

# CACAO CELL CULTURES: A CONTROLLED SYSTEM TO STUDY FLAVONOID PRODUCTION

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**PennState**

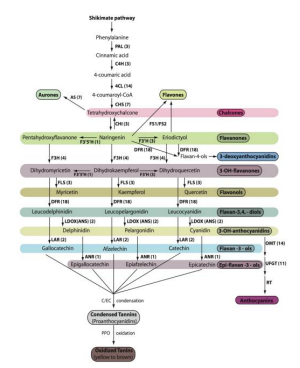
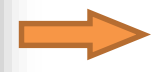
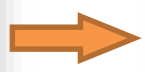


# Cacao flavonoids are important in plant defense and human health, but are lost during manufacturing



30% decrease flavonoid content

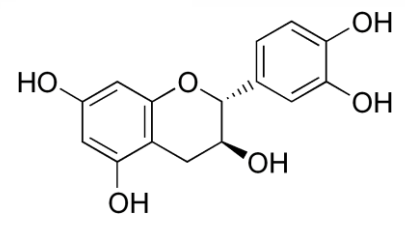
45% antioxidant activity



Simple and controlled



Traditional chocolate

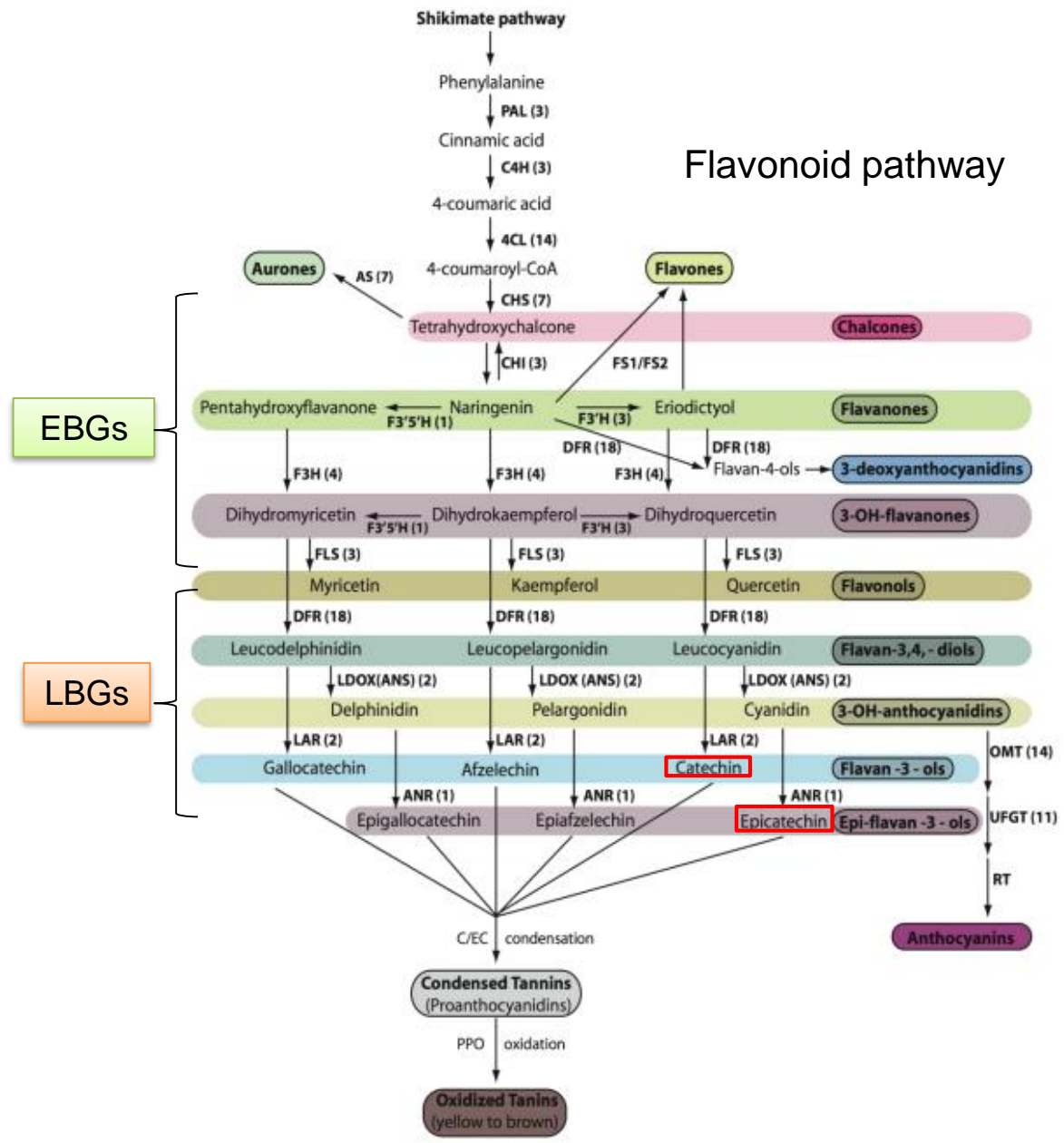
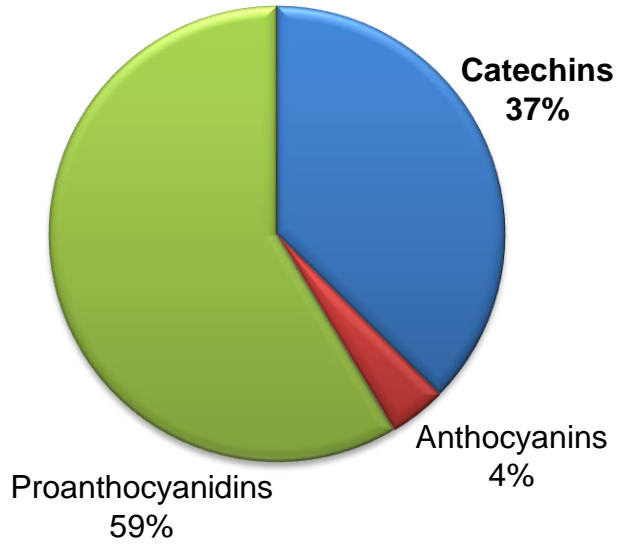
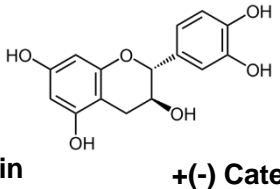
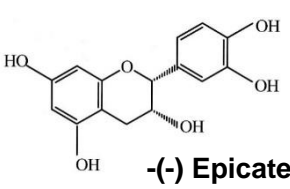


