The root tip

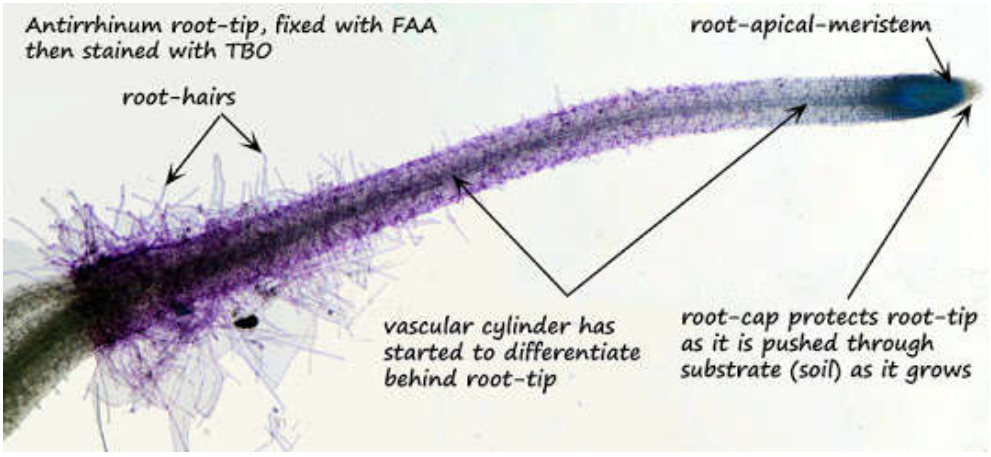
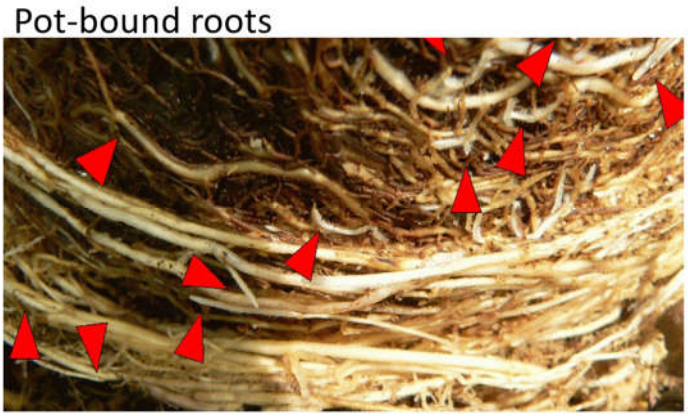
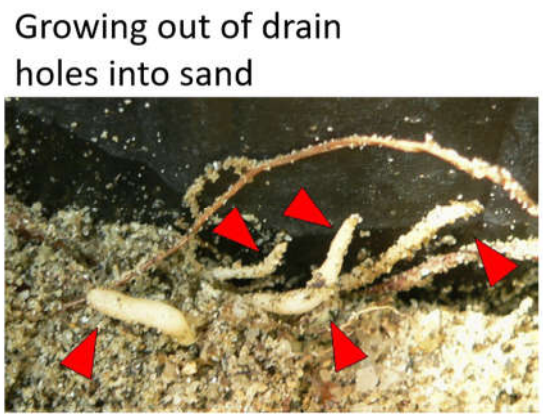


Figure 1. http://www.microbehunter.com/microscopy-forum/viewtopic.php?t=4985

Collecting tips





- Need tissue with a high mitotic index– root tips
- Plants must be growing vigorously
- Harvest about 1 cm of root on a sunny morning following a sunny day
- Only thick, white roots
- Avoid spindly or brown roots

- Tips break off if pulled from soil
    - Meristem limited to the area right behind the root cap

Harvest about 1 cm of root length:

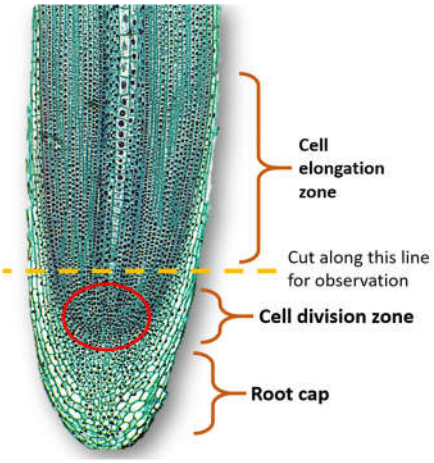


Figure 2. http://www.bio.miami.edu/dana/151/151F13_mitosis3.pdf & http://www.uri.edu/cels/bio/plant_anatomy/18.html

