# Metabolic and transcriptomic shifts during tobacco leaf post-harvest senescence

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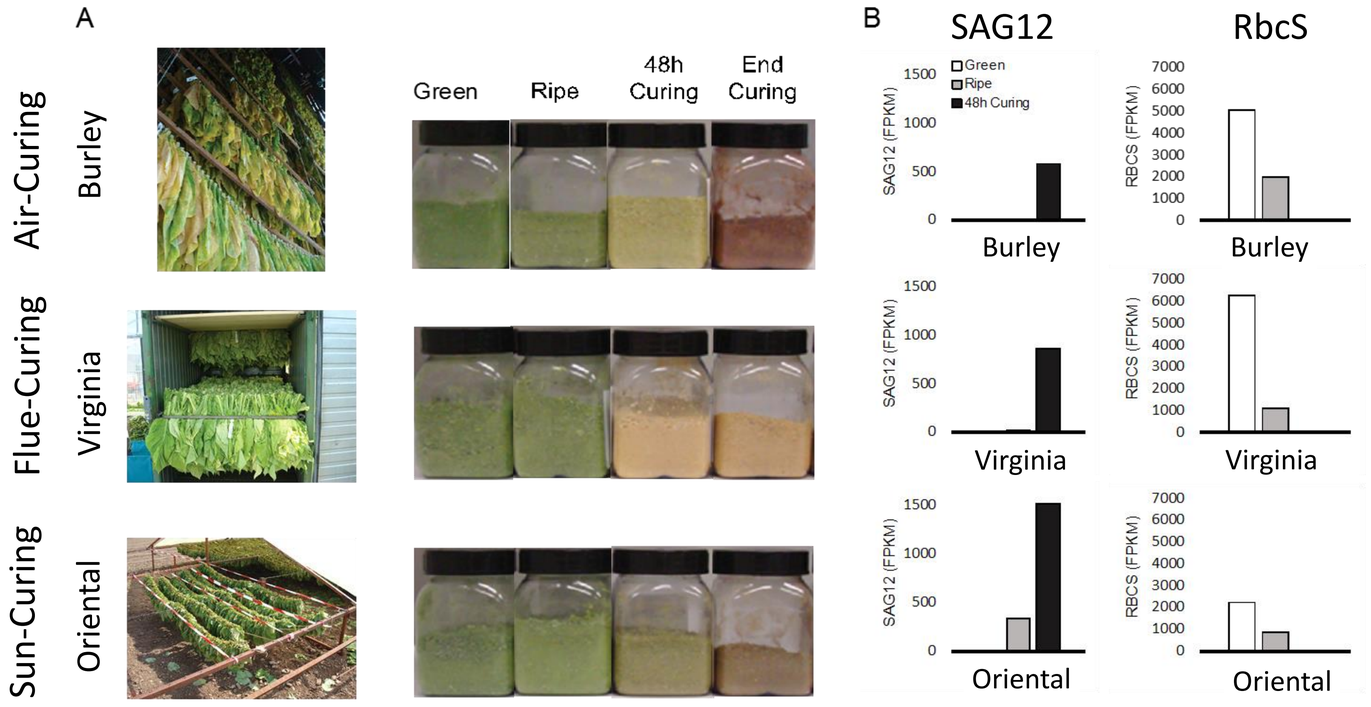
During leaf curing, the process of senescence is initiated when leaves are detached from the tobacco plant, leading to "leaf yellowing" and followed by a drying step. The chemical composition changes drastically upon yellowing. While this process occurs in all cultivars, there are major differences between tobacco varieties, such as the amount of reducing sugars and produced amino acids. In this study, we characterized the transcriptomic and metabolic changes occurring upon leaf detachment of the three main commercial tobacco varieties: Virginia, Burley, and Oriental.

