Diversity in type and location of phytochemical accumulation across … species of *Scutellaria*

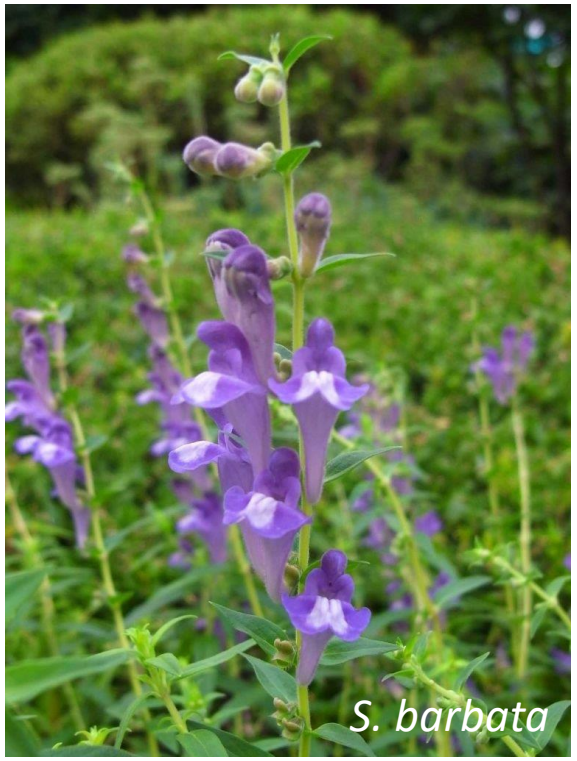
Bryce Askey

Lab Meeting 12-5-19

Background

- *Scutellaria baicalensis, barbata,* and *lateriflora* are medicinal plants

Root extracts used in traditional Chinese medicine



Aerial extracts used in traditional Chinese & Korean medicine

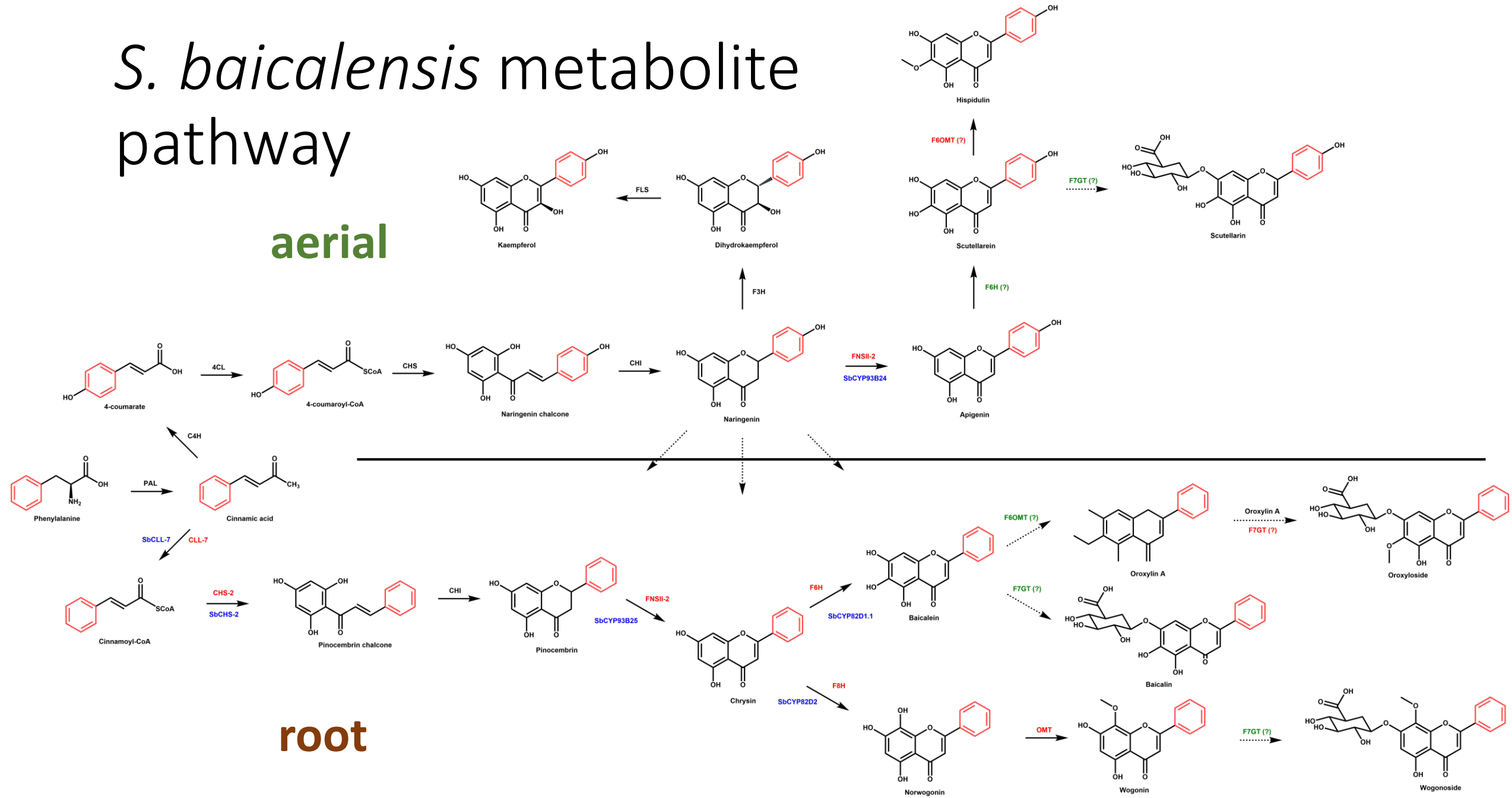
Aerial extracts used in traditional Native American medicine

Background

- *S. baicalensis*, *barbata*, and *lateriflora* are well-studied
  - Anticancer, anti-Alzheimer, and antimicrobial phytochemicals identified

Phytochemical	Species	Property	Study
Baicalin	*S. baicalensis*, *S. lateriflora*	Anti-cancer, anxiolytic	Zhao et al., 2016; Awad et al., 2003; Cole et al., 2007
Baicalein	*S. baicalensis*, *S. lateriflora*	Anti-Alzheimer's, anxiolytic	Shang et al., 2005; Gasiorowski et al., 2011; Awad et al., 2003; Cole et al., 2007
Wogonoside	*S. baicalensis*	Anti-cancer	Zhao et al., 2016
Wogonin	*S. baicalensis*, *S. lateriflora*	Anti-cancer, anti-Alzheimer's	Zhao et al., 2016; Cole et al., 2007
Oroxylin A	*S. baicalensis*	Anti-Alzheimer's	Shang et al., 2005; Gasiorowski et al., 2011
Scutellarein	*S. barbata*	Anti-cancer	Wang et al., 2018; Guo et al., 2019
Scutellarin	*S. barbata*, *S. lateriflora*	Anti-microbial	Tsai et al., 2018; Islam et al., 2011; Cole et al., 2007
Apigenin	*S. barbata*	Anti-microbial	Tsai et al., 2018