Biomass rich in flavonoids from cell cultures



Healthier chocolate rich in flavonoids

# Regulation of flavonoids is very complex

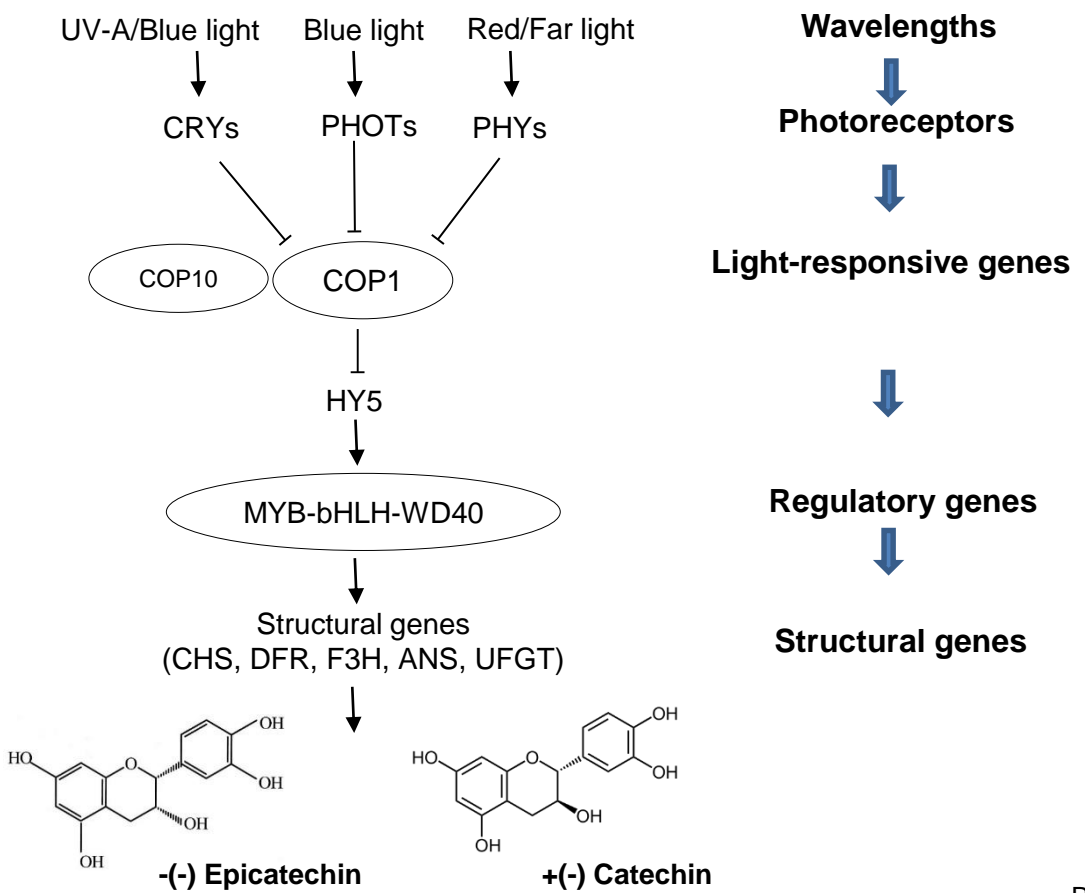


# Light is one the main factors affecting growth and flavonoid production in cell suspensions



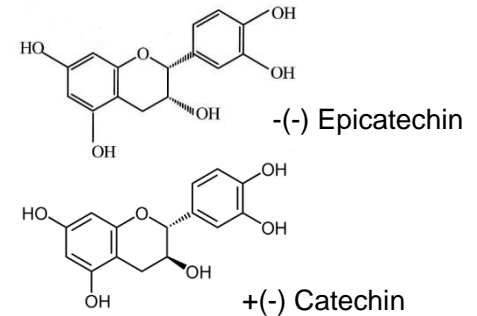
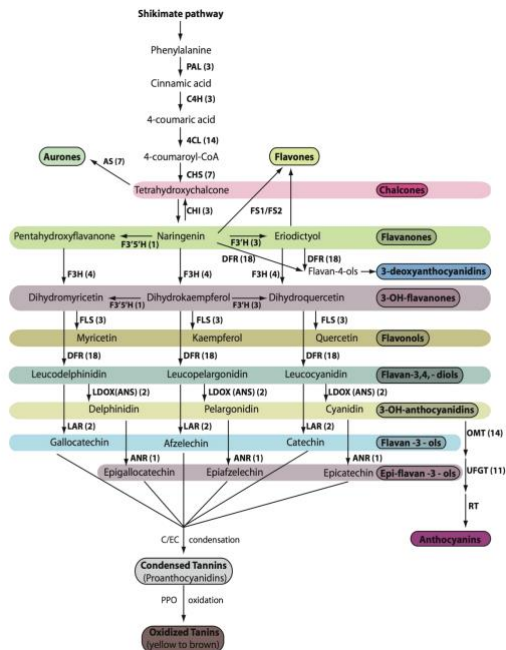
LEDs lights (Light Emmiting Diodes)

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# Main Objective

To determine the link between transcriptional regulation underlying flavonoid accumulation in response to light changes in cacao cell suspensions.



