Lignin Modification via Expression of a Tyrosine Rich Cell Wall Peptide in Hybrid Poplar

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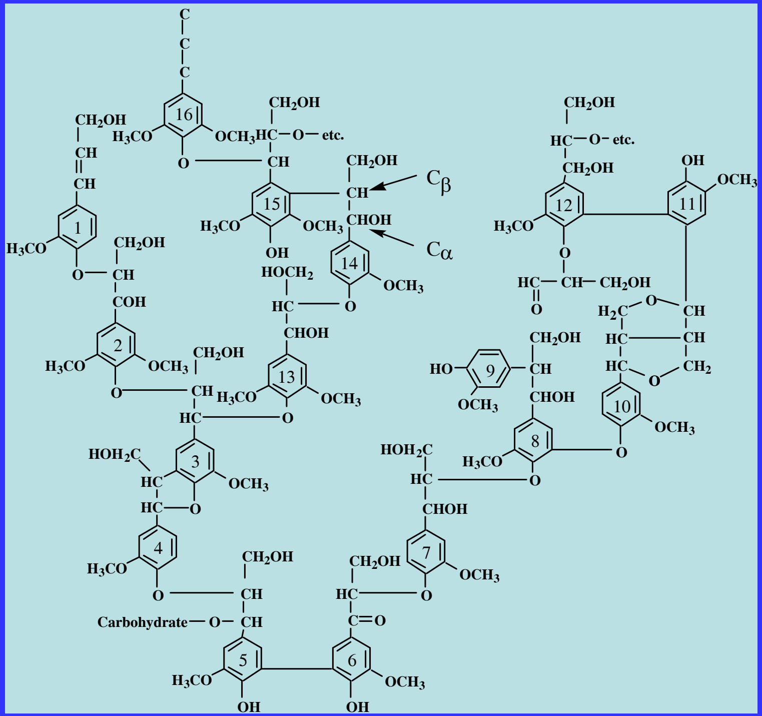
Importance of lignin

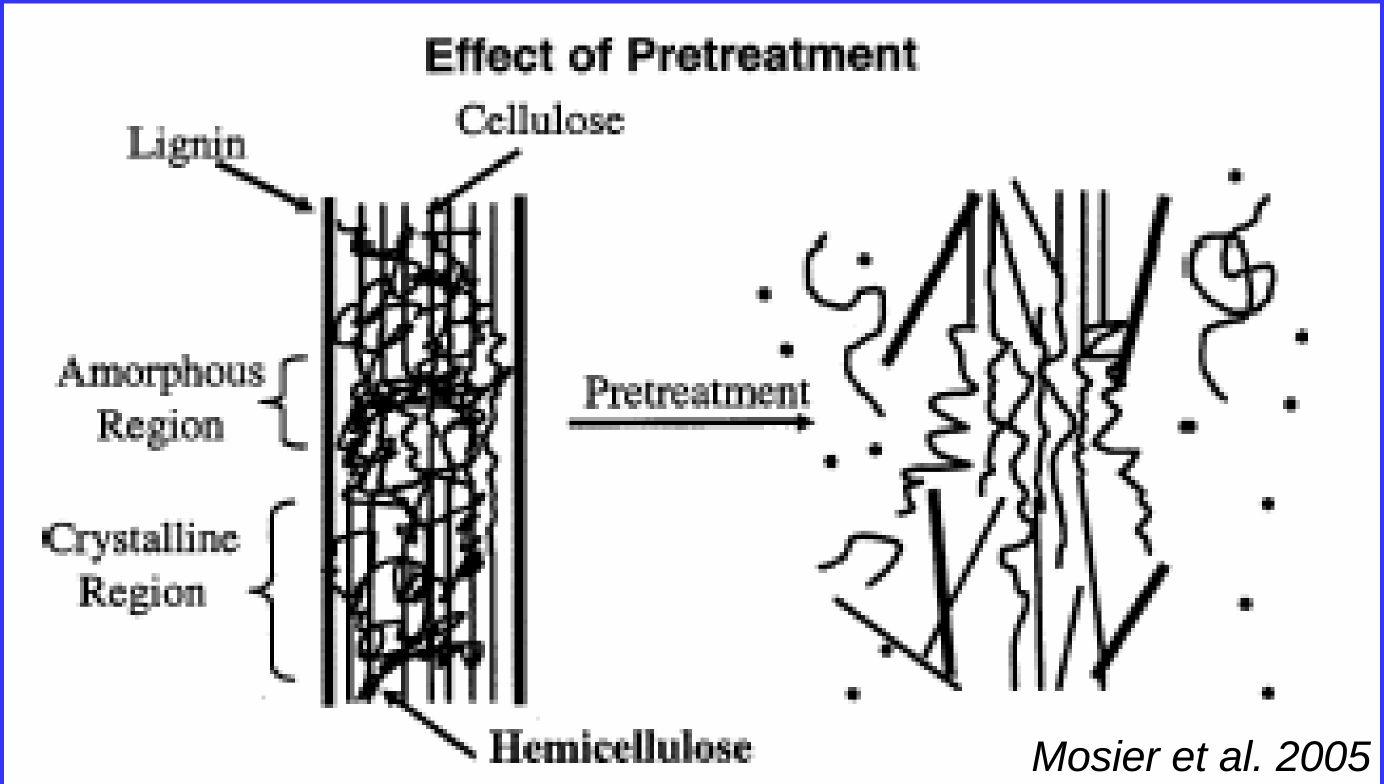
- Approximately 20% of all carbon is fixed in lignin
- Lignin provides plants with structural rigidity
- Lignin is removed from wood for paper production
  and interferes with ethanol production



