Plant Tissue Culture: Challenging Micro-Organisms©

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Plant tissue culture is aimed at propagation of plant material; this is done by growing plantlets under sterile conditions. The major problem is to get plant material to grow under sterile conditions. To achieve this stage one has two stumbling blocks; firstly the plant material needs to be surface sterilised to the extent that no contaminants will occur on the culture medium and secondly a culture medium needs to be found that encourages the plant material to grow!

For step one, various agents are used and they all have their advantages and disadvantages. The general principle is that the fungi and bacteria which are on, and sometimes in, the plant tissue are being killed, while the plant material itself stays alive. There are no strict rules for this treatment; it depends on the size and structure of the desired plant material.

Once the material is growing without any visible infecting agents, the propagating of the material can start. This requires finding a culture medium that will encourage side-shoot formation and at a later stage, root-induction. The plant material will be "fed" by supplying macro and micro elements, vitamins and sugars in the culture medium.

The final product from the laboratory will need to be hardened off; which means that the plant material, that we so carefully manipulated to become free of micro-organisms, needs to be introduced to these organisms again, while at the same time it needs to be "taught" to use its own roots and photosynthesis for growth.

INTRODUCTION

This presentation is aimed to give you a better understanding about the process they call plant tissue culture. In principle, the procedure is nothing else than taking cuttings of plant material and by doing so; increasing the amount of plant material. Mainstream tissue culture does not do anything with the product itself; what you put in, will come out.

In tissue culture there are four main problems:

1) To get the plant material clean enough to grow on culture medium
2) To figure out which type of culture medium the crop requires
3) To keep the plant material free of fungi and bacteria during this process
4) To get the plant back from the tube into the soil

Firstly, before anything else, one needs to realise that, in theory, one single plant cell will be able to produce a complete plant structure. Failing to have a plant, or cutting, to respond in the anticipated way is basically our ignorance, not the plant's fault! However, since sometimes we are not able to figure out the plant's requirements, we cannot propagate that plant material.

TO GET THE PLANT MATERIAL CLEAN ENOUGH TO GROW ON CULTURE MEDIUM

The principle of tissue culture is very simple; get plant material, surface sterilise that plant material, propagate and multiply the material, get it to form roots and deliver it to the nursery.

However the first problem at this stage is to figure out what the correct plant tissue for the so-called initiation will be. Basically we are trying to get a growth-point transferred from the plant into a tube. This growth point will simply grow out again, this time under sterile conditions.

Another problem is how to get the product delivered to the laboratory when the complete plant structure cannot be made available, e.g., the material is overseas or a shrub that is planted out in the soil. The packing is essential for the end product. The difference between damp, moist and wet might have a huge impact on the final results. Placing all the cuttings in water and letting the material absorb the liquid (including bacteria!) will often cause all the material to become unsuccessful at initiation.

Lastly a major problem is the time frame. Sending plant material just before a weekend or public holiday will obviously cause a delay. However, many nurseries only "get around" to cutting the material towards the end of the week, expecting the laboratory to simply do it.

The next step is to surface sterilise the plant tissue. There are many options that we have for this procedure. Most commonly used are alcohol, peroxide and chlorine. However, sometimes a household detergent supplies the right combination of chemicals as well. In this process the type of plant tissue determines the concentration and the time period that the tissue is exposed to the solution. One can understand that a woody stem of a tree requires a more harsh treatment than the soft tissue of plants like for example *Agapanthus*. Particularly difficult are the plants where the growth point is underground. This subjects the growth point to a constant infection pressure and makes it is much harder to get it "cleaned up" as often organisms live around the growth point.

Once this procedure is finished the plant tissue is free of any immediate visible infectants, but please note that viruses, fungi and bacteria in low concentrations and the so-called endophytes could still be present in the material.

Viruses could be eliminated through an intensive tissue culture procedure; however this involves lifting the growth point out of the tissue and subsequently culturing it into a plant again. Viral testing must be done to check if the virus has been eliminated. This method is very costly and commercially only applied on high-value crops.

Fungi and bacteria in low concentrations; the tissue might look clean, but over time the build up of the pathogens will cause a visible infection and the cultures are lost.

