# Virus Elimination from Grape Selections Using Tissue Culture

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Microshoot tip tissue culture is the method of choice to eliminate virus(es) and other pathogens from many plant species. This method has the advantage of regenerating a single plant from a single, minuscule (approximately 0.5 mm) shoot. The technique also avoids the production of plants from callus which can lead to regeneration of an off type plant. The combination of low hormone levels combined with a minimum time in culture reduces the chance of mutation and regeneration of an off-type plant. At the same time, many pathogens, including viruses, are eliminated by this technique. It is thought that this is because the meristem is growing faster than it can be infected by pathogens that may be present in the older plant tissues.

The FPS grape program was founded in the 1950s and is the largest of the FPS commodity programs. At FPS, growth chamber heat therapy was the technique of choice until the late 1980s, but worldwide, tissue culture techniques were being developed and used extensively for grapevines. FPS first began applying this technology to grapes at FPS in 1988 with support from an industry grant. Further work at FPS throughout the 1990s has resulted in improvements in survival and the rate of virus elimination to the extent that this process is now routine and reliable (Golino et al., 2000). Molecular detection techniques for the grapevine viruses have improved, making it possible to screen young plants regenerated from tissue culture, greatly speeding up the virus screening process (Rowhani, 1992).

Rapidly growing shoots in the spring and early summer provide the best tissue for excision. We prefer to use terminal buds because they are larger, easier to excise and more vigorous than axillary buds. Both field and greenhouse grown plants perform well as sources of material. Shoot tips about 2 cm long are harvested and brought to the lab. If material from the field is especially dusty, it is rinsed under running tap water for 1 hour with the addition of a drop of dishwashing liquid every 20 minutes. Tissue is then surface sterilized by submersion in 10% commercial bleach plus 1 drop (~0.1 ml) of dishwashing liquid for 10 minutes. Tissue is removed under aseptic conditions and serially transferred through three rinse containers containing sterile distilled water.

Microshoot tips are excised aseptically in a transfer hood under 10–50X magnification with the aid of a zoom binocular dissecting scope. Individual leaf scales are removed to expose the shoot tip; after each cut, the forceps and scalpel are flame sterilized and cooled to prevent contaminating younger, inner tissues with virus particles from older tissue which might be transferred by the blade. When the meristematic dome becomes visible, a final cut is made just at the base of the last several leaf primordia, and the tip is gently placed on the surface of the initiation medium. If the cut was made at the correct place, the shoot tip will come off easily with a slight touch of the scalpel to the medium surface. It should not be too sticky and the dome should remain turgid and dome-shaped. Microshoot tips are approximately 0.4 to 0.5 mm and include 1 to 3 pairs of leaf primordia.

Freshly cut microshoot tips

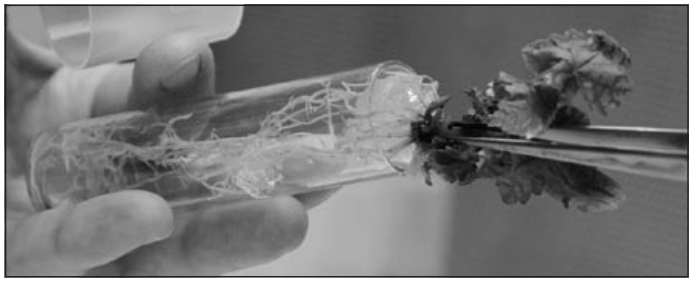
The initial and maintenance medium is Murashige and Skoog (MS) salts and vitamins with 1.0 mg/l of the cy-

| Medium name | BA, mg/l | IAA, mg/l | MS Basal Salts and Vitamins g/l | Sucrose g/l | Uses |
|---|---|---|---|---|---|
| **MSB** | 1.0 | 0 | 4.43 | 30 | Grape initiation and maintenance |
| **MSB-2** | 1.0 | 0 | 2.22 | 30 | Selected varieties, especially certain grape rootstocks including 101-14 Mgt, Schwarzmann, Riperia Gloire |
| **RM** | 0 | 1.0 | 2.22 | 15 | Rooting |

