Investigation of the CEP / CEPR1 / CEPD interaction in *arabidopsis thaliana* as a potential method for alerting farmers of the presence of nitrogen stress in the plant.

Abstract

My science fair project is about plants and how they can communicate with farmers when they are deficient in nitrogen. I chose this project because I think the idea of crops growing was interesting and I wanted to better understand the importance of nitrogen to the growing process. Nitrogen and other nutrients cost money. Adding more nitrogen than the plants need is bad for the environment. So farmers do not want to waste nitrogen, but there is no easy way to tell when a plant lacks nitrogen. My results demonstrated a potential method for monitoring plants for nitrogen stress. My project could help farmers not waste money on excessive use of nitrogen-rich fertilizer, and reduce the risk of nitrogen related problems in the environment.

This area of research is part of the work being done in the [redacted] lab at the University [redacted].

Introduction

Plants need certain elements to grow these include potassium, nitrogen, and phosphorus. Of these nitrogen is considered the most important and impactful. Farmers add these nutrients to their fertilizers which creates a better outcome for the crops. However, when you add more of these elements than can be used by the crop it can be harmful to the environment. This is especially true of nitrogen. How can we add enough nitrogen to maximise crop growth without damaging the environment? One answer could be engineering the plant to communicate with the farmer.

This project investigates one possibility. Using genes in arabidopsis thaliana (A.thaliana) we will attempt to show a way for A.thaliana. to communicate a deficiency in nitrogen. If successful the A.thaliana. plant will turn its veins red when it's deficient in nitrogen. This would be a great way for a farmer to create a balance in their fields by there not being too much nitrogen nor a lack.

Problem

The problem I am trying to solve is that when farmers are trying to make money off of their crops they put nitrogen into their fields to help boost production. Nitrogen can be harmful to the environment (Johnson and Laney). Excess nitrogen in the environment can lead to water pollution allege blooms and other harmful outcomes. Giving farmers away to maximise their crop yields without polluting the planet.

The [redacted] lab has successfully modified the CEPD gene so that it is visible in the shoot veinage under microscopic analysis. This modified CEPD gene will be denoted as CEPD+.

Major areas of future research will be focused on (a)transferring techniques from A.thaliana to crops; (b)development of devices to monitor nitrogen stress signals in crops.

Investigative method

Research question

Variables

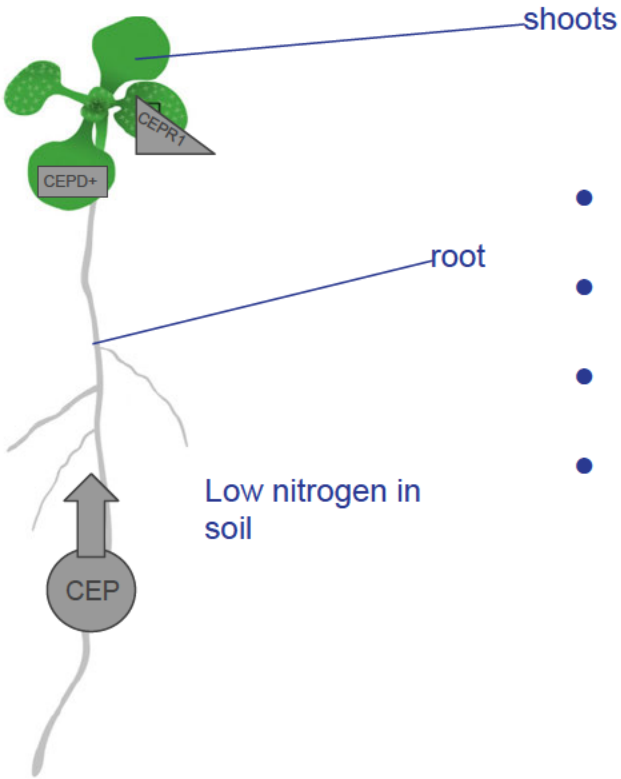
- First we took extracted DNA from six plants. The DNA from these six plants had been modified in the Beilstein lab to vary the occurrence of CEPD+, but we were not informed which sample was which before the next step.
- Extracted DNA and controlled known DNA were subjected to polymerase chain reaction (PCR)
- After PCR, all DNA samples were subjected to agarose gel electrophoresis
- Source plants for the extracted DNA were examined with a fluorescent stereoscope at 18.6x magnification and images were recorded
- Images were compared with gel electrophoresis results to determine if the communication method works as expected

