Directing Photosynthesis towards the Production of Useful Chemicals in Plants

Lily Ha[1], Lauren F. Cole[2], Shannon A. McCallan[3], Erin J. Cram[3], and Carolyn W.T. Lee-Parsons[4]

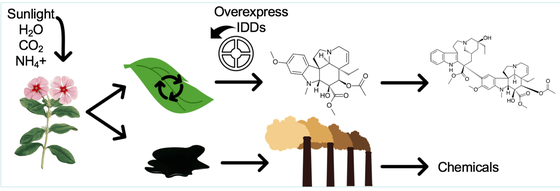
University of Maryland College Park – Bioengineering Department[1], Northeastern University - Bioengineering Department[2], Biology Department[3], Chemistry and Chemical Engineering Department[4]

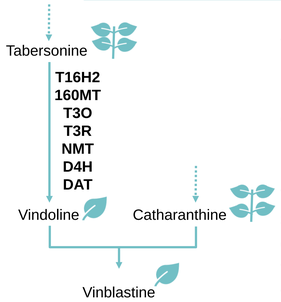
Abstract

Fossil fuels remain the most important energy source but the supply is being depleted. From 1990 to 2010, net emissions of greenhouse gases increased 35% worldwide with carbon dioxide accounting for three-fourths of total emissions. Instead of deriving chemicals from petroleum, our goal is to direct the sugars produced in plants from photosynthesis towards producing useful chemicals. To address the depletion of fossil fuels and consume carbon dioxide, our research engineers switches in the *Catharanthus roseus* plant to produce chemicals used in chemotherapy.

Introduction

Vinblastine is an anti-cancer drug produced by the *Catharanthus roseus* plant, commonly known as the Madagascar periwinkle. Vinblastine is only produced in low concentrations in the leaves of *Catharanthus roseus*. Vinblastine is formed from vindoline and catharathine. In order to increase production of vinblastine, we will investigate how to increase the production of the limiting precursor, vindoline. Optimizing vinblastine production will meet the demands of chemotherapy and the energy market.





Our focus is to understand the transcription factors involved in regulating the pathway. Our research is to characterize the role of the indeterminate-domain (IDD) family of transcription factors in regulating vindoline biosynthesis. The expression of the IDDs are positively correlated with the expression of the vindoline pathway genes. We will characterize specific IDDs through gene-silencing and over-expression methods. Overall, are IDD transcription factors important in turning on the vindoline pathway?

Research Aims

1. Clone plasmids to be used in VIGS and EASI
2. Overexpress IDDs, monitor expression of vindoline pathway
3. Silence IDDs, monitor expression of vindoline pathway

Experimental Order

General overview:

Acknowledgements

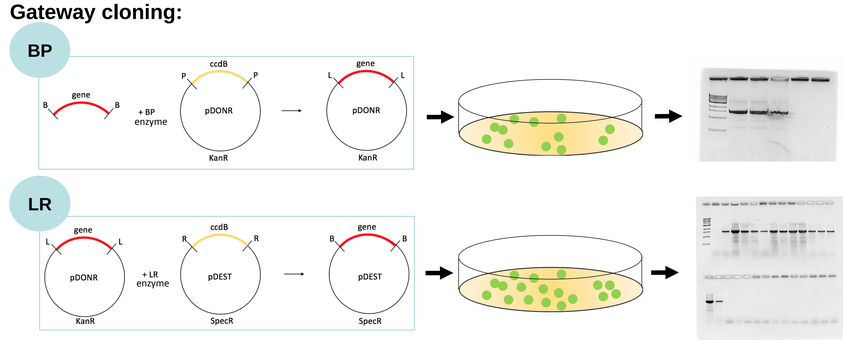
National Science Foundation: CBET Award # 1033889 (CLP, EJC), MCB Award # 1516371 (CLP, EJC).
Carolyn Lee-Parsons Lab: Carolyn Lee-Parsons, Lauren Cole, Samuel Mortensen, Krystyna Farrell, Shannon McCallan, Olga Prifti. **Center for STEM Education & REU POWER Program:** Brad Lehman, Claire Duggan, Mary Howley, Camara Johnson, Emily Chernich, Nicolas Fuchs.