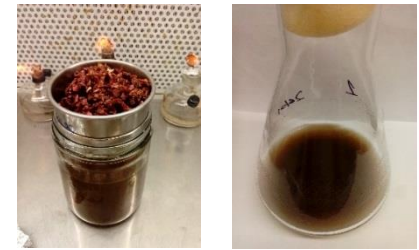
# Methodology



Plant material



Enzymatic treatments of seeds



Cell suspension establishment

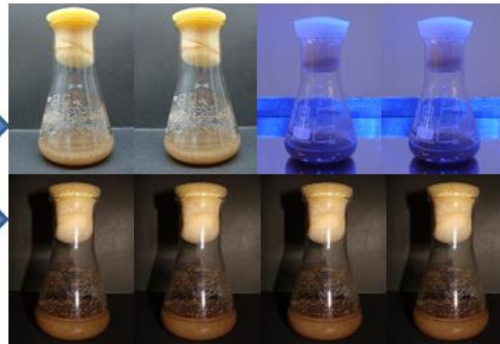


10 Subcultures

Add media



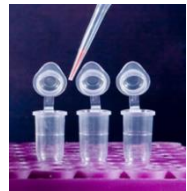
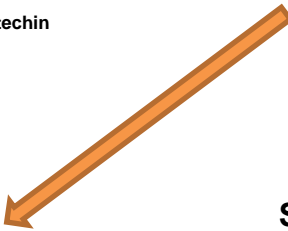
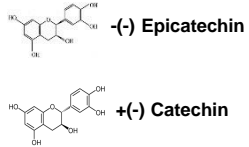
1 d    7 d    8 d    14 d



0 d

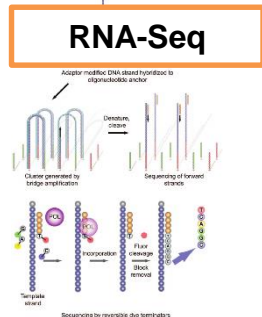


Polyphenols, catechins

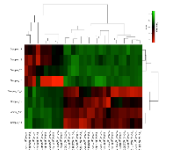
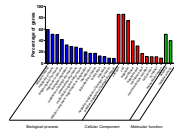


