived by leucine-rich repeat receptor kinases that induce cell expansion or elongation [9]. Genetic study and exogenous peptide application revealed that PSK-α mediated cell expansion or elongation in the root, hypocotyl, and leaf promotes their growth.

Additionally, PSK-α regulates in vitro regeneration of recalcitrant legumes, somatic embryogenesis, immune responses, and drought-induced flower drop [10]. PSK-α is a positive regulator of nodule number and promotes nodule maturation from the primordium [11].

Yu et al., 2022 searched the genomes of legume species using reported PSK-α sequences as a query [11–14]. During their search, they identified legume-specific genes encoding pentapeptides differing in one amino acid, i.e., YIYTN instead of YIYTQ, and named as PSK-δ. Only one gene encoding PSK-δ was identified in *Medicago truncatula* and *Lotus japonicus.* In contrast, two genes were identified in *Glycine max.* The PSK-δ genes were identified from the three legumes; however, the experiments were performed in *M. truncatula* and *L. japonicus.* PSK-δ precursor proteins were ~80 amino acids long with an N-terminal secretory peptide and the pentapeptide motif at C-terminus. The expression pattern analysis revealed that the PSK-δ gene showed nodule-specific expression in all three species. Simultaneously, the nodule/root-specific peptide nature of PSK-δ was also established. Temporal expression pattern analysis in nodule tissue revealed that the PSK-δ gene was majorly involved in nodule development. Further, a promoter: *GUS* assay using *pMtPSKδ:GUS* transformation into hairy root displayed a GUS signal from the developing primordia, mainly in meristematic and infection zones.

Since the PSK-δ expression was mainly localized in nodules, the authors decided to perform *in planta* functional characterization using overexpression and *Tnt1*-insertion mutant analysis. Constitutive overexpression of PSK-δ resulted in an increased pink and white nodule number and a slight increase in primary root length. Moreover, the number of nodule primordia increased. However, infection thread formation remained unaffected, suggesting that *MtPSKδ* enhances nodule organogenesis, thereby promoting nodulation. To rule out the role of shoot signaling in the regulation of nodule numbers because of constitutive expression using 35S promoter, authors expressed the gene in the root by hairy root transformation. The expression in root tissue resulted in an increase in nodule number by 54% compared to the wild type, indicating a direct role of *MtPSKδ* in promoting nodulation. In addition to overexpression, the exogenous application of pentapeptide to *M. truncatula* and *L. japonicus* seedlings increased the nodule numbers. Thus, *MtPSKδ* has a conserved promotive effect across different legume species irrespective of determinate or indeterminate nodulation types.

It was expected that the mutant of *MtPSKδ* should exhibit a phenotype opposite to one observed upon overexpression or exogenous application of *MtPSKδ.* Interestingly, the homozygous *Tnt1*-insertion mutant of *MtPSKδ* (*mtpskδ*) showed similar parameters in terms of shoot height, root length, nodule number, shape, size, and color compared to wild type control. In a previous investigation by the same authors, they found that the *MtPSKα* family has three members, *MtPSKα1*, *MtPSKα2*, and *MtPSKα3*, with *MtPSKα1* showing the highest expression [13]. The authors performed RNA interference of the *MtPSKα1* gene to check the functional relevance of *MtPSKα1* during nodulation. In the RNAi lines, the expression of *MtPSKα1* was decreased; however, no change was observed in the expression of *MtPSKα2*, *MtPSKα3*, and *MtPSKδ.* The nodule number in *MtPSKα1*-RNAi lines remained the same as in the control. Therefore, the authors hypothesized that the unexpected phenotype of *MtPSKδ* knockout might be due to *MtPSKα* expression, and there is a functional redundancy between these two. To check this hypothesis, *MtPSKα1*-RNAi was performed in the *mtpskδ Tnt1* mutant line. The *MtPSKα1*-RNAi in *mtpskδ Tnt1* mutant background resulted in decreased shoot height, fresh weight, and several pink and white nodules. Additionally, the number of infection threads remained unaffected; however, a ~41% decrease in nodule primordia was observed. Therefore, these results prove that nodule organogenesis in *M. truncatula* is redundantly regulated by PSK-α and PSK-δ (Figure 1).

