Biotechnology at the Arnold Arboretum

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Biology has made enormous progress during the last 30 years in understanding the chemical reactions that characterize living things. This burst of scientific discovery largely has resulted from two fundamental findings: (1) the identification of deoxyribonucleic acid (DNA) as the genetic material and (2) the elucidation of its detailed molecular structure. Because of these discoveries and the perfection of powerful chemical techniques for altering DNA molecules, biology has reached a stage at which it is now theoretically possible to manipulate the genetic makeup of organisms in specific ways. The term *biotechnology* is used to describe practical applications of this capability in medicine, agriculture, and forestry.

Although most discussions of biotechnology focus on the essential role of DNA biochemistry, biotechnology actually requires input from virtually every field of biology. The full realization of the potential of plant biotechnology, for example, will undoubtedly depend on a multidisciplinary effort, combining knowledge from biochemistry, physiology, morphology, anatomy, genetics, ecology, and systematics.

With the conversion of facilities at the Dana Greenhouses into scientific laboratories, the Arnold Arboretum has begun a new program of research in the use of tissue culture to gain further knowledge of the physiology of woody plants. The program is also expected to provide valuable information pertinent to the development of biotechnology for woody species.

Tissue Culture

Tissue culture involves the control of development in isolated parts of an organism placed under defined conditions of nutrient supply and physical environment. It was first employed as a basic research tool to identify chemicals that normally nourish and regulate development in plants. The ultimate objective of our research is to obtain a better understanding of plant hormones (phytohormones) and other factors that control plant growth generally.

As far as propagation is concerned, tissue culture is a relatively new technique, having been used extensively only during the last 15 years. To date, it is estimated that tissue-culture methods have been devised for over 300 plant species, although the technique is used commercially for only about 30 species. That is not to say that the impact of tissue culture has been minor. As a matter of fact, tissue culture is already an extremely valuable process for propagating plants with superior characteristics rapidly and for producing plants (via meristem-culturing) that are free of virus and fungal infections. Undoubtedly, these applications will continue to be important to agriculture and forestry in the future. Indeed, if the true potential of tissue culture in combination with DNA bio-

chemistry can be realized, tissue culture may very well lead to revolutionary advances in applied plant biology.

Our program of tissue-culture research involves comparative studies of woody species in about 35 different families that represent a considerable degree of the diversity in the plant kingdom. The table below summarizes the families, orders, and superorders under study. Experimentally, we will investigate several of the physiological characteristics of these woody plants in tissue cultures. In this manner, we will be able to assess each species for its suitability for tissue-culture propagation and to study the factors that regulate growth and development in plants generally.

The families, orders, and superorders of plants under study at the Arboretum to determine their suitability for propagation by tissue culture.

Superorder Magnoliidae
- Order Magnoliales
  - Family Magnoliaceae (Magnolia)
  - Family Annonaceae (Annona)
- Order Laurales
  - Family Calycanthaceae (Calycanthus)
  - Family Lauraceae (Laurel)
- Order Ranunculales
  - Family Ranunculaceae (Buttercup)

Superorder Caryophyllidae
- Order Polygonales
  - Family Polygonaceae (Buckwheat)

Superorder Dilleniidae
- Order Theales
  - Family Theaceae (Tea)
  - Family Guttiferae (Garcinia)
- Order Malvales
  - Family Tiliaceae (Linden)
  - Family Sterculiaceae (Sterculia)
- Order Urticales
  - Family Ulmaceae (Elm)
  - Family Moraceae (Mulberry)
- Order Ericales
  - Family Actinidiaceae (Actinidia)
  - Family Ericaceae (Heath)
  - Family Clethraceae (White Alder)

Superorder Rosidae
- Order Rosales
  - Family Rosaceae (Rose)
- Order Fabales
  - Family Leguminosae (Pea)
- Order Cornales
  - Family Cornaceae (Dogwood)
- Order Myrtales
  - Family Melastomataceae (Melastoma)
- Order Proteales
  - Family Proteaceae (Protea)
- Order Euphorbiales
  - Family Euphorbiaceae (Spurge)
- Order Sapindales
  - Family Aceraceae (Maple)
  - Family Staphylaceae (Bladdernut)
  - Family Sapindaceae (Soapberry)
  - Family Anacardiaceae (Cashew)
  - Family Rutaceae (Rue)
  - Family Meliaceae (Meliaceae)

Superorder Asteridae
- Order Gentiales
  - Family Oleaceae (Olive)
- Order Polemoniales
  - Family Boraginaceae (Borage)
- Order Lamiales
  - Family Verbenaceae (Vervain)
- Order Scrophulariales
  - Family Bignoniaceae (Bignonia)
- Order Rubiales
  - Family Rubiaceae (Madder)