RNA extraction

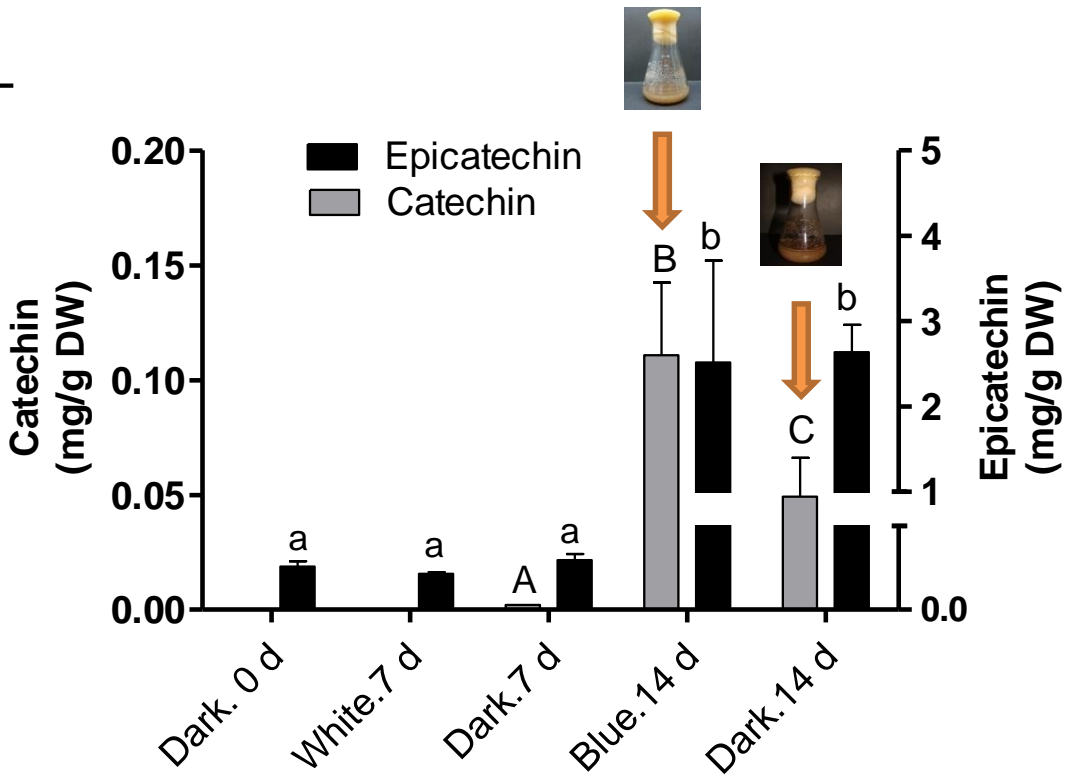
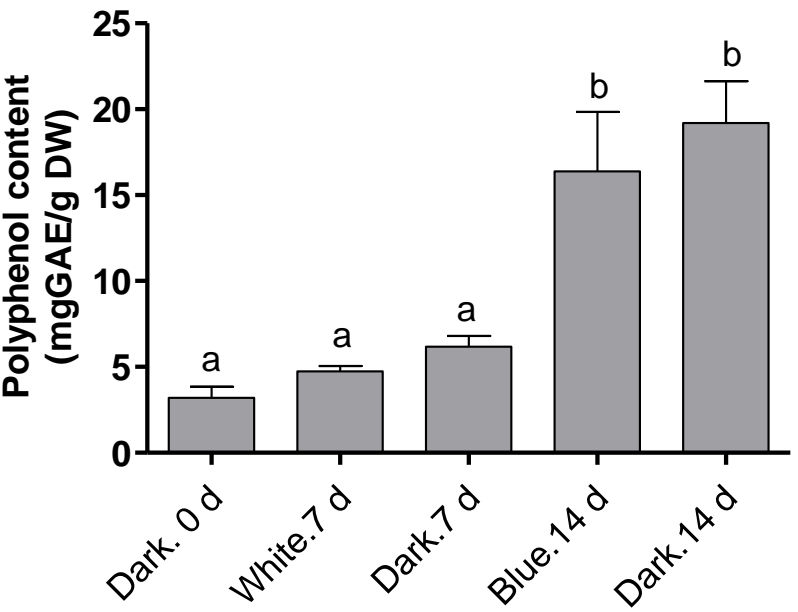
Sequencing



