



TISSUE CULTURE DISEASE PREVENTION

WHAT IS THE ROLE OF TISSUE CULTURE IN
PREVENTING THE MOST PREVALENT DISEASES
IN CANNABIS CULTIVATION?



CONCEPTION NURSERIES

INTRODUCTION TO TISSUE CULTURE

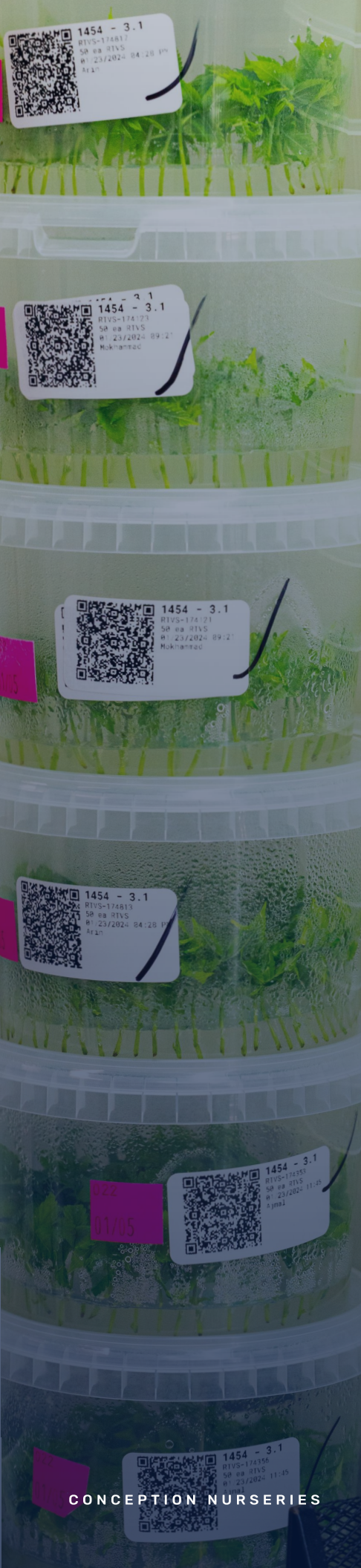
Conception Nurseries has implemented an effective procedure that upholds the highest standards of purity and quality in cannabis growing. This approach is based on the principals of the agricultural tissue culture and micropropagation industries. The process begins by carefully choosing and confirming that our initial plant materials are free from any disease-causing organisms and are of the utmost excellence.

This procedure involves the meticulous choice of unique plant varieties, along with the examination of viruses and viroids carried out by a certified third-party laboratory. This core approach **emphasizes the methods for guaranteeing the genetic purity, strength, and cleanliness of initial plant materials**, encompassing all aspects from initial selection to continuous testing and improvement. Our unwavering commitment to upholding the utmost standards in every facet of our operations demonstrates industry excellence and our passion for delivering robust clones to our clients.

AUTHORS

Dr. Maria Rojas PhD, Austin Widdershoven, Joe Derr, Jesse Davis





EXPLANT SELECTION & STERILIZATION

At Conception Nurseries, our product development team carefully chooses unique cultivars by **evaluating their genealogy, agronomic qualities, and performance attributes** in a thorough study. These cultivars are subjected to meticulous sampling and testing for viruses and viroids at a reputable third-party laboratory to verify their pathogen-free condition. Prior to accessing our premises, we mandate the submission of a certificate of analysis (COA) obtained from external collaborators, which provides comprehensive information regarding the outcomes of these examinations.

The testing panel should encompass all crop-specific pests, including many viruses and viroid known to affect cannabis. These include:

- **Hop Latent Viroid (HLVd)**
- **Lettuce Chlorosis Virus (LCV)**
- **Alfalfa Mosaic Virus (AMV)**
- **Tomato Mosaic Virus (TMV)**
- **Beet Curly Top Virus (BCTV)**

These microorganisms can have a substantial impact on the overall health and functioning of plants. HLVd, for example, has a particularly negative impact, resulting in slowed growth, deformed leaves, shorter spaces between nodes, and infected plants producing fewer cannabinoids (Punja et al., 2019). In outdoor environments, the transmission of BCTV by leafhoppers can result in both stunted growth and reduced crop yields (Melgarejo et al., 2022).