References:

Catharanthus terpenoid indole alkaloids: Biosynthesis and regulation - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/ndoline-biosynthesis-pathway-T16H-tabersonine-16-hydroxylase-OMT-O-methyltransferase_fig2_225655949 [accessed 23 Jul, 2019]
A vacuolar class III peroxidase and the metabolism of anticancer indole alkaloids in Catharanthus roseus: Can peroxidases, secondary metabolites and arabinogalactan proteins be partners in microcompartmentation of cellular reactions? - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Biosynthesis-of-vinblastine-from-the-monomeric-precursors-catharanthine-and-vindoline_fig1_26764651 [accessed 23 Jul, 2019]
Singh, Prof Bharat Raj & Singh, Onkar. (2012). Global Trends of Fossil Fuel Reserves and Climate Change in the 21st Century. 10.13140/2.1.1079.8084.
"Climate Change Indicators: Greenhouse Gases." *EPA*, Environmental Protection Agency, 22 Feb. 2017, www.epa.gov/climate-indicators/greenhouse-gases.
Vo ̀lz R, Kim S-K, Mi J, Rawat AA, Veluchamy A, Mariappan KG, et al. (2019). INDETERMINATE-DOMAIN 4 (IDD4) coordinates immune responses with plant-growth in Arabidopsis thaliana. PLoS Pathog 15(1): e1007499. https://doi.org/10.1371/journal. ppat.1007499
Aerts, Rob J., and Vincenzo De Luca. "Phytochrome Is Involved in the Light-Regulation of Vindoline Biosynthesis in Catharanthus." *Plant Physiology*, vol. 100, no. 2, 1992, pp. 1029–1032., doi:10.1104/pp.100.2.1029.
Norton, E. H. *Brazilian Flowers Drawn from Nature in the Years 1880-1882, in the Neighbourhood of Rio De Janeiro; Viz. Larangeiras, Tijuca, Paquetá, Petropolis, Many of the Specimens Gathered in Primeval Forests*. 1893.

Methods

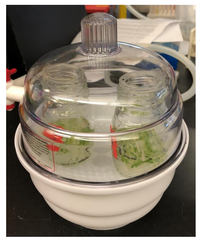
Gateway cloning:



Gateway cloning is a strategy for transferring and overexpressing any gene in the desired pDEST plasmid through recombination reactions based on natural bacterial enzymes that recognize B,P,L, and R sites. The desired pDEST is then transformed into Agrobacteria.

Efficient Agro-mediated Seedling Infiltration (EASI)

Purpose: Overexpressing transcription factors to monitor the effect on the expression of genes in the vindoline pathway
Time: 14 days old
Infiltrate seedlings:

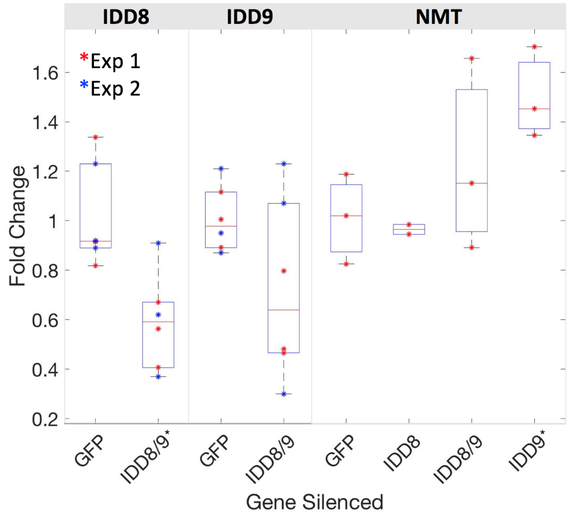


Virus-induced gene silencing (VIGS)