S. baicalensis metabolite pathway



Problem statement and goal

- Phytochemical profiles of *S. baicalensis*, *barbata*, and *lateriflora* are well characterized
  - Published pathway of *S. baicalensis*
- However, ~350 species of *Scutellaria* exist around the world
  - Majority of phytochemical profiles not well studied/not studied at all

Goal: Identify similarities and differences between organ-specific phytochemical profiles of a wide range of *Scutellaria* species

Objectives

1. Use HPLC analysis to measure phytochemical concentrations in leaves, shoots, and roots of different species of *Scutellaria*
2. Identify species of interest for untargeted metabolite analysis with LC-MS
3. Compare phytochemicals profiles for different species against:
   - Genome size
   - Published *S. baicalensis* pathway
   - Phylogenetic tree
4. Identify species with interesting phytochemical profiles for additional study

Significance

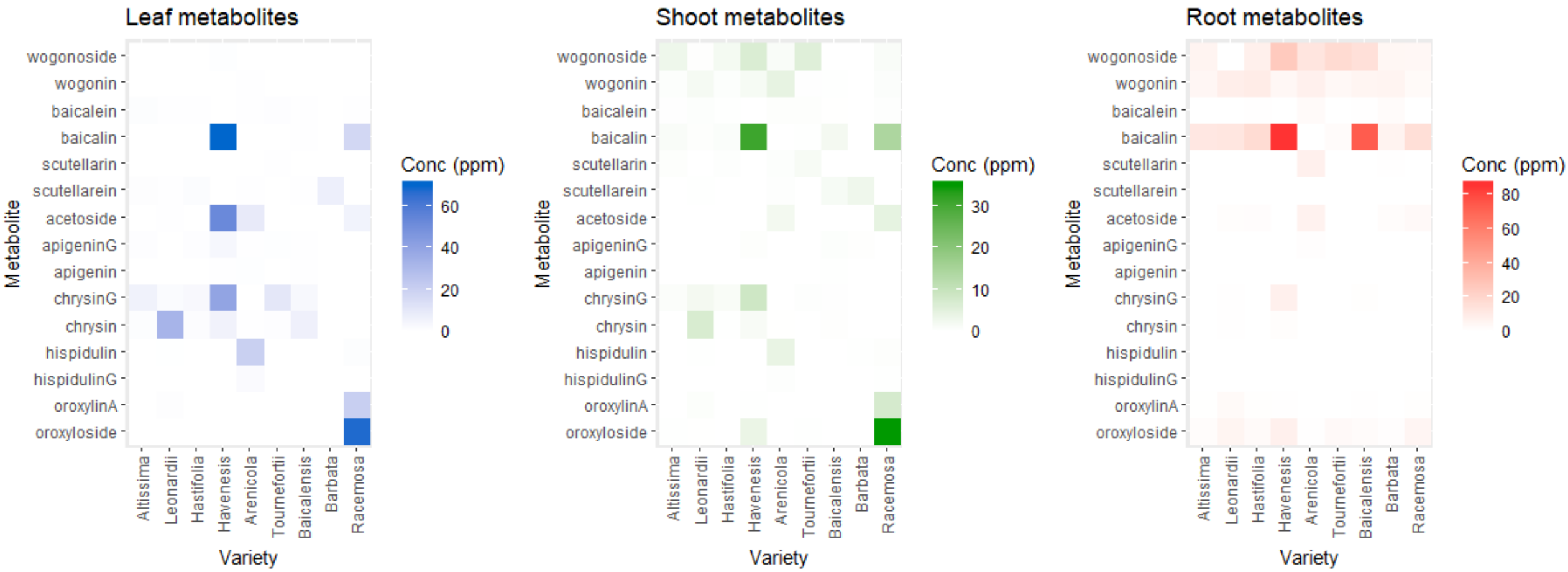
Intellectual merit:

- Identification of species with interesting phytochemical profiles
- Untargeted metabolite analysis to characterize unknown compounds

Broader impacts:

- Better understanding of health-beneficial phytochemicals in not well-studied species
- Aid in development of new drugs/treatments from extracts of these species