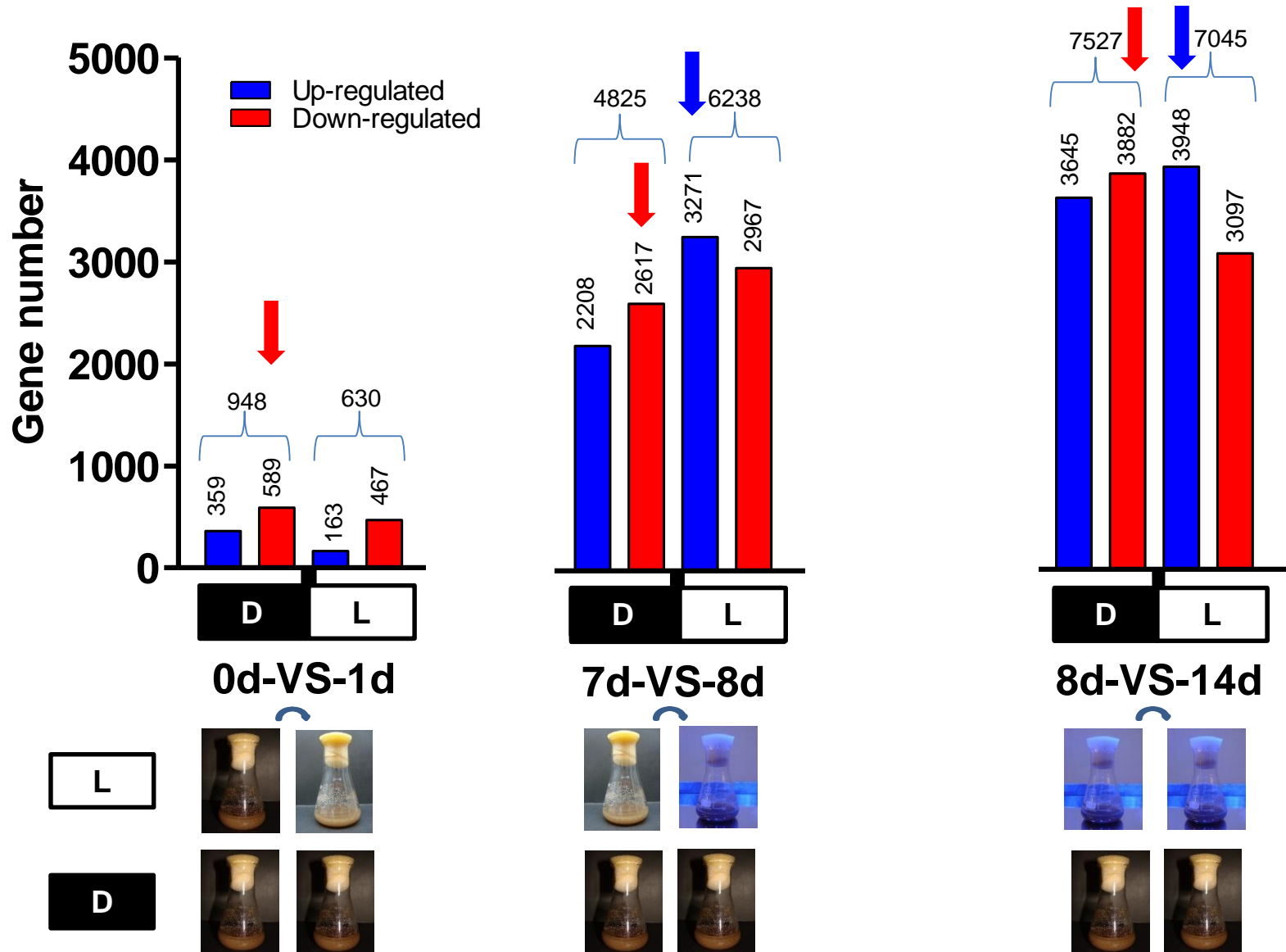
Bioinformatic analysis



# Catechins profile is differentially regulated by light treatments

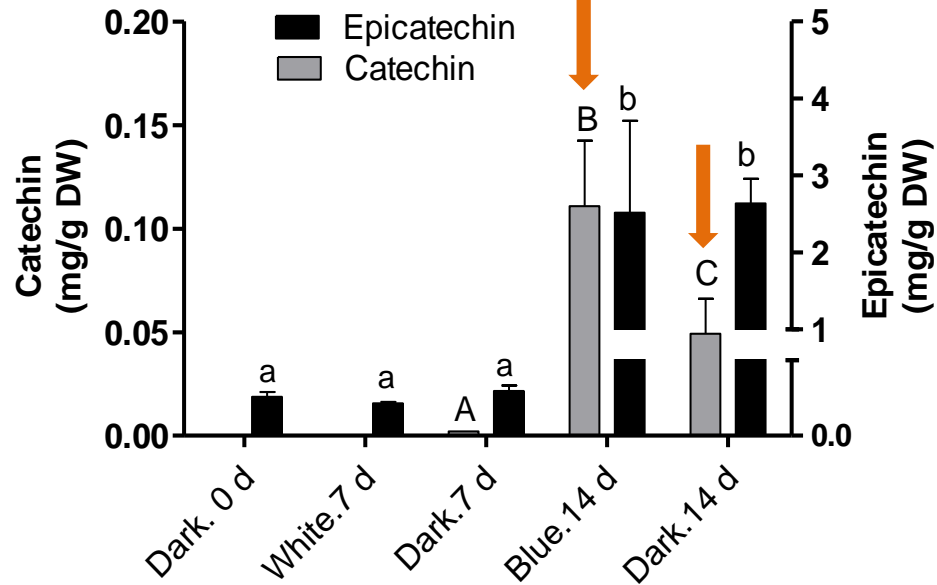


# DGEs showed a **dynamic pattern** across the time course experiment under