

Metabolic and transcriptomic shifts during tobacco leaf post-harvest senescence

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Curing

- Post-harvest processes aim to obtain a dry and storable material
- Changes during curing contribute to the **quality** of the tobacco leaf
- Curing generally involves two phases:
 - **Yellowing**, when leaf senescence occurs (cell death, remobilization)
 - Drying, when residual water is removed from the lamina and mid-rib
- Research question: while curing outcome is strongly dependent on process, what are the biological/genetic factors at play during this particular step?



Curing time course of three major tobacco types

0h

R

G

Harvest



AIR-CURED

FLUE-CURED VIRGINIA

SUN-CURED

BURLEY





Yellowing

12h 24h 48h 96h 8d

Time course: Chemistry Gene expression

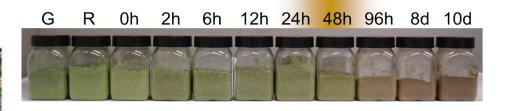
different: environment varieties practices

Drying

16d 31d 45d



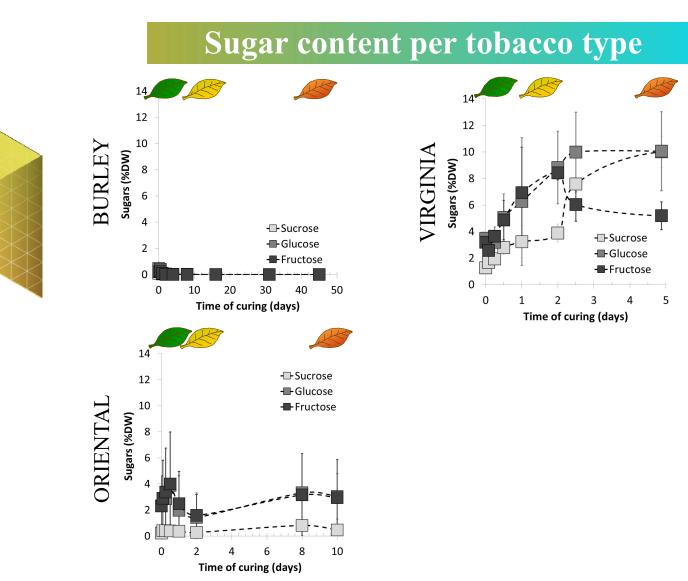






- Free amino acid and sugars changes during curing
- Overall gene expression changes
- Correlations

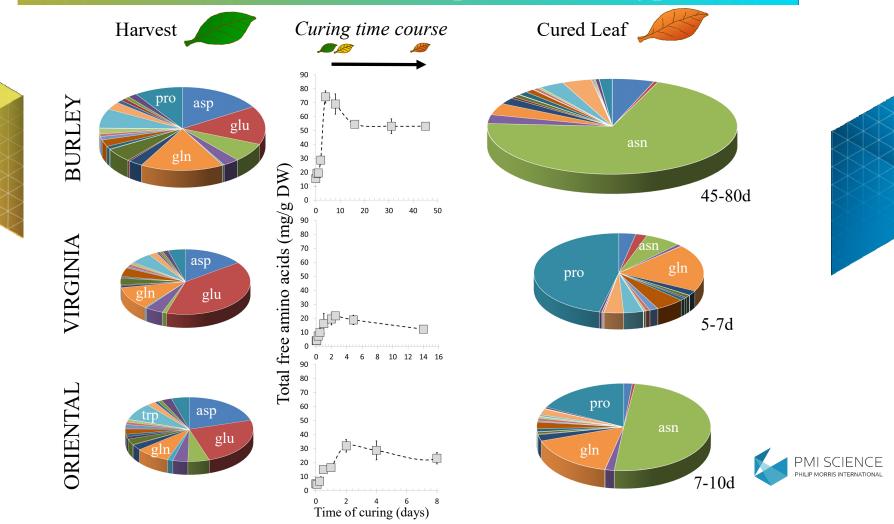




Suc



Total free amino acid content per tobacco type

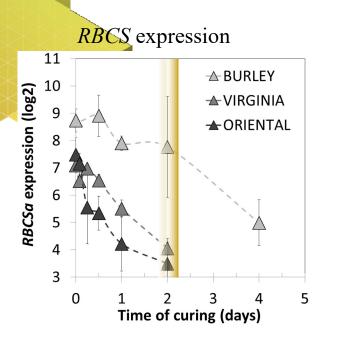


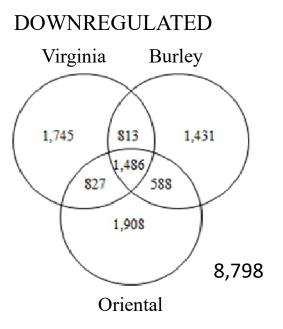


- Free amino acid and sugars changes during curing
- Overall gene expression changes
- Correlations