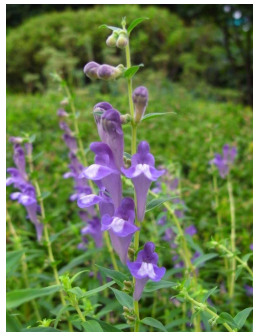
Metabolite profiles

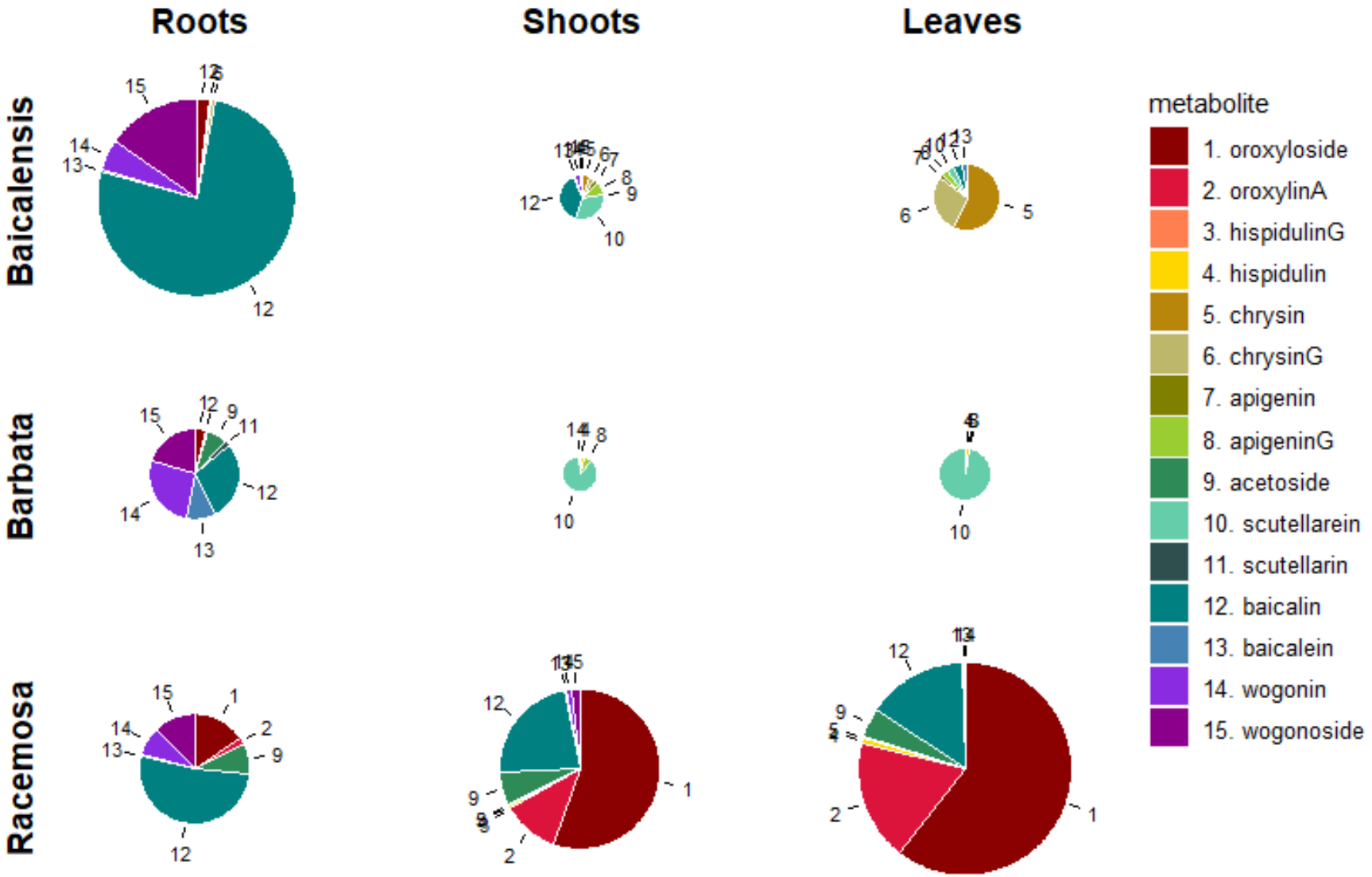


Comparison to genome size

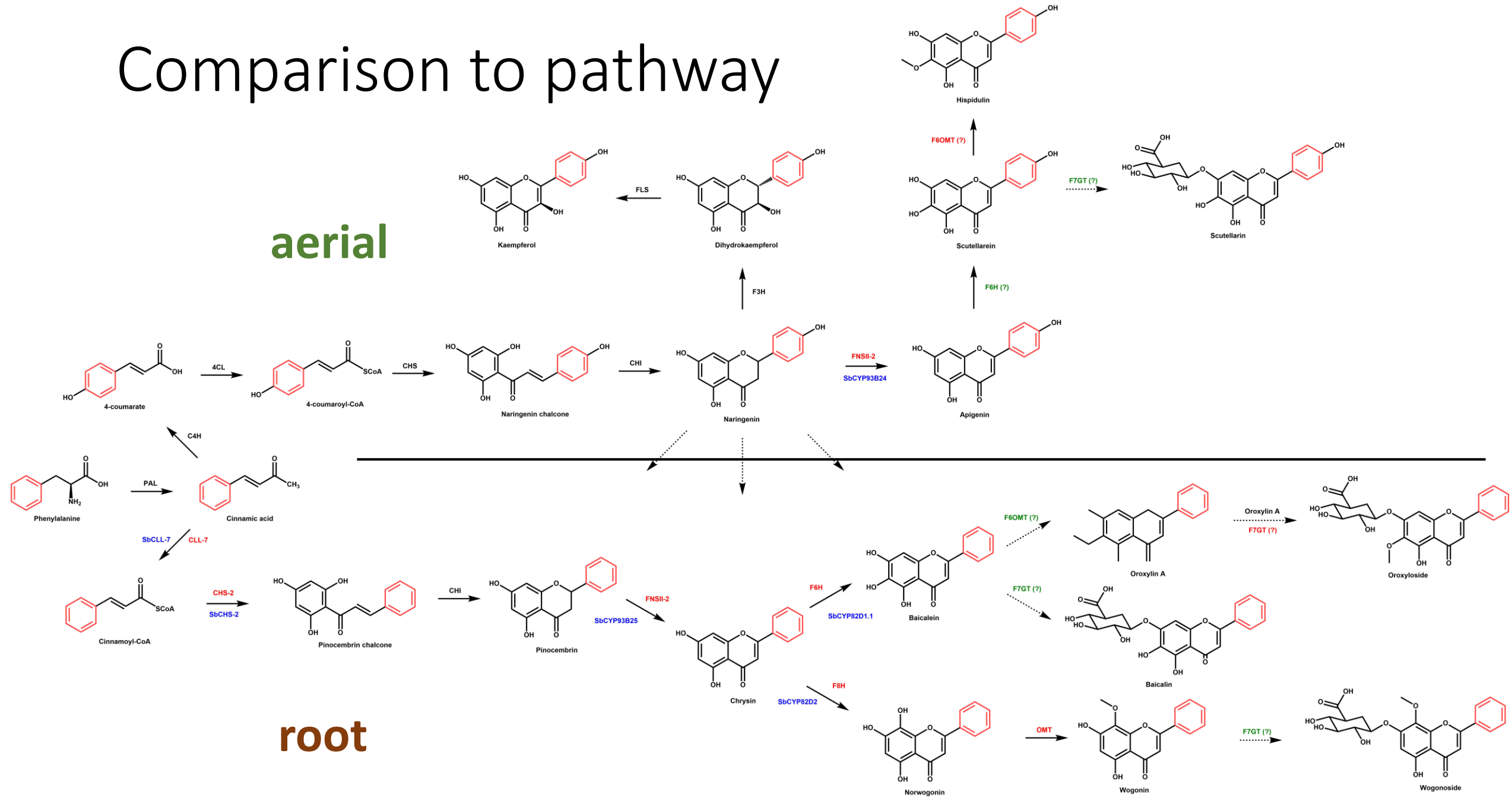
No.	Taxa	Standard Plant	1C (Mean)	SD	Genome Size (Gbp)
1	*S. altissima*	*Solanum*	0.40	0.02	0.39
2	*S. leonardii*	*Glycine*	0.51	0.02	0.50
3	*S. hastifolia*	*Solanum*	0.39	0.04	0.39
4	*S. havanensis*	*Solanum*	0.38	0.03	0.37
5	*S. arenicola*	*Glycine*	0.87	0.02	0.85
6	*S. tournefortii*	*Solanum*	0.40	0.01	0.39
7-1	*S. racemosa*	*Solanum*	0.44	0.03	0.44
8	*S. baicalensis*	*Solanum*	0.55	0.00	0.54