The Importance of Comparative Physiology

Understandably, most current efforts worldwide to propagate woody plants in tissue cultures have concentrated on economically important plants, primarily in two families, the Rosaceae (which includes the roses, apples, and blackberries) and the Ericaceae (which includes the rhododendrons and mountain laurels). As a result, only a limited number of systematic groupings have been studied. It is because of this

that we believe that the Arboretum can play a unique role in physiological research by conducting fundamental comparative studies on a broad range of woody species. This research will increase knowledge of growth regulation in plants and will also result in new technology for propagation, conservation, and improvement of these species.

From the perspective of basic plant physiology, comparative studies are particularly important now. Although much is known about the metabolism and physiological effects of the five major classes of phytohormones, most of the research on these subjects is based on experiments with just a few types of plants. The obvious question is whether concepts based on a limited number of species can be extrapolated to all plants. For example, our understanding of the apical-dominance phenomenon (the tendency of a single shoot to inhibit the growth of others) is based on extensive research with beans, peas, and tobacco. Comparative studies in tissue cultures will determine whether the same controls are operating in other species. Another subject of interest is cellular proliferation and the factors in plants that regulate it.

Studies on comparative physiology will also broaden the understanding of growth regulation and its evolution. It is already evident that plants vary in the ways they control their growth, and this variability can be documented and characterized through comparative physiology. This is an essential first step in understanding the evolution of growth regulation in plants.

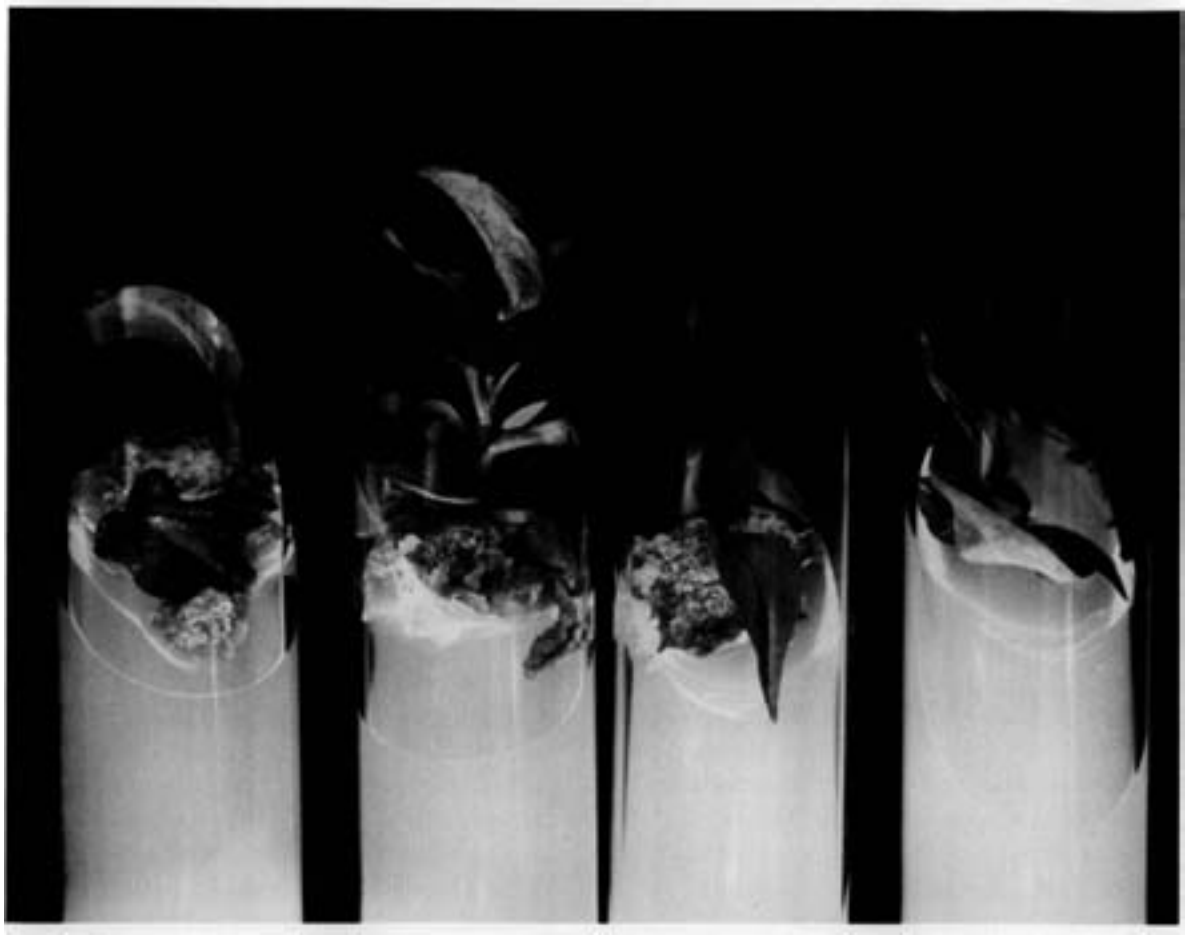
Professor G. L. Stebbins, an evolutionary biologist with the University of California, has pointed out that different characteristics in plants evolve at different rates and that characteristics associated with essential aspects of plant life are the most slowly evolving. Because growth regulation is central to plant development, one would expect that it would evolve slowly. One would also expect that it would distinguish large systematic groupings rather than individual species within a genus. In this sense, comparative physiology potentially could become a complement to systematics, especially in addressing questions of the relationships of families and orders to each other.