STERILIZATION METHODOLOGY



MERISTEM CULTURE INITIATION



INDEXING PROCESS

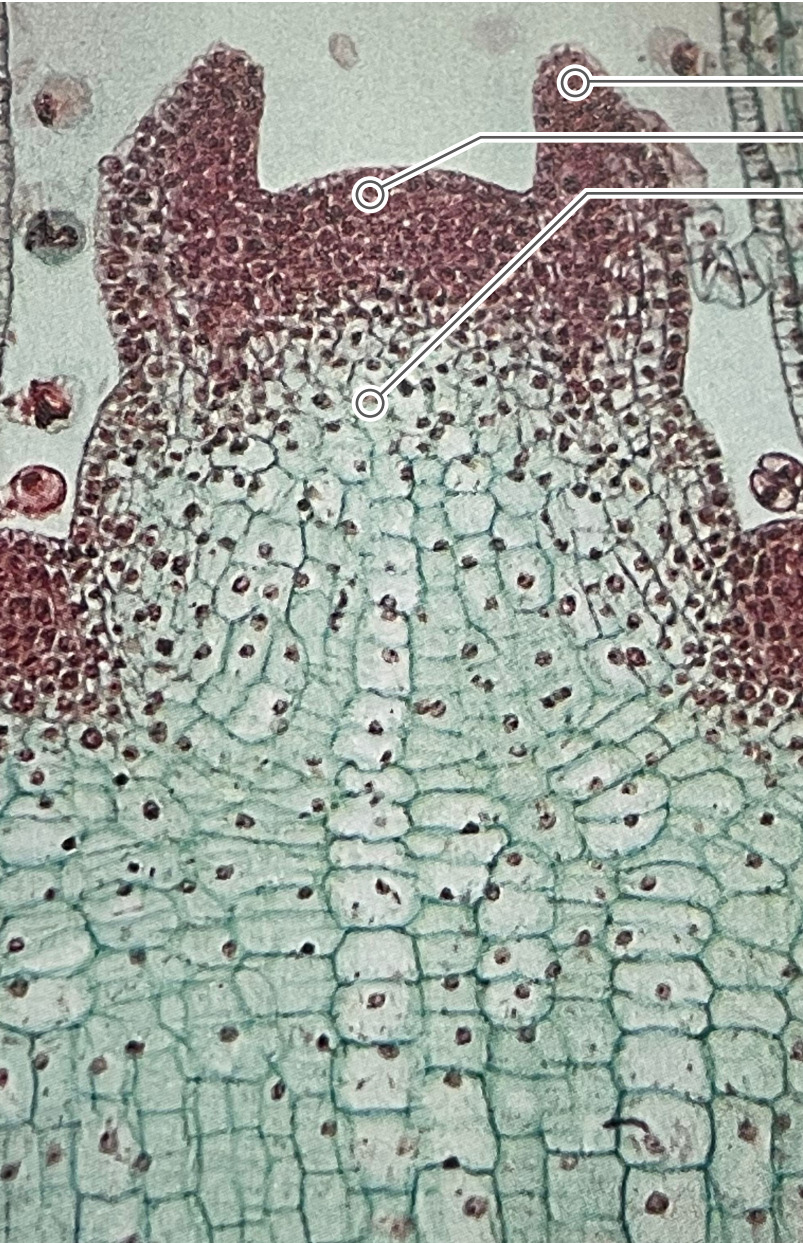


**CULTIVAR OPTIMIZATION &
AGRONOMIC PERFORMANCE**



MERISTEM CULTURE INITIATION

Tissue culture procedures commence with the establishment of a biomass derived from the mother or source plant. This initial stage is commonly known as the intake stage. During this phase, apical and lateral cuttings are acquired from a fully grown plant that is still in the vegetative phase of growth. The initial stages of the intake procedure are carried out at the client's or breeder's premises.