Representative Structure of Lignin

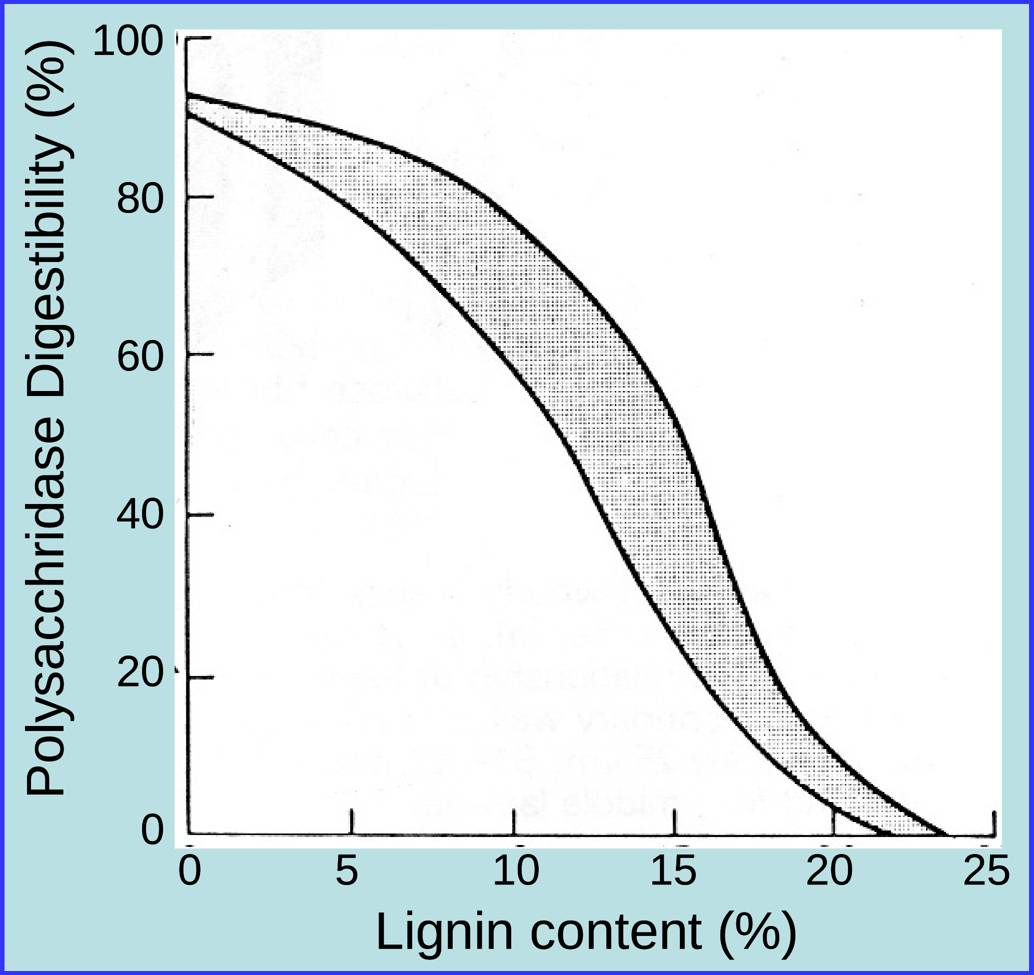
Adapted from Adler

Lignin Degradation is of Central Importance in Biomass Utilization



- Pretreatment (acid and/heat treatment) is the most expensive part of lignocellulose utilization for biofuels.
- Pretreatment disrupts the lignin encasement around the cellulose and disrupts the crystallinity of cellulose (increase access of hydrolytic enzymes.

Impact of Lignin on Polysaccharide digestibility



Strategies for dealing with the lignin barrier

- Post Harvest
  - Treat lignocellulosic material with fungal enzymes or whole fungi (area of on-going research)