The practical implications of comparative physiology, of course, are also significant. In a general sense these studies will define a framework of knowledge that relates propagation technology to systematic botany. Therefore, it will help to make propagation by means of tissue culture a predictable rather than a hit-or-miss procedure. Beyond this, the research will lead directly to new technology as illustrated by the examples in this article. It could also result in the identification of germ-plasm resources within important plant groups that could be valuable to biotechnology. In the family Leguminosae, for example, if species that are particularly amenable can be identified, then the characteristics that render them amenable potentially could be transferred by plant breeding into soybeans or other important legumes. For agricultural technology, the implications of this research are far-reaching.

Botanical Interpretation of Growth and Development

Knowledge of some botanical terms and principles is necessary in order to understand tissue-culture propagation.

The term *monopodial* refers to the condi-



Lilacs (*Syringa vulagaris* × *hyacinthiflora* 'Excel') being propagated in four different tissue-culture mediums. The medium in the second test tube from the left contains a high concentration of cytokinin, a hormone that is found in all plants and stimulates the most vigorous growth.

tion in which a single growing tip produces an unbranched stem from year to year. Extreme examples of monopodial growth can be seen in several palms in which a single stem constitutes the entire above-ground part of the plant. The contrasting condition is *sympodial* growth, which involves stem growth resulting from different growing tips. Sympodial growth is exhibited by elms, in which the terminal shoot tips abort from branches at the end of each growing season. As a result, shoot growth during the subsequent year always begins from a lateral bud. The sympodial growth condition is also evident in many tropical tree species in which both main stems and branches from lateral buds grow simultaneously.

Growth of lateral branches concurrent with growth of the main stem is also referred to as an example of *weak apical dominance* in contrast to *strong apical dominance*, a

condition in which a growing shoot tip effectively inhibits growth of lateral buds in the axils of leaves below it. Physiologically, apical dominance is believed to involve two phytohormones: *auxin*, produced in the growing tip and transported to lateral buds, where it inhibits growth; and *cytokinin*, which stimulates shoot growth. According to the major scientific hypothesis on apical dominance, the relative levels of auxin and cytokinin in lateral buds determine whether the buds will or will not grow out. If auxin is in excess, the lateral buds will not grow, and a monopodial shoot will emerge. On the other hand, if cytokinin is predominant the lateral buds will grow, and a sympodial shoot will emerge. (An article by Michael Donoghue in *Arnoldia* [January/February 1981, volume 41, number 1] contains further information on terminology.)

Multiplying Plants in Tissue Culture

The most common procedure for multiplying woody plants in tissue culture is to add a high concentration of cytokinin to a complex nutrient medium. This environment stimulates shoot growth and overcomes apical dominance. The sympodial shoots that result are then subdivided into individual branches, and these are either recultured on the same medium, to increase the number of shoots, or treated with auxin to stimulate rooting. Rooted plants can then be transplanted to soil.

If the objective is rapid, clonal propagation, each branch can be excised and subcultured on the same medium. For example, at a multiplication rate of five shoots produced from one every month, this procedure theoretically would generate well over a million plants within nine months.

At the Arboretum rapidly expanding shoots of *Syringa vulgaris* × *hyacinthiflora* 'Excel' are taken from the plants in spring and disinfested with detergent and bleach. When these shoot tips are transferred to a medium with the cytokinin thidiazuron, within six weeks monopodial shoots develop and inhibit lateral buds at three to five nodes. A surprising characteristic of *Syringa*, which is shared by other genera in the Oleaceae, such as *Ligustrum* and *Forsythia*, is that shoots in tissue culture exhibit strong apical dominance that cannot be overcome by cytokinin. Shoot multiplication with these plants therefore requires a different strategy.