Genes commonly downregulated during yellowing





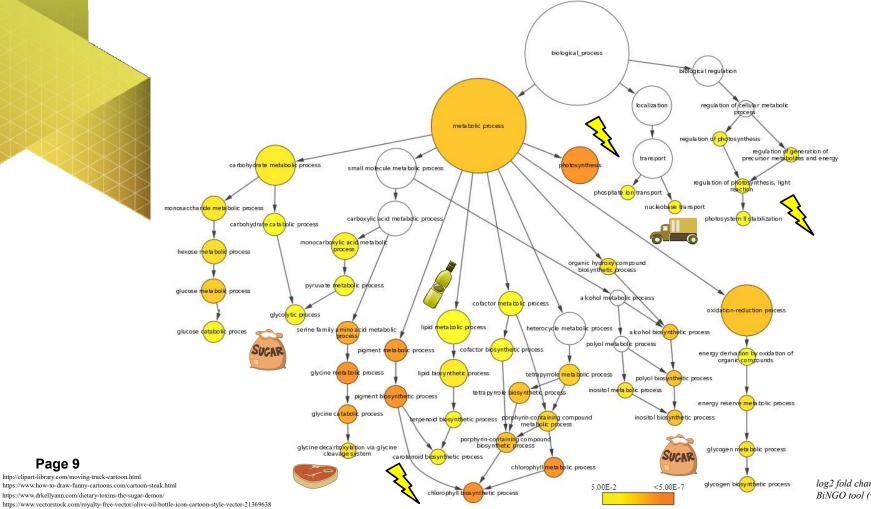
Number of genes: 81,404RNAseq data log2 fold change ≤ -2 , adjusted p value ≤ 0.05 Gene Ontology: main
downregulated gene productsOxidation-reduction processesCarbohydrate metabolismLipid metabolismPhotosynthesis (RBCs)

>10 gene products per family



Yellowing - downregulated genes

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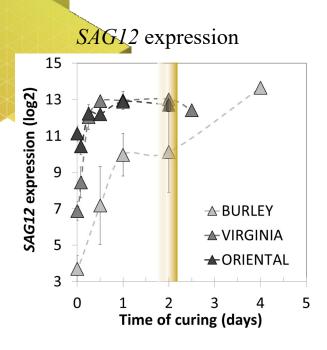


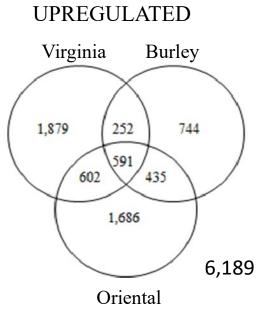


n = 1486**PMI SCIENCE** PHILIP MORRIS INTERNATIONAL

log2 fold change \geq -2, adjusted p value \leq 0.05 BiNGO tool (version 3.0.3; Maere et al. 2005)

Genes commonly upregulated during yellowing



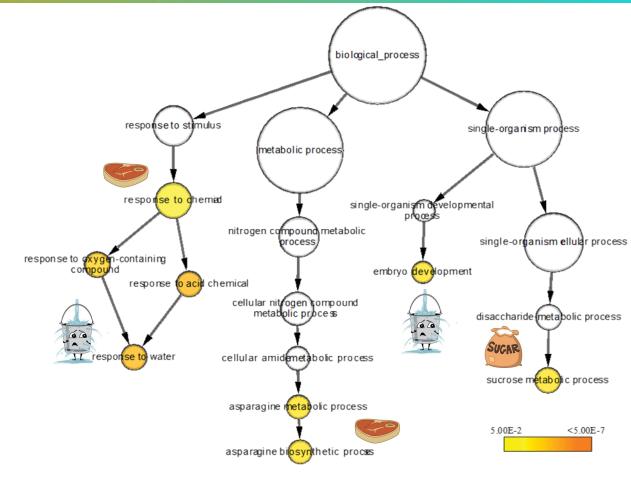


Number of genes: 81,404 RNAseq data log2 fold change ≤ 2 , adjusted p value ≤ 0.05 Gene Ontology: main
upregulated gene productsOxidation-reduction processesOxidation-reduction processesZinc ion bindingTranscription factorsTransmembrane transportProtein bindingProtein phosphorylationProteolysis (*SAG12*)DNA bindingIron ion bindingHydrolase activity

