The Application of Tissue Culture for Grapevine Disease Elimination

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My experience working with tissue culture techniques on grapevine dates from my first job (after my post-doctoral fellowship at UC Berkeley) at Agritope, Inc., a biotechnology company that was based in Oregon. The company owned a grapevine nursery and I was hired to start a disease testing and eradication program for their elite clones and varieties.

More recently, I have produced tissue culture plants from heritage Cabernet Sauvignon clones that were acclimated and planted in California vineyards. This spring (Argentina's fall) I spent over a month working at a biotechnology company ClonAr in Mar del Plata in Buenos Aires province (Argentina). The laboratory is interested primarily in the micropropagation of different plant species such as garlic, kiwi, fruit, ornamentals and vegetable crops. Recently the lab started working with woody plants

Reprinted from The Grapevine Magazine – July-August 2023

such as grapevines and invited me to spend time working and training their staff on grapevine tissue culture and diagnostic techniques.

Plant Tissue Culture

Plant tissue culture technology (growing plants *in vitro*) can be used for the quick propagation of grapevines but also is a technique that is used for the elimination of plant pathogens (disease causing agents). The propagation material may start in a greenhouse using either dormant grapevine cuttings or in the lab using nodal sections (portions of a shoot with at least a bud) from an actively growing plant.

In the laboratory, a process called surface sterilization is used to remove external bacterial or fungal species that can affect plant growth in culture. The cleaning process involves treating the nodal portions of the vine in a detergent and bleach solution.

After this process, the nodal sections are placed in a special media that contains nutrients and hormones that sustains plant growth. These plants are grown in sterile (autoclaved or baked) vessels at ideal light and temperature conditions. This process is known as plant tissue culture initiation. All the work is done under aseptic conditions in a laminar flow hood that keeps the environment clean and free of airborne microorganisms. If the purpose of initiating plants in tissue culture is the elimination of plant pathogens, the meristematic tissue from each of the plants can be isolated.

Plant Meristem Isolation

The plant meristem is a portion of the plant with cells that has not differentiated into specialized tissues (e.g., leaves, stems, roots) and are capable of producing a new plant (the tissue is totipotent). The meristematic tissue is also responsible for keeping the plant growing. The advantage of using the apical (the uppermost meristem dome) is that the vascular tissue has not been differentiated and it is expected that viruses (or other pathogens) are not yet present in an infected plant. By isolating the smallest possible meristematic tissue (generally the meristem dome plus a couple of leaf primordia), and growing it in tissue culture, it is expected that the new plant that is regenerated will be free of pathogens. The meristem tissue culture technique has been used for decades to produce healthy grapevine plants as well as plants from many other species. The smaller the meristem (0.1- 0.3 mm), the higher is the chance of viral elimination. However, these small meristems are more difficult to regenerate. The trick is to isolate many meristems of various sizes (0.1 – 0.5 mm) and test each regenerated plant to determine if the pathogens that were present in the original plant were eliminated. Because of the small size of the meristems, the work must be done using fine dissecting tools with the aid of a dissecting microscope under aseptic conditions. Research in my laboratory has shown that tissue culture plants have a higher virus concentration than plants grown in greenhouse or field. Therefore, the testing of plants to determine their virus status, can start very early in the process, collecting small amounts of tissue during the first transfer to fresh media.

Photos: Meristem extraction with a dissecting microscope under sterile conditions (left); tissue cultured plant in tube with isolated meristem in plate with dissecting tools in the background (right)

Plant Growth and Propagation

Once a clone is obtained (always best to produce more than one) that has tested free of the pathogens of interest, the plant propagation starts. Once the regenerated plants are grown, these must be transferred to fresh media regularly (every 3-4 weeks) to replenish nutrients. With each transfer it is possible to produce 5-6 new micro-propagated plants. At some point the plants will be transferred to the greenhouse to be acclimated. Because there would be a shock for the plants to be moved from the pampered conditions in test tubes, initially the plants must be grown under misting conditions. In the growth chamber or greenhouse, the humidity must be kept very high to allow the plants to acclimate as they were "spoiled" growing in culture media. Up to this point, the plants were grown in a clean environment to ensure that pathogens or environmental contaminants were not present. The plants will continue to grow in the greenhouse up to a certain point and brought to the nursery to further propagate and/or start the grafting process. Because the potential of re-infection, it is important that plants are always protected. This is accomplished by potting the plants in clean soil and growing in a greenhouse or screenhouse with a mesh that offers insect protection.