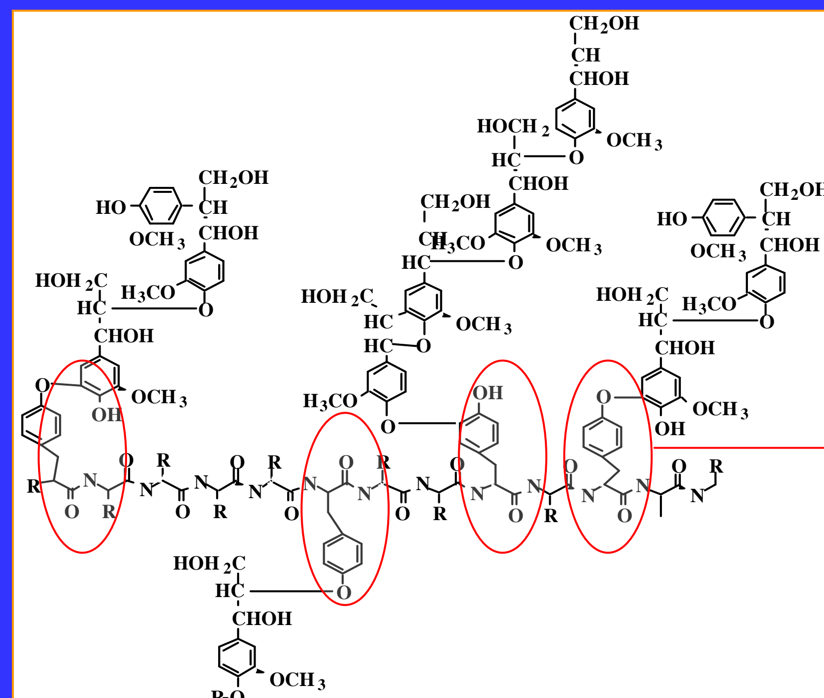
- Feedstock Modification
  - Much effort has been expended in decreasing lignin content
  - And in modifying lignin composition, i.e. increase in the syringyl to guaiacyl monomer ratio

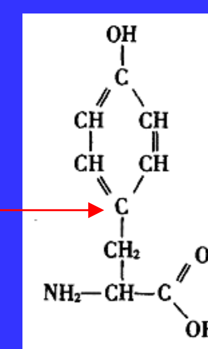
A Novel Strategy for Lignin Modification in Poplar

Our Hypothesis:

Free radical coupling between lignin subunits and TYR will result in a lignin structure that can be partially hydrolyzed with protease pretreatment. This could permit more efficient extraction of lignin and enzymatic conversion of wood to ethanol.

Model for free radical coupling between the lignol precursors and the phenolic TYR.





tyrosine

Our Approach:

Transform hybrid Poplar with a TYR-rich gene construct.

- Design TYR-rich peptides differing in length and sequence
- Express peptide transgenes in lignifying tissue in poplar
  - Populus x Euramericana cv. Neva at Beijing Forestry University
  - Populus x Ogy hybrid at Penn State University
- Characterize transgenic plants
  - Plant fitness
  - Lignin structure
  - Ethanol production

