Tall Trees and Test Tubes

Wayne Loescher

A RESEARCH program recently begun at the Arboretum involves growth of plants under unusual conditions (Figures 1 through 5 illustrate some of these conditions). Small pieces of tissue are removed from intact plants and placed in sterile culture flasks and test tubes containing complex mixtures of minerals, sugars, vitamins, and other substances. The intent is to determine the conditions necessary for continued growth of the isolated tissues and also to regulate the direction and extent of that growth. Our objective in doing this here at the Arboretum is to use this and related procedures to develop new techniques for plant propagation and to improve cultivated plants. The procedures being used and the techniques being developed represent applications of a field of plant science called plant cell and tissue culture.

Although plant cell and tissue culture has only in recent years begun to attract support and to show potential in solving problems in plant propagation, the field is not new to botanical research. It was shortly after the turn of this century that the German botanist, Gottlieb Haberlandt, published a paper entitled, "Experiments on the culture of isolated plant cells." In that paper, he described attempts to culture isolated or single vegetative cells from plants in simple mixtures of mineral salts. He felt that the results of such culture experiments should give some interesting insights into the properties of cells, and he also felt that the experiments would provide information about the interrelationships and influences to which cells within multicellular whole organisms are exposed.

Haberlandt's experiments were not entirely successful. Although his isolated cells did survive for a short time and occasionally increase in size, they did not go on to divide or to develop further. Nonetheless, his paper was a milestone in botanical research, for it represented a first attempt that opened the way for development of what is now a useful tool for probing basic biological questions, and for what promises to provide solutions to important horticultural and agricultural problems.

For two decades after Haberlandt's paper appeared, there was little success in plant cell and tissue culture. But in the early twenties, two researchers, one of them a student of Haberlandt, independently succeeded in growing isolated roots for some weeks in mixtures of mineral salts in solution. In the thirties, Philip R. White, an American, in another study of the nutritional requirements of isolated roots, demonstrated that with the use of carbohydrates and vitamins in addition to mineral nutrients, isolated tomato roots could be grown potentially indefinitely. The roots that he started were continued in culture for three decades.

At about the same time, White, in another study, and two Frenchmen, Gautheret and Nobécourt, all independently began studies of the growth requirements of masses of cells isolated from carrot roots. In the late thirties, each developed complex mixtures of nutrients which permitted indefinite growth of the tissues in

culture. The unorganized tissues, or callus as they are sometimes called, were the first true tissue cultures in the strictest sense of that term. Some of these callus cultures are still being maintained in laboratories around the world.

Since these early pioneering studies, a great deal of work has been done on the nutritional requirements of isolated plant tissues. Many more tissues from a wide variety of species have been cultured. Improvements in techniques and composition of the nutrient media—the mixtures of substances on which the tissues grow—have permitted smaller and smaller pieces of plant tissues to be cultured, making it possible, over half a century later, to accomplish what Haberlandt set out to do in 1902, namely, to induce single isolated plant cells to grow and divide. That success was not achieved sooner is not surprising if one considers the state of botanical knowledge in Haberlandt's time. The essentiality of some of the inorganic elements or minerals required for growth of plants was not recognized, nor had studies yet revealed the existence of a large number of organic compounds of various degrees of complexity that are involved in the growth of plant tissues. When these compounds were recognized, and as they became available, researchers were able to test their effects on growth of isolated plant tissues. Many were found to be essential for growth and their use facilitated further progress.

One result was that single cells not only could be induced to grow and divide, but also to regenerate intact plants. Such experiments elegantly confirmed the hypothesis that almost every cell in a plant under proper conditions was totipotent, or capable of regeneration into a whole organism. Such experiments also represented the ultimate in vegetative propagation, with nearly every cell in a desirable plant having the potential to become another intact plant.

Although for some these experiments seemed little more than laboratory curiosities, bordering on science fiction, they were more than that. The experiments illustrated how certain chemicals, plant hormones called auxins and cytokinins, regulated the direction and extent of plant development. Unorganized tissues treated with relatively high concentrations of auxin and low concentrations of cytokinin would develop roots, while those treated with relatively high concentrations of cytokinin and low concentrations of auxin would develop shoots. Intermediate levels of both hormones would result in continued growth of the unorganized tissue. Similar experiments provided insight into how other compounds were involved in the growth of plant tissues.