>10 gene products per family



Yellowing - upregulated genes





n = 591 PMI SCIENCE

log2 fold change >=2, adjusted p-value <=0.05 BiNGO tool (version 3.0.3; Maere et al. 2005)

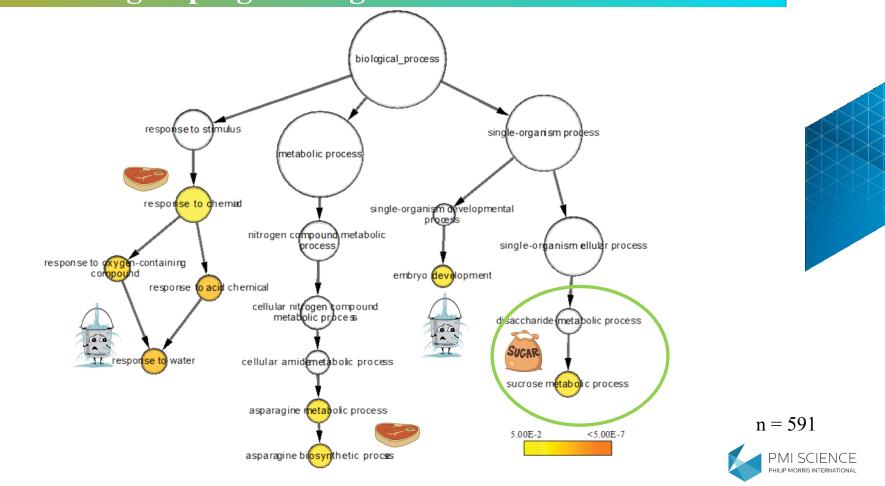
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- Free amino acid and sugars changes during curing
- Overall gene expression changes
- Correlations