Metabolite profiles



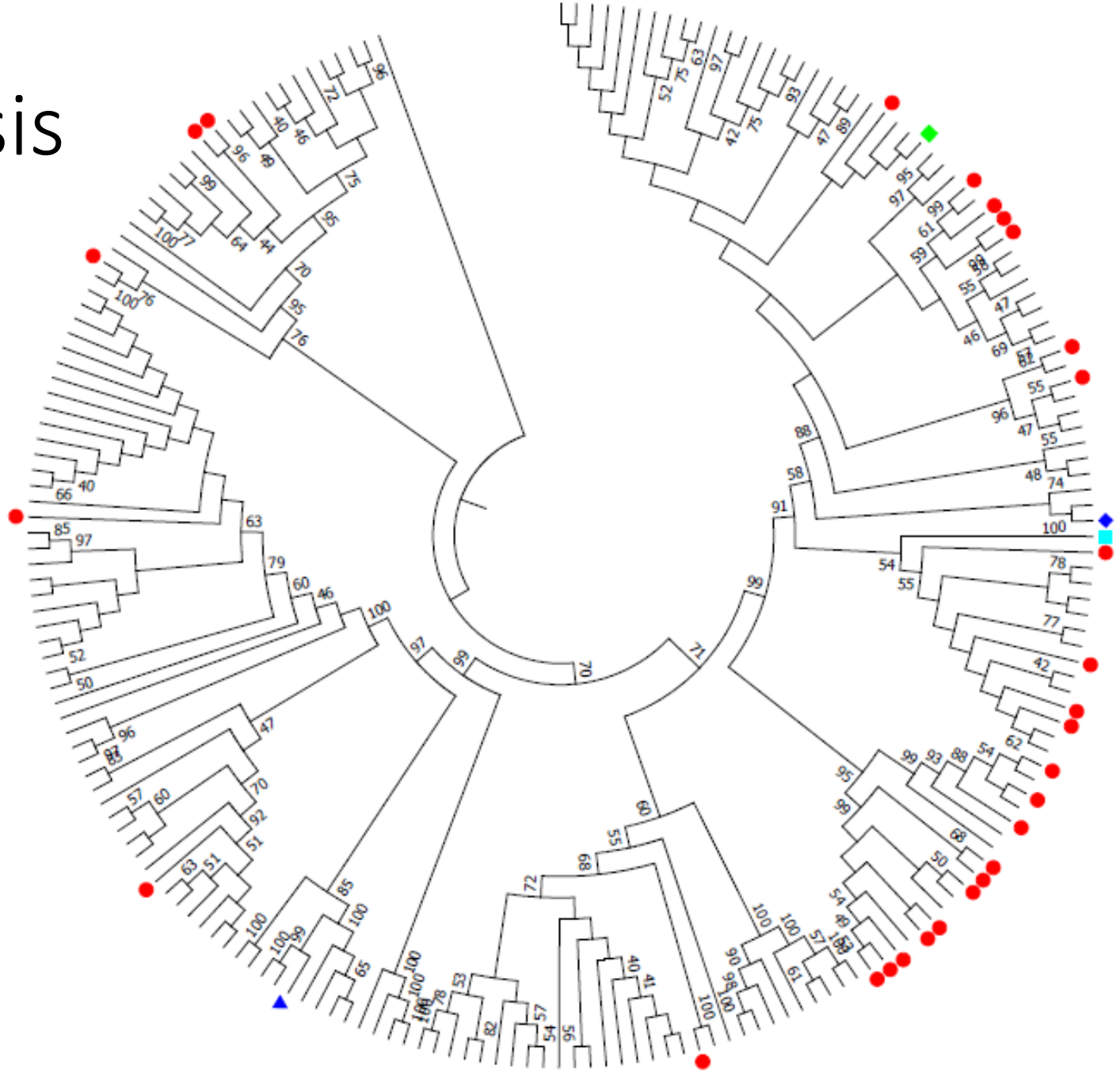


Comparison to pathway



Phylogenetic analysis

- Find clades with similar phytochemical profiles
  - Identify species with potential for further study
- Match branches in tree to differences in phytochemical profiles



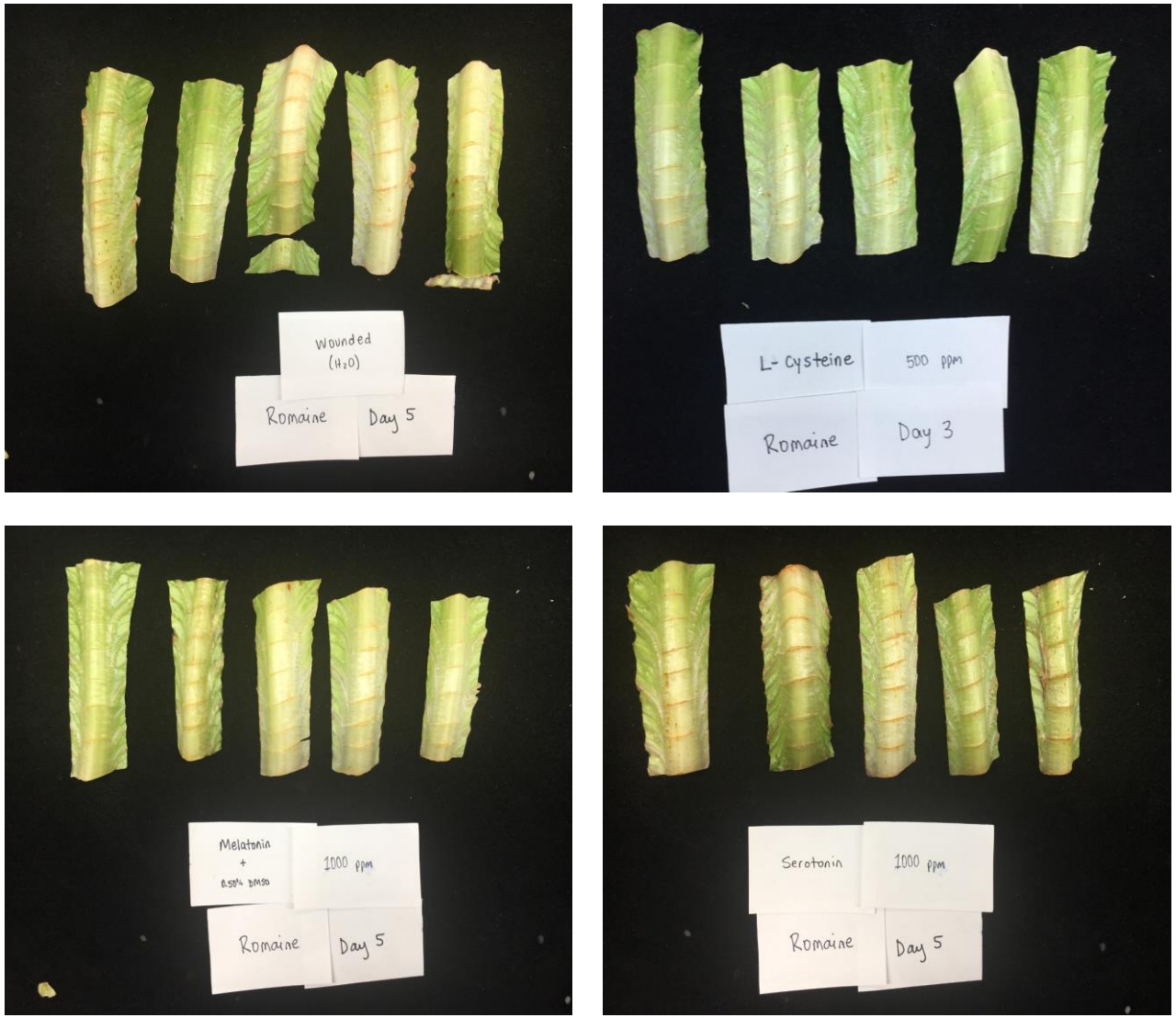
Methodology for quantifying wounding response in lettuce

