

# E X E R C I S E

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# 10

## Comparison of Root Tip Squashes Grown in Different Sterile Conditions

### PURPOSE

- 1) To sterilize seeds for growth in culture.
- 2) To grow seedlings sterilely.
- 3) To take root tips from seedlings.
- 4) To stain root tips and make chromosome squashes.
- 5) To determine any differences between varieties or species of plants.

How do seed companies determine exactly what variety of crops are from which batch(es) of seed? For instance, how can you tell one cultivar of tomato from another just by looking at the seeds? Cherry from Beefsteak? It often is not easy. All carrot cultivars are variations of one species; therefore, they have the same number of chromosomes. Theoretically, each variety, or cultivar, should have different chromosomal markers, or banding patterns, to distinguish them.

Carrots and parsnips, or bell peppers and habenero peppers, will be used as a pair. Both sets of seeds look the same in size and shape.

Why are the seeds grown sterilely? Why not grow them in soil or other potting medium?

Why are root tips a good choice for chromosomal squashes?

### PROCEDURE

1. Obtain a set of seeds from the instructor.
2. Sterilize seeds by placing 1 variety of seeds in 1 tea strainer, and the other variety in the second strainer. Sterilize for 5 min. in 70% Ethanol and 5 min. in 20% bleach. Then rinse 2 x 5 min. in sterile ddH<sub>2</sub>O.
3. When sterile, shake all excess water out of strainer, then place half of the seeds from 1 strainer on a plate of solid MS medium and the other half of the seeds from the same strainer in a bottle of liquid MS. The bottle of medium must be placed on a shaker to aerate the liquid; this can be at room temperature. The plates can be left at room temperature on the bench top.

4. Repeat Step 3 for the other strainer of seeds.
5. Leave for 1-2 weeks so the seeds can germinate.
6. Make sure all plates and bottles are labeled.

The following week:

1. Carefully remove each seedling from the plate, taking care not to break off roots. Place seedlings in a sterile Petri plate to cut off the root tips. Place root tips on a glass slide and stain with Geimsa stain.
2. Repeat Step 1 with the other plate of seedlings.
3. Repeat Step 1 with one bottle of liquid medium.
4. Repeat Step 1 with the second bottle.

## MATERIALS NEEDED

4 sterile, autoclaved beakers per pair

70% Ethanol

20% bleach

Sterile ddH<sub>2</sub>O

Sterile forceps

2 plates of MS medium

2 bottles of Liquid MS medium

Parafilm, shaker

Tea strainers

Seeds of carrot and parsnip, or bell pepper and habenero pepper

Glass slides

Geimsa stain

## HINTS

- It can take up to 10-18 days for germination of seeds outdoors in the soil. It should take less time indoors under warmer temperatures. It really depends on how old the seeds are, what variety, and light/temperature conditions. If the lab is planned for mid-winter, it would be best to have grow lights over the seeds (or an incubator) with light for 16 hrs./day.
- Seeds were chosen for this lab based on size comparison, and likeness of crop type. Carrot and parsnip are both root crops, with small hard seeds. Even though they have taproots, they do have lots of root tips when young.
- Bell pepper and habenero pepper seeds are whitish and flat, again about the same in size and shape.

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- Depending on varieties available, chromosome number may be different, and banding patterns may be similar or very different.
  - Root tips are good to use, as they are a site of great mitotic activity in a plant. Chromosomes are quite condensed and easily stained and observed. Cells in metaphase will be the best for chromosome counts.
  - Root tips grown in sterile conditions are best to use, as the tips are much less likely to get torn off when being pulled out of agar-based medium than being pulled out of soil. Root tips at tip-end are a millimeter or two of a root, and are easily lost in soil.
  - Growing seeds in liquid and solid media is just an exercise to see if media type has an effect on seedling growth. Seeds may or may not germinate, depending on cultivar used, or seeds may rot. Liquid media is exactly the same as solid, except agar is not added.

