

# Production of Permanent Crops Using Tissue Culture Techniques, Does it Make Cents?®

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## INTRODUCTION

The term “tissue culture” has come to encompass many different techniques associated with the clonal reproduction of plants. Terms such as somatic embryogenesis, meristem culture, embryo rescue, protoplast fusion and micropropagation are all specialized techniques that are often referred to as “tissue culture” when discussing the propagation of plants. Any one of the above techniques would ultimately give rise to a new plant, but micropropagation has probably come to be the most widely adopted, since its relative ease in training novices in the technique.

At Duarte Nursery, in Hughson, Calif., micropropagation is used primarily to clonally reproduce rootstocks used in the production of stone fruit, nut, and citrus trees for commercial plantings. The technique allows us to produce elite, “clean” plant material, but not without substantial costs and investments.

With that being said, let’s look at what micropropagation has to offer the nurseryman and examine the question: “Does it make Cents?”

## WHAT IS MICROPROPAGATION?

Micropropagation is a specialized technique used to propagate plants using small pieces of a plant stem, leaf, or growing tip. It is particularly useful on plants that are otherwise difficult to propagate by regular means. The technique generally is performed in a sterile environment due to possible contamination by bacteria, fungi, or insects such as mites or thrips.

### There are Four Distinct Stages of Micropropagation:

- 1) Initiation
- 2) Multiplication
- 3) Rooting
- 4) Acclimatization

**Stage 1: Initiation.** A segment (explant) is cut from the parent plant, disinfected to kill bacteria and mold spores that may be present, and then placed in sterile medium. During this stage, if the parent plant material is found to contain a virus, the plant could undergo thermotherapy treatment or meristem culture to remove the presence of the virus before being placed in culture.

**Stage 2: Multiplication.** Explant is placed in a medium containing cytokinins (and maybe auxin) and nutrients to encourage cell division, shoot initiation, and lateral bud growth. The objective is to maximize shoot production.

**Stage 3: Rooting.** Shoots arising from Stage 2 are transferred to a medium containing auxins to induce roots. During this stage, the correct ratio of hormones and nutrients becomes more precise in order to induce root formation.

**Stage 4: Acclimatization.** The rooted shoots are removed from the sterile containers, transplanted into cell trays filled with soilless medium, and placed in a greenhouse under high humidity for rooting out. The high humidity is necessary to keep the transplants from desiccation, as the leaves' stomata are not fully functioning like those of plants grown in a natural environment.

**Sounds Pretty Straight Forward Doesn't It?** So, why aren't we all as propagators using micropropagation? Answer is \$\$\$\$ capital investment!\$\$\$\$

To do micropropagation on a commercial scale, you would need to invest in:

- Facility: large enough to fit your needs, with heating and cooling system to keep temperatures fairly constant.
- Specialized equipment: such as autoclaves, laminar flow hoods, instrument sterilizers, dissecting microscope, medium mixer, etc.
- Media components: comprised of macro and micro nutrients, vitamins, amino acids, sugars, agar (solidifying agent), and growth regulators.
- One of the most important factors governing the growth and morphogenesis of plant tissues in culture is the composition of the culture medium. The basic nutrient requirements of cultured plant cells are very similar to those of whole plants!
- Containers: size, shape, and type will be dependent on your individual needs.
- Trained staff: critical that they be trained in proper techniques to minimize contamination, but still work efficiently to keep labor costs down.
- Grow room / lighting: racks to support containers and lights for growth.
- Acclimation area: extracted plants will need a growing environment with adequate temperature, light, and humidity controls for acclimating, rooting, and hardening off.

## TRADITIONAL PROPAGATION

Compared to traditional propagation methods where all one needs is an area with a mist bench and mist controller/timer, proper heating and cooling, healthy stock plants, propagation trays with media and various rooting hormones, micropropagation seems expensive. So, what's the advantage?

## ADVANTAGES OF MICROPROPAGATION

Clean Disease Free Plants.

- Plants are maintained in a sterile environment.
- Provides an avenue to more easily clean up plants that have virus or disease issues.
- Once plants are certified virus free and disease free, the possibility that they will become diseased while in culture is virtually zero.

**Ability to Grow Large Number of Plants in a Relatively Small, Well-Controlled Environment.**

- Seasonality issues that normally occur with traditional vegetative propagation are eliminated.
- Currently producing 4,000,000 + plants yearly.

**Alternative Clonal Propagation Method for Difficult-to-Propagate Plants.**

- Especially useful for hybrids, where propagation by seed introduces a large amount of variability.

A good example is UCB-1 pistachio rootstock, a cross between *Pistacia atlantica* × *P. integerrima*. The resulting hybrid has better cold tolerance, salinity tolerance, better overall disease resistance, and produces higher yields. It is difficult to propagate from cuttings and is thus propagated primarily by seed. The problem with UCB-1 pistachio rootstock grown from seed is the variability that grafted trees exhibit in the field. By making careful selections of seedling performance, a single selection was made of the best performing seedling and is now being sold as the "Duarte clone."

**Quicker Response to Market Demand.** When new varieties are introduced into the industry, micropropagation allows us to multiply the material much more quickly and made available to growers much sooner.

- Clean, disease free plants that are container grown are available for planting year round, thus giving the grower more flexibility in establishing their plantings.

**CONCLUSION**

Micropropagation of permanent crops such as walnuts, pistachio, almond, stone fruits, avocado, and citrus, to name a few, have a distinct advantage to the grower over traditionally produced field grown plants. In general, micropropagation allows the nurseryman to make available to the grower, trees free of viruses and diseases. Trees that are clonally produced, eliminate the variability observed in seedling populations and can lead to higher production yields. Coupled with a containerized growing system, new varieties can be brought to the market much sooner in larger numbers and available year round for planting. When you consider the advantages, micropropagation does make "cents" for both the nurseryman and our grower/customer!