MERISTEM DIAGRAM

Leaf Primordium

Apical Meristem

Central Mother Cells

We carefully analyze the meristems, which are areas of undifferentiated cells located in the rising tips of shoots and are responsible for active cell division and growth. The meristems are positioned adjacent to the vascular tissues, with a separation of 2 or 3 cell layers. The undifferentiated cell layers generate the cells and tissues of plants that are now devoid of pathogens transmitted by the vascular system.

Pathogens, such as viroids, viruses, bacteria, and endophytic fungi, cannot be completely eliminated from non-meristem nursery practices.

Consequently, initiating a cultivar tissue culture using meristems ensures the production of plants that are free from contamination and have minimal risk.

Reference for Meristem Diagram:

Raven H. Peter; Evert F. Ray; Curtis, Helena. (1981). *Biology of Plants, Third Edition.*Worth Publishers, INC. 686p.



INDEXING PROCESS

In order to further reduce the risk of contamination in a tissue culture facility, it is necessary to carry out the full intake procedure in separate departments. To minimize the possibility of contamination, it is important to maintain separate growth of meristems and manufacturing material. These methods reflect a dedication to rigorous quality control throughout all operations.

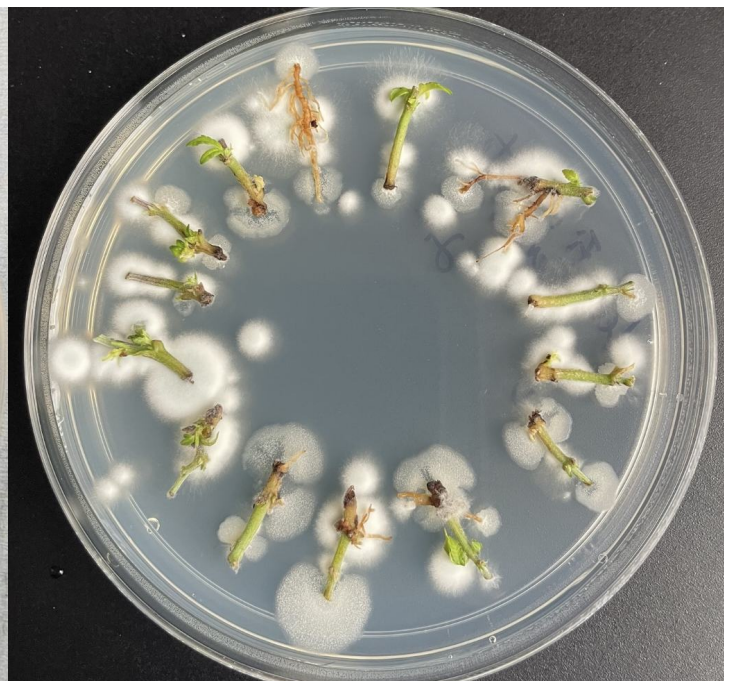
Intake processes are inherently delicate, so it is advisable to have a specialized team in charge of overseeing and executing these activities to maximize the likelihood of successful new intakes. After the initial intake stage, plants are placed in test tubes, and samples are periodically collected and analyzed to verify their absence of pathogens.

Indexing refers to the process of organizing and categorizing information in a systematic manner. The Tissue Culture facilities are at the forefront of the industry, and they also analyze materials to identify prevalent fungal contamination.

It is essential to **identify and remove pests that are present in traditional cannabis growing** in order to ensure the success of later phases of the process.

We employ the technology of successful meristem intakes to effectively eradicate these impurities and ensure the production of plants that are free from pathogens.

In order to verify this, samples of the intake material are collected and meticulously sliced into small, accurate fragments. These fragments are positioned on a Petri dish that is filled with a nourishing agar medium. Following the incubation period, the samples are analyzed to detect the presence of fungal and bacterial growth. Each meristem sample undergoes a meticulous quality assurance screening process.





INDEXING PROCESS [CONTINUED]

Indexing is crucial for identifying endophytic microbes, such as fungus or bacteria, that live inside plant tissues without giving obvious symptoms. This method is necessary to avert possible disease situations. Any materials discovered to be tainted should be promptly eradicated. Through early identification and elimination of these dangers, we may avert possible disease outbreaks and ensure that plants maintain optimal health, growth, and output. This proactive strategy not only ensures the integrity of laboratory processes but also demonstrates a strong commitment to upholding impeccable standards in all areas of operations.

