Optimization of Genome size Estimation protocol for Thimbleberry (*Rubus parviflorus*)

Stirling Todd, Michael Rotter, Carl Hjelmen, Yu-Ya Liang

Department of Biology, Utah Valley University, Orem, Utah

Plant Breeding & Genetics

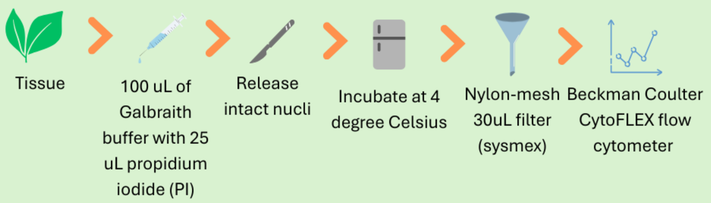
Introduction

- Thimbleberry (*Rubus parviflorus*) is a perennial woody shrub with white flowers and raspberry-like fruit, native to the western temperate regions of North America, ranging from Alaska to California and the Rocky Mountains.
- Genomic information for thimbleberry is extremely limited, with no available reference genome and only a single genome size estimate of 2C = 0.55 pg.
- The *Rubus* genus exhibits a wide range of ploidy levels, ranging from diploid to dodecaploid (2n = 2x = 14 to 2n = 12x = 84) in blackberries (*R. argutus*). The long-term goal of this study is to estimate the genome size and ploidy variation of thimbleberry populations collected across the United States.

Research Question

What is the optimal protocol of thimbleberry for genome size estimation using flow cytometer?

Materials and Methods



- Standards:
  - Tomato leaf (*Solanum lycopersicum* cv *'Stupickle'*) (2C = 1.96pg)
  - Napa Cabbage leaf (*Brassica rapa subsp.* Pekinensis*)* (2C = 1.60pg)
- Tissues and treatments:

Tissue:
- Leaf bud
- Young leaf
- Old leaf
- Petal
- Petiole
- Old Petiole

Treatment:
- Incubation time for tissue.
- Incubation time Etraction buffer (with PI)
- Frozen

Results

Table 1. Summary of results across different tissues, treatments, and species.

Species	Tissue	Treatment	Outcome
Tomato	Leaf	-	:D
	Leaf	Frozen	x(
Cabbage	Leaf	-	:D
	Leaf	Frozen	x(
Thimbleberry	Leaf	Young	:|
	Leaf	New bud	x(
	Leaf	Old	:(
	Leaf	Frozen	x(
	Leaf	Day old	:)
	Leaf	3 week old	:|
	Leaf	6 week old	:(
	Petiole	Young	:)
	Petiole	Old	:(
	Petiole	10min incubation	:(
	Petiole	20min incubation	:(
	Petiole	24hr incubation	x(
	Petal	-	x(

Figure 1. Genome size estimation of tomato leaf.

Figure 2 . Genome size estimation of frozen thimbleberry leaf from Arizona White Mountains.

Figure 3 . Genome size estimation of a young thimbleberry petiole from McLane Creek, WA, co-prepared with tomato leaf.

Figure 4. Genome size estimation of thimbleberry leaf from Evergreen, WA, co-prepared with cabbage leaf.

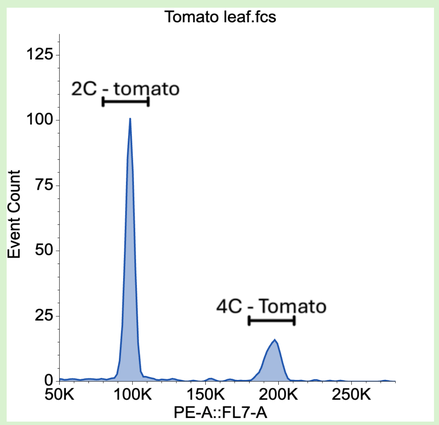


Figure 1

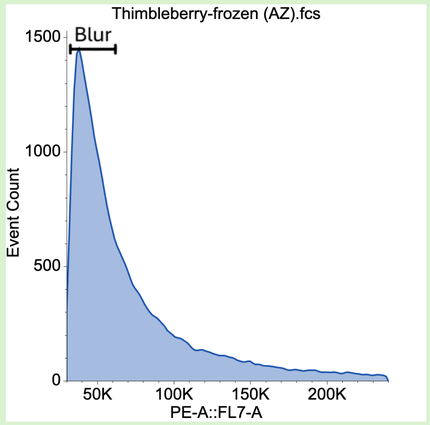


Figure 2

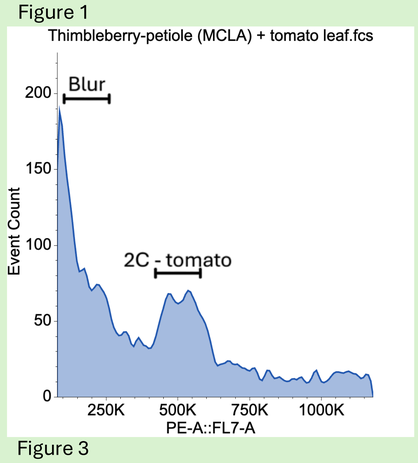


Figure 3

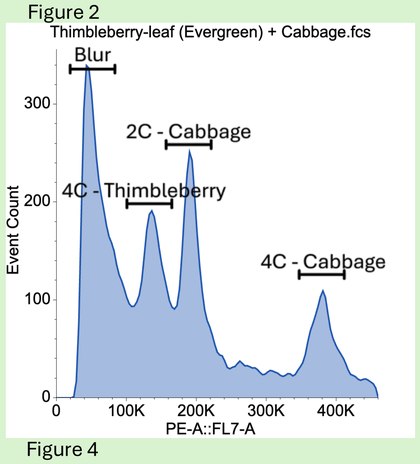


Figure 4

Discussion

- Freezing the tissue before flow cytometry preparation causes vacuolar expansion, which disrupts the nucleus and affects the results.
- Thimbleberry tissues have high phenolic content, which interacts with PI and interferes with flow cytometry results.
  - Young, fully expanded leaves and petioles have lower phenolic levels and are better suited for sampling.
  - Small tissue samples (≈0.5 × 0.5 cm) and light chopping reduce the release of phenolic compounds.
  - Keeping tissue samples refrigerated overnight before extraction helps slow phenolic oxidation and can improve flow cytometry results.
- DAPI does not require incubation and does not interact with phenolic compounds like PI. Using both PI and DAPI together can provide more accurate and complementary genome size estimates.

Conclusion

- Avoid freezing tissue samples to prevent damage and inaccurate results.
- Petiole tissue provides more reliable results compared to leaves.
- Keep samples refrigerated for up to 24 hours before processing for improved quality.
- Incubate samples with PI for 20–30 minutes for optimal staining.

Thimbleberry

Thimbleberry - Flower

Thimbleberry - Fruit

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