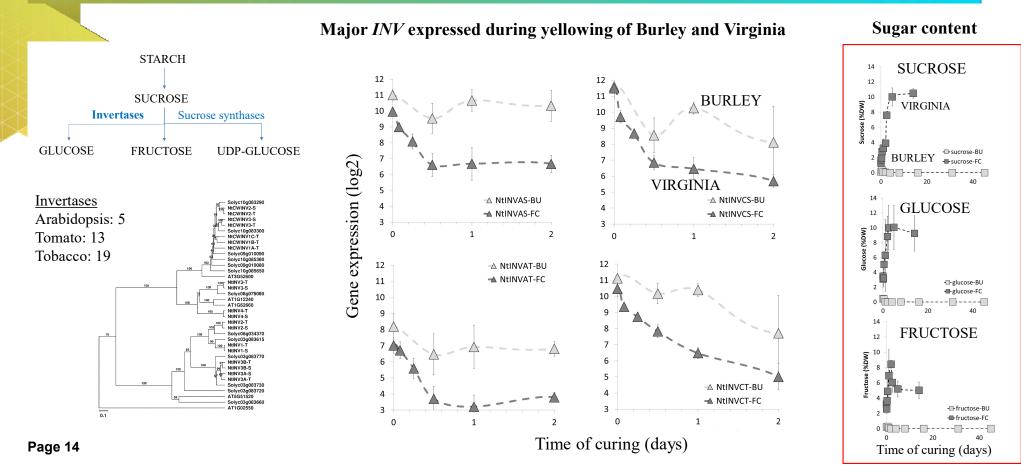




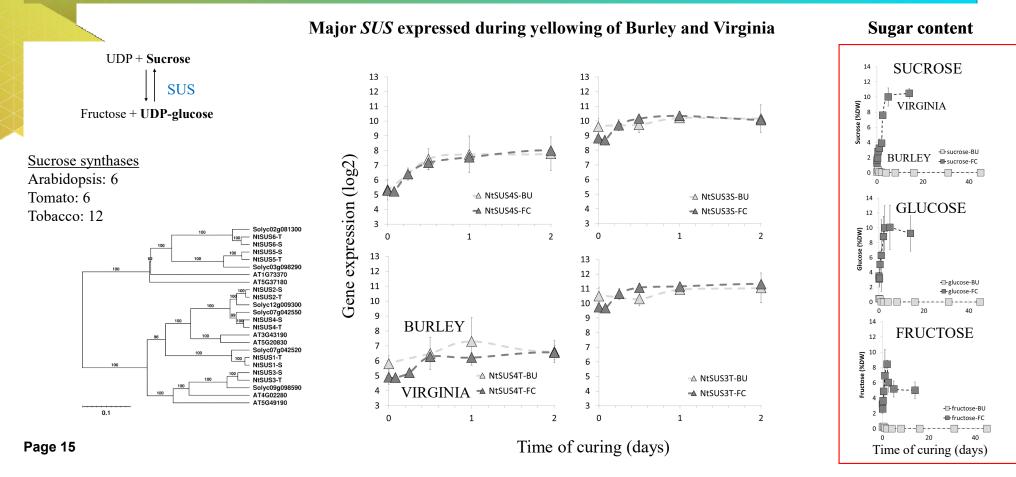


log2 fold change >=2, adjusted p-value <=0.05 BiNGO tool (version 3.0.3; Maere et al. 2005)

Most of the *invertases (INV)* have different expression profiles during yellowing in Burley and Virginia



Sucrose synthase (SUS) expression profiles during yellowing are similar in Burley and Virginia

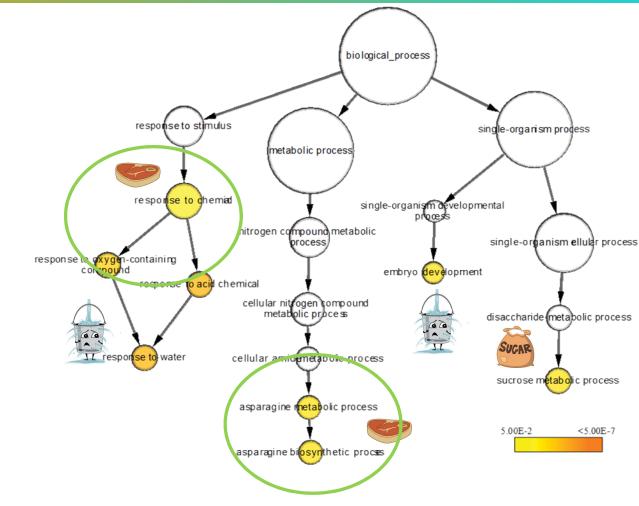


Remarks

 Sucrose synthase genes appear to be expressed similarly in Burley and Virginia tobacco during yellowing, independent of the growing and curing conditions, which attests to a key senescence-activated genetic program for sugar metabolism



