A Literature Review of the Effects of Nitrogen Availability on Cell Wall Development in Woody Bioenergy Crops

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Introduction

- Pressure on the agricultural industry to increase production on decreased acreage has resulted in excess nutrient runoff in local waterways causing eutrophication (Rosa, et al. 2017)
- Planting agricultural and riparian buffer strips is one method to mitigate this issue.
- Bioenergy crops, like shrub willow (*Salix spp.*), are good candidates for buffer strips because they can produce large amounts of biomass with minimal inputs and grow on marginal soil
- It is critical to understand shrub willow's responses to excess nitrogen in these buffers
- Increases in nitrogen availability have shown significant impacts on cell wall development and biomass composition (Camargo, et al. 2014; Euring, et al. 2018)

Goal and Objectives

- To understand how excess nitrogen affects cell wall ultrastructure and gene expression in key genes involved in cell wall development.
  - Review data from literature on the impacts of nitrogen on cell wall development
  - Clone specific genes from *Salix eriocephala* for future gene expression studies

Cloning of Genes from *Salix eriocephala*

Performed TOPO TA® cloning using the plasmid pCR 2.1- TOPO (Invitrogen; Carlsbad, CA)
- Amplified and ligated PCR product
- Transformed ligation into *E. coli*.
- Selected colonies and isolated plasmid DNA
- Selected 5 plasmids for sequencing

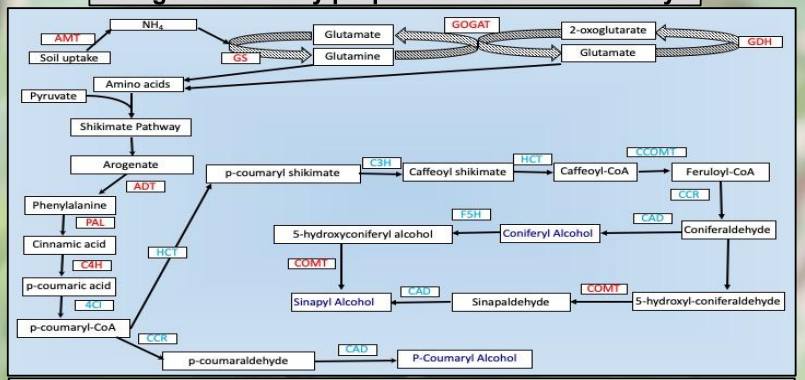
Gene	Clone #	% Similarity
WRKY 33	1	92.593
	3	92.593
	4	92.593
	5	92.593
WRKY 40	3	82.161
	4	81.855
C4H	1	98.742
	2	98.239
MYB 70	1	75.114
	2	77.856
	4	76.987
	5	78.493

Cloning Results

- The sequences clones of WRKY 33 showed high similarity with the *S. purpurea* gene
- WRKY 40 had a lower similarity because of a possible alternative transcript (further annotation required)
- C4H had the highest identity between the clones and the *S. purpurea* gene
- MYB 70 had the lowest % similarity because there was a consistent missing region 135 bp long in all of the clones
  - This could mean that it was a different MYB transcription factor cloned

Alignment in WRKY 33 clone, demonstrated that there was a high correlation to the WRKY gene

Nitrogen and Phenylpropanoid Metabolic Pathways



Nitrogen metabolic pathway in plants. AMT: Aminomethyltransferase;GS- Glutamine synthetase; GOGAT- Glutamine synthase; GDH- Glutamate dehydrogenase; ADT – arogenate dehydratase; PAL- phenylalanine ammonia lyase; C4H - cinnamate-4-hydroxylase; COMT - Caffeic acid O-methyltransferase. Genes encoding the enzymes in red have been isolated and amplified from shrub willow cDNA. (Kusano, et al 2011; Xu, et al. 2012)

MYB and WRKY Transcription Factors

- WRKY genes regulate gene expression in response to external stress.
- MYB genes regulate cellular processes including lignin biosynthesis. (Camargo, et al. 2014)
- MYB transcription factors that specifically control the biosynthesis of lignin were up-regulated by a decrease in N conditions. (Fataftah, 2018)

arogenate dehydratase (ADT):

- This gene is located upstream of the shikimate pathway, which is up-regulated in response to limited N conditions.
- ADT enzymes are able to modulate carbon flux into lignin biosynthesis
  - Reflects reorganization of the anabolic metabolism in response to decreased amounts of N (Camargo, et al. 2014)
- Ability to catalyze the transformation of arogenate to phenylalanine in the shikimate pathway.

phenylalanine ammonia-lyase (PAL):

- This is the first committing step into the phenylpropanoid pathway, converting phenylalanine to cinnamic acid.
- It was shown to up-regulate in response to high N levels (Pitre et al. 2010; Camargo, et al. 2014)
- Increases in *PAL* gene expression leads to increased lignin in the cell wall