Modifications of the procedures involved, and use in more applied problems, has resulted in a technique called shoot apex culture. In this procedure, a shoot tip consisting of the tiny mass of dividing cells at the shoot's apex and a few of the very small undeveloped leaves adjacent to the apex, is placed on a complex nutrient medium. Under these conditions, the shoot tip is induced to continue growth and to develop roots. This technique, sometimes incorrectly referred to as meristeming or mericloning, has been used to propagate such economically important plants as strawberry, asparagus, orchid, gladiolus, and carnation. In orchids the technique has revolutionized the marketing of certain rare and valuable clones. For example, shoot apex culture of a single orchid plant, and subsequent monthly subdivisions of the resulting tissue into four new plants, can give rise at the end of a year to well over a million progeny, each one identical to the original plant. Conventional propagation would yield only two or three plants.

There is considerable interest in adapting these and related procedures to more

plant species. Woody plants, including many ornamentals and most economically important forest trees, often cannot be propagated from cuttings; outstanding specimens must be propagated from seeds, and there is no guarantee that seed-propagated progeny will have all the desirable characteristics of their parents. Also, some species before they begin producing seeds may be 30 to 50 years old, a long time to wait for a plant breeder or forester who has only one lifetime to make significant progress in the improvement of a species.

Some problems remain, however, before these techniques can become routine. Often each plant species and sometimes each variety within a species has to have specific requirements met before it may grow in tissue culture. Unfortunately, progress in this field has not yet reached the point where one can predict with certainty how a particular plant will respond. Thus, it is not only necessary to attempt to adapt existing techniques to new species, but also to investigate the very basic problems of how a plant grows and develops. If the exact conditions were known that cause undifferentiated tissues to become transformed into highly organized tissues like roots, shoots, and leaves, one could utilize this information in directing the differentiation of tissues taken from whatever desirable plants that one might wish to propagate.

The future potential of tissue culture in plant propagation may not be nearly as important as its potential in plant breeding. The basic techniques in plant breeding have changed very little in the past 40 years. In that time, however, those techniques have permitted tremendous increases in the yields of many economically important plants. With the use of hybrids, yields of many crop plants have more than doubled. Unfortunately, such rates of increase are slowing, and many say that we are approaching what is called a yield plateau. For example, when hybrids first came onto the agricultural scene, potential yields of some crops increased at the rate of ten to twenty percent every few years. Nowadays, yields of those same crops are increasing, at most, at the rate of one to two percent annually, or somewhat less than the rate of increase of the world's population. Plant breeders are now at the point where their major efforts are concerned with modifying existing varieties in response to better and better adapted pests. The result is not an increase in yield, but instead an attempt to maintain current yields with the use of new and resistant crop varieties as the old varieties become susceptible to new strains of fungi and insects.

Plant breeders thus feel the need for an infusion of new techniques and knowledge. Bypassing the traditional methods and utilizing plant cell and tissue culture may be the answer. Plant tissue culture has already been of some value. For a number of years, when some potentially valuable plant hybrids were difficult to obtain due to incompatibility between the prospective parental stocks, removal and culture of the developing hybrid embryo as a kind of test tube offspring permitted obtaining a viable hybrid.

More recently, techniques have been developed which allow not just cultivation of single cells, but the isolation and culture of naked protoplasts, plant cells from which the cell wall has been dissolved by enzymes. Such naked protoplasts can reform new cell walls and go on to make entire plants. They can also be induced to fuse or to engulf particles like viruses(chloroplasts, and DNA strands. The implications are immense. Fusion of protoplasts from different plant species, thus bypassing the normal sexual process and all its incompatibility barriers, could result in new and valuable hybrids. This is not as far-fetched as it

