Cannabis Tissue Culture: Expectations and Reality

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When I started my first cannabis tissue culture lab ten years ago, I assumed I could draw on my previous experience with tobacco. Cannabis is easy to clone and grow so how could it be difficult? Tissue culture protocols for mass propagation and genetic modification were available for many crops. A few publications already reported methods and results for Cannabis.

At the first Cannabis industry convention I attended in 2015 many of the attending growers announced their tissue culture programs and how it would revolutionize cannabis propagation. Today we are still cloning, and many tissue culture laboratories have gone out of business.

Cannabis turned out to be difficult to culture. Protocols that were producing consistent results for other crops just did not work for Cannabis. Others that did were complicated, expensive, time-consuming, or required highly skilled personnel. Shoot and root induction, strain-by-strain variations, contamination, rooting, and hardening remain challenging for anyone in the field. It

took me a long time to develop methods that were dependable and simple enough to be applied at large scale.

Tissue culture has rebounded but many of the initial claims remain unfulfilled:

TC is an economical way of mass-producing starter plants.
Tissue culture propagation basically "cloning deluxe." The resulting plants are genetically identical to the mother plant. The difference is that it starts from smaller cuttings and the initial stages happen in a sterile environment. In most other crops mass propagation is achieved in sterile culture through a process called somatic embryogenesis. This has not been established beyond the laboratory stage in Cannabis and mass-propagation is far less efficient. Producing starter plants for an entire cultivation operation is still too expensive and time-consuming for most cultivators.

TC will eliminate the need for a mother room.
Not practical. Strains can be maintained in sterile culture for extended periods of time under slow-growth conditions. Individual clones also can grow and branch in culture and produce multiple micro cuttings. This

requires repeated cycles of sub-culturing, produces fewer clones than a few mother plants, and exposes the cultures to the risk of contamination. Keeping a collection of tissue-culture propagated stock mothers remains the most reliable way of maintaining valuable strains.

TC will help generate genetically modified cannabis soon.

True. Stable genetic transformation of Cannabis has recently been shown and patented. The process involves introducing custom designed genes into meristems of seed embryos, grow these in culture, flowering the resulting plants, cross pollinate, and then screening the seeds for the desired trait. Researchers at Wisconsin Crop Innovation Center have produced 0% THCA hemp using this method. However, preserving the exact genetic makeup of a specific strain requires somatic embryogenesis, which still needs to be established.

TC will restore strains to their original vitality.

True. We have run hundreds of strains through our tissue culture process and produced healthy, strong copies of mother plants that had grown poorly and produced weak clones.

TC can reverse genetic changes.
Partially true. Tissue culture cannot reverse changes to the DNA sequence that have accumulated over time. However, the recovery of strong clones from "tired" moms through tissue culture suggest that some (epigenetic) modifications that affect gene activity but not DNA sequence can be reversed.

TC can cure any pathogen infection or pest infestation.
Depends. The cuttings (explants) from which cultures are started range from microscopic meristems to stem sections containing one or more nodes. Surface sterilization removes most fungal pathogens, such as mildew and fusarium and pests like spider mites but not viruses and fungi that are contained deep inside the tissue. Starting from meristems is the best way of producing disease-free plants but is technically difficult and time-consuming. Smaller explants are more likely to produce healthy plants than bigger ones. Hop latent viroid (HLVd) is one of those pathogens that can be easily transmitted through tissue culture. We could recover most infected strains by combining tissue culture with in-house PCR testing.

The present and future of Cannabis tissue culture

The best way to assess the current state of Cannabis tissue culture and the capability of the many service providers is to look at images on websites and Google search. One will find mostly images of cute little plantlets in culture vessels, graphics of how the process is supposed to work, and calluses in Perti dishes. Much less frequently are pictures of explants and initial stages of the cultures. Pictures of explants mostly show node segments that include stem sections.

The publication of peer-reviewed scientific articles has accelerated rapidly over the last few years. Cannabis tissue culture is gradually catching up to other species. However, the published methods are often difficult to reproduce at commercial scale. I had so far no success with somatic embryogenesis trying every published process I could find. Like with other publications I recommend looking at the images before trusting numbers and tables. What you see is what you get!

Cannabis is not easy to culture but current micropropagation methods can deliver great benefits to cultivators. The term tissue culture is used for many different protocols and to be successful these must be scalable, simple, economical, and dependable. The risk of losing valuable genetic assets to pests, pathogens, and

human errors in maintaining mother plants has now increased with the arrival of HLVd and will continue with other upcoming pathogens. Combining tissue culture with (in-house) qPCR testing is not expensive compared to losing yield and potency in only one or several harvests.

To learn how to build your own laboratory and manage your strains go to https://tcworkslab.com/.