Materials

- DNA analysis
  - Pipettes 0.1 - 2.5 microliters and 2.5 - 20 microliters
  - CEPR1 wild type
  - 2406 F primers 9 microliters
  - 2405 R primers 9 microliters
  - GoTAQ Green 90 microliters
  - CEPR1 mutant
  - 2572 primer F 9 microliters
  - 2406 primer R 9 microliters
  - Extracted arabidopsis thaliana dna (6 samples 4 microliters each)
  - H2O 36 microliters
  - Eppendorf vapo protect mastercycler polymerase chain reaction machine
  - Agarose gel as needed
  - Gel electrophoresis frame
  - BioDoc-It ultraviolet imaging
- Leaf vein observation
  - Zeiss fluorescent stereo microscope
  - 6 Arabidopsis Thaliana plants being investigated
  - Image J image analysis software

Results and Data Visualization

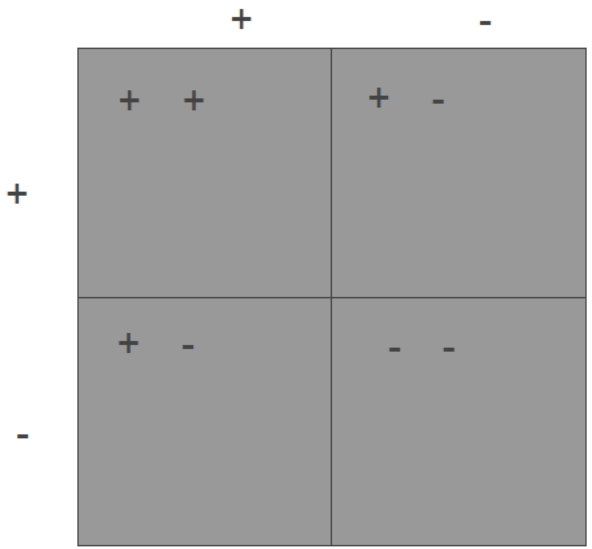
Arabidopsis thaliana



- The project investigates the interaction between CEP, CEPR1, CEPD with red fluorescent marker(CEPD+)
- When A.thaliana is under nitrogen stress (low nitrogen) CEP is generated in the roots and moves to the shoots and looks for CEPR1
- The CEP/CEPR1 interaction generates CEPD+ in the shoots and CEPD+ moves through the veinage to the roots to help nitrogen uptake
- The more CEPD+ generated in the shoots, the brighter the red marker should appear in the veins of the shoots

Results and Data Visualization (continued)

Punnett square for CEPR1



- As illustrated by the Punnett square to the left, A. thaliana can have these types of outcomes in nature
- The project will assess the performance of CEPD+ with red marker in any of these three outcomes