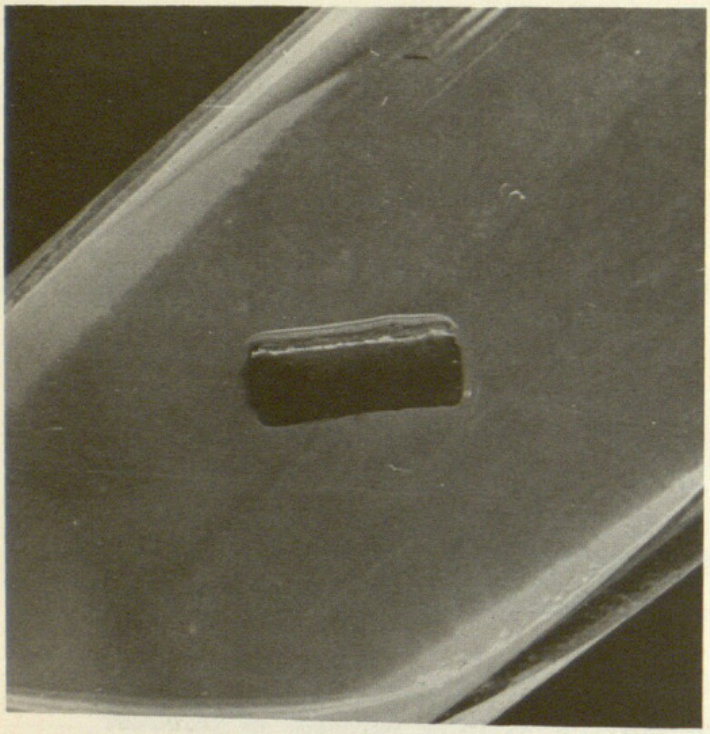

Figure 1. *A small piece of tissue, in this case a section of twig, is treated to remove bacteria and fungi, and then placed in a test tube with a nutrient gel containing the minerals essential for growth, a carbohydrate for an energy source, one or more plant growth hormones, and a number of cofactors, i.e., vitamins and other complex substances.*

Figure 2. *If the right minerals, carbohydrates, growth substances, and cofactors are present, and if they are present in the right amounts, cells in the tissue will divide and grow forming a callus.*

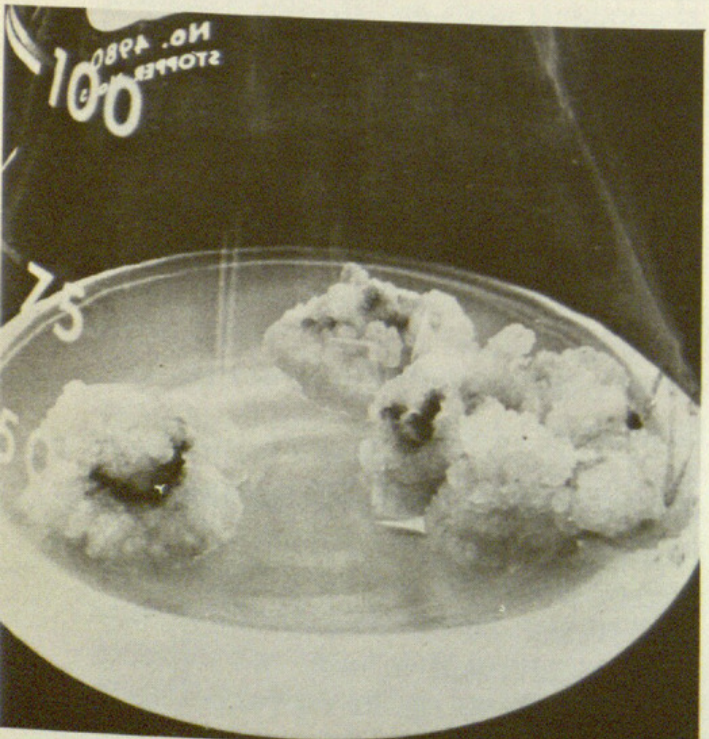

Figure 3. *The callus can then be subcultured, i.e., transferred to fresh mixtures of nutrients which will stimulate further growth.*

