top is cut off just above the bud. We do this by hand with loppers. The tops are ground up and distributed in the field as a bit of organic matter added to the soil.

As the new plant starts to grow, the whole field is "mowed" about 5-inches high to "pinch" the plants back and encourage branching. This procedure is repeated 2 or 3 times early in the growing season.

During the growing season the field is cultivated repeatedly, weeded, fertilized in early April and early June, then sprayed with a fungicide weekly.

When the plants go dormant in the late fall, budwood is cut, and the plants are dug, graded, tied in bundles, labelled, treated with a fungicide, and placed in cold storage at 34°F. The plants must be watered daily in cold storage and are marketed either as bareroot plants or as processed or potted plants.

The growing of field roses is a lengthy and costly process involving a tremendous amount of time-consuming manual labor. Some of the processors have worked out technology for processing the roses for marketing in an economical manner, but there is a real need for economic improvements in producing the plant itself.

WOODY TISSUE CULTURE RESEARCH

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Plant tissue culture is a term used to describe *in vitro* plant propagation in a nutrient medium. Tissue culture uses the totipotency capability of plant cells to differentiate, develop, and grow into a plant (plantlet) from excised plant tissues (explants) (Figure 1). The first requirement for successful tissue culture is obtaining aseptic or sterile condition of the explants, laboratory, and medium to produce clean uncontaminated cultures. This is referred to in the literature as Stage I or "Establishment of Cultures". The type of explants (leaves, roots, shoots, etc.), the conditions of growth of the stock plants (indoors, outdoors, healthy), and the chemical used (sodium or calcium hypochlorite, benzalkonium chloride, ethanol) as sterilizing agent with the interaction of concentration-time of treatment, have a direct effect on the success of the establishing stage.