**Table 1.** Tissue culture media used for 0.5 mm shoot tip culture for virus elimination in FPS grape programs. Salts and vitamins are as described by Murashige and Skoog (MS).

tokinin growth hormone 6-benzylaminopurine (BA), 3% sucrose, and 6.0 g/l gum agar adjusted to pH 5.8 (MSB). The rooting medium is half-strength MS salts and vitamins with 1.0 mg/l of the auxin growth hormone indole-3-acetic acid (IAA), 1.5% sucrose, and 6.0 g/l gum agar adjusted to pH 5.8 (RM) (**Table 1**). Murashige and Skoog (MS) salts and vitamins are a standard mixture of specific nutrients developed for plant tissue culture in 1962 by two scientists, T. Murashige at the University of California, Riverside and F. Skoog at the University of Wisconsin. MS salts and vitamins are available premixed from many sources. We use PhytoTechnology Laboratories, Shawnee Mission, Kansas catalog #M519.

Explants are incubated in a growth chamber at 25ºC, 70% relative humidity, 16-hour days, under cool white fluorescent and incandescent bulbs. They are transferred to fresh medium every 3 weeks. When the explants develop a shoot about 2 cm long and 4 to 5 well developed leaves (a minimum of 6 to 8 weeks after excision), they are transferred to rooting medium. When roots are well-developed and the shoot has reached the height of the tube (a minimum of 3 to 9 weeks), the plants are ready to be introduced to soil and greenhouse conditions—a process that takes about 3 weeks. Medium is rinsed off of the roots, roots are trimmed if necessary and plants are transplanted to sterilized potting mix in 2-inch pots. The pots are placed inside a clear plastic Magenta box with the lid on.



Rooted grape plant, being removed from tissue culture medium, is ready to be planted into soil. *Photo by Bev Ferguson*

Over the next two weeks, the plants are gradually acclimatized to ambient humidity by leaving the box lid slightly ajar; then removing it. Finally, the plants are transplanted to 4-inch pots and taken to the greenhouse

Varieties vary tremendously in how well they grow in tissue culture. Usually, more vigorous varieties in the field are also more easily established in tissue culture and less vigorous varieties are more difficult to establish. For this reason, Cabernet Sauvignon is relatively easy to tissue culture and Pinot noir relatively difficult. Many grape rootstocks are difficult to tissue culture. We have found that reducing the MS salts to half-strength will work for some of them; this is usually the first medium variable we try when a selection fails to thrive in tissue culture. Much progress can be made by careful observation and adjustment of specific medium components. For instance, if explants develop vitrified tissue (stiff, distorted leaves), the next time the selection is excised, we would start with a reduced salt medium; if too much callus develops, the BA level would be reduced. There are almost endless variations of medium components that can be tried, and we are continually experimenting based on the plants' response.

Grape transplants (inside Magenta boxes) will be gradually acclimated to ambient humidity. *Photo by Bev Ferguson*

We normally expect that 10 to 30% of the meristem pieces survive tissue culture and become rooted plants. Of those that survive tissue culture, usually 70–100% will test virus negative, depending on the virus type in the source plant. For example, if we cut 100 microshoot tips, we expect anywhere from 7 to 30 of them to grow into healthy plants. Survival, however, is very variable and can be much less or even 0% for certain varieties. The whole process from excision of a <0.5mm shoot tip to a plant in a 4-inch pot takes a minimum of 4 months— and can take a year or longer if the variety is recalcitrant.

**References:**

Golino, D.A., S.T. Sim, J. Bereczky, and A. Rowhani, 2000. The Use of Shoot Tip Culture in Foundation Plant Materials Service Programs. Proc. Int. Plant Propagation Soc. 50:568-573.

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