Background

- Pinking occurs when lettuce leaves are damaged during harvesting, shipping, or packaging
- Considered undesirable by consumers
  - Reduces value of crop
  - Increases amount of food waste

Preventing pinking

- Testing with various concentration of cystine, melatonin, and serotonin
- Traditional method – evaluate by visual inspection and rate 1-5
- Need method to objectively quantify degree of pinking



General approach

1. Analyze pixel RGB values @ wounding site
2. Use pixels from control treatment to develop RGB pixel threshold
3. Apply threshold to wounding sites in all images to determine % of pink pixels

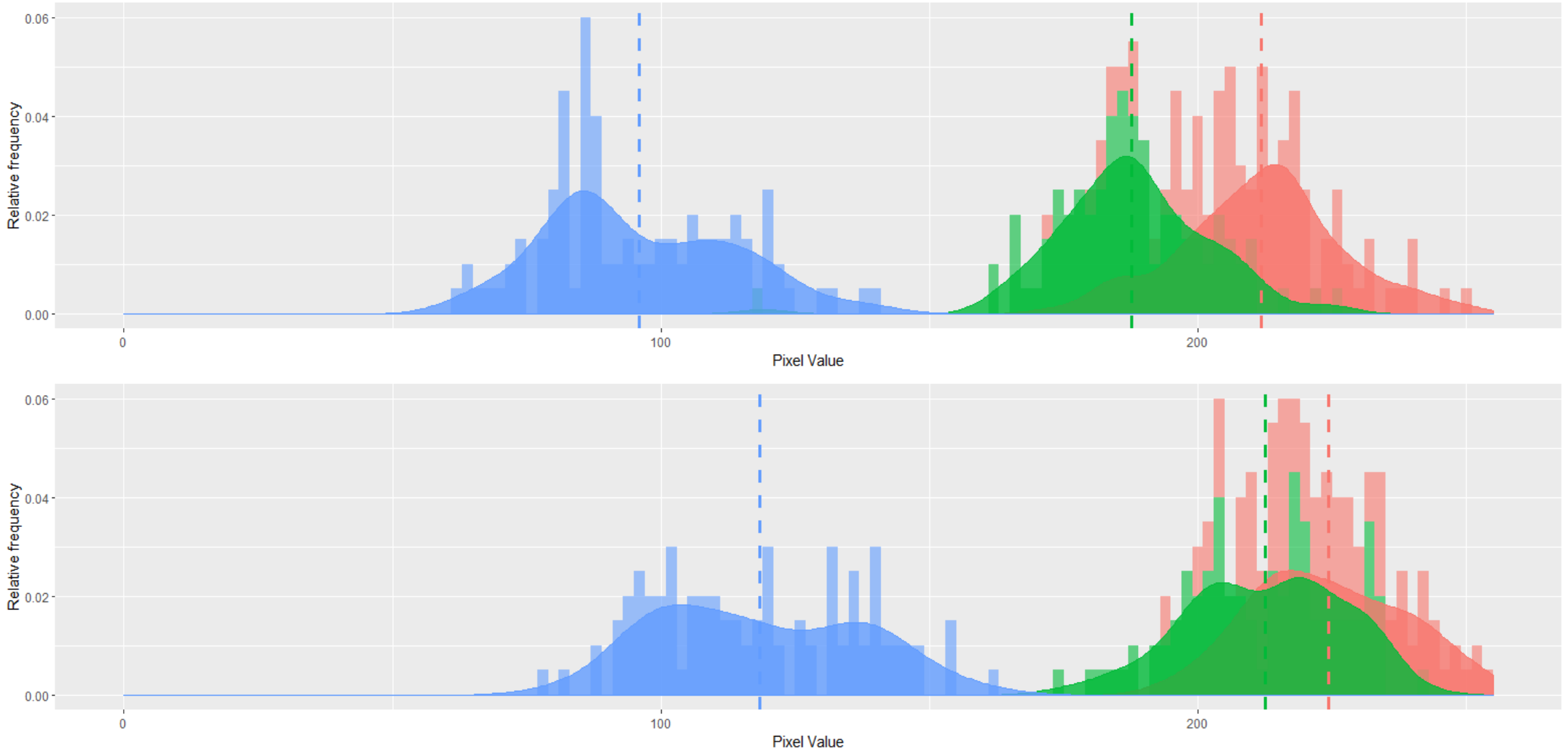
OR

1. Convert image to L*a*b* color space
2. Calculate average a* value at wounding sites in all image

Differences in RGB profiles

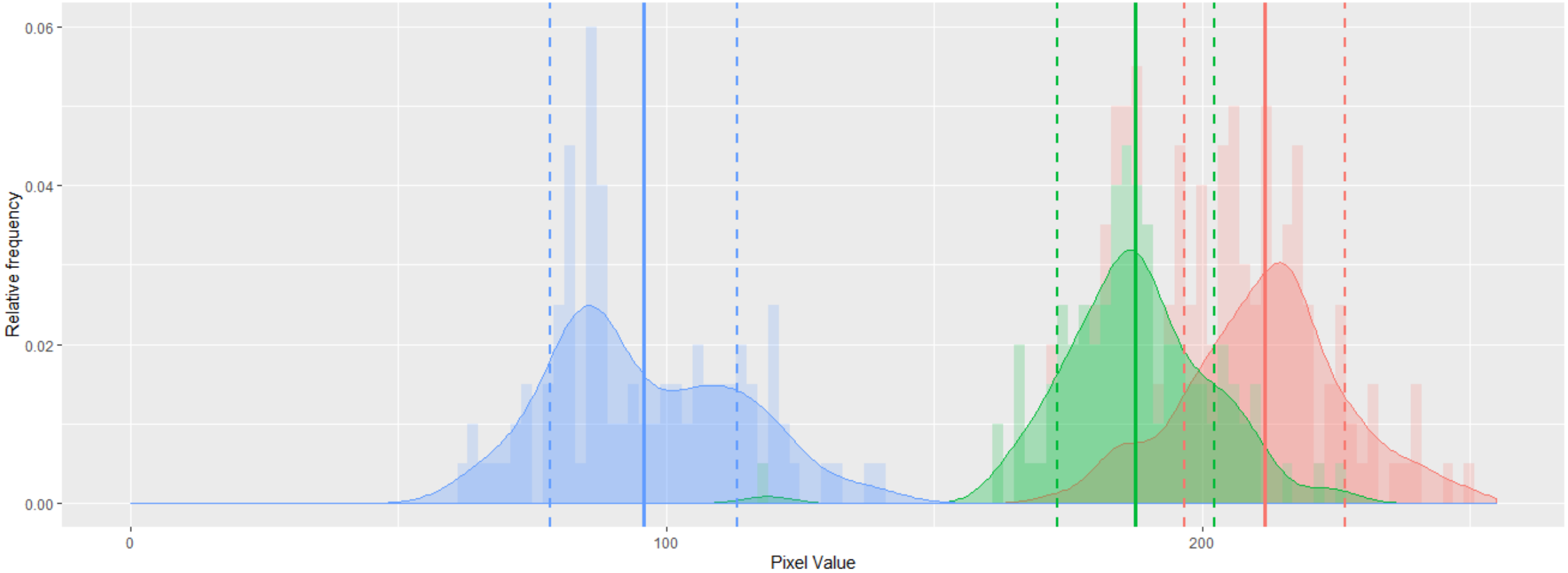
Control

Melatonin
1000 ppm



Threshold RGB values

- 1 standard deviation above and below mean value



Quantifying pinking response

Calculate % of pixels that meet threshold to be considered pink:

- Standardize area considered
- Count pixels in area that meet threshold

Image processing in GIMP

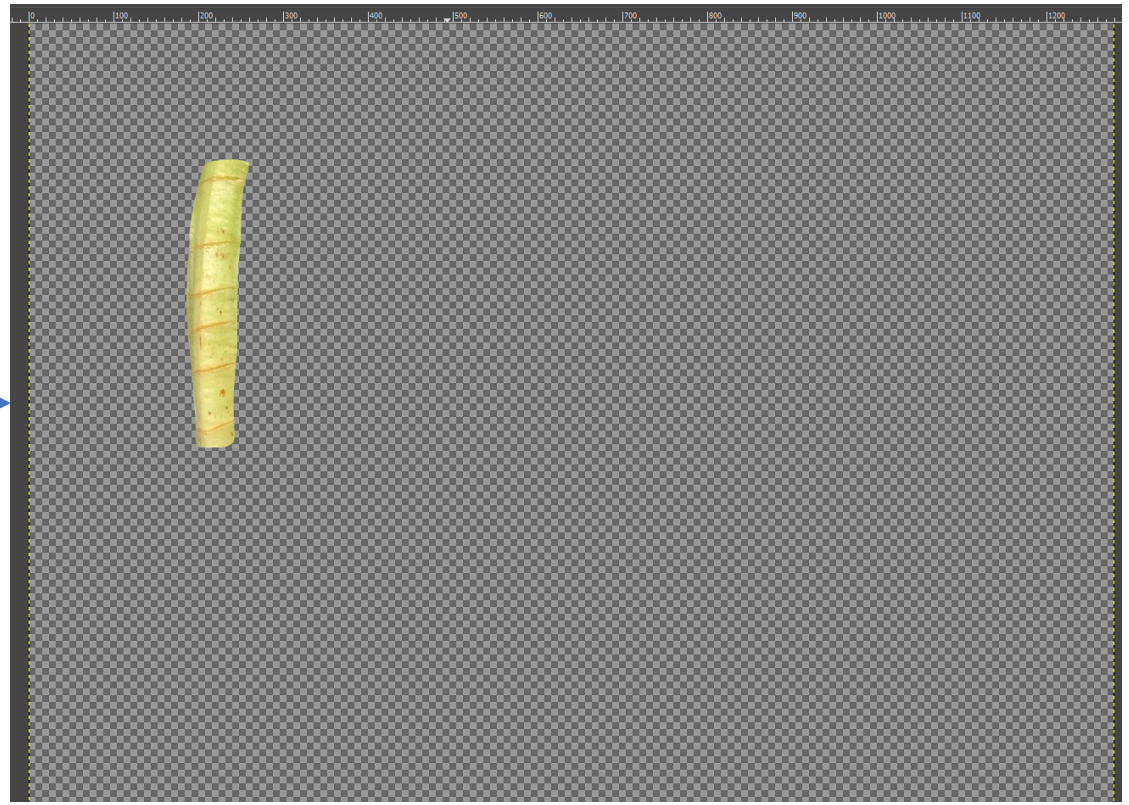


