*By Tim Spann, PhD*
Spann Ag Research & Consulting

# Avocado Tissue Culture Within Reach

Tissue culture — the growing of plant cells under sterile culture usually for the purposes of producing clones of a plant — has been the Holy Grail of avocado propagation for decades. But until now, no one has been able to develop a technique that could be broadly applied to most avocado cultivars. A few years ago, one California nursery developed techniques to propagate a couple of avocado rootstocks in a semi-tissue culture environment, but the trees produced proved to be unthrifty and did not perform well in the field. Others have been able to develop robust techniques for a single cultivar but have not been able to get those techniques to translate to a broad range of cultivars. And some techniques have been developed privately and are not available to the public. Enter the Huntington Botanical Gardens.

*Photo credit: Raquel Folgado, Cryo-preservation Research Botanist, The Huntington.*

## Huntington Botanical Gardens

The property that is today the Huntington Botanical Gardens in San Marino was home to some of the earliest avocado plantings in California. As a result, the Huntington has long had an interest in preserving avocado varieties. That said, traditional plant collections — especially of trees — take up considerable land area and are vulnerable to ever changing threats such as pests, diseases and now climate change. Thus, the Huntington, as part of its mission to preserve various plant species, has a robust research laboratory to develop the techniques necessary to carry out their mission of preservation.

One group of plants the Huntington is working to preserve is magnolias. To secure their magnolia collection for generations to come, the Huntington looked to cryopreservation – the process of cooling cells to very low temperatures (-320 °F) to maintain their viability. However, to use cryopreservation successfully one needs the ability to go from a few frozen cells back to a living tree. This is where tissue culture comes in. The magnolias, like avocados, were notoriously difficult to maintain in tissue culture successfully, but the Huntington research team, led by Dr. Raquel Folgado, solved that mystery. And, as it turns out, the magnolias and avocados are closely related both belonging to the clade Magnoliids.

In 2018, the Huntington approached the California Avocado Commission (CAC) to see if there was interest in avocado tissue culture research. The Huntington staff believed they could translate what they learned from magnolias to avocados without too much difficulty. Equally appealing for CAC was the fact that central to the Huntington's mission is sharing of information, which meant whatever

*Photo credit: Raquel Folgado, Cryo-preservation Research Botanist, The Huntington.*

they learned would be in the public domain. The Huntington had no interest in patenting their techniques or profiting from them. That said, by partnering with the Commission, the Huntington would be able to serve their own interests – to preserve their avocado collection via cryopreservation. This seemed like a win-win situation and an ideal opportunity for CAC to fund.

## Avocado Tissue Culture

Although simple in theory, plant tissue culture is a road strewn with potholes. The tissue culture environment must be kept sterile since the media on which the plant cells are established is full of nutrients, sugars, vitamins and other additives that fungi and bacteria find ideal to grow on. Thus, the first step in tissue culture is surface sterilization of the plant material being used to start a culture.

The Huntington has developed reliable techniques to surface sterilize tissue collected from mature avocado trees so it can be brought into culture.

After sterilization is achieved, tissue culture can be broken down into three phases: initiation, establishment, and multiplication. Initiation involves putting sterilized mature plant tissue in a culture media and getting those mature cells that exist as some tissue (e.g., leaf cells) to de-differentiate — cells change from a given differentiated state into a stem cell-like state where they can divide and multiply — and grow as a mass of undifferentiated cells known as callus.

In the establishment phase, cells of callus tissue begin to differentiate and grow into small stems and leaves. In the multiplication phase, the established cultures are physically divided so they produce more stems and leaves.

A fourth phase, rooting, can sometimes be done in tissue culture or the tiny stems also may be removed from the tissue culture environment and rooted by more standard methods used for any stem cutting.

The transition from each of these phases to the next is achieved by manipulating the culture media, typically by adjusting the ratio of different plant hormones and nutrients. This is often the challenging part of tissue culture as has been the case with avocados in the past. For example, several different labs have had success getting avocados to establish in culture but could not get them to multiply. Still others have been able to get them to multiply just to find they could not successfully get the shoots to produce roots.

The Huntington, while still having their share of challenges, has been able to successfully bring avocado tissue into culture, move it through all phases of culture, and regenerate a small plant. To date they have established cultures of 10 avocado varieties as well as two *Persea* species (*P. indica* and *P. podadenia*). In addition, they have developed several different culture media that work for groups of avocado varieties.

