Application of synthetic peptide CEP1 increases nutrient uptake rates along plant roots

Sonali Roy[1,1], Marcus Griffiths[2,2], Ivone Torres-Jerez[3,3], Bailey Sanchez[3,3], Elizabeth Antonelli[3,3], Divya Jain[1,1], Nicholas Krom[3,3], Shulan Zhang[3,3], Larry York[4,4], Wolf-Rüdiger Scheible[3,3], and Michael Udvardi[5,5]

[1]Tennessee State University
[2]Donald Danforth Plant Science Center
[3]Noble Research Institute LLC
[4]Oak Ridge National Laboratory
[5]University of Queensland

January 20, 2023

Abstract

The root system of a plant provides vital functions including resource uptake, storage, and anchorage in soil. The uptake of macro-nutrients like nitrogen (N), phosphorus (P), potassium (K), and sulphur (S) from the soil is critical for plant growth and development. Small signaling peptide (SSP) hormones are best known as potent regulators of plant growth and development with a few also known to have specialized roles in macronutrient utilization. Here we describe a high throughput phenotyping platform for testing SSP effects on root uptake of multiple nutrients. The SSP, CEP1 (C-TERMINALLY ENCODED PEPTIDE) enhanced nitrate uptake rate per unit root length in Medicago trun-catula plants deprived of N in the high-affinity transport range. Single structural variants of M. truncatula and Arabidopsis thaliana specific CEP1 peptides, MtCEP1D1:hyp4,11 and AtCEP1:hyp4,11, enhanced uptake not only of nitrate, but also phosphate and sulfate in both model plant species. Transcriptome analysis of Medicago roots treated with different MtCEP1 encoded peptide domains revealed that hundreds of genes respond to these peptides, including several nitrate transporters and a sulfate transporter that may mediate the uptake of these macronutrients downstream of CEP1 signaling. Likewise, several putative sig-naling pathway genes including LEUCINE-RICH REPEAT RECPTOR-LIKE KINASES and Myb domain containing transcription factors, were induced in 1

NAPPN 2022 Conference Proceedings Manuscript Sample

Application of synthetic peptide CEP1 increases nutrient uptake rates along plant roots

Sonali Roy[1,2#], Marcus Griffiths[1,3#], Ivone Torres-Jerez[1,4], Bailey Sanchez[1], Elizabeth Antonelli[1], Divya Jain[2], Nicholas Krom[1], Shulan Zhang[1], Larry M. York[1,5], Wolf-Rüdiger Scheible[1]*, Michael K. Udvardi[1,6]*

[1] Noble Research Institute LLC, Ardmore, OK, 73401, USA

[2] Tennessee State University, College of Agriculture, Nashville, TN, 37209, USA

[3] Present address: Donald Danforth Plant Science Center, St. Louis, MO, 63132, USA

[4] Present address: Institute of Agricultural Biosciences, Oklahoma State University, Ardmore, OK, 73401, USA

[5] Present address: Biosciences Division and Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, TN, 37830, USA

[6] Present address: Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Australia

#Authors contributed equally

*Author for correspondence tomales2013@yahoo.com and m.udvardi@uq.edu.au

Email Address of presenting author: `sroy3@tnstate.edu`

ABSTRACT

The root system of a plant provides vital functions including resource uptake, storage, and anchorage in soil. The uptake of macro-nutrients like nitrogen (N), phosphorus (P), potassium (K), and sulphur (S) from the soil is critical for plant growth and development. Small signaling peptide (SSP) hormones are best known as potent regulators of plant growth and development with a few also known to have specialized roles in macronutrient utilization. Here we describe a high throughput phenotyping platform for testing SSP effects on root uptake of multiple nutrients. The SSP, CEP1 (C-TERMINALLY ENCODED PEPTIDE) enhanced nitrate uptake rate per unit root length in *Medicago truncatula* plants deprived of N in the high-affinity transport range. Single structural variants of *M. truncatula* and *Arabidopsis thaliana* specific CEP1 peptides, MtCEP1D1:hyp4,11 and AtCEP1:hyp4,11, enhanced uptake not only of nitrate, but also phosphate and sulfate in both model plant species. Transcriptome analysis of Medicago roots treated with different MtCEP1 encoded peptide domains revealed that hundreds of genes respond to these peptides, including several nitrate transporters and a sulfate transporter that may mediate the uptake of these macronutrients downstream of CEP1 signaling. Likewise, several putative signaling pathway genes including LEUCINE-RICH REPEAT RECPTOR-LIKE KINASES and Myb domain containing transcription factors, were induced in