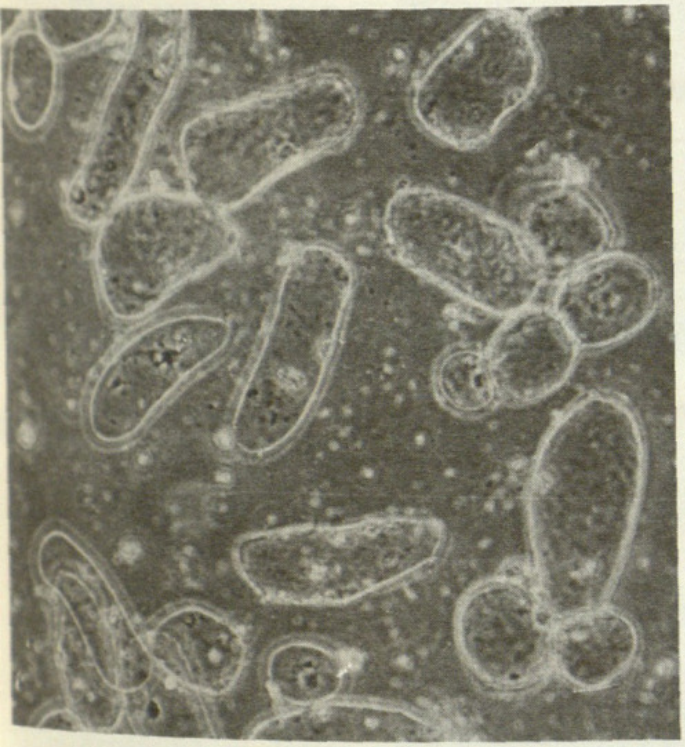

Figure 4. *Adjustment of the kinds and amounts of nutrients added will determine how the plant grows. In this case the tissue has been induced to grow as single cells.*

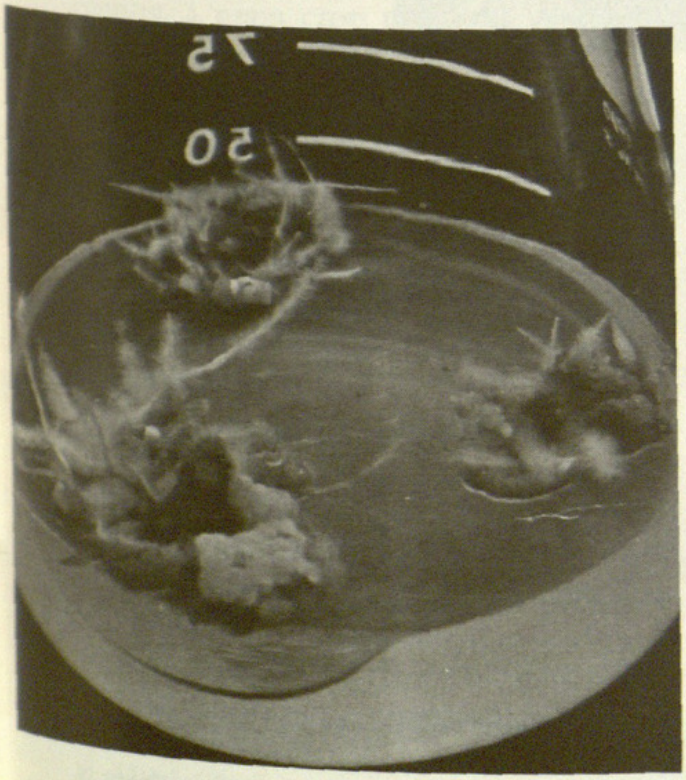

Figure 5. *Other combinations of nutrients may result in initiation of organized tissues like shoots or, as in this case, roots.*

Figure 6. *The intent is to obtain an intact plant with the leaves and roots that can be transplanted to soil to develop normally.*

may seem; it has already been achieved between two different species of tobacco.

Instead of transferring all the information from one species to another and creating a new hybrid, it may become possible to improve existing species by selective transfer of only part of the genetic information of another species. Plant physiologists now know that some plants are photosynthetically much more efficient than others. Among the efficient plants are corn and crabgrass; inefficient plants include wheat and soybeans. Since naked protoplasts have the capacity to engulf chloroplasts and mitochrondria, some have suggested replacing the photosynthetic apparatus of inefficient species with that from more efficient species. Others have suggested that instead of transferring the entire photosynthetic machinery it may only be necessary to transfer strands of DNA. This genetic information would allow the recipient cells to synthesize for themselves the more efficient forms of cellular machinery or enzymes. There has been a step in this direction. Tomato tissues, for example, cannot utilize the sugars galactose and lactose. But if strands of DNA are isolated from a bacterium that can utilize these sugars, and if the strands of DNA are then incorporated into the tomato cells, the cells develop the ability to grow using the two sugars.

Although we have not as yet been able to transfer functional strands of DNA from one higher plant to another, there is considerable impetus to do this. One application could involve the major food crops: corn, wheat, rice, and sorghum.