Populus x Ogy

***Populus*×*euramericana* cv.'Neva '**

Glycine-rich protein (from pea)

```
 MATIHRLPSL VFLVLLALGV CSARRALLTL DAGYGLGHGT GGGYGGAAGS 50
YGGGGGGGSG GGGGYAGEHG VVGYGGGSGG GQGGGVGYGG DQGAGYGGGG 100
GSGGGGGVAY GGGGERGGYG GGQGGGAGGG YGAGGEHGIG YGGGGGSGAG 150
GGGGYNAGGA QGGGYGTGGG AGGGGGGGGD HGGGYGGGQG AGGGAGGGYG 200
GGGEHGGGGG GGQGGGAGGG YGAGGEHGGG AGGGQGGGAG GGYGAGGEHG 250
GGAGGGQGGG AGGGYGAGGE HGGGAGGGQG GGAGGGYGAG GEHGGGAGGG 300
QGGGAGGGYG AGGEHGGGGG GGQGGGAGGG YAAVGEHGGG YGGGQGGGDG 350
GGYGTGGEHG GGYGGGQGGG AGGGYGTGGE HGGGYGGGQG GGGGYGAGGD 400
HGAAGYGGGE GGGGGSGGGY GDGGAHGGGY GGGAGGGGGY GAGGAHGGGY 450
GGGGGIGGGH GGNVP
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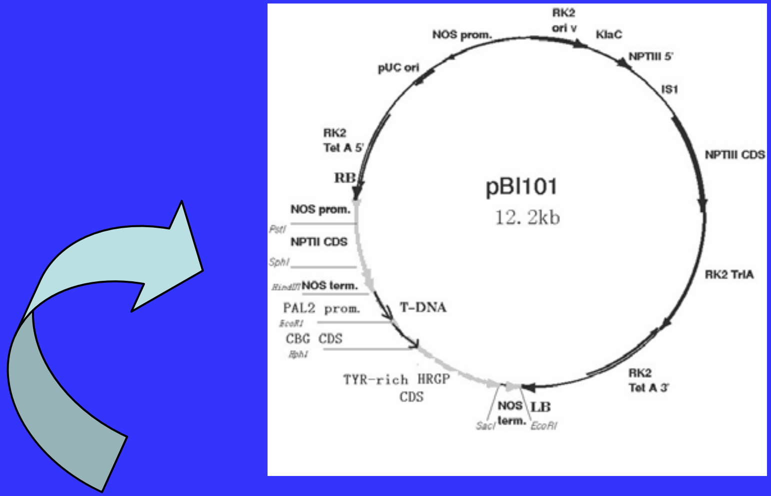
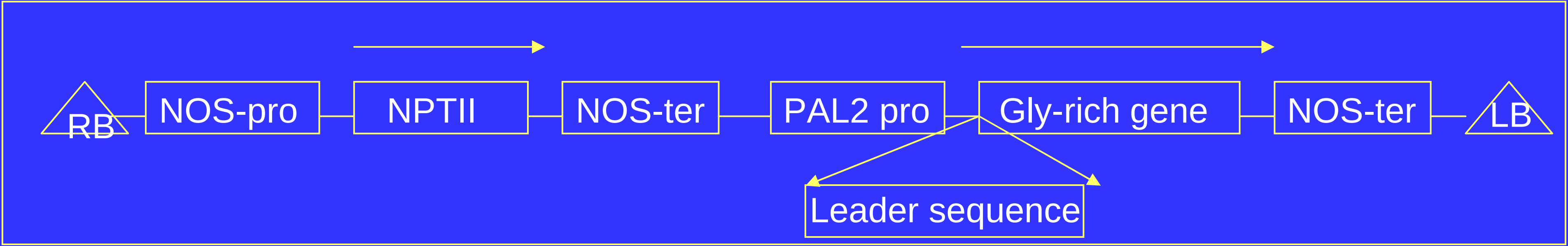
- Has elevated Tyrosine level of 7%.
- Second generation peptides have over 40% Tyrosine

Binary Vector Construction



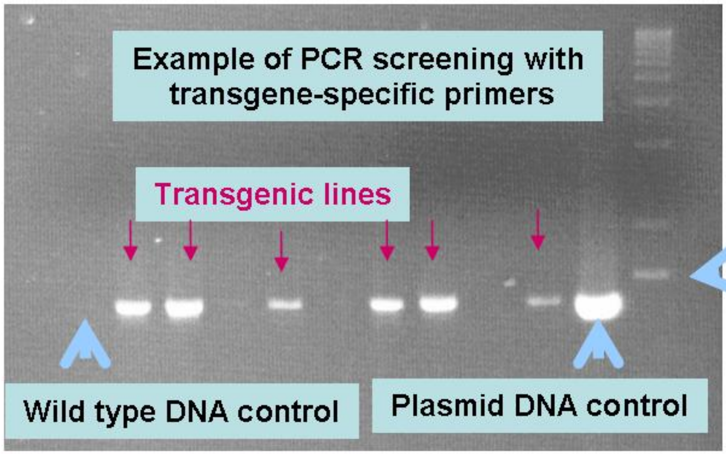


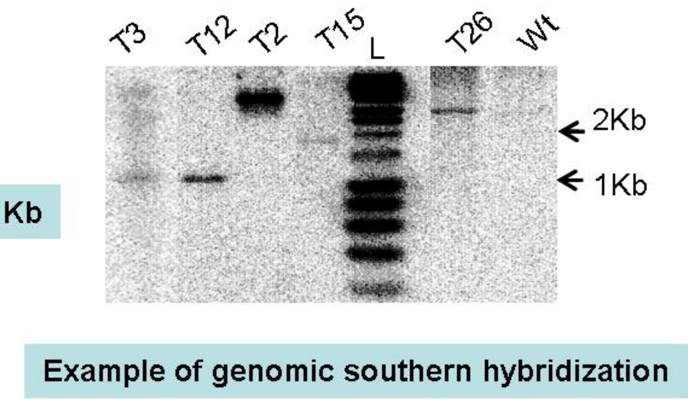
- Poplar phenylalanine ammonia-lyase (*PAL2)* promoter
- Leader sequences – Pine cell wall Glucosidase signal peptide