both treatments L and D

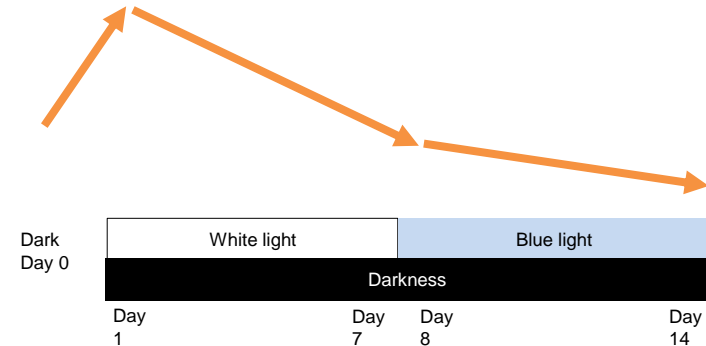
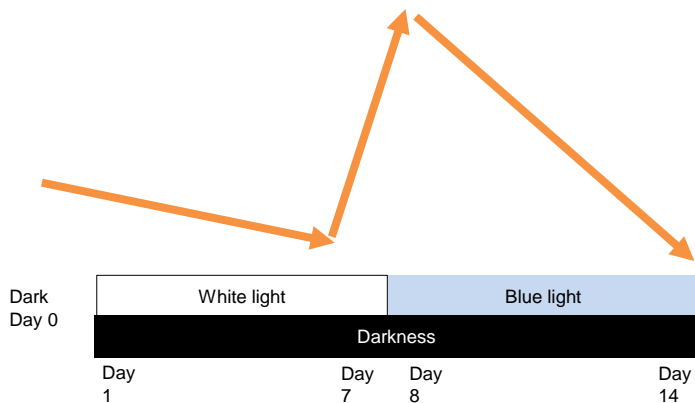




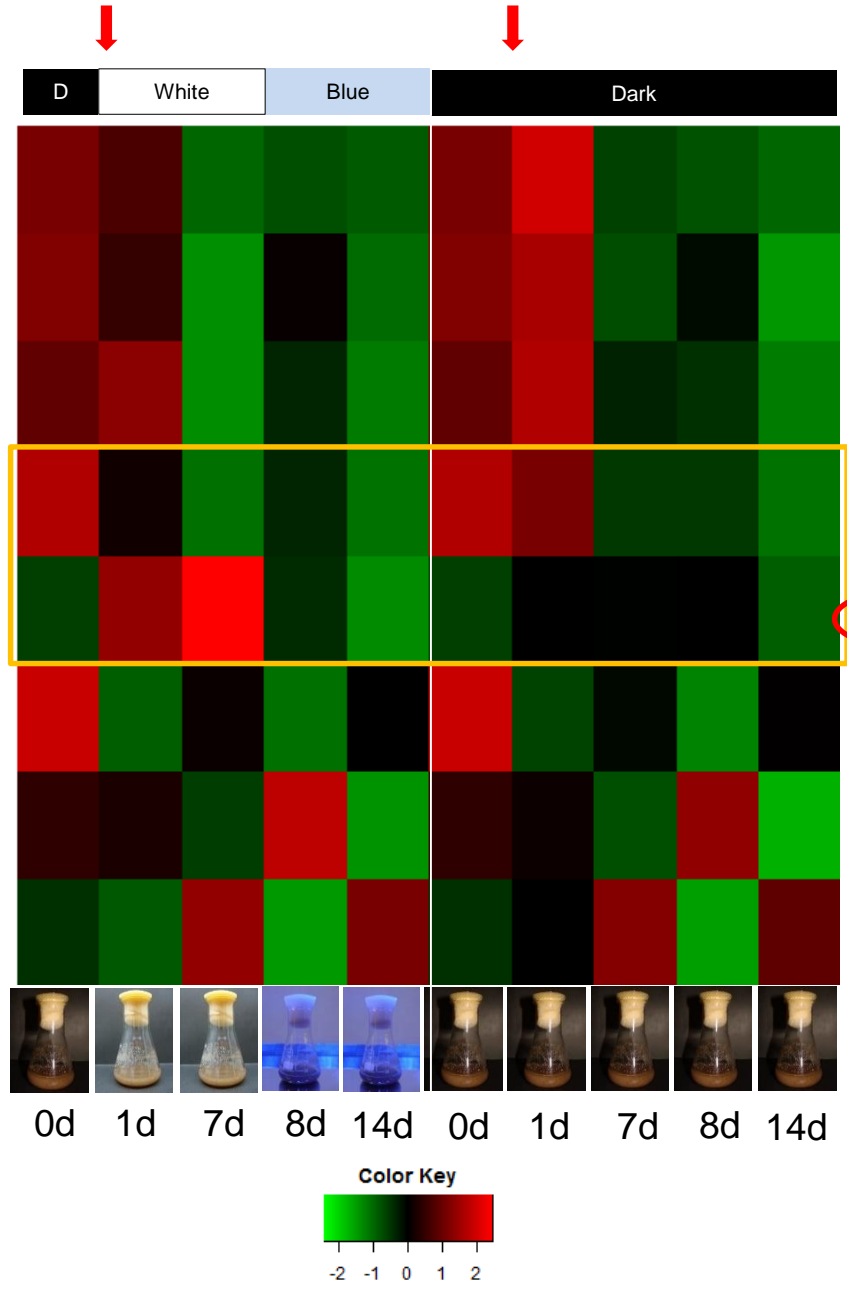
# Structural genes of the flavonoid pathway were **differentially regulated** by light treatments



