A FEW ACADEMIC APPROACHES TO PROBLEMS IN TISSUE CULTURE PROPAGATION

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Commercial tissue culture propagation is not as simple as following a published recipe. The use of tissue culture for cloning ornamentals is expensive and too often the high price is a consequence of one or different misadventures during or following the tissue culture procedure.

The major problems encountered in most tissue culture laboratories are of two kinds: pathological and physiological.

PATHOLOGICAL PROBLEMS

The most common contaminations in cultures are due to fungi, bacteria or mites.

Problems of fungal contaminations are in most cases the less serious, as they can usually be solved by an appropriated sterilization of the startmaterial and/or the preparation of the motherplants under hygienic conditions. Fungal contaminations can however also be caused by mites, and this can be presumed by the at random distribution of dots of fungal mycelium in a limited or large number of containers.

The most severe problem however is bacterial contamination. Still a lot of people believe that all bacterial contaminations in tissue cultures are exogenous in origin and they claim that the contamination is due to unappropriated hygienic techniques. There is however a lot of evidence to prove that endogenous bacteria too are

involved in this problem. After testing a lot of antibacterial products with different modes of action we have the impression that most of this classical products have only a bacteriostatic effect but that none is able to completely eliminate bacterial contaminations. There is an urgent need for new antibiotics and/or for good test media which should allow to detect the presence of bacteria in a very early stage in culture. But this is apparently not an easy task.

PHYSIOLOGICAL PROBLEMS

In vitro propagated plants have very often an abnormal physiological condition as a consequence of the culture in a closed container. Such abnormalities are also manifest when field grown plants are cultured in a closed atmosphere (e.g. under plastic film). The most important factor responsable for this abnormality is probably the saturation of the container atmosphere with water vapour.

In the worst situation those physiological disorders can result in complete loss of the plants, they become necrotic after a phase where the material looked vitrified. The tissues are as it were translucent and apparently gorged with water, they glister. In the early stages of the disorder apparently healthy shoots have at first glance normal looking leaves. But appearances are deceptive, indeed very often those leaves have no wax layer and the stomata are not functional. This is one of the major problems upon transfer from such plants from the culture container to the greenhouse. In other words the final stage *in vitro* and the early stages *in vivo* are crucial.

If healthy shoots with functional leaves are produced at the end of the tissue culture stages a lot of problems are solved in the greenhouse. A lot of apparently minor differences between laboratories can be responsable for the production of good or bad quality plants: e.g. agar brand and concentration, the type of container and the type of closure, the construction of the shelves in the culture room (distance of the lamps, aeration, level in the shelve). Indeed a lot of factors can have a direct or indirect effect on the ecology of the container. All interventions which are susceptible to lower somewhat the relative humidity at leaf-level will improve the quality of the plant.

Another physiological disorder is apex necrosis. Probably there too a major factor controling this phenomenon is the ecology in the container. In an atmosphere saturated with water vapour there is no active transport of the nutritional elements and actually a lot of research workers agree to make a link between apex necrosis and lack of calcium in the apex. The creation of a gradient in vapour pressure deficit stimulates active transport and helps to overcome the problem.

Tissue culture conditions can also be responsable for other types of aberrations. In some cases true-to-type plants are produced but with an aberrant growth pattern. A few typical examples are Gerbera's with too many leaves and not enough flowers and moreover with a

short flower stalk, strawberries producing too much runners and too much small fruits. Rhododendrons which do not elongate and continue to bifurcate... All those symptoms are a consequence of too much subcultures *in vitro* or an unappropriated cytokinin treatment in the propagation stages. This happens most often with N^6-benzyladenine, a strong and cheap cytokinin. In those cases the laboratory is responsable for the misadventures later on under normal culture conditions.

The major cost in plant propagation by tissue culture is manual labour. This is especially pronounced in the latter stages (elongation and rooting) when individual shoots are manipulated. In order to save manual labour, we tried adding liquid media to established, exhausted cultures, instead of transplanting the tissues to a fresh medium. This has been investigated for the elongation and the root induction stages with different plants (herbaceous and woody). The salt concentration and the application time are important factors, as well as the hormones, the presence of charcoal, the light and temperature conditions. The method opens new opportunities for automation of some stages in tissue culture production of plants. Some preliminary experiments with injection of liquid media through the stopper of a penicilline bottle placed in the polypropylene screwlid gave good results.

As soon as good quality shoots are produced *in vitro* there are a lot of possibilities to perform rooting of those shoots under *in vivo* conditions. A root induction treatment can be given *in vitro,* and the induced shoots can be treated as cuttings and placed in a normal or artificial substrate. In many cases the growers favour plants which can be manipulated as units, especially to allow sorting and calibration of the material, because it is an utopy to believe that tissue culture is a guarantee for the production of homogeneous plants.

FURTHER READING

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