  \- Poplar Laccase signal peptide

PCR and of genomic Southern to show transformation

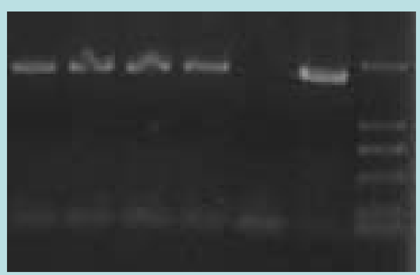
transgenic Ogy:





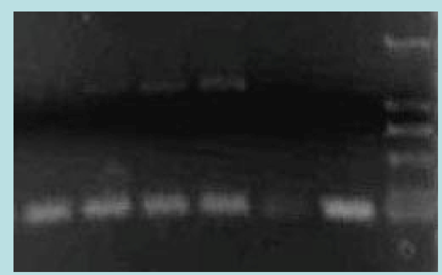
transgenic Neva:

1 2 3 4 ck-ck+M



***HRGP* gene**

1 2 3 4 ck-ck+M



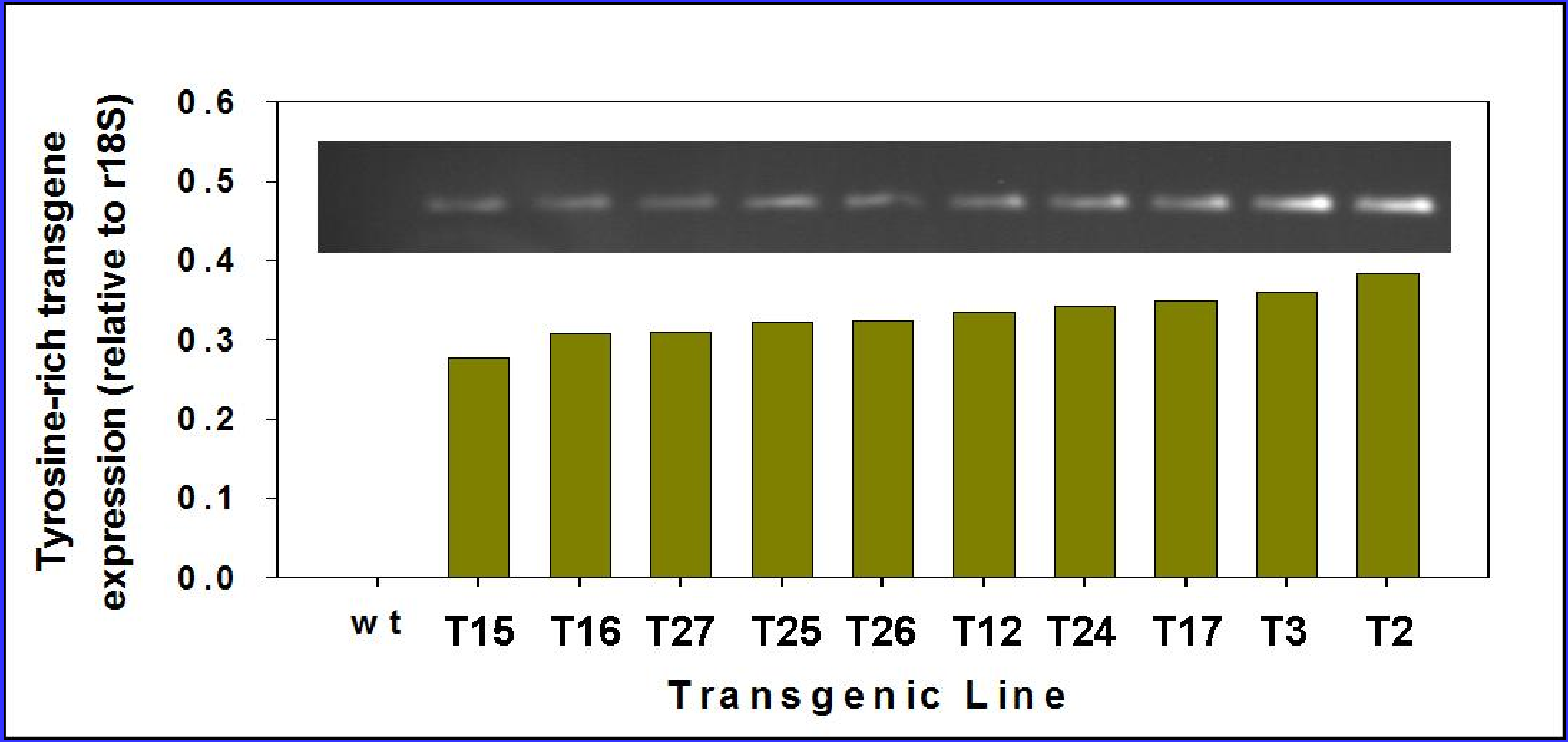
***NPT II* gene**

Transgenic poplar plants

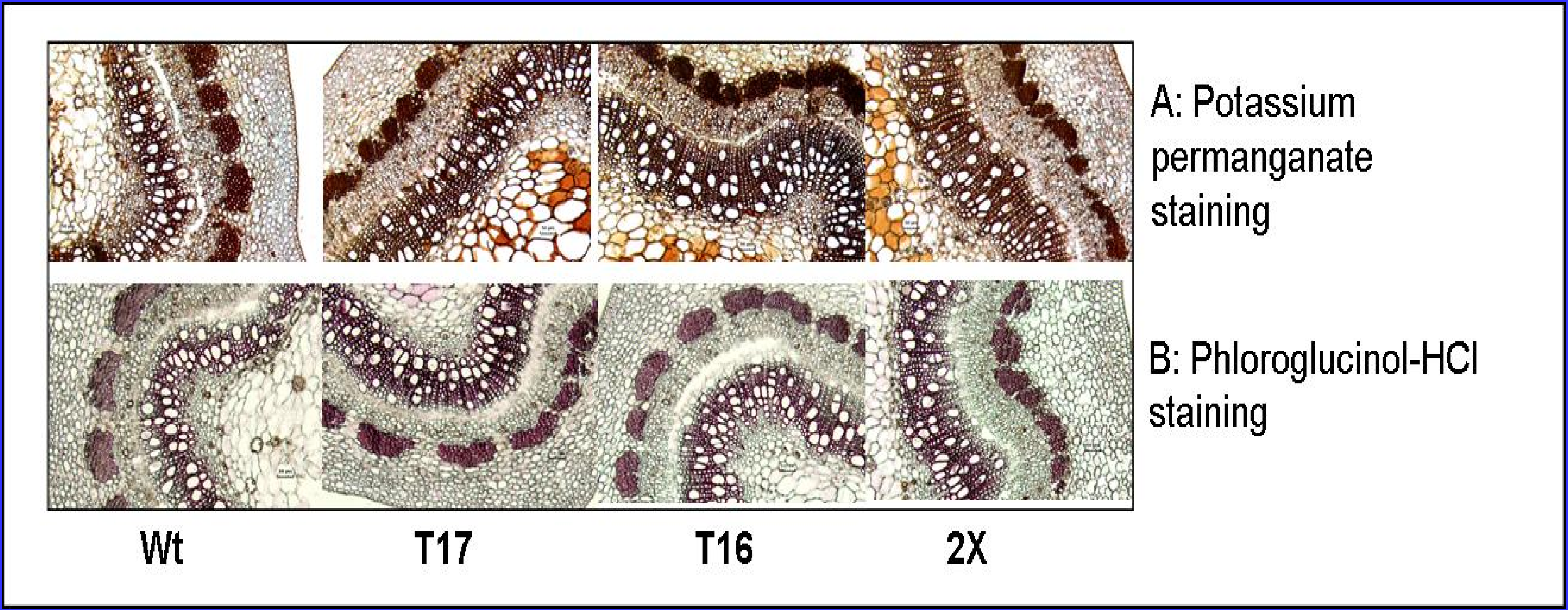
transgenic Neva:

transgenic Ogy plants in the green house

Detection of transcriptional expression of Gly-rich protein construct by Real-time PCR