Image processing in GIMP

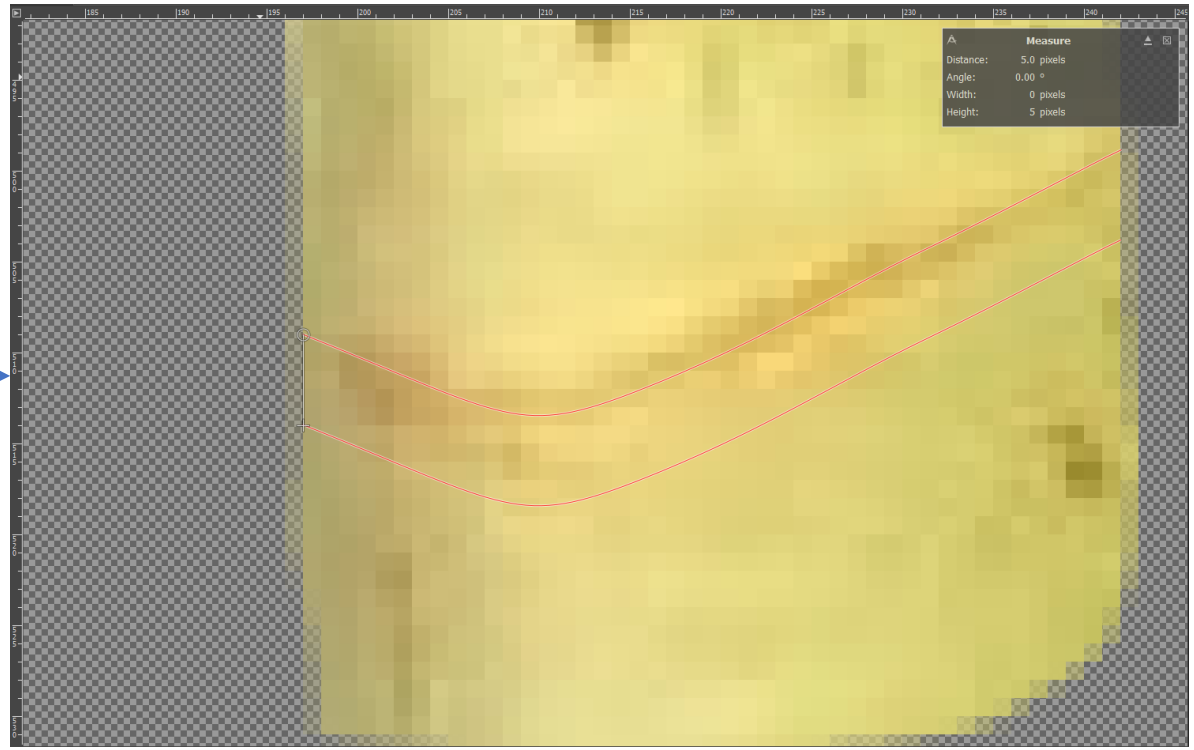
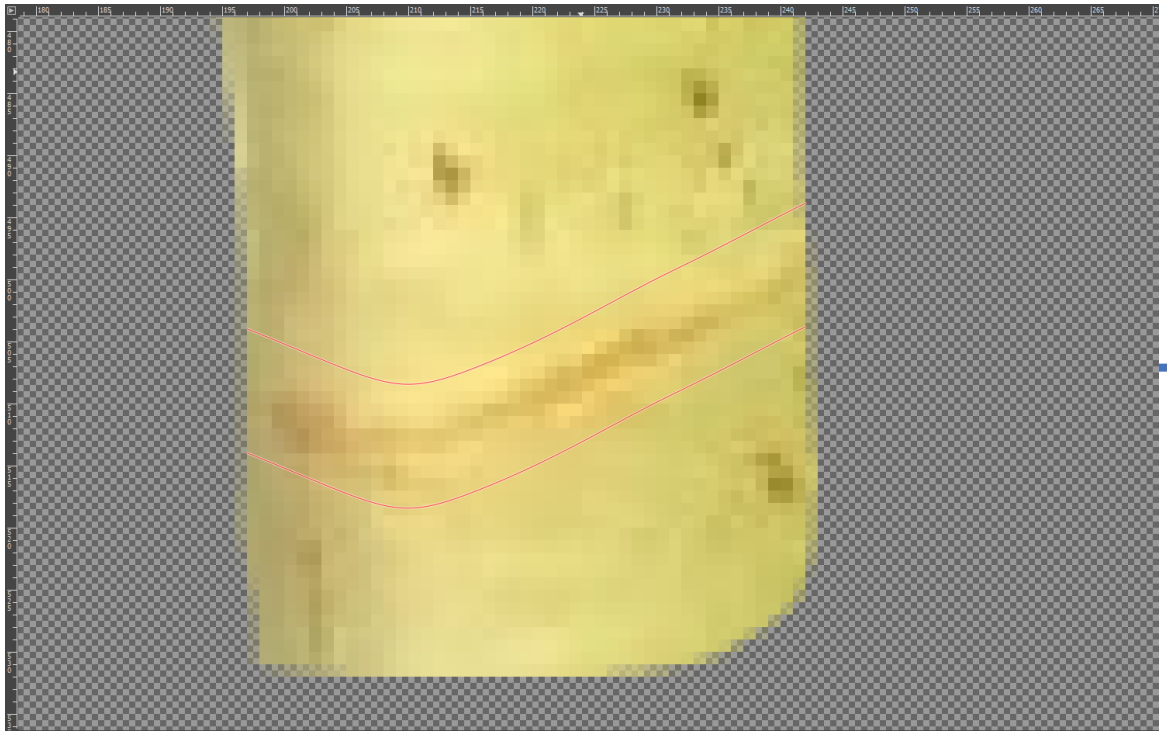
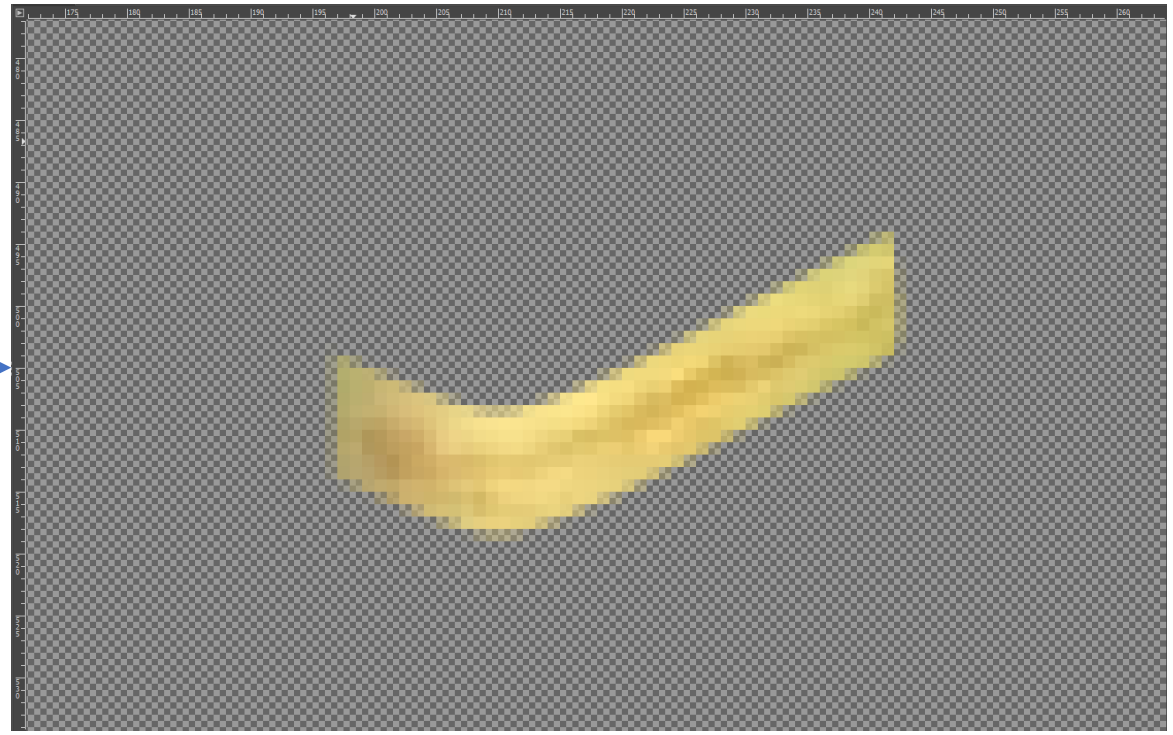