Yellowing - upregulated genes



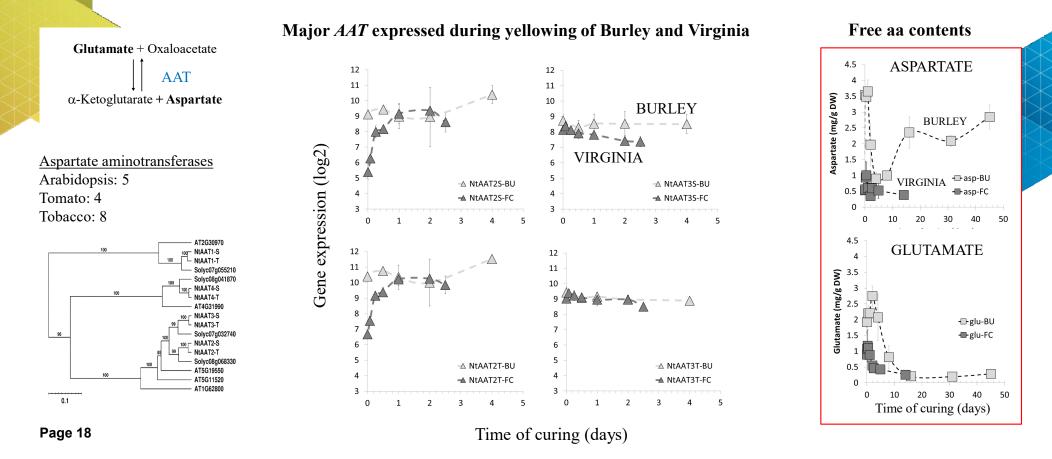


n = 591 PMI SCIENCE PHILIP MORRIS INTERNATIONAL

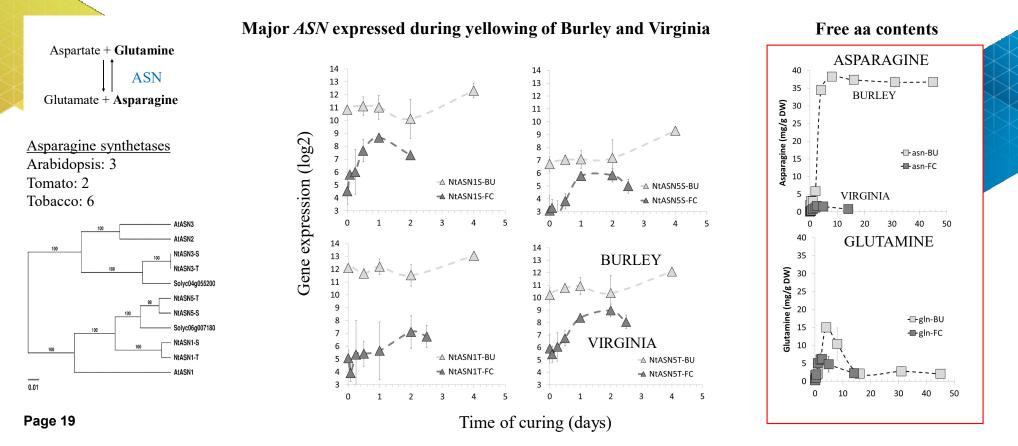
log2 fold change ≥ 2 , adjusted p value ≤ 0.05 BiNGO tool (version 3.0.3; Maere et al. 2005)

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Aspartate aminotransferase (AAT) expression profiles during yellowing are similar in Burley and Virginia



Asparagine synthetases (ASN) are up-regulated during yellowing in Burley and to a lesser extent in Virginia



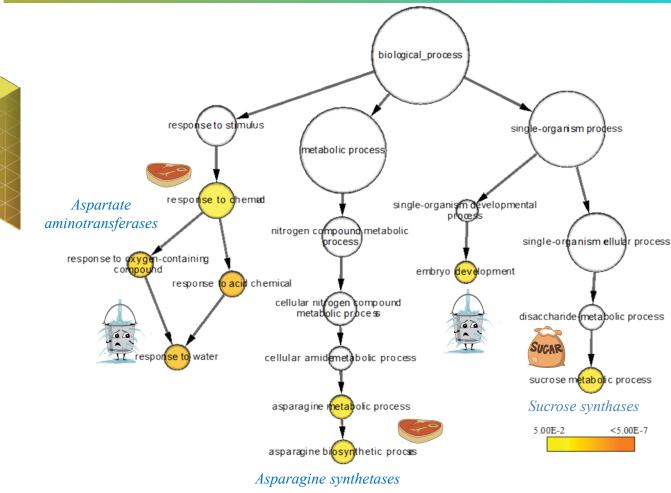
Asparagine is a source-sink nitrogen transport form (Masclaux-Daubresse, 2006)

Remarks

- Aspartate aminotransferase genes appear to be expressed similarly in Burley and Virginia tobacco during yellowing
- Asparagine synthetase genes are upregulated in Burley and Virginia during yellowing. However the gene expression level is lower in Virginia than in Burley, in correlation with the asparagine content in cured leaf



Yellowing - upregulated genes





n = 591 PMI SCIENCE PHILIP MORRIS INTERNATIONAL

log2 fold change ≥ 2 , adjusted p value ≤ 0.05 BiNGO tool (version 3.0.3; Maere et al. 2005)

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Conclusion

- Tobacco types reflect different genetics environments and practices. The variable chemical content is an opportunity to learn about the biological process occuring during leaf yellowing.
- Transcriptomic analyses of three cultivated tobacco types (Burley, Virginia, and Oriental) revealed an active gene regulation processes
- It is possible to identify regulated gene networks associated with the changes in tobacco leaf chemistry
- Panel of genetic elements potentially influencing quality