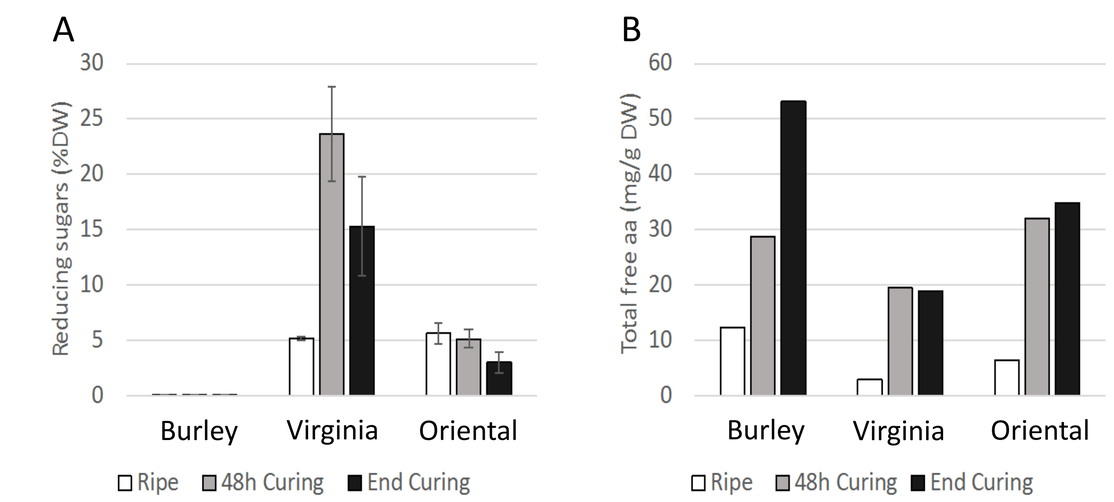
## Curing practices of the three main tobacco types: Burley, Virginia, and Oriental




**(A)** Burley (Stella) leaves were air-cured in a barn (CH), Virginia (ITB683) leaves were flue-cured in an oven (CH), and Oriental (Katerini) leaves were sun-cured (GR). Leaves were collected at mid-stalk position from field-grown tobacco plants (green), at harvest time (ripe), after two days of curing (48-hour curing), and at the end of curing. The yellowing phase starts after leaf harvest and can be monitored by gene expression; it corresponds to a typical senescence process including chlorophyll degradation.

**(B)** The tobacco frozen material was either freeze-dried for chemical analyses or subjected to RNA sequencing (RNAseq) analyses (except for the material collected at the end of curing). From RNAseq analyses, the marker *genes senescence-associated gene 12* (*SAG12*) and *RuBisCO small subunit* (*RbcS*) are upregulated and downregulated, respectively, after two days of curing in all three tobacco varieties (data are expressed in fragments per kilobase million [FPKM]).

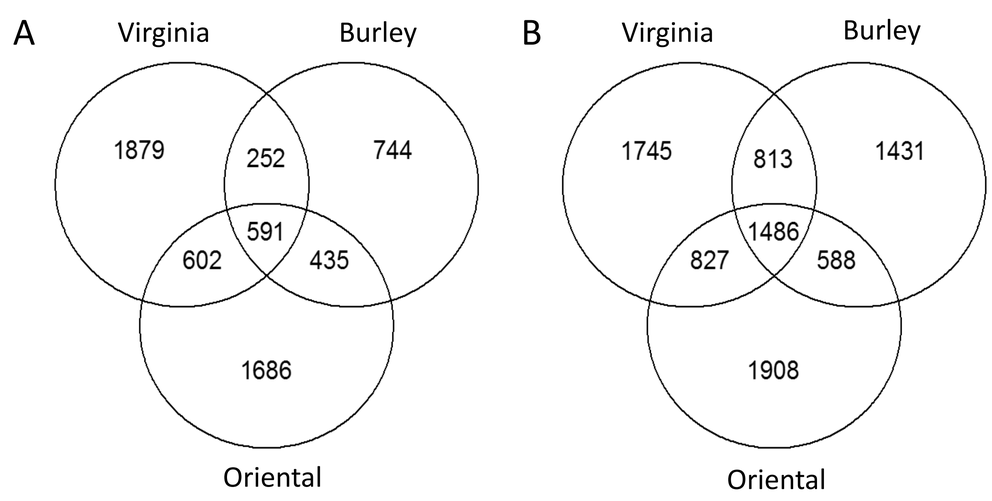
## Evolution of reducing sugars and total amino acids during curing




The leaf content of reducing sugars **(A)** and total free amino acids **(B)** increased after harvest (ripe), two days of curing (48-hour curing), and at the end of curing (except for reducing sugars in Stella).

