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Chromosome Counting: Aceto-orcein Technique Using Hydrochloric Acid for Root Tips

1. Pretreat root tips in 0.002 M 8-hydroxyquinoline for 4–8 hrs. Place root tips in the refrigerator at 4ºC.
   LCM Note: Treatment time is dependent on the species. I've left *Opuntia* root tips in for 24 hours and they still worked fine. Somatic cell division is also dependent on the species, so this has to be determined for the taxa under study.

2. Wash root tips in distilled H_2O and transfer to Carnoy's Solution. Fix for at least 2–4 hrs. Wash roots before transferring to 70% EtOH. Root tips can now be stored indefinitely at 4°C (in 70% EtOH).
   LCM Note: Fixing for longer tends to yield better results.

3. Wash root tips in distilled H_2O and place in 40% HCl (to digest cell walls; alternatively glusulase or pectinase can be used to digest cell walls for 15–30 min) for 2.5+ minutes depending on the size of the root tip and the species. Digested roots can be stored in 70% EtOH until use.
   LCM Note: I normally digest *Opuntia* root tips for up to 10 min at room temperature, but this is taxon and root size dependent. HCl+ root tips can also be heated at 40°C for up to 5 min to speed up the digestion process.

4. Wash root tip in distilled H_2O, dab off water with Kimwipe, and place directly on a slide in 10– 20ul of 60% acetic acid. Tease apart root tip (removing outer epidermal layer and root cap) and macerate cells.

5. Add 5ul of 1% aceto-orcein dye and squash.
   LCM Note: Alternatively, a 2% aceto orcein solution can be used. Acetocarmine also can be used for this procedure.

6. Place clear fingernail polish around the cover-slip to reduce evaporation of the squash (I only do this when I want to maintain the slide for a longer period of time).

7. Count at least five cells per squash with easily viewable chromosomes to make sure no endomitosis cells are being counted.

Publications using these methods

- Soltis, D.E., 1980. Karyotypic relationships among species of *Boykinia, Heuchera, Mitella, Sullivantia, Tiarella*, and *Tolmiea* (Saxifragaceae). *Systematic Botany* 5: 17–29.
- Majure, L.C., and E. Ribbens. 2012. Chromosome counts of *Opuntia* (Cactaceae), prickly pear cacti, in the Midwestern United States and environmental factors restricting the distribution of *Opuntia fragilis. Haseltonia* 17: 58–65.
- Majure, L.C., W.S. Judd, P.S. Soltis, and D.E. Soltis. 2012. Cytogeography of the *Humifusa* clade of *Opuntia* s.s. Mill. 1754 (Cactaceae: Opuntioideae): Correlations with geographic distributions and morphological differentiation of a polyploid complex. *Comparative Cytogenetics* 6: 53–77.
- Majure, L.C., R. Puente, and D.J. Pinkava. 2012. Miscellaneous chromosome counts in Opuntieae DC. (Cactaceae) with a compilation of counts for the group. *Haseltonia* 18: 67-78.

Recipes for reagents used

- **0.002 M 8-hydroxyquinoline**
  - Used for the contraction and spreading of chromosomes as well as halting mitosis.
  - 0.029 g of 8-hydroxyquinoline
  - 100 mL of diH_2O
  - Note: Can use a small amount of 100% ethanol to make 8-hydroxyquinoline go into

solution.

- **3:1 Carnoy's solution**
  - Used for fixing chromosomes
  - 3 Ethanol : 1 Glacial Acetic Acid
- **6:3:1 modified Carnoy's solution**
  - Used for fixing meiotic material (e.g., flower buds, etc.)
  - 6 Chloroform : 3 EtOH : 1 Acetic Acid
- **1-2% solution aceto-orcein dye**
  - 100 mL 45% acetic acid
  - 1–2 g orcein dye
  - Place 1–2 g orcein dye in 45% acetic acid and heat to dissolve. Then cool, filter, and store in a glass vial.