Preserve tissues

- Condense chromosomes
  - Place harvested tips on ice
  - Maintain at 4ºC while in pretreatment
- Stop mitosis
  - Pretreat with a c-mitotic agent (8-hydroxyquinoline)
  - Stop protein synthesis with cycloheximide (optional)
- Kill & clear tissues
  - Fixatives (Farmer's, Carnoy's)
- Store– room temperature in 70% EtOH

Prepare for viewing – can be very species specific

- Dissolve pectin so cells can be spread in a single layer
  - Acid or enzymatic hydrolysis
- Prepare the DNA for staining
  - Acid hydrolysis
  - Base treatments

The study organism

Tradescantia pallida

2n = 2x = 12
2n = 4x = 24

Figure 3. https://albania.desertcart.com/

A genus from the western hemisphere

Figure 4. Named after the father/son botnanists who described it, John Tradescant the Elder and the Younger. Wikimedia commons

The modified carbol fuchsin stain
(also works with acetocarmine)

Hydrolyze the root tips

- Take a watch glass
- Using a Pasteur pipet, add
    - 3 squirts 95% EtOH
    - 1 squirt concentrated HCL
- Add a root tip about 5 seconds
    - It will turn waxy/translucent
    - The meristem remains white

- Place a microscope slide over a dark surface
- Place a root tip over on the slide

- Remove all but the very tip of the root
- Will be a whitish color, while the rest of the root will be translucent
- Blot dry the excess HCl : EtOH mixture
- Add a drop of stain over the tip

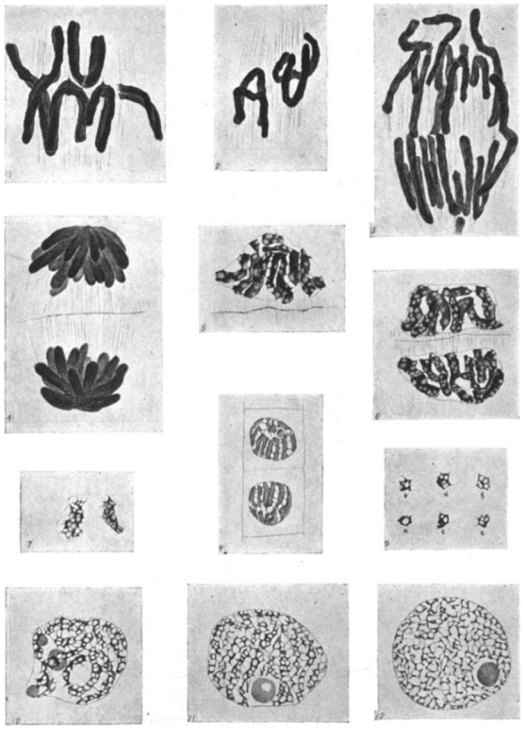
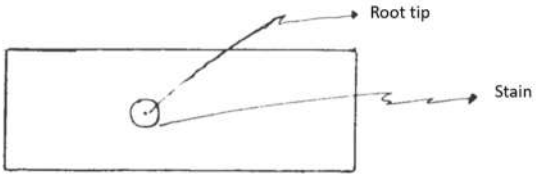
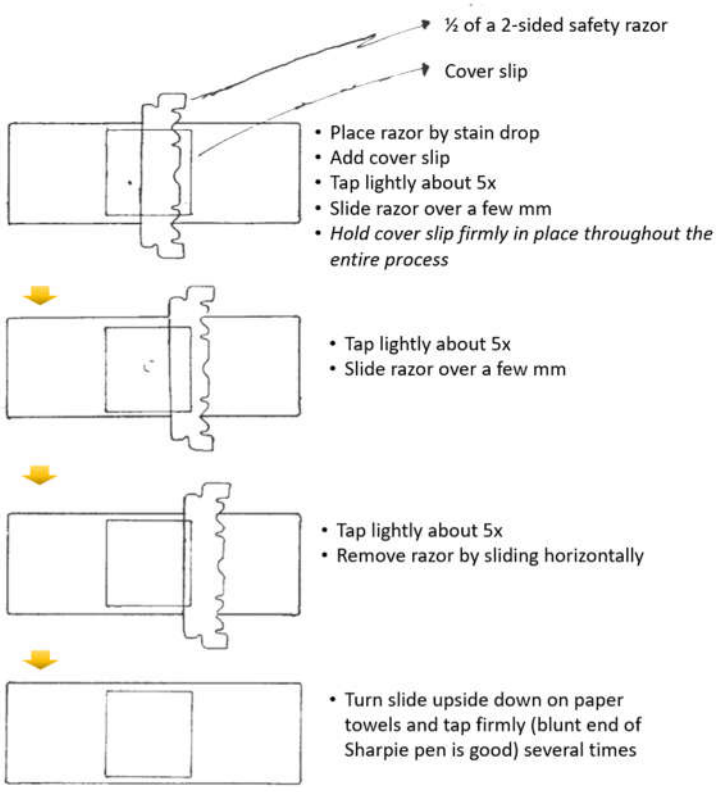