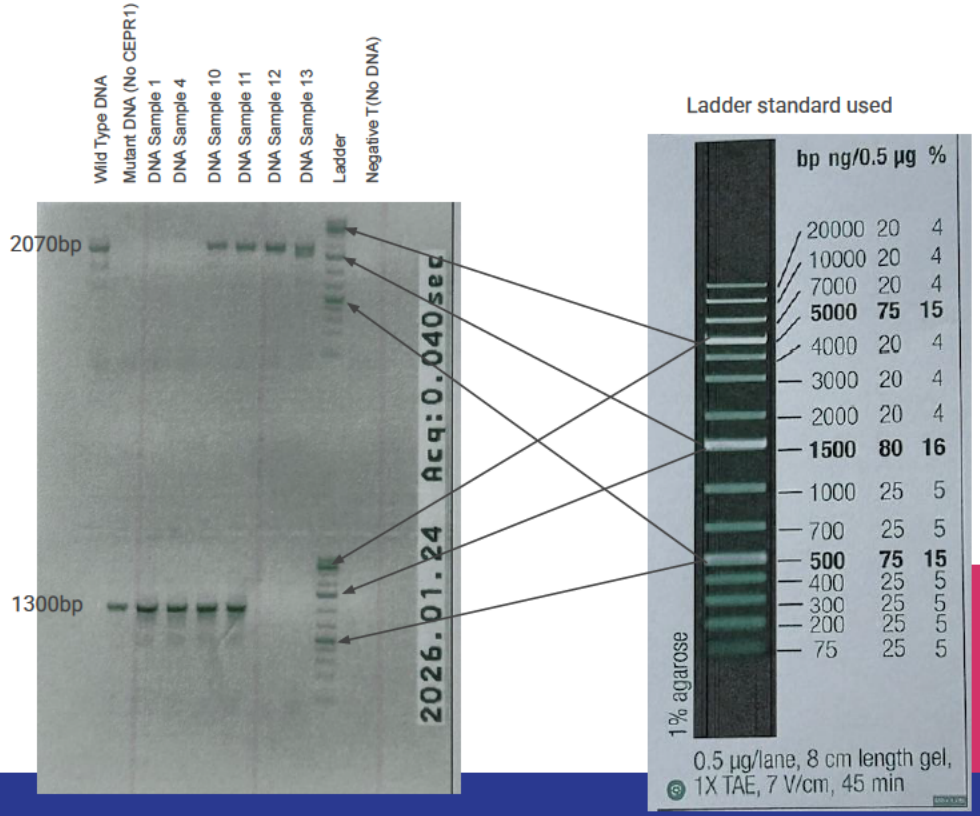
\+ + 2 CEPR1 genes - most CEPD+

\+ - 1 CEPR1 genes - some CEPD+

\- - 0 CEPR1 genes - least CEPD+

Results and Data Visualization (continued)

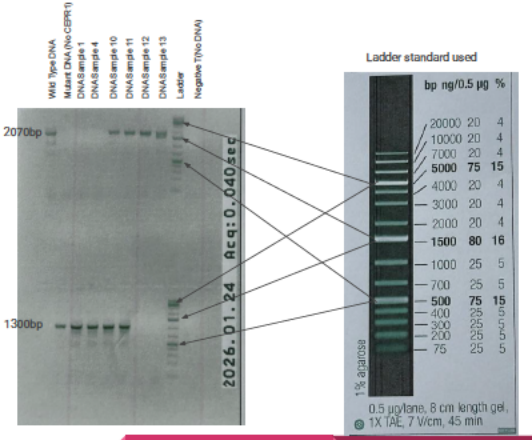
- After PCR we have two samples from each of the possible outcomes
- Using Agarose gel electrophoresis, we can compare the unknowns samples to the two known samples
- The images to the right show the results after electrophoresis



(bp = base pairs, a way of measuring the relative size of a molecule or DNA strand. Smaller numbers are smaller molecules and will fall faster through the gel during electrophoresis.)

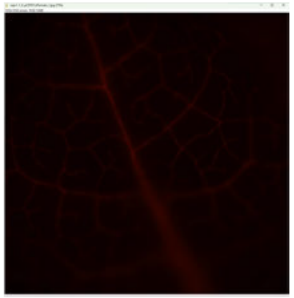
Results and Data Visualization (continued)

- After PCR, subjecting the data samples to agarose electrophoresis allows us to measure the size of each known sample and compare them to the known sample.
- Wild type DNA - known normal CEPR1(+ +)2070bp
- Mutant DNA-known modified DNA with no CEPR1 (- -)1300bp
- DNA sample 1- matches mutant DNA only (- -)1300bp
- DNA sample 4 - matches mutant DNA only (- -) 1300bp
- DNA sample 10 - matches both wild type and mutant DNA (+ -)2070bp and 1300bp
- DNA sample 11- matches both wild type and mutant DNA (+ -) 2070bp and 1300 bp
- DNA sample 12 - matches wild type DNA only (+ +) 2070
- DNA sample 13 - matches wild type DNA only (+ +)2070bp

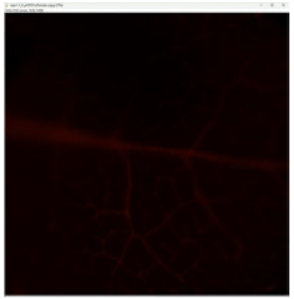


Results and Data Visualization (continued)

