Tissue Culture Acclimation©

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There are very few articles to date in the literature on tissue-culture acclimation (acclimatization), however, there are a few paragraphs in *Plants from Test Tubes, An Introduction to Micropropagation* (Lydiane, 1983) on "hardening off" and a chapter in Pierik's (1987) *In Vitro Culture of Higher Plants* called "The transfer from nutrient medium to soil".

This year's I.P.P.S. Western Region meeting should go a long way towards rectifying the situation with two pages at the Eastern Region, including one by Deb McCown and three papers from this conference.

The first consideration in this topic is that of economics. The book, *Plants from Test Tubes, An Introduction to Micropropagation*, tells us about costs and labor in the lab, how to analyze costs through crop planning, space scheduling, etc., however, the grower on the receiving end of the tissue-culture labs scheduling has an entirely different set of cost factors to weigh before an analysis of profitability can be performed.

YOUR PLANT LIST

The choice of plants to be grown on has an influence on production costs. The grower must learn to maximize output of healthy liners with the tricks of this new trade. Some of these plants will be propagated by tissue culture always and with others it is just a means to an end where they are bulked up then produced by conventional cuttings. Some cultivars can be produced either by softwood cuttings or tissue culture (TC), but TC plants are consistently easier to root all season long while the softwood cuttings demand perfect timing, attention to detail, and the whole crop could easily be an irretrievable bust. Some species acclimate well from TC but need to be staked as a liner, e.g., *Pyrus communis* "Old Home Farmingdale". They can be propagated as a hardwood cutting as long as the stock plants get just enough but not too many chilling days. Both of these methods of propagation demand expenses that need to be reflected in their cost. Other plants are grown by the TC method because they are difficult to produce economically by bud grafting. New techniques may change this situation but the grower still needs to compare costs before investing in TC acclimation of a hot clone.

FACILITIES

The basic idea is to harden the plant off from the environs of the particular tub used by the lab to a manageable greenhouse situation and to create a functional root system from whatever you find at the bottom end of your explant.

These facilities can be "low tech" — in my first attempt at acclimation the head grower designated some lumber, plastic, pots, and potting soil, in a poly tunnel and insisted that a hose end mist nozzle be employed manually every 15 min. That company didn't get too many 'Autumn Brilliance' liners to sell. Since then I have found that it can be less stressful on the staff and easier on the plants to use more sophisticated tent/mist systems.

Basically you need mist bottles for the time between the plants leaving their shipping container and arrival in their misted tent. The staff does not have to be subjected to the hot, humid conditions the plants need to produce roots, although many growers still do that to their people.

The mist system should be hooked to a timer. The timer will need to be adjusted for weather conditions and the stage of the crop.

GREENHOUSE ENGINEERING

Don't let your builder/pseudo engineer have the last laugh. Double check grades, vent directions, irrigation spacing, and materials with the various manufacturers. Make sure that the water drains somewhere and that your recycling of it and your heat has been designed intelligently.

LABOR/STAFF

The transfer of TC plantlets to rooting media can be difficult. Mechanization can certainly help. A dibbled flat on a belt in front of an average worker is much more efficient than a good worker with a pile of trays, potting media, and a stick to poke the holes. Remember, however, to design a facility according to the basic rules of the engineering bible *Critical Path Analysis of Repetitive Man-Machine System Operation*. That means that the inputs to the production process arrive at their designated points of entry with minimal effort. The worker is not forced to walk back and forth all day to achieve his task and materials and handling is designed to match the potential output. Production cannot exceed the output of the slowest component of the process.

The staff of the facility must be able to keep the equipment in good order, encourage the workers to do a good job safely in the time parameters determined. Richey explains the use of reasonable expectancies or ('REs') in his 1989 article, "Costing Variables in Propagation Techiques". Some type of time study is essential to budget labor requirements for each crop.

The staff must also monitor the mist environments of the different crops according to their stage of growth, weather, etc. This must be performed 7 days a week during the growing season. One hour can make the difference between success and failure if the mist goes off or a shade house lets in too much sunlight. Go ahead and write up all the protocols for different crops, but in the end it is the grower's sixth sense that make the crop marketable.

POTS/MEDIA/WATER

Bench space is expensive, so if there is a way to optimize the rotation from high tech bench space to supposedly less expensive hardening off structures then that will help the bottom line. Plug trays, then, can be a great way to save space, however, not everything that comes out of the Mason jar can handle a 406 tray. The trick is to find the optimum medium, tray size, plug shape, depth, and mist cycle for each individual crop. The problem occurs when medium changes from one batch to another. If a peat manufacturer changes bogs or grade of perlite, it can have devastating effects on a crop that needs good drainage to handle the amount of water the mist cycle produces. Soil porosity is an easy thing to measure. It is as important as the EC meter becomes, once fertilizer is added to the crop later on. It is also a good idea to check the salts in the media (and your water).