Figure 5. Sharp, 1920





If using acetocarmine, before the final squash

- Heat the slide over an alcohol lamp, so it feels warm to the touch
- Do not boil it

- Examine the slide under a microscope
- If the cells are well spread and mitotic figures are apparent
    - Seal the edges with paraffin or nail polish

Reference

Sharp LW. 1920. Somatic chromosomes in *Tradescantia*. American Journal of Botany, 7: 341-354

Appendix

Preparation of plant root tips for mitotic analysis

- Collect only root tips that are actively growing. These will be large, white and "healthy" in appearance.
- Collecting the morning after a sunny day helps ensure cells are actively undergoing mitosis.
- Best root tips are obtained from root-bound pots-- simply removing the pot reveals excellent roots growing around the perimeter of the root ball.
- Alternatively, pots can be placed on sand or vermiculite. Roots grow out of the drainage hole and into the sand or vermiculite, from which they can be easily collected.

1. Harvest the terminal 1 to 1.5 cm of each root tip.
2. Pretreat root tips in 8-hydroxyquinoline at 4°C for 4-6 hour. The hydroxyquinoline helps dissolve the spindle, and the cold helps condense the chromosomes. (4 hours for plants with small genomes; 6 for plants with large genomes)
3. Place in a fixative, such as Farmer's or Carnoy's, and leave overnight at room temperature.
4. Place in 70% EtOH for at least 10 minutes. Store in refrigerator.

The acetocarmine squash

1. Hydrolyze the root tips in 5M HCl at room temperature for 5 minutes, or 1M HCl 15 60°C for 20 minutes. This softens the cell walls, and permits the cells to be flattened out on a slide.
2. Place a microscope slide over a dark surface, then place a root tip over on the slide. Remove all but the very tip of the root, which will be a whitish color, while the rest of the root will be translucent.
3. Add a drop of stain over the tip, then place a cover slip over the drop. Heat the slide over an alcohol lamp, so it feels warm to the touch. Do not boil it.
4. After this point, ensure that the cover slip does not slide over the slide, or it will roll and crush the cells. Place a paper towel over the slide, and press down firmly with your thumb. Turn the slide over (cover slip down) and tap gently but firmly with a pencil eraser or a Sharpie pen.
5. Examine the slide under a microscope. If the cells are well spread and mitotic figures are apparent, seal the edges with Hoyer's or with paraffin.

The modified carbol fuchsin stain (also works with acetocarmine)

1. Hydrolyze the root tips by placing them in 1 part concentrated HCl : 3 parts 95% EtOH (v/v) for about 5 seconds.
2. Place a microscope slide over a dark surface, then place a root tip over on the slide (see figure at end of handout). Remove all but the very tip of the root, which will be a whitish color, while the rest of the root will be translucent.

3. Add a drop of stain over the tip, then place half of a double-sided razor blade by the stain. Add a cover slip over the stain and the razor blade.
4. Holding the blade and cover slip firmly in place, tap the root tip about 5 times with a flat pencil eraser or the end of a Sharpie. Holding the cover slip in place, slide the razor a mm away from the tip. Repeat the tapping-razor sliding sequence until the razor blade has been completely removed from under the cover slip.
5. Turn the slide over onto a paper towel, and tap firmly several times.
6. Examine the slide under a microscope. If the cells are well spread and mitotic figures are apparent, seal the edges with Hoyer's or with paraffin.

The Feulgen stain

1. Hydrolyze the root tips in 5M HCl at room temperature for 5 minutes, or 1M HCl 15 60C for 20 minutes. This softens the cell walls, and permits the cells to be flattened out on a slide.
2. Rinse in H_2O.
3. Place the tips in Feulgen stain, until stain develops at tip. This can take an hour or more.
4. Place 1 tip on a glass slide, and cut away the unstained portion of the root tip.
5. Add a drop of acetocarmine as a counterstain over the tip, then place a cover slip over the drop. Heat the slide over an alcohol lamp, so it feels warm to the touch. Do not boil it.
6. After this point, ensure that the cover slip does not slide over the slide, or it will roll and crush the cells. Place a paper towel over the slide, and press down firmly with your thumb. Turn the slide over (cover slip down) and tap gently but firmly with a pencil eraser or a Sharpie pen.
7. Examine the slide under a microscope. If the cells are well spread and mitotic figures are apparent, seal the edges with Hoyer's or with paraffin.