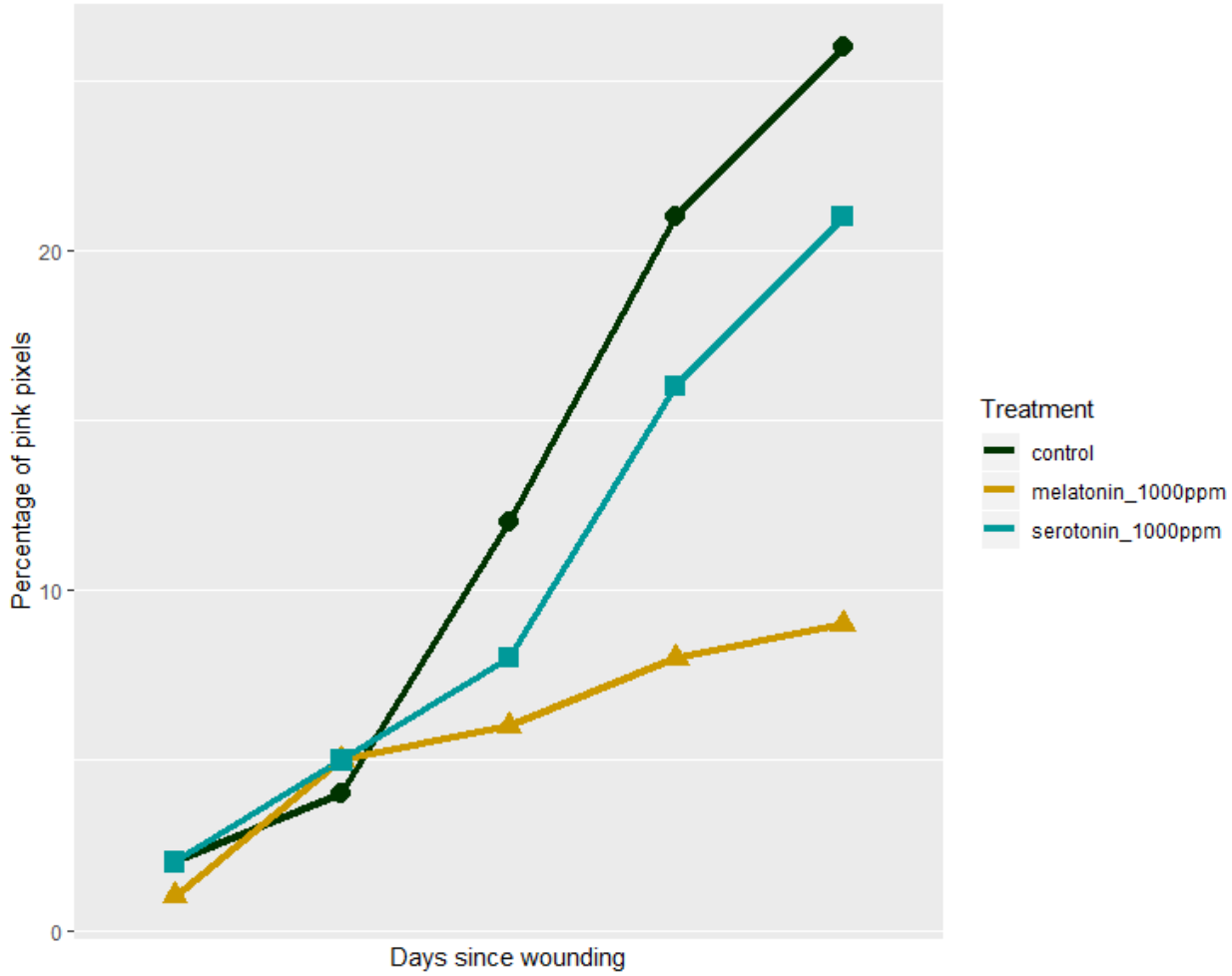
Image processing in GIMP





Output

- Calculate % pink pixels for each wounding site
- Standard deviation and significance of treatments



a* method

- Calculate average a* value at each wounding site
- More pinking -> greater a*
- **Drawback** – ignores all data in the L* and b* components

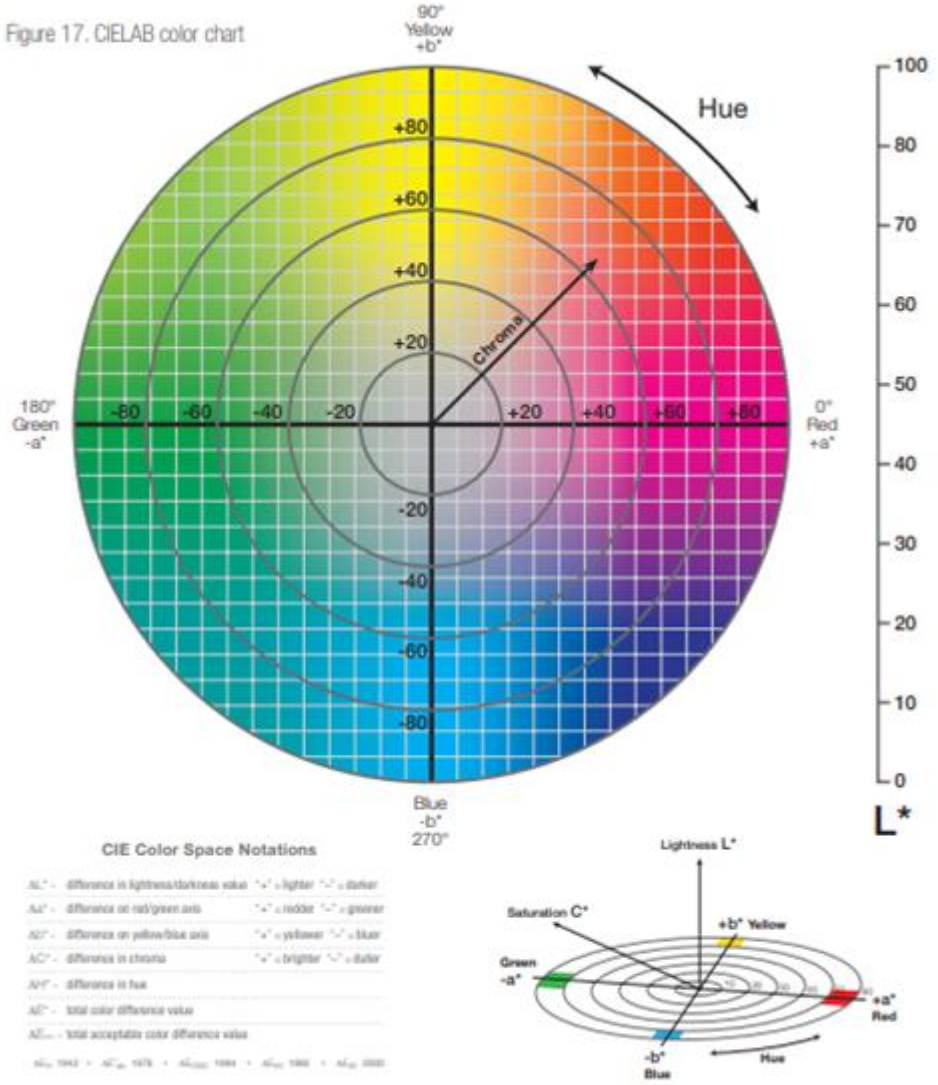


Figure 18. The L* value is represented on the center axis. The a* and b* axes