

## Plant Propagation of *Solanum tuberosum* Using Tissue Culture<sup>®</sup>

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### INTRODUCTION

A rapid technique to rapidly increase the number of potato plants is by using the tissue culture method. The first step is getting clean plantlets. Mother plants may be purchased from universities or private tissue culture "banks." These are virus-free and ready to multiply. This is the fastest and easiest way to begin. Unfortunately, many of the new selections in field trials get viruses and have to be cleaned up before clean seed stock is available for multiplication. This is time consuming and may take more than a year.

### MATERIALS AND METHODS

Before starting with a potato plantlet you will need to make medium. This may be purchased from various labs in ready-made packages and all you do is add de-ionized water and agar. Alternatively, it may be made from scratch (Table 1). After cooking the medium until the agar dissolves, you pour ¼ in. into Magenta<sup>®</sup> vessels or baby food jars and cap them. They are then autoclaved and, once cooled, ready for new plantlets.

The tissue culture technique is performed in a laminar air-flow hood. Using forceps, remove the plantlet from its container. Cut the plant with a small pointed scissors between the leaf nodes leaving a small stem on each to place into the new medium, making sure the leaf node rests on the top of the agar in the new vessel. Each vessel can hold 15–20 new nodes to grow into plantlets. Sterilize the tools in a glass bead sterilizer or ceramic sterilizer for 15 sec. Place under fluorescent grow room lights giving them a 16/8-h light/dark photoperiod. In a few days the nodes will callus and new shoots between the leaf and stem segment will begin to grow. In 4–5 weeks you will be ready to repeat the process giving you five to eight times the plant material, depending on the selection.

### METHODS AND MATERIALS

**Using a Tuber.** When starting with a tuber, wash it with soapy water, lightly scrubbing the eyes to remove dirt. Place into a paper bag and let it sprout. This may take up to 2 months if the potato has been freshly dug.

In the laminar flow hood cut the sprouts into a beaker containing a 10% bleach solution and 15 drops of Tween 20 (disinfectant) stirring for 15 min. Place sprouts into a 70% ethanol solution for 1 min and rinse twice in sterile water for 3 and 12 min, respectively. Take out the sprouts and let them dry on a sterile paper towel. Put them into growing medium under the grow-room lights with a 16/8-h light/dark photoperiod, keeping the temperature around 70 °F. Make enough plantlets to have back-ups since the potato will shrivel and die. If these plantlets are tested, the chance of virus is high and the clean-up process will have to be done again.

**Table 1.** Potato medium recipe for 8 Liters.

Sucrose	240 g
Inositol	800 mg
Sulfate (CaSO <sub>4</sub> )	2752 mg
Nitrate solution	80 ml
Sulfate solution	80 ml
Halide solution	80 ml
Phosphate solution	80 ml
Iron (Fe) solution	80 ml
Thiamine solution	80 ml
Kinetin solution	80 ml
GA <sub>3</sub> solution	80 ml
Bring to 7500 ml dH <sub>2</sub> O	
pH to 5.7	
Add 500 ml dH <sub>2</sub> O (up to 8 L)	
Agar	56 g
Heat till clear (1 h or so)	
Add 50 ml dH <sub>2</sub> O per L for evaporation	
Fill vessels with 1/4 in. medium	
Autoclave filled vessels for 15 min	

**Virus Eradication.** After making six to eight vessels of plants, keep a couple in the grow room for back-ups and place the others into an incubator. Each day of the week, the incubator is set to 35 °C with the lights off at 2 pm and then to 30 °C with the lights on at 2 am. This makes the plants grow rapidly and also “cooks” out the diseases. After 4 weeks of this heat and light therapy (or until the plant is nearly wilted), remove to the hood. Within 1 h out of the incubator, remove the apical bud from the upper nodes using a microscope and put it into a test tube of therapy medium containing Ribavirin and gibberellic acid (GA<sub>3</sub>). Ribavirin is an anti-viral drug. Transfer the small plant tissue every 2 1/2 weeks to new medium. After 5 weeks, GA<sub>3</sub> is deleted and a growth regulator, IBA, is used to promote root development. Transfer again to new medium at 2 1/2 weeks. The next medium at 5 weeks contains no Ribavirin and is renewed after 2 1/2 weeks. Finally, after about 4 months of this process you will hopefully have some plant material to cut and put into regular growing medium.

When a plant grows six to eight nodes, take out that plant and tissue culture the bottom four nodes into one vessel and the top nodes into another. Allow these to grow 4–5 in. and test the bottom nodes for potato viruses and bacteria. Keep the top plant nodes for your own increase. If the test results are clean and free of

disease, discard all plant material and back-ups of that clone except what is clean from the top of the tested plant. This is considered the “mother plant” from which the clean plant stock will be made of.

**Saving Clones for Future Use.** Putting tissue culture nodes into media to create microtuber potatoes instead of plant tissue is the best way to keep selections for future needs and backups. They take up a very small place in the refrigerator and can be kept for up to 3 years. When the selection is needed again, the microtubers are cut in half and placed cut-side down on regular growing media. A plant will emerge out of the eye of that tuber.

## SUMMARY

Many different plants can be propagated quickly using tissue culture. Potato is one of many plant species for which this technique is useful. New stock is clean of disease in a sterile environment and takes very little space to keep year after year.

## QUESTIONS AND ANSWERS

**Steve McCulloch:** When you use ribavirin are you filter sterilizing it or autoclaving it?

**Marlys Bedlington:** It's added to the medium before autoclaving.

**Gayle Suttle:** Once a commercial grower gets their planting started, how often can they continue to use the stock?

**Marlys Bedlington:** We have commercial growers who buy our “seed” every year because potato gets infected with viruses quickly. That's why we start fresh every year even though we sell the same cultivar. Commercial growers are primarily focused on cosmetics, not so much disease. We try to keep our fields as disease-free as possible by rouging the summertime.

**Kristen Yanker-Hansen:** How many varieties of potato do you grow?

**Marlys Bedlington:** We have 28 in the greenhouse this year. I switched varieties 25 times 2 days ago and 5 times yesterday in the field. We eliminate those that don't work for us and/or are not of interest to our growers.

**Anonymous:** Have you considered starting them as plantlets rather than mini-tubers?

**Marlys Bedlington:** We've tried that. I have had to cover our plants with Styrofoam cups because of the weather. They are very fragile at that stage. You can harden them off somewhat, but I would have to have a separate greenhouse.