Cultivars undergo various indexing processes that only the most robust and healthy material is selected for further production. Therefore, before entering the production phase, the cultivar will also be subjected to an additional round of virus/viroid testing. **Further testing is necessary because endophytic pollutants of plant material are difficult to detect and eliminate.** Our techniques effectively minimize the presence of contaminants and pathogens in our Gen 0 clones. Furthermore, this stringent series of tests for a cultivar guarantees that the central production laboratory maintains a hygienic environment, devoid of any potential impurities that could be introduced during the intake process.

Laboratory Production parameters are also assessed at this step, and meristem lines that do not perform well are removed. By the conclusion of this step, the plants will have undergone several iterations of both endophyte indexing and, pathogen testing.





CULTIVAR OPTIMIZATION & AGRONOMIC PERFORMANCE

Optimization of methods is necessary for all commercial tissue culture crops, frequently at the cultivar level, where distinct growth characteristics are observed in *in vitro* populations. The objective is to **enhance the quality of tissue culture populations to ensure consistent performance while upholding pathogen-free settings.**

Growers understand that different starting material can respond differently to inputs, especially across different cultivars. Conception adheres to the industry norm by applying two unique tactics, namely handling and media optimization, to guarantee the success of each cultivar. In order to achieve production benchmarks, the optimization process starts by making adjustments to the semi-solid medium mixture. More precisely, the modification of the proportion of nutrients and hormones in accordance with findings from prior studies. A cultivar is subjected to thorough evaluation across a wide range of medium types before being incorporated into production.

Experienced Tissue Culture Technicians receive the meristem initiations in order to ascertain the optimal methods for processing and incorporating them into manufacturing.

It is crucial to conduct **comprehensive observations of plant quality, proliferation factor, and harvest rate for each cultivar**, as they have distinct requirements.

Once the optimal methods for increasing the number of plants have been identified, the population is transferred to the production stage, where the cultivar is replicated.

This multiplication technique has two purposes: **it facilitates the creation and maintenance of a robust stock, and it enables the production of individual cloned plants for commercial purposes.** At this point, the number of plants derived from a single new meristem is sufficiently abundant to satisfy the demands of our customers, filling numerous containers or even hundreds. Regularly performing quality assurance processes upon the plants' arrival at our primary production region is the recommended approach to ensure that cleanliness is maintained. Incorporating the aforementioned indexing method.





RESULTS OF TISSUE CULTURE

By utilizing our certified pest- and disease-free beginning clones, a wise grower can expect notable enhancements in facility hygiene and plant performance throughout the growing cycle. Customers can be confident that Conception does not require the use of any pesticides. The nurseries subject the tissue culture clones to meristem culture and a rigorous validation process, guaranteeing that all materials are verified as being free from diseases.

Traditional nurseries in the horticulture sector have extensively recorded that **the absence of sterility in tissue culture methods might result in elevated losses, greater expenses, and increased work requirements.** This assertion is particularly accurate in the cannabis sector, as highlighted by the National Cannabis Industry Association.

Furthermore, it is widely recognized that tissue culture methods have the ability to increase crop yields. Numerous cannabis growers encounter difficulties in acquiring genetically authentic strains from conventional plant nurseries. Conception Nurseries ensures the delivery of healthy and robust plants to customers by employing genetic and genomic techniques to verify the presence of 100% desired traits.

REFERENCES

Punja Zamir K. , Collyer Danielle , Scott Cameron , Lung Samantha , Holmes Janesse , Sutton Darren (2019). Pathogens and Molds Affecting Production and Quality of Cannabis sativa L. *Frontiers in Plant Science*, 10, <https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2019.01120>

Tomas A. Melgarejo, Li-Fang Chen, Maria R. Rojas, Annemiek Schilder, and Robert L. Gilbertson (2022). Curly Top Disease of Hemp (Cannabis sativa) in California Is Caused by Mild-Type Strains of Beet curly top virus Often in Mixed Infection. *Plant Disease*, 106(12), 3022-3026 <https://doi.org/10.1094/PDIS-04-22-0856-SC>