Finally, the authors tried to place the gene in the Nod-factor signaling pathway. Treatment of the seedlings with PSK-δ peptide for 12 and 24 h, followed by qRT-PCR expression analysis of three early nodulation genes, *MtNIN*, *MtERN1*, and *MtENOD40*, revealed no change in expression, indicating that PSK-δ does not function upstream of Nod-factor. How-

ever, the expression of *MtPSKδ* increased only in wild-type plants but not in Nod-factor signaling mutants *nfp*, *dmi3*, *nsp1*, and *nin*, suggesting that Nod-factor signaling induces *MtPSKδ* expression. The authors checked the expression of Nod-factor-inducible genes after PSK-δ treatment and found that only *MtENOD11* expression was elevated. Hence, it was proposed that PSK-δ functions downstream of Nod-factor signaling and regulates nodule formation through *MtENOD11* expression. The researchers concluded that the PSK-δ is a novel kind of PSK that is widespread in different legume species and is essential for many functions related to development, growth, and legume plants' immune response. Overall, the study highlights the importance of PSKs in plant growth and the potential for these small peptide hormones to be utilized for practical applications in agriculture.

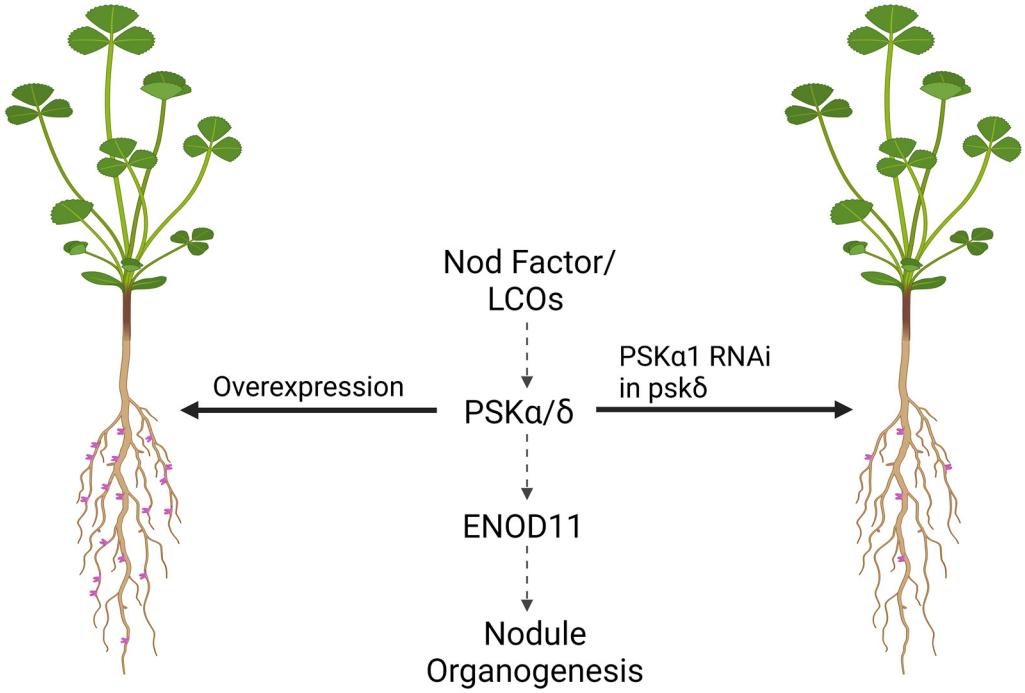

Figure 1. A schematic representation showing the role of PSKs in nodule organogenesis. PSKα and PSKδ are activated downstream of Nod-factors or LCOs perception and activate ENOD11 expression. The overexpression of PSKα/δ results in increased nodulation (left). PSKα/δ act redundantly towards each other, with decreased nodulation phenotype observed only in RNAi lines of PSKα in pskδ mutants (*Tnt1* lines) (right). Dashed arrows indicate the proposed activation of genes in the common symbiotic pathway. The figure compares only the nodule numbers. Figure created using Biorender.