Purpose: Silencing transcription factors to monitor the effect on the expression of genes in the vindoline pathway
Time: ~5 weeks old
Infect leaves:

VIGS Results

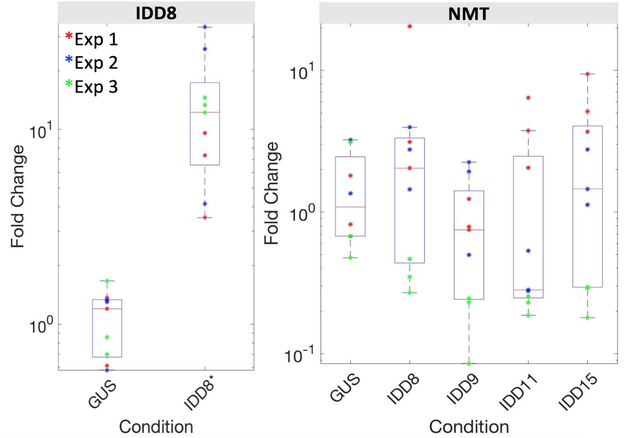
Successful Double-Silencing with VIGS did not strongly affect NMT expression



Results show that expression of IDD8 and IDD9 was reduced about half when IDD8/9 was silenced. NMT expression not strongly affected by IDD8 silencing, and IDD9 silencing showed increase of NMT expression which could mean it is a repressor, * = t-test, $p < 0.05$.

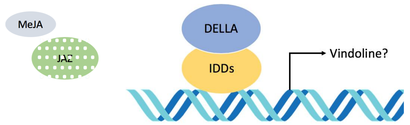
Overexpression Results

Overexpressing IDDs 8, 9, 11, & 15 did not effect pathway gene expression

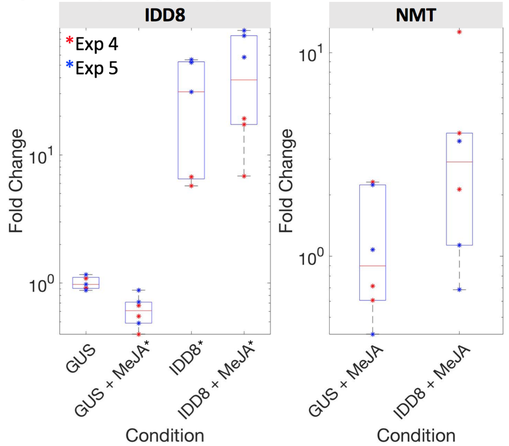


From all three experimental replicates, the fold change of NMT was similar to that of the negative control. One explanation is that IDDs can act as activators or repressors depending on the presence of other cofactors. All were under 9-hour light conditions, * = t-test, $p < 0.05$.

New hypothesis: If JAZ inhibits DELLAs while DELLAs interact and activate IDDs, then adding MeJA will increase DELLA-IDD complex & increase expression



Overexpressing and adding MeJA has a modest, positive effect



When comparing IDD8+MeJa to GUS+MeJA, NMT expression increased modestly with p=0.16. All were under 24-hour light conditions,* = t-test, $p < 0.05$.

Conclusion

- Successful double silencing with VIGS
- Results from overexpressing IDDs are inconclusive, but suggests a strict environment & time condition
- Adding MeJA with IDD8 showed a modest, positive effect on NMT but not statistically significant

Future Direction

- Fuse activation or repression domain to IDD
- Run more trials with MeJA, but maybe need to look more upstream at mitogen-activated protein kinase cascades (MAPKs)
- Perform light-sensitivity experiments with varying times

NSF REU-Site: Research Experience for Undergraduates site to create "Pathways Opening World Energy Resources" (REU - POWER)

This work was supported by the National Science Foundation Grant #1757650