Endophytes are organisms, mainly bacteria, that happily and harmlessly live in the cells of plant material. Since the do not cause any symptoms, they can only be detected through intensive research. Unfortunately, it has been known that stress sometimes can change these harmless organisms to pathogens. In tissue culture the step from multiplication, using cytokinins as hormones, to root-induction, using auxins as hormones, is such a major stress factor. Often at this stage a whole culture "turns up infected."

TO FIGURE OUT WHICH TYPE OF CULTURE MEDIUM THE CROP REQUIRES

The multiplication of the plant material is the "easiest" step. When we finally ended up with one or two visibly clean shoots, we place these shoots on the culture medium. In our laboratory we stay clear of using callus in any form. Callus, being an undifferentiated mass of cells, can grow back into a complete plant; however there is a significant chance of getting a minor or major change in the plant material through that phase. Hence our preference to avoid callus and use a normal growth point as the start of our cultures.

"Luckily" for the tissue culture laboratory there are many variables that we can work with:

1) The type and concentration of our culture medium including: macro elements, micro elements, vitamins, hormones, sugars, and agars
2) The pH of the culture medium
3) The temperature at which the culture is grown
4) The light intensity and day length of the cultivation light

Having listed all the options again, it makes one wonder how we ever manage to cultivate anything at all! Luckily we do find combinations that work and over time we are able to fine-tune the composition and improve on the appearance of the final product. Although our environment of cultivation is highly controlled, there are so many options that we can forever improve on our methods and our products.

In some instances it takes us quite a while to figure out the requirements of a crop and the speedy production that was anticipated does not materialize. One of the major disappointments for our customers is the realisation that it usually takes 1 to 1 ½ year from initiation to final product.

TO KEEP THE PLANT MATERIAL FREE OF FUNGI AND BACTERIA DURING THIS PROCESS

Once the product is clean and multiplying, the propagation is done by cutting the plant material under sterile conditions. Having a good discipline in the work areas will reduce the chance of infection during this process. Over the years we have found that the operators need to understand the concept of sterility before being able to work adequately. We found that the laminar flow-bench itself provides everything one requires to keep the cultures sterile and in our setting we stay clear of "confusing" items as hairnets and latex gloves.

In our opinion, the staff is not supposed to bring their heads into the flow bench, and we prefer to have that problem sorted out (often by sending the staff member to the optician!) than fighting the symptoms by providing hairnets. Also the use of latex gloves creates the impression that the staff member's hands are sterile, which in fact they are not! All the measurements create a false sense of security, while making the person who needs to wear them quite uncomfortable.

TO GET THE PLANT BACK FROM THE TUBE INTO THE SOIL

The finished product for the laboratory is a small rooted plant that resembles a seedling of that crop. This plant has been "spoiled" and although it has formed roots, our opinion is that these are hormonally induced and not really functioning as roots yet. Also the stomata have not been "taught" how to operate properly as inside the tubes, the plants are in a very high relative humidity. Lastly, the photo-

synthesis process has not yet been operation, supplying the plant with a lot of sugar in the culture medium has made the material "lazy" and quite inactive. Except for growth that is!

Now that the plant is taken out of its sterile and optimal environment, it needs to adjust to the harsh condition outside. This transfer is often a bottle neck in the process. While trying to cope with the reality of feeding itself and regulating their stomata and root system, the plants are also exposed to various fungi and bacteria. As soon as they are touched by human hands, the first "load" of organisms is re-introduced to the plants. Most of them quite harmless, but some might be very harmful. The common damping off fungi; *Fusarium*, *Pythium,* and *Rhizoctonia*, can cause major losses in the cultivation of the tissue culture products.

At this stage it is important that the optimal combination of growth medium, moisture, temperature and light is found and seeing that the plants come from a completely different environment, the requirements for the plant material change rapidly during the first few days out of the flask! In the first few weeks outside the laboratory these "babies" require a lot of attention!

CONCLUSION

In theory it is possible to cultivate all plant species in tissue culture, however it is often not economical to do so. And, of course, sometimes we are still not capable of figuring out the requirements of a product! In overcoming all the obstacles that we described above, we found that experience improves the chance of success.

The process of tissue culture is often described as quick and easy, but although that sometimes is true, there are more problems than solutions and this will keep us entertained for years to come.