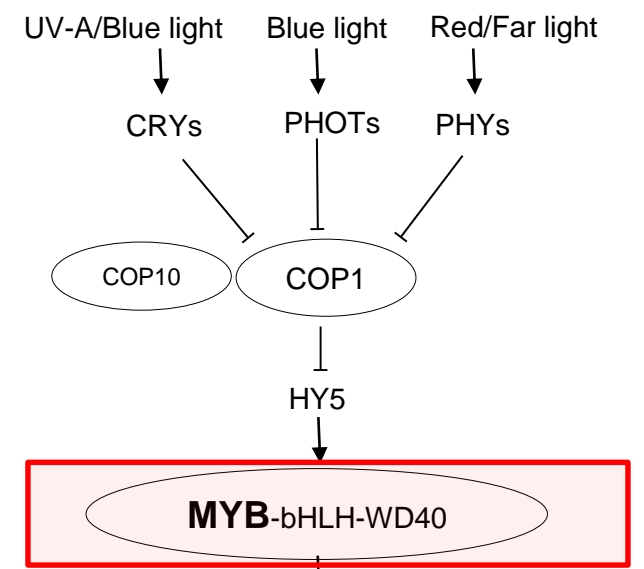
Anthocyanins



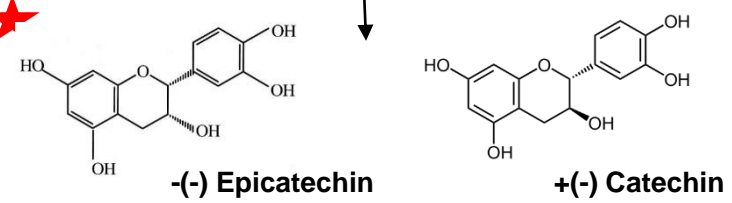
# MYB genes associated with flavonoids were enriched in different times under light/dark