Stains and Solutions

Pretreatment Solutions

1. Cycloheximide-hydroxyquinoline: In one liter of water, dissolve 70 mg cycloheximide and 438 mg 8-hydroxyquinoline. Heat and stir until dissolved, which may take several hours. Do not let it boil. Store in the dark and under refrigeration. *Note:* Addition of the cycloheximide is optional.

2. Monobromonapthalene: Mix a saturated, aqueous solution of this material in distilled H_2O.

Fixatives

1. Farmer's (3:1): Mix 3 parts 100% EtOH with 1 part glacial acetic acid (v/v). As an option, add enough of a saturated aqueous solution of ferric chloride to result in a pale yellow or straw-

colored solution. This acts as a mordant. However, the tools used (forceps, spears, etc.) have enough iron in them that adding the ferric chloride is usually unnecessary.

2. Carnoys (6:3:1): Mix: 6 parts 100% EtOH, 3 parts of chloroform, and 1 part of glacial acetic acid (v/v/v). Addition of chloroform can help dissolve fats and oils that would otherwise interfere with observation. However, this mixture is not stable, so it must be used immediately after mixing.

When there is little known about the species, I would play it safe and use Carnoy's rather than Farmer's.

3. FAA:

- 90 ml 70% EtOH
- 5 ml formaldehyde (remember formaldehyde is a carcinogen!)
- 5 ml acetic acid

Wait 24 hours before using.

Stains for Root-tips

ACETOCARMINE:

- 2 g carmine
- 100 ml 45% AcOH

Heat slowly until the color turns. Then boil gently for 5 minutes. Let cool, then filter.

FEULGEN:

- 1 g basic fuchsin
- 200 ml boiling water
- 2 g $K_2S_2O_5$ (potassium metabisulfite)
- 10 ml 1M HCl
- 0.5 g activated carbon

Dissolve the basic fuchsin in the water. Filter and cool to 50C. Add the potassium metabisulfite and the HCl. Let it bleach for 24 hours, under refrigeration and in the dark. Add the activated carbon, shake 1 minute, and filter.

Store in the refrigerator in the dark. Discard when it becomes tinted.

FEULGEN (QUICK FEULGEN):

- 1 g basic fuchsin
- 1.9 g potassium metabisulfite
- 18 ml 1M HCl
- 0.5 g activated carbon
- 200 ml distilled water

Mix all the ingredients except the charcoal. Stir for at least 2 hours. Add the charcoal and stir for at least 2 minutes. Filter. If the filtrate is not clear, repeat the previous step. Store in a brown bottle under refrigeration.

MODIFIED CARBOL FUCHSIN: (Kao, 1976)

- 3.0 g basic fuchsin in 100 ml 70% EtOH (solution A) -- Store indefinitely
- 10 ml solution A in 90 ml of 5% phenol (in distilled water) (solution B) -- use within 2 weeks
- 45 ml solution B in 6 ml glacial AcOH and 6 ml 37% formaldehyde (solution C = carbol fuchsin)
- 10 ml solution C in 90-98 ml 45% AcOH and 1.8 g sorbitol

Age at least 2 weeks. May even take a month before it is ready and becomes inky.

Hydrochloric acid:

- 1 M = 88 ml concentrated HCl/L
- 5 M = 440 ml concentrated HCl/L

Always add acid to water!!

Alcohol dilutions: Formula is X = (desired %)/0.95 EtOH

		100 ml	500 ml	1 L	2 L
25%	EtOH	26 ml	130 ml	260 ml	520 ml
	Water	74 ml	370 ml	740 ml	1480 ml
50%	EtOH	53 ml	265 ml	530 ml	1060 ml
	Water	47 ml	235 ml	470 ml	940 ml
70%	EtOh	74 ml	370 ml	740 ml	1480 ml
	Water	26 ml	130 ml	260 ml	520 ml
85%	EtOH	89 ml	445 ml	890 ml	1780 ml
	Water	11 ml	55 ml	110 ml	220 ml

Note: Distilled EtOH is 95%