SPECIAL EFFECTS

Spun fiber-type, frost-control fabric can have a place, especially for sensitive material that comes as unrooted cuttings. Bench heat can also be useful, but it is rarely essential. Capillary mats on a bench can ease the mist cycle and may really help crops that can't handle a lot of water from above. Lights to extend the photoperiod are not necessary as long as you acclimate everything in midsummer. Carbon dioxide enrichment is mentioned in the literature as is mycorrhizal inoculation for certain in-vitro-produced plants.

PLANTING AIDS

It is nice to have a tray in front of you with a hole the correct diameter dibbled in the middle of each cell. This is considered an extra by the potting machine manufacturers with whom I have dealt. (H.J. Kern of Perrysburg, Ohio at 419-874-2844 makes a full range of dibbles.) Often a plantlet is so small that tweezers can be an essential to the process.

JIFFIES

Jiffy 7's are mentioned in the literature for tissue culture acclimation. Our experience has been with forestry pellets. Some things didn't do terribly well, but there are some propagators using them successfully. Hort pellets, which have a higher pH, may be better for some species. Jacquemonti birch (*Betula utilis* var. *jacquemontii)* loved Jiffy 7's and is normally difficult to acclimate in 406 trays. The resultant peat plugs can be transplanted into larger Jiffies creating an elegant containerless plant. Customer acceptance is the only drawback. People are not used to a product that doesn't arrive in a plastic pot, even though the forestry industry plants billions every year worldwide.

CHEMICALS

There are some excellent new fungicides that can control botrytis and other greenhouse diseases with minute dosages. Zerotol injected into the water has eliminated most foliar disease problems and we supplement this with cultural practices to control any sources of contamination. Insects such as shoreflies, fungus gnats, and whiteflies can all be controlled biologically, but as new media is brought into the greenhouse every week, it is essential to get a regular supply of fresh beneficials in place. Occasional spot sprays of hard chemicals may be necessary if the insect pests flare up, so sticky cards and scouting are important.

Algae spells trouble. Floors should drain, white pipes should be painted, and a good algaecide may need to be injected. If algae and liverworts build up, so will insect pests.

Once liners are outside you will need to instigate biological and chemical controls of your pet pests. In the west this would include root weevils.

ACCLIMATION

On receipt of the shipment from the lab: open the package, check the temperature, and look to see if anything is showing stress or symptoms of overheating. Make permanent nametags for the number of trays you will be planting. The tags that accompany plants from the lab are rarely permanent and you need to be sure that the cultivars are correctly identified for life. Place the package of microplants in refrigerated (not freezer) storage until planted. Some species store longer than others. A few, such as pears, actually benefit from a period of cold storage (to break dormancy).

Keep the cuttings moist during the planting process using a misting bottle of clean water. Some species require the total absence of agar media by washing or cutting. Lightly water in to seal the soil, then place in the tunnel for gradual acclimation.

OUTPLANTING/UPPOTTING

The explants have been rooted and are now in the containers deemed best for field outplanting or potting up. Depending on the location and time of year, these plants may be dormant or carry leaves. They may be plugs, containerized liners or bare root. They should obviously be alive and look healthy or a phone call should alert the supplier immediately of the problem. Check the roots for health and any potential insect/disease problems, then do what any good gardener does—cut some roots off. The size, shape, and stage of the root system determines the severity and style of the root pruning but if you do nothing, in my opinion, you planted it incorrectly. In the time allotted here I do not plan on saying much more than this—leave the main root skeleton (tap and branches) alone, but do trim any circling roots in such a way that there are none that do U-turns in the planting hole.

Tissue-culture liners often have immature bark. Do not plant them too deep or they will rot. Some will need misting more often than others. Obviously outplants in the high desert require different rules than in the valley. Fall planting of TC liners is ideal but they, and the site, must be ready together.

ECONOMICS II

You did your homework, figured out how many, how much, how to, and then the lab delivered 2 months late. The plants didn't size up by October. Do you (A) turn up the heat, lights, and fertilizer, or (B) tell the customer he will need to wait until May 1 for green plants? How do you put a cost analysis to that?

Tissue culture has all the difficulties of the softwood rooting process (and more), yet the plants have the characteristics of seedlings. If the caliper, root-to-shoot ratio, and nutrition aren't just right on planting day, your grower will take a hit. The main difference is that it costs a lot more to produce a TC liner than most seedlings or cuttings, so you need to dot the "Is" and cross the "Ts" at all stages.

Did I mention the word "grade out"? "Cull"? We've already got well over a dollar in the liner and there's going to be a royalty charge on top. They are like gold at the trade show and you want to throw 10% away because they are crooked just above the soil line? That's the difference between a good liner nursery and the rest, but you have to include the estimated grade out in your costs and check grades often. There may be something the lab can do to improve things, perhaps some tweaking of the hormone balance would lower the cull rate in the nursery. Call the lab and describe your observations of specific cultivars.

LITERATURE CITED

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