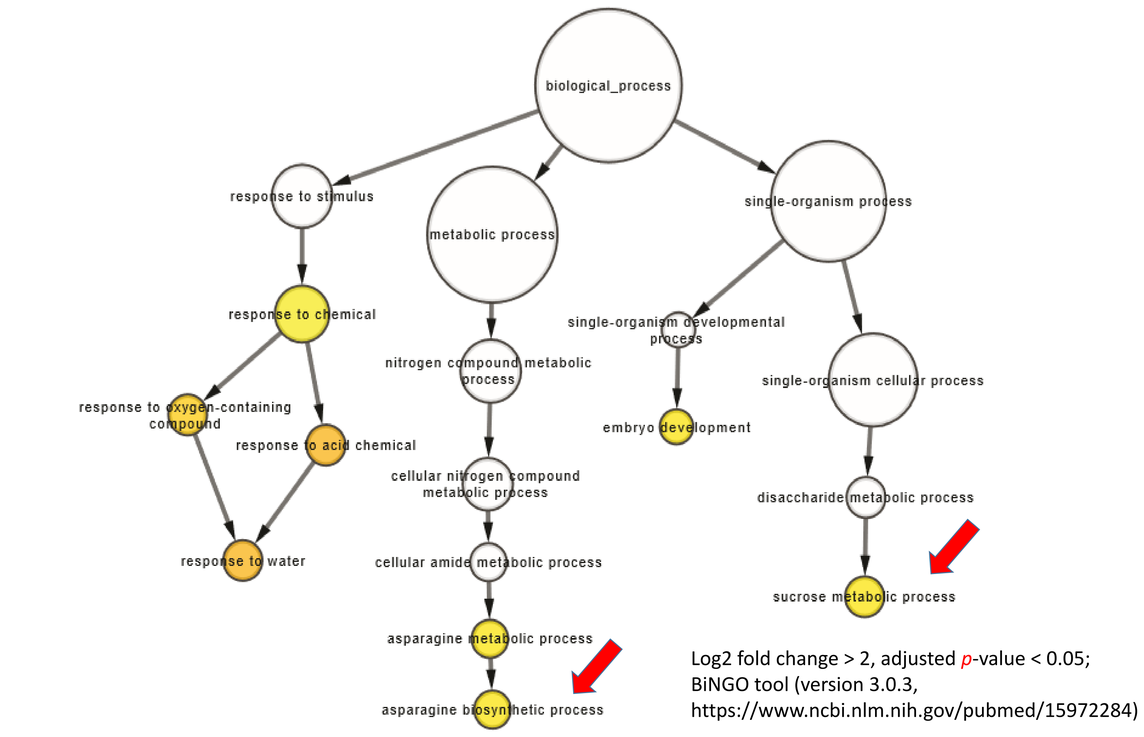
The data attest that during curing (leaf senescence), cellular activities led to the formation of reducing sugars and free amino acids as a common activity in commercial tobacco varieties.

## Upregulated and downregulated genes after 48-hour curing




Among all the genes, about 8% were found to be upregulated after 48-hour curing in the three tobacco varieties. 591 genes were activated in common after 48-hour curing, independently of the curing process and the origin of the tobacco cultivars **(A)**. On the other hand, about 12% of the genes were found to be downregulated after 48-hour curing. 1,486 downregulated genes were in common in Burley, Virginia, and Oriental **(B)**. All data are calculated based on log2 fold change > 2, adjusted *p*-value < 0.05).

## GO term overrepresentation analysis for genes upregulated upon 48-hour curing in all three tobacco varieties




Log2 fold change > 2, adjusted *p*-value < 0.05;
BiNGO tool (version 3.0.3,
https://www.ncbi.nlm.nih.gov/pubmed/15972284)

| GO terms | Tobacco transcripts | Tomato orthologs |
|---|---|---|
| response to chemical | 13 | 6 |
| sucrose metabolic process | 4 | 3 |
| response to acid chemical | 4 | 1 |
| response to oxygen-containing compound | 4 | 1 |
| response to water | 4 | 1 |
| asparagine metabolic process | 3 | 1 |
| asparagine biosynthetic process | 3 | 1 |
| embryo development | 2 | 1 |

The most GO term overrepresented functions are related to 37 upregulated tobacco genes corresponding to 15 tomato orthologs. Interestingly, several transcripts involved in sugar and amino acid (asparagine) metabolism are identified in the GO terms list. This observation is in line with the increase of reducing sugars and free amino acids observed during tobacco leaf curing, thus providing some gene targets to identify novel metabolic pathways.

The curing process common to all major tobacco varieties is related to leaf senescence involving a set of genes linked to sugar and amino acid metabolisms. Such activated genes play certainly a role in a more global carbon and nitrogen remobilization process occuring during the leaf yellowing phase.

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