The procedure we devised is as follows: we cut each monopodial shoot into sections consisting of a node with two lateral buds and a piece of stem. We then culture individual sections on the cytokinin medium, where they each, in turn, produce a monopodial shoot that also can be separated into sections for the next tissue-culture passage. This procedure, when used repeatedly, can produce a million shoots from a single bud within one year. These can be treated with indole butyric acid and rooted in vermiculite.

A third method of tissue-culture multiplication is used with an uncommon amaryllis (*Hippeastrum striatum* 'Fulgidum'). This plant (see photo on page 33), a native of the tropical rain forests of Brazil, exhibits several characteristics that make it an excellent house plant. It blooms at least twice a year, producing many umbels of showy orange flowers. (Most commercially available *Hippeastrum* cultivars produce only one umbel with four flowers.) Its evergreen foliage re-

mains vigorous and healthy throughout the year. Tolerant of low light and low humidity, the plant requires little care.

In using tissue culture to multiply this desirable *Hippeastrum* clonally, we adapted methods that had been used successfully for *Narcissus*, a member of the same family as the *Hippeastrum*, the Amaryllidaceae. We first cut the bulb of the plant into sections, each containing a piece of stem and the bases of at least two leaves. (A bulb is a compact shoot system with a short stem and several scalelike leaves.) Next each section is placed with its stem side down on a medium supplemented with powdered charcoal but lacking phytohormone. The charcoal apparently absorbs chemicals produced in response to the wound made in cutting the bulb. After about four weeks of incubation in the dark, each section forms a new bulblet in the axil of the two leaves. At this stage the bulblets are removed and cut longitudinally into two equal parts, each containing a piece of stem and at least two leaves. Within another four weeks each of these explants in turn will regenerate a new bulblet, which also can be cut in two and recultured as often as needed. Depending on the number of bulbs required, the tissue-culture method can be scaled up. We estimate, for example, that 1,000 *Hippeastrum* plants can be produced from a single bulb in six months.

Goals and Prospects

In all probability the variation among strategies of growth regulation in plants is a product of evolution just as any other plant characteristic is. Our rationale is that the mechanisms of growth regulation can be characterized, and their evolution can be described, by a systematic, comparative study of physiological expression in tissue cultures. We believe that over time this experimental approach will improve the understanding of developmental regulation in plants generally and will also point the way for new methods in biotechnology.

A second and equally important aspect of our research at the Arboretum is the direct analysis of the physiology and biochemistry of two phytohormones, cytokinin and ethylene, which are crucial to tissue-culture manipulations. By obtaining a better understanding of these substances, we hope to gain further knowledge of comparative physiology.

At present rapid progress also is being made in several areas related to plant biotechnology. In the last few years, for example, two completely new methods for hybridizing plants have been discovered. The first of these involves *protoplast fusion*, a process in which cells from two different plants are treated with enzymes to dissolve their cell walls, and the protoplasts then are mixed together under special conditions that stimulate them to fuse and produce a hybrid cell. Once this has been accomplished, tissue-culture techniques are used to produce a whole plant from that cell. Protoplast fusion was first achieved with species of tobacco, but it has since been used with potato and tomato plants and two species in the Brassicaceae (cabbages).

A second technique for genetically altering plants is one of the most elegant procedures in DNA biochemistry, involving the injection of bacterial DNA into plant cells. In the most sophisticated versions of this technique, a gene conferring resistance to a poison is isolated from bacteria and introduced via a bacterium into protoplasts from

The evergreen amaryllis (*Hippeastrum striatum* 'Fulgidum') produces many flowers, in contrast to the usual four of most commercially available amaryllis cultivars. By means of tissue-culture techniques, 1,000 of these plants could be produced from a single bulb in only six months. Peter del Tredici photo

cells of petunia, tobacco, and carrot plants. Tissue-culture techniques are then used to produce poison-resistant plants.

The most desirable woody species for genetic modification at this time are those that permit the regeneration of whole individuals from single hybrid cells. Although few species are amenable to this type of manipulation at present, it is expected that more will become so as the factors that regulate development become better understood. In our research on woody species from the Arboretum's collection, we are especially interested in identifying such species. Not only are these of interest from the point of view of comparative physiology, but they also may represent valuable genetic resources for the biotechnology of plants. With the advent of biotechnology, the consolidating work of the Arnold Arboretum in botany and horticulture will have a profound impact.

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