roots by CEP1 treatment. Thus, a scalable method has been developed for screening synthetic peptides of potential use in agriculture, with CEP1 shown to be one such peptide.

Keywords: Nutrient Uptake, Small signaling peptides, Legume roots, Medicago, Arabidopsis

1. INTRODUCTION

Nitrogen (N) is essential to all living organisms being a critical component of amino acids and nucleotides, building blocks of proteins and the genetic code. It is the first nutrient that can become limited in soils which affects plant growth and agricultural productivity. On the other hand, addition of manufactured Nitrogenous fertilizer can boost plant growth in any ecosystem. This largely fueled the green revolution of the 1970s, making many countries agriculturally self-sufficient (*1*, *2*). Production of N-fertilizers, however, requires fossil-fuel based energy that is harmful to the environment. N-fertilizer that is not taken up by plant roots leaches into the water table or into the atmosphere and can be detrimental to animals. Moreover, excess nitrate in drinking water has been linked to cancer in humans (*3*). Therefore, nitrogen in agriculture has to be used cautiously so that the benefits outweigh the detrimental effects caused by its overuse. One method of reducing the current dependance on added N-fertilizers for optimal plant growth is to improve the N-use by plant roots given that almost 40% of Nitrogen that is applied to plants in agricultural fields is not taken up by roots (*4*). Therefore, optimizing root architecture for nutrient uptake and understanding processes controlling nutrient acquisition are key to ensuring sustainability of agroecosystems.

Legume roots are excellent models not only for the study of plant-microbe interactions, but also to understand how root architecture and regulated nutrient uptake can be optimized to enhance N-acquisition for improved agricultural sustainability (*5–7*). Synthetic versions of plant hormones such as auxin (2,4-Dichlorophenoxyacetic acid) have been exploited by farmers to selectively control growth of weeds in cereal fields. Until thirty years ago, plants were thought to encode only nine families of classical hormones including auxin, cytokinin, ethylene, abscisic acid. Research over the past 10 years shows that small signaling peptides also act as hormones and that synthetic versions of these peptides retain their biological activities (*8*) . Peptide hormones are genome encoded fragments cleaved at the C-terminal of an amino acid chain. These bioactive peptides can signal locally as well at long distances to mediate plant immune responses as well as nodule formation. The Arabidopsis CEP1 peptide acts as a N-hunger signal that induces expression of nitrate transporters in N-rich patches (*9*). In Medicago, application of the synthetic version MtCEP1 represses lateral root development but increases number of symbiotically formed nodules upon infection with soil rhizobia. Studying this repertoire of powerful growth modulators thus has the potential to discover new chemicals that can be exploited

to address problems of agriculture. By investigating biological activity of peptides, we can identify peptide hormones that can be used in the future as seed treatments or sprays to optimize N-uptake and modulate beneficial relationships with symbiotic bacteria (*10*).

Here we describe the development and use of a platform to measure the simultaneous uptake of multiple macro- and micro- nutrients. This platform allowed us to test the effect of synthetic peptides on root nutrient uptake, a trait that is understudied but is of significant economic importance. We found that a synthetic peptide corresponding to the 15 amino acids at the C-terminal end of the CEP1 peptide is sufficient to induce nitrate uptake in two model systems *M. truncatula* and *A. thaliana*. Further, not only nitrate but also phosphate and sulfate uptake were enhanced by CEP1 application. We further investigate the molecular basis of peptide mediated nutrient uptake by analyzing the root transcriptome RNAseq with three different peptide domains provides insights into the molecular mechanism of peptide activity on Medicago roots. These findings will be of interest to root physiologists, nutrient biologists and legume molecular geneticists.