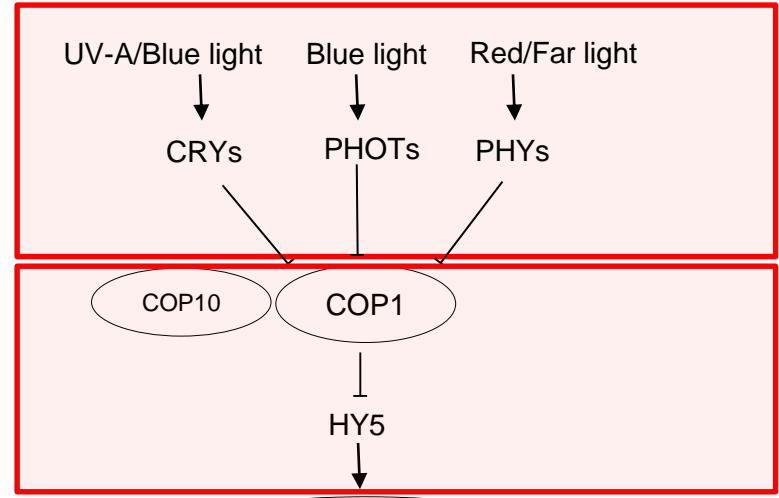
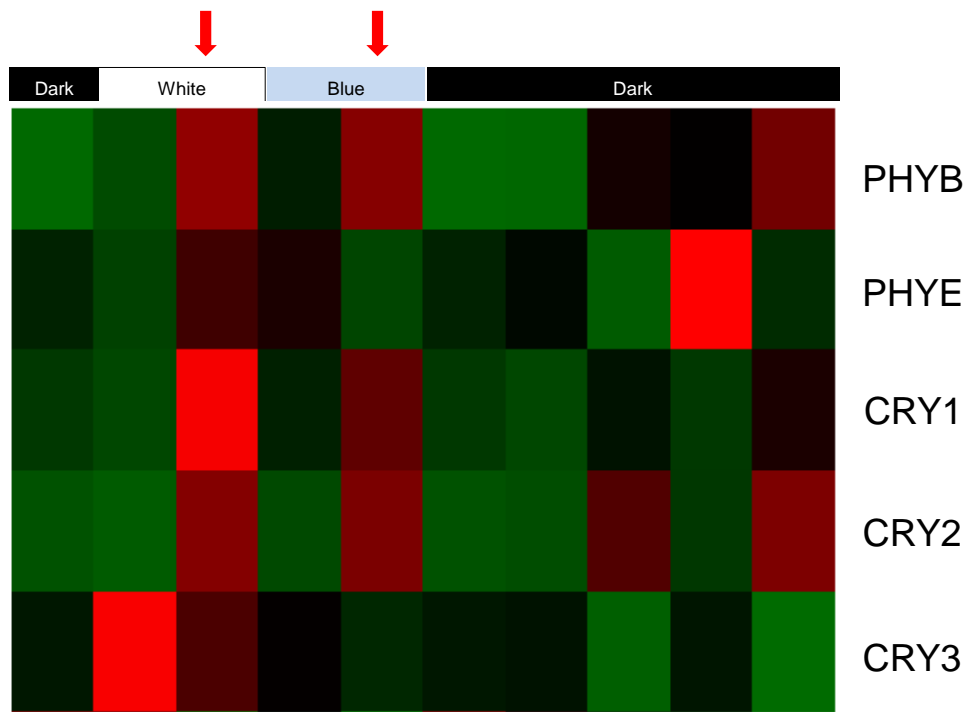
- TT2 ★
- Putative TT2 ★
- Putative TT2 ★
- MYB111 ★
- MYB12 ★
- MYB3 ★
- MYB4 ★
- MYB4 repressor ★



Structural genes  
(CHS, DFR, F3H, ANS, UFGT)

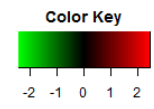
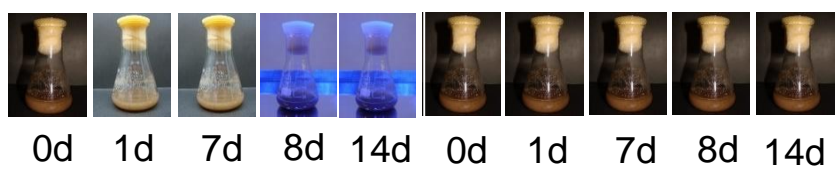
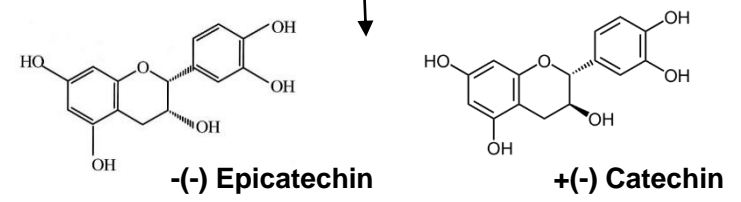


# Light signaling genes were enriched in different times under light/dark



MYB-bHLH-WD40

Structural genes  
(CHS, DFR, F3H, ANS, UFGT)



# Conclusions

## In cacao cell cultures:

1. Light treatments dramatically affect gene expression at all regulatory levels (i.e. structural, regulatory and light signaling genes).
2. Major shifts in gene regulation occur from white to blue light treatments.
3. Opposite expression of photoreceptors and downstream effectors known to occur in the pathway can be completely recovered in vitro.
4. From the MYB transcription factors, only MYB12 is upregulated in light treatments
5. Light can effectively regulate flavonoid profiles as it changes catechin to epicatechin ratios.

# Acknowledgements



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Penn State University



Penn state University, USA



Grupo Evo-Devo,  
Universidad de Antioquia



Compañía Nacional de  
Chocolates, Colombia