In their final year of work, the Huntington researchers will be working to refine the entire process to maximize efficiency of the process. They also will be working to establish additional cultivars in culture. Finally, they will be publishing their methodologies so anyone who would like to propagate avocados by tissue culture will have a solid foundation to start from.

## Potential Benefits of Avocado Tissue Culture

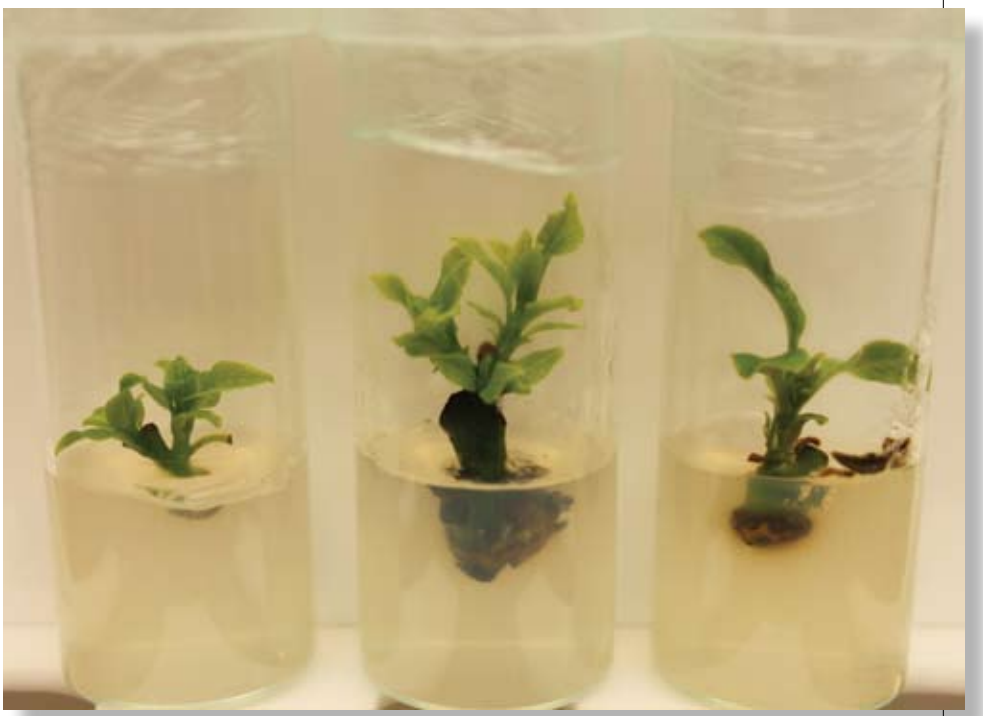
There are many potential benefits to the California avocado industry of having sound avocado tissue culture methods worked out. First is germplasm preservation. Germplasm, both what is in curated collections around the world as well as wild avocados and their relatives that may only exist in the wild, are in danger of being lost. Curated collections are expensive to maintain due to the land requirements and personnel needed to curate them. Disease threats, such as laurel wilt disease for which there is no known treatment or cure, could easily wipe out collections. And threats such as climate change and deforestation threaten wild avocados and their relatives. Being able to move material into tissue culture and potential

cryopreserve could save these genetic resources for generations to come.

Perhaps the most obvious benefit of tissue culture is the potential to propagate avocado trees more rapidly for commercial production. Current techniques are labor intensive and slow. In addition, the use of nurse seeds has always posed the threat, albeit very small, of introducing some unknown pest or pathogen. Being able to generate rootstocks in tissue culture and produce large quantities of clonal rooted cuttings ready for grafting should, in time, reduce the propagation time for trees and possibly reduce the cost of propagation.

A less obvious benefit of having viable avocado tissue culture techniques is the benefit to other avocado research projects. For example, in current avocado rootstock breeding programs, seeds are collected from mother trees, germinated and the seedlings are exposed to phytophthora root rot or salinity. If they survive, those seedlings are propagated by conventional means to produce a handful of trees for the next phase of testing. Imagine if, instead of a handful of trees, several hundred could be produced, and more quickly, for the second round of testing. Data collection and potential selection of new rootstocks could be sped up considerably. Or screening could even move into the tissue culture environment — imagine a culture media with various salt levels to test for salinity tolerance.

These are just a few of the potential benefits that will arise from having robust tissue culture methods available for avocados. The Huntington research team is making great strides and will be concluding their research at the end of 2021. Stay tuned for their final report and the doors that will be opened.



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