II. RESULTS

The ion uptake platform was used to measure root uptake rates of multiple nutrients simultaneously. In addition to enhancing nitrate uptake rates ($p<0.01$), application of 1 µM of the Arabidopsis AtCEP1 peptide significantly enhanced phosphate and sulfate uptake in Arabidopsis thaliana ($p<0.05$, c.f. *12*). For *M. truncatula*, both AtCEP1 and Medicago MtCEP1 domain 1 peptide significantly enhanced the nitrate uptake rate ($p<0.05$ and $p<0.001$, respectively; c.f *12*). Unlike MtCEP1, that enhanced uptake of both phosphate and sulfate in Medicago, AtCEP1 peptide application did not enhance uptake of these two nutrients in M. truncatula, ($p<0.001$ and $p<0.1$, respectively; Figure 3B). Thus, MtCEP1 had a greater effect than AtCEP1 on uptake rates of nitrate, phosphate and sulfate in Medicago (Figure 3B). In contrast, the CEP1 peptides had no effect on ammonium or potassium uptake rates in *M. truncatula*.

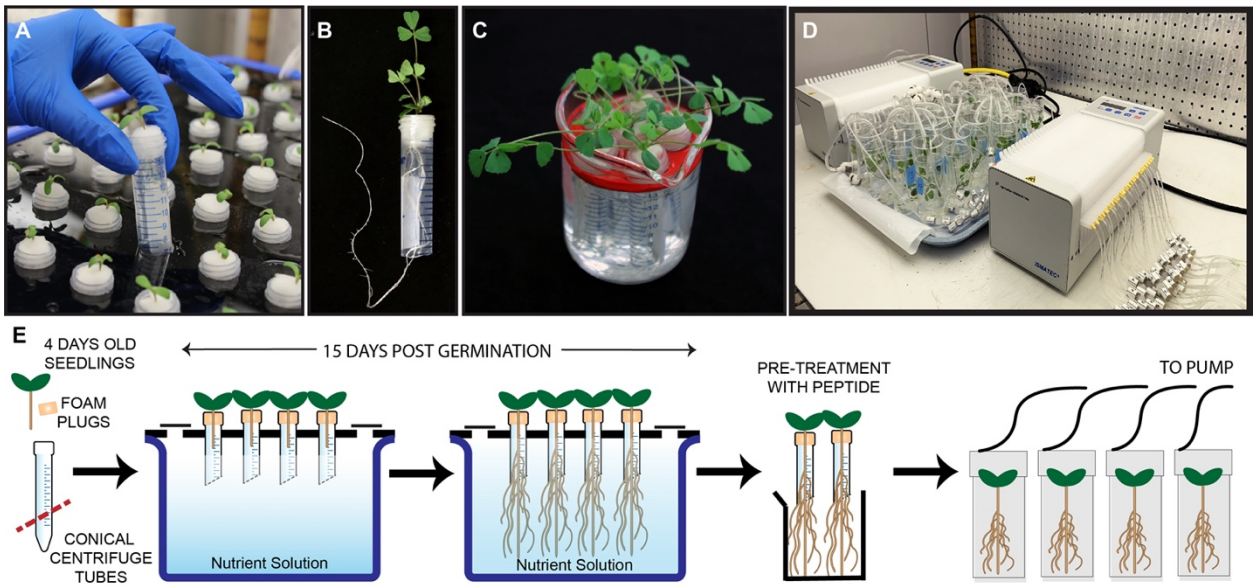

Figure 1: Overview of platform to measure nutrient uptake by *Medicago truncatula* roots upon application of synthetic peptides. The platform was adapted from Griffiths M. et. al., 2020 for smaller plants such as *M. truncatula* and *A. thaliana*. **A.** Transfer of 4-day old M. truncatula seedlings encased in foam plugs into Broughton and Dillworth growth media. **B.** Representative root after 11 days of growth in hydroponics chambers. **C.** Pretreatment with 48 hours of peptide in nutrient deprived media. **D.** Simultaneous sampling and measurement of nutrient content in solution provided to measure uptake **E.** Diagrammatic representation of all above mentioned steps are depicted in Panel E.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in the preprint "Application of synthetic peptide CEP1 increases nutrient uptake rates along plant roots". doi: https://doi.org/10.1101/2021.10.11.463963