Photo Credit Larry Smart

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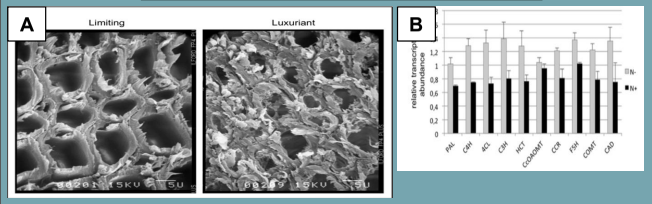
cinnimate-4-hydroxylase (C4H):

- Catalyzes the transformation of cinnamic acid to p-coumaric acid in the phenylpropanoid pathway
- Has been shown to be up-regulated in limited N conditions (Camargo, et. al. 2014)
- Changes in *C4H* gene expression impact lignin content in the cell wall

caffeic O-methyltransferase (COMT):

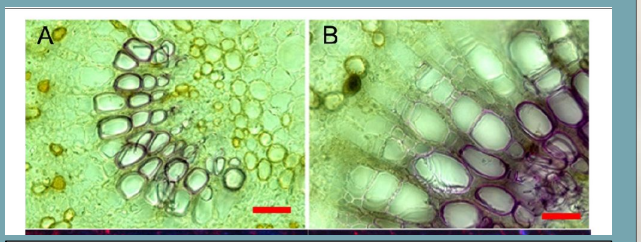
- Can catalyze multiple reactions in the final steps in forming Sinapyl Alcohol
- Down-regulation in limiting nitrogen levels of transcripts linked in the formation of lignin (Camargo, et. al. 2014)
- C1 metabolism (seen in limited N conditions) is very active in lignin producing tissues, and is closely connected to lignin biosynthesis through COMT

Response to N Availability in Eucalyptus



A: Structure of cell walls exposed to N limiting (left) and N luxuriant (right) conditions. Plants that were exposed to lower levels of nitrogen had a stronger cell wall structure. The cells had more lignan and the cell wall remained rigid.
B: Bar graph of transcript abundance of specific lignin biosynthetic genes in response to N
(Camargo, et al. 2014)

Response to N Availability in Poplar



Stem cross-sections from second internode in *P. trichocarpa* stems. A: low N levels; B: high N levels. Increased stem elongation and reduced lignin content in developing xylem in response to N content (Euring, et al. 2014)

Conclusion

- Gene cloning from S. eriocephala was successfull with high identity to the annotated genes in *S. purpurea*. This will allow for future gene expression studies with willow.
- In studies with both eucalyptus and poplar, the phenylpropanoid pathway was altered with increased nitrogen levels, causing gene expression to vary depending on the gene
- N supply impacts secondary cell wall composition and structure.
  - High levels of nitrogen → decreases lignin production (and vice versa)
  - This could have significant impacts on biomass composition of biofuel crops grown as buffers
- Nitrogen availability impacts altered gene expression
  - *MYB, ADT, C4H* were up-regulated with limiting levels of nitrogen
  - *PAL* and *COMT* upregulated in excess nitrogen availability

Future Directions

- Perform different nitrogen experiments with various species of shrub willow used for bioenergy production and as buffers
- Peform gene expression analysis with isolated stem tissue from nitrogen experiments
- Further annotation of genes (MYBs and WRKYs)
- Begin to understand how MYB and WRKY transcription factors play a role in lignin biosynthesis

References

- Camargo, ELO et al., **2014**. Contrasting Nitrogen Fertilization Treatments Impact Xylem Gene Expression and Secondary Cell Wall Lignification in Eucalyptus. *BMC Plant Biology* **14**: 256
- Euring, D et al. **2018**. Nitrogen-Driven Stem Elongation in Poplar Is Linked with Wood Modification and Gene Clusters for Stress, Photosynthesis and Cell Wall Formation. *BMC Plant Biol* 18, 77
- Fataftah, et al. **2018**. Changes in nitrogen availability lead to a reprogramming of pyruvate metabolism. *BMC Plant Biol* 18, 77
- Kusano, et al. **2011**. Metabolomic Approaches toward Understanding Nitrogen Metabolism in Plants. *Journal of Experimental Botany* **62**: 1439-1453
- Rosa, et al. **2017**. Water Quality Changes in a Short-Rotation Woody Crop Riparian Buffer. *Biomass and Bioenergy* 370–375
- Tzin, Galili **2010**. The Biosynthetic Pathways for Shikimate and Aromatic Amino Acids in Arabidopsis thaliana. *Arabidopsis book* vol. **8**e0132
- Xu, Guohua, et al. **2012.** Plant Nitrogen Assimilation and Use Efficiency. *Annual Review of Plant Biology*, vol. 63, no. 1,153–182