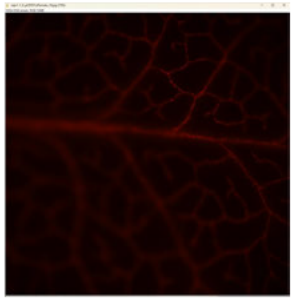
DNA Sample 1



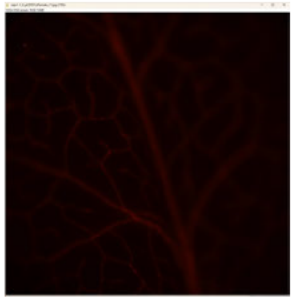
DNA Sample 4



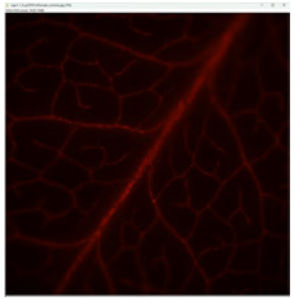
DNA Sample 10

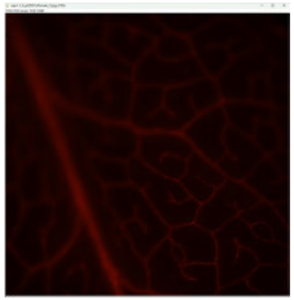


DNA Sample 11



DNA Wild Type Control





DNA Sample 12

DNA Sample 13

	DNA Sample 1	DNA Sample 4	DNA Sample 10	DNA Sample 11	DNA Sample 12	DNA Sample 13	DNA Wild Type Control
Average Red intensity	16	13	23	18	3	5	26
Max Red Intensity	92	71	198	117	152	255	190

Discussion and Interpretation

- We extracted DNA from plants that represented each of the three possible outcomes
- We used polymerase chain reaction and agarose gel electrophoresis to verify we actually had each of the three possible outcomes
- Using a fluorescent stereomicroscope we examined leaves from each of our examples and collected images
- Each image was analyzed for red intensity using Image J software
- The red intensity data was encouraging
    - The (- -) examples with no CEPR1 genes had the lowest max red intensity
    - The (+ +) examples had two of the three highest max red intensities
    - The (+ -) examples fell mostly in between the (- -) and (++) red intensities
- The result of this project demonstrates that A. thaliana with CEPD plus a red fluorescent marker does provide an indication of low nitrogen stress in the plant
- This technique has the potential to help farmers monitor nitrogen levels in their crops

Implications for Further Research

- All of the work presented in this project was performed on a well understood plant called arabidopsis thaliana. In the future the technique would need to be translated to crops that farmers grow
- It was apparent from our red intensity measurements that CEPD genes were being produced even when the CEPR1 gene was not present. Further research to understand what other gene interactions are generating CEPD is warranted
- Few farmers own fluorescent stereomicroscopes. Further research is warranted in two areas
    - Creation of a hand held fluorescent analyzer would allow farmers to more easily assess the condition of their crops
    - Rather than using a marker that requires fluorescent light, use a marker visible to the naked eye
    - For either of these two methods of assessment, use of robotic equipment to cover large areas could be useful

Works Cited

Mas, Joseph. “How does Nitrogen Help Plants Grow? - Phoslab Environmental Laboratories Phoslab Environmental Laboratories.” *Phoslab Environmental Laboratories*, 7 May 2013, https://www.phoslab.com/how-does-nitrogen-help-plants-grow/. Accessed 25 January 2026.

“Why do plants need nitrogen?” *OCI Global*, https://oci-global.com/products-solutions/agricultural/fertilizers-agronomy/why-nitrogen/. Accessed 25 January 2026.

Taleski, M., Chapman, K., Novák, O. *et al.* CEP peptide and cytokinin pathways converge on CEPD glutaredoxins to inhibit root growth. *Nat Commun* 14, 1683 (2023). https://doi.org/10.1038/s41467-023-37282-6

Works Cited (continued)

National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Environmental Health Matters Initiative; Johnson A, Laney K, editors. Reducing the Health Impacts of the Nitrogen Problem: Proceedings of a Workshop—in Brief. Washington (DC): National Academies Press (US); 2021 Sep 30. Available from: https://www.ncbi.nlm.nih.gov/books/NBK574300/ doi: 10.17226/26328

Nagano M, Ueda H, Fukao Y, Kawai-Yamada M, Hara-Nishimura I. Generation of Arabidopsis lines with a red fluorescent marker for endoplasmic reticulum using a tail-anchored protein cytochrome *b5* -B. Plant Signal Behav. 2020 Sep 1;15(9):1790196. doi: 10.1080/15592324.2020.1790196. Epub 2020 Jul 7. PMID: 32633191; PMCID: PMC8550181.