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References

1. Tiwari, M.; Bhatia, S. Expression Profiling of MiRNAs Indicates Crosstalk between Phytohormonal Response and Rhizobial Infection in Chickpea. *J. Plant Biochem. Biotechnol.* **2020**, *29*, 380–394. [CrossRef]
2. Tiwari, M.; Pandey, V.; Singh, B.; Yadav, M.; Bhatia, S. Evolutionary and Expression Dynamics of LRR-RLKs and Functional Establishment of KLAVIER Homolog in Shoot Mediated Regulation of AON in Chickpea Symbiosis. *Genomics* **2021**, *113*, 4313–4326. [CrossRef] [PubMed]
3. Tiwari, M.; Yadav, M.; Singh, B.; Pandey, V.; Nawaz, K.; Bhatia, S. Evolutionary and Functional Analysis of Two-Component System in Chickpea Reveals CaRR13, a TypeB RR, as Positive Regulator of Symbiosis. *Plant Biotechnol. J.* **2021**, *19*, 2415–2427. [CrossRef] [PubMed]

4. Roy, S.; Liu, W.; Nandety, R.S.; Crook, A.; Mysore, K.S.; Pislariu, C.I.; Frugoli, J.; Dickstein, R.; Udvardi, M.K. Celebrating 20 Years of Genetic Discoveries in Legume Nodulation and Symbiotic Nitrogen Fixation. *Plant Cell* **2020**, *32*, 15–41. [CrossRef] [PubMed]
5. Rush, T.A.; Puech-Pagès, V.; Bascaules, A.; Jargeat, P.; Maillet, F.; Haouy, A.; Maës, A.Q.; Carriel, C.C.; Khokhani, D.; Keller-Pearson, M.; et al. Lipo-Chitooligosaccharides as Regulatory Signals of Fungal Growth and Development. *Nat. Commun.* **2020**, *11*, 3897. [CrossRef] [PubMed]
6. Oldroyd, G.E.D. Speak, Friend, and Enter: Signalling Systems That Promote Beneficial Symbiotic Associations in Plants. *Nat. Rev. Microbiol.* **2013**, *11*, 252–263. [CrossRef] [PubMed]
7. Suzaki, T.; Ito, M.; Kawaguchi, M. Genetic Basis of Cytokinin and Auxin Functions during Root Nodule Development. *Front. Plant Sci.* **2013**, *4*, 42. [CrossRef]
8. Roy, S.; Müller, L.M. A Rulebook for Peptide Control of Legume-Microbe Endosymbioses. *Trends Plant Sci.* **2022**, *27*, 870–889. [CrossRef] [PubMed]
9. Kutschmar, A.; Rzewuski, G.; Stührwohldt, N.; Beemster, G.T.S.; Inzé, D.; Sauter, M. PSK-α Promotes Root Growth in Arabidopsis. *New Phytol.* **2009**, *181*, 820–831. [CrossRef] [PubMed]
10. Di, Q.; Li, Y.; Zhang, D.; Wu, W.; Zhang, L.; Zhao, X.; Luo, L.; Yu, L. A Novel Type of Phytosulfokine, PSK-ε, Positively Regulates Root Elongation and Formation of Lateral Roots and Root Nodules in *Medicago truncatula*. *Plant Signal. Behav.* **2022**, *17*, 2134672. [CrossRef] [PubMed]
11. Wang, C.; Yu, H.; Zhang, Z.; Yu, L.; Xu, X.; Hong, Z.; Luo, L. Phytosulfokine Is Involved in Positive Regulation of *Lotus japonicus* Nodulation. *Mol. Plant-Microbe Interact.* **2015**, *28*, 847–855. [CrossRef] [PubMed]
12. Yu, L.; Di, Q.; Zhang, D.; Liu, Y.; Li, X.; Mysore, K.S.; Wen, J.; Yan, J.; Luo, L. A Legume-Specific Novel Type of Phytosulfokine, PSK-δ, Promotes Nodulation by Enhancing Nodule Organogenesis. *J. Exp. Bot.* **2022**, *73*, 2698–2713. [CrossRef] [PubMed]
13. Yu, L.; Zhou, W.; Zhang, D.; Yan, J.; Luo, L. Phytosulfokine-α Promotes Root Growth by Repressing Expression of Pectin Methylesterase Inhibitor (PMEI) Genes in *Medicago truncatula*. *Phyton (Buenos Aires)* **2020**, *89*, 873–881. [CrossRef]
14. Yu, L.; Liu, Y.; Zeng, S.; Yan, J.; Wang, E.; Luo, L. Expression of a Novel PSK-Encoding Gene from Soybean Improves Seed Growth and Yield in Transgenic Plants. *Planta* **2019**, *249*, 1239–1250. [CrossRef] [PubMed]