ACKNOWLEDGMENTS

We would like to thank David Huhman for help with ion content measurement, Lynne Jacobs for help growing plants, and Kim Spiering for technical support. This work was funded by the National Science Foundation award #1444549 to Wolf R. Schieble and M.K Udvardi; USDA-NIFA award #2017-67007-25948 to Larry M. York; the Center for Bioenergy Innovation, a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science; and the Lloyd Summer Noble Summer Scholar grant to S. Roy and M. Griffiths and a USDA-Evan's Allens grant to S. Roy.

REFERENCES

1. J. J. Elser, M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, J. E. Smith, Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* **10**, 1135–1142 (2007).

2. N. D. Mueller, J. S. Gerber, M. Johnston, D. K. Ray, N. Ramankutty, J. A. Foley, Closing yield gaps through nutrient and water management. *Nature.* **490**, 254–257 (2012).

3. M. A. Sutton, O. Oenema, J. W. Erisman, A. Leip, H. van Grinsven, W. Winiwarter, Too much of a good thing. *Nature.* **472**, 159–161 (2011).

4. M. Griffiths, L. M. York, Targeting Root Ion Uptake Kinetics to Increase Plant Productivity and Nutrient Use Efficiency. *Plant Physiol.* **182**, 1854–1868 (2020).

5. S. Gonzalez-Rizzo, P. Laporte, M. Crespi, F. Frugier, Legume root architecture: A peculiar root system. *Annual Plant Reviews online* (2018), pp. 239–287.

6. J. D. Burridge, H. Rangarajan, J. P. Lynch, Comparative phenomics of annual grain legume root architecture. *Crop Sci.* **60**, 2574–2593 (2020).

7. N. A. Mohd-Radzman, C. Laffont, A. Ivanovici, N. Patel, D. Reid, J. Stougaard, F. Frugier, N. Imin, M. A. Djordjevic, Different Pathways Act Downstream of the CEP Peptide Receptor CRA2 to Regulate Lateral Root and Nodule Development. *Plant Physiol.* **171**, 2536–2548 (2016).

8. A. Kereszt, P. Mergaert, J. Montiel, G. Endre, É. Kondorosi, Impact of Plant Peptides on Symbiotic Nodule Development and Functioning. *Front. Plant Sci.* **9**, 1026 (2018).

9. R. Tabata, K. Sumida, T. Yoshii, K. Ohyama, H. Shinohara, Y. Matsubayashi, Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. *Science* (2014) (available at https://science.sciencemag.org/content/346/6207/343).

10. M. W. Lee, A. Huffaker, D. Crippen, R. T. Robbins, F. L. Goggin, Plant elicitor peptides promote plant defences against nematodes in soybean. *Mol. Plant Pathol.* **19**, 858–869 (2018).

11. M. Griffiths, S. Roy, H. Guo, A. Seethepalli, D. Huhman, Y. Ge, R. E. Sharp, F. B. Fritschi, L. M. York, A multiple ion-uptake phenotyping platform reveals shared mechanisms affecting nutrient uptake by roots. Plant Physiol. 185, 781–795 (2020).

12. S. Roy, M. Griffiths, I. Torres-Jerez, B. Sanchez, E. Antonelli, D. Jain, N. Krom, S. Zhang, L. M. York, W.-R. Scheible, M. K. Udvardi, Application of synthetic peptide CEP1 increases nutrient uptake rates along plant roots. bioRxiv (2021), p. 2021.10.11.463963.