

# Transcriptomic approach to unveil the molecular basis underlying apple sunscald development during cold storage

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## Background

Sunscald is a significant post-harvest physiological disorder in apples, induced during cold storage after the fruit has been exposed to high heat and sunlight prior to harvest. This condition results in browning of both the peel and the cortex of the fruit. 'Granny Smith' apples are particularly susceptible to sunscald due to their physiologically immature harvesting and low starch levels. The goal of this research project is to identify genes that may contribute to the development of sunscald and to formulate hypotheses for subsequent lab-based validation and testing.

## Methods

This project involved field-work for sample collection, lab-work for sample preparation and sequencing and computational analysis to identify genes and their function. The process is described as follows:

### Step 1: RNA Collection

- Peels from sun-facing and shade side of apples
- Apples stored in refrigerator storage and sampled every few weeks (for 24 weeks)
- Three biological replicates each

### Step 2: RNA Sequencing

- Illumina short-read sequencing

### Step 3: Gene Expression Quantification

- Read mapping and quantification: GEMmaker
- Outcome: Quality reports and gene expression matrix

### Step 4: Differentially Expressed Gene (DEG) Analysis

- Identify genes whose expression is statistically different between sun and shade exposure

### Step 5: Network Analysis

- Identify groups of genes that are working together

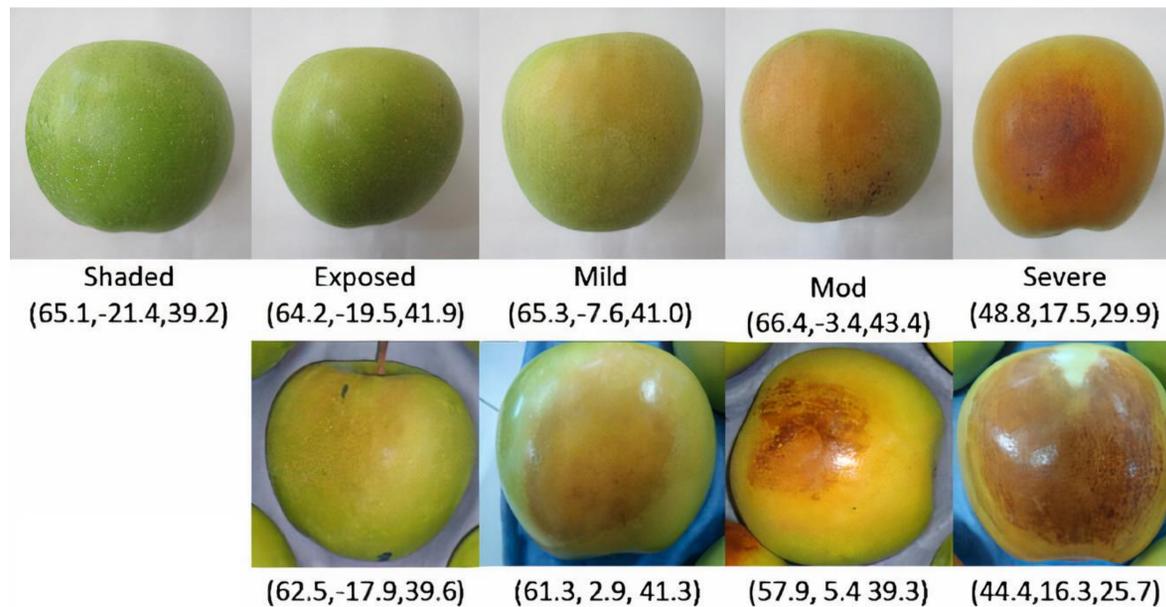
### Step 6: Functional Analysis

- Identify the function of DEGs and genes in network modules

### Step 7: Develop Hypotheses

- Are there genes that underlie differences in sun exposure that may be resulting in sun scald in storage?