Reprinted from The Grapevine Magazine – July-August 2023

Virus Detection and Evaluation of Candidate Selections

Many vineyards and wineries are interested in propagating their own heritage selections or clones. In some instances, these clones were introduced hundreds of years ago by families who immigrated from Europe. At that time, quarantine and plant introduction programs were not as reliable as they are presently. Consequently, many of the imported grapevine plant material carried important and deleterious pathogens. In addition, over the years of being planted in the field, even healthy vines may become infected with numerous pathogens due to the spread of pathogens from vineyard to vineyard.

Every project starts with the testing of the plant material the grower wishes to treat. Based on the results, the laboratory can opt to apply meristem tissue culture or other disease elimination techniques. Thermotherapy (heat treatment) and cryotherapy (cold treatment) are other methods that have been described and can be used in combination with meristem tissue culture and micropropagation. But by far, the meristem tissue culture is the most applied method when it comes to disease elimination for grapevines. This is because the meristem tissue culture is a technique widely accepted by growers. Although, thermotherapy is a very old and successful method, some growers do not accept its use as they are suspicious that deleterious mutations can occur in the plant material. This concept has not been scientifically proven though.

Although generally, the meristem tissue culture technique is applied for viral elimination, a bonus result is the eradication of pathogenic bacteria and fungi. Because disease diagnostics have been covered in other articles I wrote, I only summarized here. Basically, the same methods that are used to detect pathogens in the field are used for the detection of viruses (or other pathogens) in tissue culture. The material being tested however, is always actively growing tissue culture plants. It is important to test the plants as soon as there is enough plant material and is generally done during the first transfer to fresh culture media (the best conservative sample are leaves with attached petioles). Because this type of tissue is sensitive to wilting (spoiling) it is important to coordinate testing with the laboratory to assure that the samples arrive in good conditions. The early testing will allow the laboratory to discard the clones that test positive for a pathogen of interest and only propagate those that are not infected.

Conclusions

The use of tissue culture propagation and meristem culture for disease eradication offers an advantage: the nursery will be starting with a clean product (not only virus tested but likely free of bacterial and fungal pathogens), grown under aseptic conditions. To preserve, this health status, it is important that plants are moved into a hermetic greenhouse (or screenhouse with small mesh) with mitigation practices to avoid the entrance of insect vectors and /or pathogens. Finally, the plants must be moved to a screened area in the field (preferably isolated from other grapevine growing vineyards).

There might be concerns from the industry that tissue culture plants could have juvenile traits (take too long or never produce fruit). These are valid concerns and the

issue varies from variety to variety or even among different clones of the same variety. There are however growing techniques that can be applied at the nursery to avoid juvenility traits from happening.

The slogan from the National Clean Plant Network is to “Start clean, stay clean”. It is important to have nursery programs that produce the cleanest (disease tested) material. However, if the plants are grown carelessly, likely these plants will become reinfected. Therefore, my hope is that this article will provide guidelines to allow the planting material to remain clean after the effort and expense of subjecting the material to disease elimination treatments.

Judit Monis, Ph.D. *provides specialized services to help growers, vineyard managers, and nursery personnel avoid the propagation and transmission of disease caused by bacteria, fungi, and viruses in their vineyard blocks. Judit (based in California) is fluent in Spanish and is available to consult in all wine grape growing regions of the word. Please visit* ***juditmonis.com*** *for information or contact* ***juditmonis@yahoo.com*** *to request a consulting session at your vineyard.*

Reprinted from The Grapevine Magazine – July-August 2023