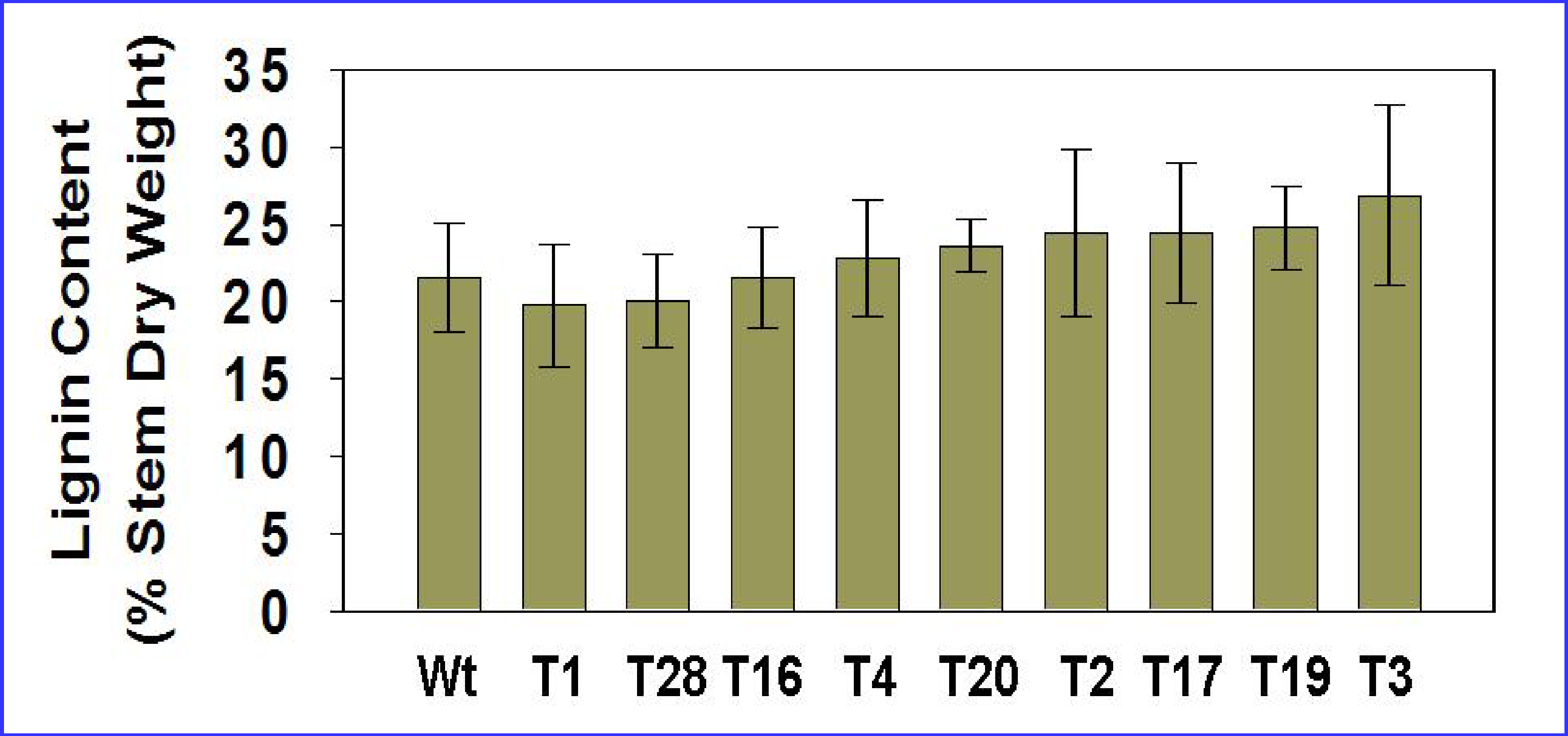


Histochemical staining for lignin

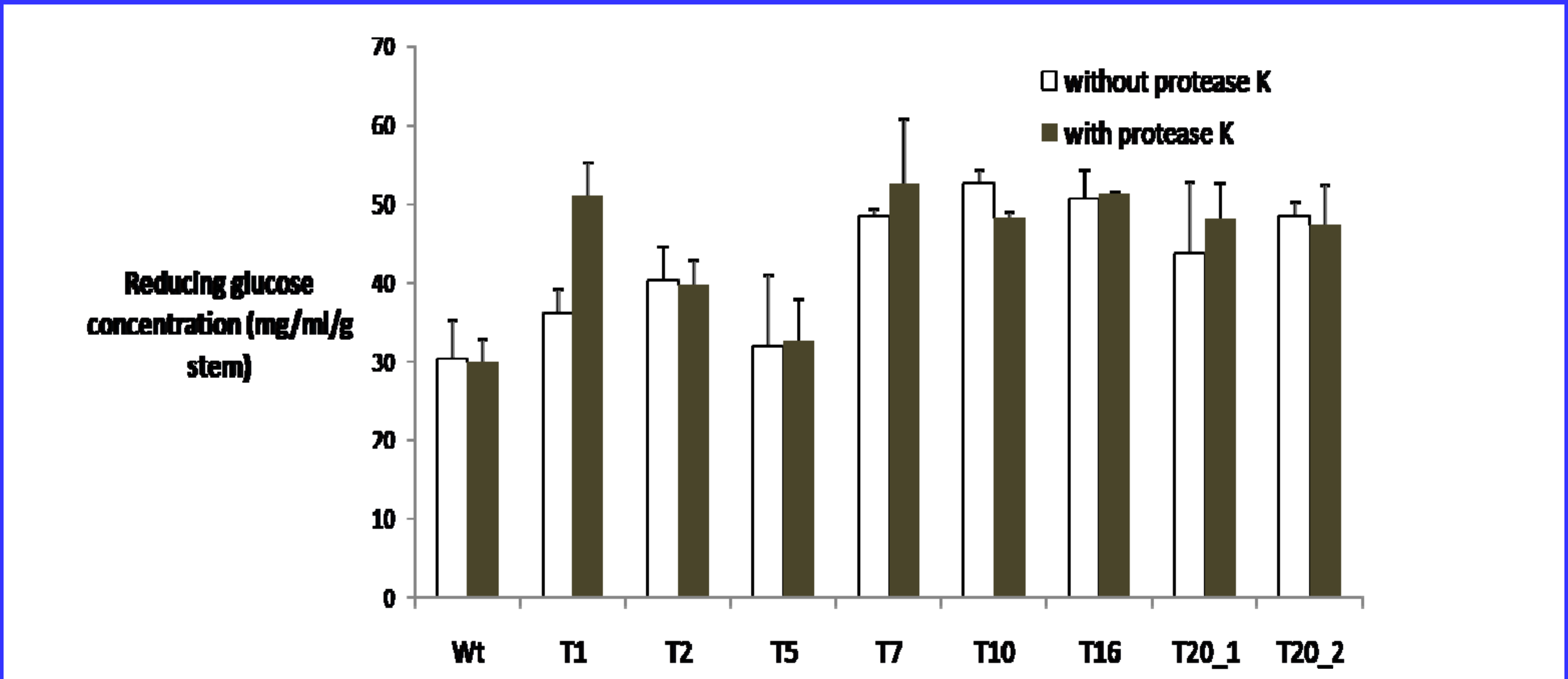


No visible differences for lignin content between Wild-type and Transformed plants

Lignin content not compromised

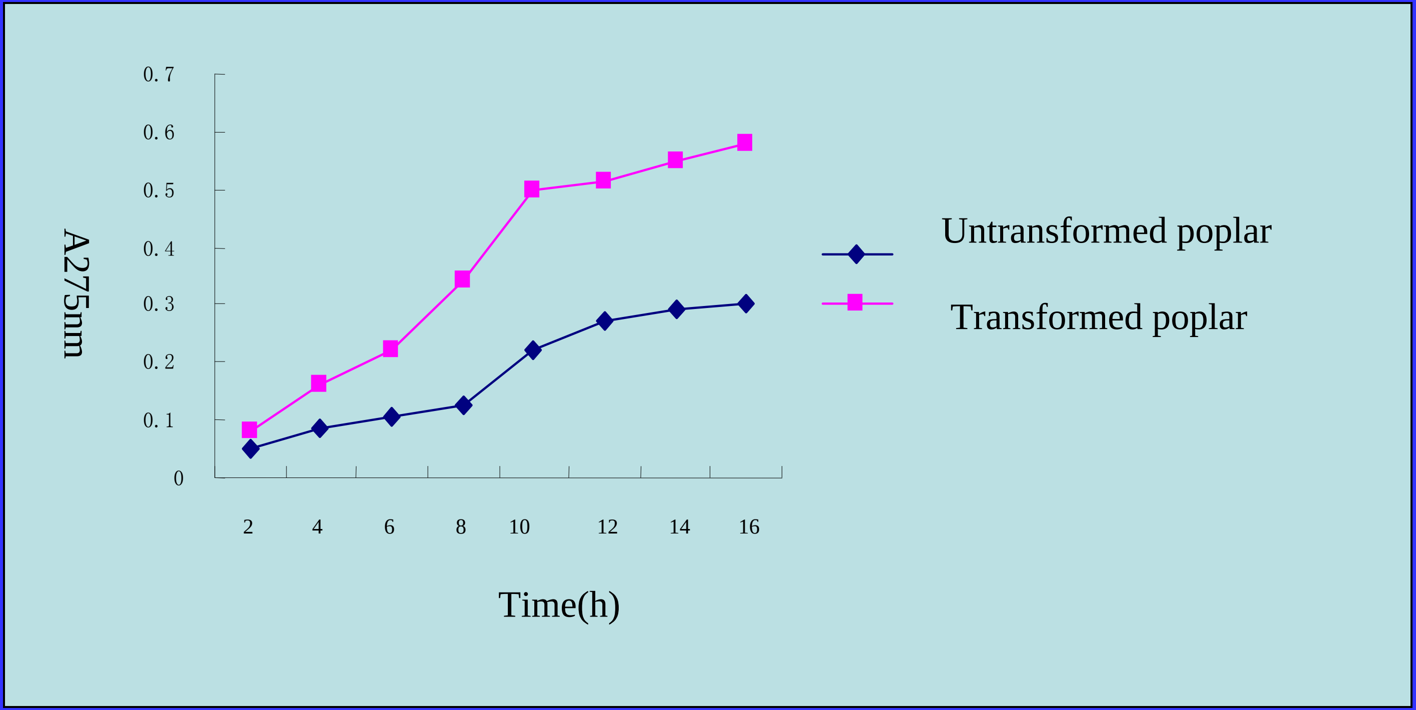


Digestibility assay with protease K



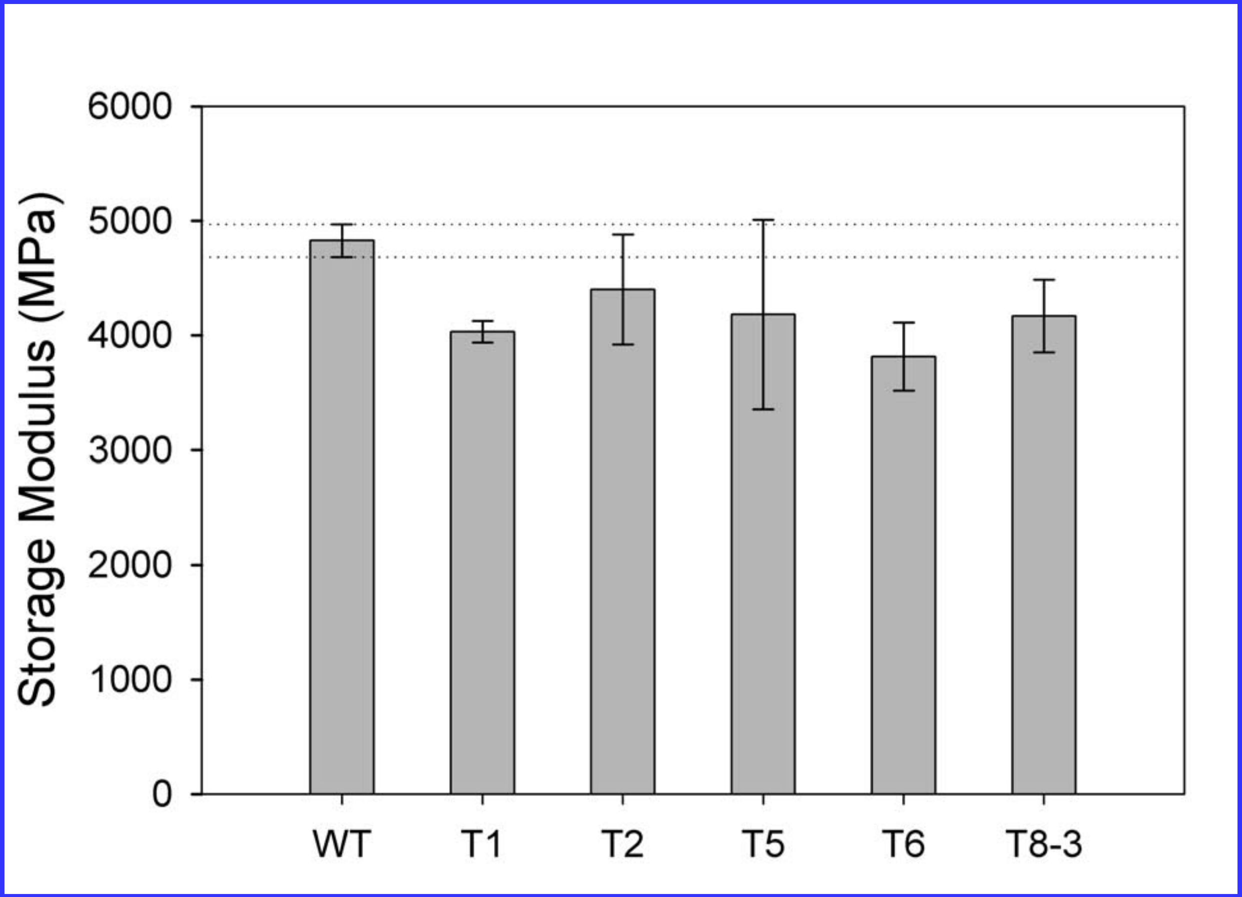
Reducing sugar concentrations in stem tissue extracts of hybrid poplar *“Ogy”* wildtypes and transgenic lines. For each line, a portion of ground tissue was incubated with sequential incubations of protease K followed by cellulase and hemicellulase (shaded bars), while another portion of tissue was incubated only with cellulase and hemicellulase (open bars). Bars are means + SD of 2-3 replicates of individual samplings.

Protease tests with 'Neva' transgenics



UV absorbance at the wavelength of 275nm over time

Dynamic mechanical analysis



Averages of 5 measurements on each of 2 samples from plants of the same transgenic line.

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