# Understanding the genes and pathways underlying the development of sunscald using transcriptome and computational biology, a step towards improving postharvest practices



Sunscald symptoms after cold storage

'Granny Smith' apples with different severity of sunscald. Lelièvre, J.-M., Latché, A., Jones, B., Bouzayen, M., & Pech, J.-C. (2013). Ethylene and fruit ripening. *Postharvest Biology and Technology*, 86, 136-144. <https://doi.org/10.1016/j.postharvbio.2013.08.003>

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## Results:

- 1210 DEGs in sun samples compared to shade across all time points
- 692 Up regulated genes
- 72 Functionally enriched terms

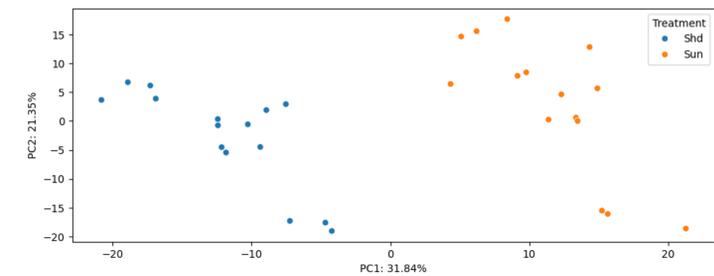


Figure 1. Principal Component Analysis for DEGs. Principal Component Analysis (PCA) graph of 1210 DEGs in sun compared to shade. The x-axis represents an unknown variable that contributes to 31.84% variation across all samples. The y-axis represents an unknown variable that contributes to 21.35% of the variation across all samples

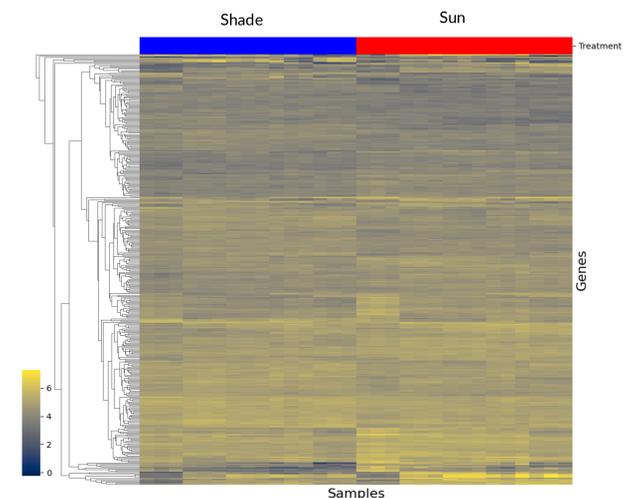


Figure 2. Heat map of expression of 1210 DEGs. The x-axis is the samples ordered by treatment and week. The y-axis are the 1210 DEGs ordered by similarity of expression. The red and blue bars group samples by treatment (blue for shade and red for sun). The shading gradient represents the log<sub>2</sub> level of expression.

Table 1. Functionally Enriched Terms. Abbreviated table of all 72 functionally enriched terms

Module <sup>1</sup>	Vocabulary <sup>2</sup>	Term	Name
Upregulated	IPR	IPR001907	ATP-dependent Clp protease proteolytic subunit
Upregulated	KEGG Ortholog	K01358	clpP, CLPP; ATP-dependent Clp protease, protease subunit [EC:3.4.21.92]
Upregulated	IPR	IPR023562	Clp protease proteolytic subunit /Translocation-enhancing protein TepA
Upregulated	IPR	IPR002068	Alpha crystallin/Hsp20 domain
Upregulated	IPR	IPR008978	HSP20-like chaperone

<sup>1</sup> A module is the list of DEGs that were grouped as upregulated or downregulated

<sup>2</sup> Controlled list of words (terms) that describe functional roles of genes.

## Discussion:

Expression in these up regulated genes gives us a glimpse of the potential factors in the development of sunscald in 'Granny Smith' apples. To find these genes I got to work with Linux and bash coding, high performance computing, and processing RNA-Seq data into quantitative data sets.