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NURSERY UNDERSTANDING OF TISSUE CULTURE

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The type of tissue culture which we are considering may be defined as the development of new plants in an artificial medium under aseptic conditions from very small pieces of plants. Propagation may be accomplished from embryos, seeds, tissues, stems, shoot tips, root tips, callus, single cells or pollen grains (1). For successful propagation, new roots and/or shoots or small embryos must develop in order to produce the new plants. The kind of growth pattern which develops depends upon the genetic potential of the plant cultured and upon the chemical and physical environment to which it is subjected.

We nurserymen are accustomed to using other methods of propagation but should keep our minds open to the the many new future possibilities of this technique. It can accomplish a much more rapid mass production of limited propagating stock, it can recover disease-free plants, and it can show us new ideas and methods which we can apply to our current ways of propagating plants.

There are many fine publications on the general premises and techniques of tissue culture which may be studied further (2). It is helpful to keep in mind a few terms which we are not normally using in the nursery industry, but which will appear when you start working with tissue culture:

IN VITRO: under artificial conditions.

IN VIVO: under natural conditions.

TRANSFER: the process of relocating cultured tissue to a fresh nutrient medium.

PLANT PIECE. The size of the plant tissue used in vitro is usually very small, ranging from the size of the head of a needle to a single cell. The general trend is to use as large a tissue as can be sterilized and kept clean. Every known condition of the tissue which might favor rooting should be closely watched. For instance, juvenility is a factor which usually enhances the chances of success. Juvenility does not mean a soft or tender cutting, but rather tissue like that from germinated seed which has not reached an adult stage.

MEDIUM. To develop a satisfactory technique for growing small pieces of tissues, scientists have adapted some of the methods used for many years in medicine and pathology. A small

piece of the plant tissue is placed in a sterile container with a small amount of a sterile medium to encourage and nourish growth. The various rates of development and growth can then be carefully measured and recorded.

The basic medium used must be sterile. It can be a liquid solution of chemicals diluted with distilled water, or agar can be added to make it solid. Agar is a gelatinous substance derived from certain sea weeds which may be purchased from chemical or medicinal supply houses.

It is important to keep the total volume of the medium small to maintain an effective chemical balance with the leachings from the small piece of tissue. After rooting has been accomplished, both in vitro and in vivo, cuttings usually increase growth by having a greater volume of the medium. Because of the small size of the plant tissue and the small amount of the medium, everything we do to it becomes more critical. The small piece of tissue does not have much storage capacity, so whatever else is needed for growth and division must be supplied by the medium.

The chemicals in the medium can range from a simple NPK formulation to one of 40 or more elements. Minerals, auxins, cytokinins, vitamins, amino acids and sugar may all be needed to form a balance to produce optimum results. Some of the needed undefined ingredients have been found in such things as coconut milk, tomato juice and brewer's yeast. Apparently, many of these ingredients are supplied on the rooting bench in the rooting medium, the water, or are already in the tissue, originating from the stock plant.

The sugar is added as a replacement for photosynthesis. It must be used with careful sanitation practices, as it also will allow a rapid spread of any contamination. The use of small containers in vitro makes it possible to completely isolate an infected tissue without infecting the other cultures.

In vitro, the pH of the media seems to be critical to the point of actually killing the tissue when the correct range is not maintained. If solid agar is used, it is also necessary that the level be kept above the point which liquifies agar. As time goes on, we may find that pH is a more critical factor in rooting plants on the bench than we had realized.

Charcoal added to the medium will cause a reaction which prevents browning of the tissue. We have used charcoal for many years in the bench rooting medium for some of the hard-to-root plants, thinking that we were getting mainly a structural change. However, it now seems that this antioxidant function may have additional value in the bench rooting medium also.

In bench propagation, we usually use only one medium and leave the plant in it until rooted. To accomplish mass production

in vitro, two and three stages are used. The first stage is that of introducing the plant tissue into a sterile growing environment. The second stage is that of causing additional shoot formation or division. And the third stage is the conversion of these shoots to root and the conditioning for transplanting into bench growing conditions.

LIGHT AND TEMPERATURE. The temperature level is close to that used on the open bench, but the light intensity is quite different. More of the lighting in stage I is furnished by supplemental light sources with an intensity of 100 to 200 foot candles. On the open bench, it is not uncommon to reach up to 2,500 foot candles when propagating under mist. Some tissue, like embryos in culture can utilize between 500 and 1000 foot candles. Many growers of tissues in stage II are using light to the top point of tolerance for increased growth and conditioning for the outside.

SUMMARY. We are involved in the chemical reactions in the tissue leading to shoot and division formation as well as root formation; the addition of cytokinins, like benzyladenine, generally increase shoot formation. Auxins like IAA, IBA, NAA, and 2,4-D stimulate root formation. Successful tissue culture requires a greater knowledge of the tissue to be propagated. At least with our present knowledge of in vitro techniques, varietal differences are critical with regard to specific photoperiod, pH, reaction to chemicals, growing peaks, juvenile stages, disease, etc. Each species and cultivar seem to require a custom mixed medium and special handling procedures.

LITERATURE CITED

1. Hartmann, H.T. and D. Kester, 1975. Plant Propagation: Principles and Practices, 3rd ed. Prentice-Hall, Englewood Cliffs, N.J., p. 509-532.
2. Murashige, T. 1974. Plant propagation through tissue cultures. *Annual Review of Plant Physiology*. 25:135-166.