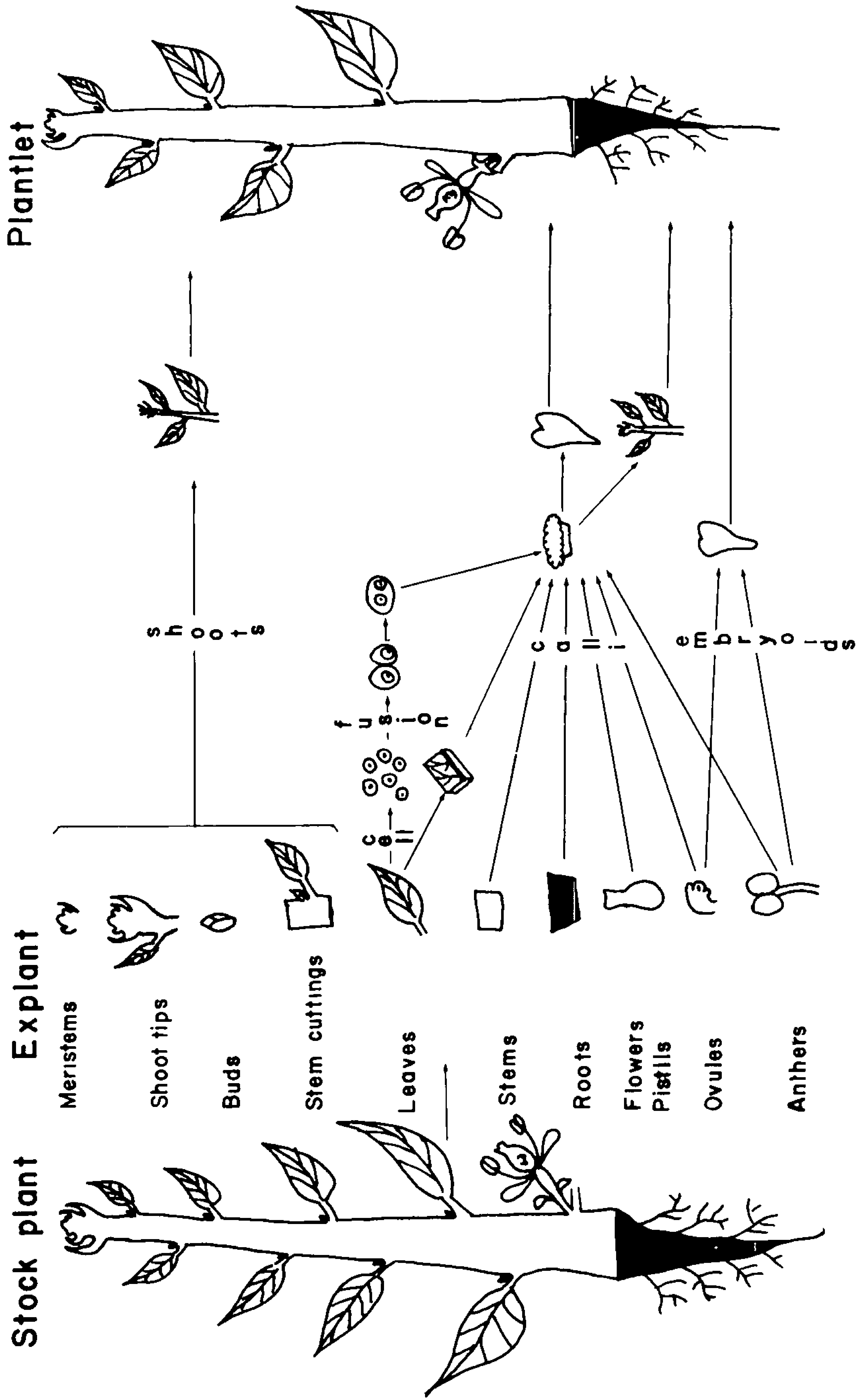


Figure 1. Diagram representing plant tissue culture

The second requirement is the type of medium to be used for culturing explants. Main components of a medium are inorganic and organic substances (Table 1, 2). Shoot, root, callus, embryoid, or plantlet differentiation or development is determined by the absence, presence, or balance of growth regulators. Mainly, auxins (NAA, IBA IAA) are used for rooting and cytokinins (kinetin, 2iP, BA) are used for shoot differentiation and development. However, a balance of these two types of growth regulators is needed for good root and shoot initiation and development. Once plantlets are developed, a last transfer to a medium without growth regulators will permit further growth of roots and shoots. The last step in any tissue culture procedure is the acclimatization of plantlets from the test tube to greenhouse and field conditions. This process requires a smooth transition which generally involves the initial use of polyethylene covers or mist systems to maintain high humidity to avoid desiccation. This humidity should be decreased with time to ambient humidity.

Table 1. Inorganic constituents of Murashige and Skoog (MS) medium and Woody Plant Medium (WPM)

Chemical	MS Murashige & Skoog) (mg/ℓ)	WPM (McCown & Lloyd) (mg/ℓ)
KNO_3	1900	—
NH_4NO_3	1650	400
$Ca(NO_3)_2$ $4H_2O$	——	556
K_2SO_4	——	990
$MgSO_4$ $7H_2O$	370	370
$MnSO_4$ H_2O	17	22 3
$ZnSO_4$ $7H_2O$	8 6	8 6
$CuSO_4$ $5H_2O$	0 25	0.25
$FeSO_4$ $7H_2O$	27 8	27 8
Na EDTA	37 3	37 3
CaCl $2H_2O$	440	96
KH_2PO_4	170	170
H_3BO_3	6.2	6 2
$NaMoO_4$ $2H_2O$	0 25	0 25

Table 2. Organic constituents of Murashige and Skoog (MS) medium and Woody Plant Medium (WPM)

Chemical	MS Murashige & Skoog) (mg/ℓ)	WPM (McCown & Lloyd) (mg/ℓ)
Thiamine HCl	1 0	1.0
Nicotinic acid	0 5	0 5
Pyridoxine HCl	0 5	0 5
Glycine	——	2.0
i-inositol	100 0	100 0
Adenine sulfate	10 0	——
Sucrose	30-50 g/ℓ	20 g/ℓ

These basic procedures make tissue culture a tool or aid to study and solve problems in propagation and basic research. Tissue culture is useful in clonal or asexual propagation, since in a relatively small growth area a high number of uniform plantlets can be produced. At present there are several laboratories, located mainly in Florida and California, which clonally propagate plants through tissue culture. Such facilities are provided with growth chambers, hoods, autoclaves, laboratory space and dishwashing areas. The larger laboratory facilities have to be operated 7 days a week and 24 hours a day in 3 shifts to justify the investment and expense. In sexual reproduction starting from the culture of anthers, microspores, or young pollen grains, tissue culture aids in the production of fully homozygous plants and in allowing recessive genes to be expressed. Contrasting, genetic variability can be obtained with protoplast fusion or *in vitro* fertilization Tissue culture of selected genotypes or mutants for genetic engineering has been the factor for the latest proliferation of tissue culture-breeding laboratories. Ideally the engineering, selection, and propagation of plants would start in the laboratory followed by a field test before increasing the "seed" for the grower. Presently the fact that embryoids (embryos developed *in vitro* without fertilization) can develop from diploid callus gives an exciting new vision of clonal propagation by asexual seed.

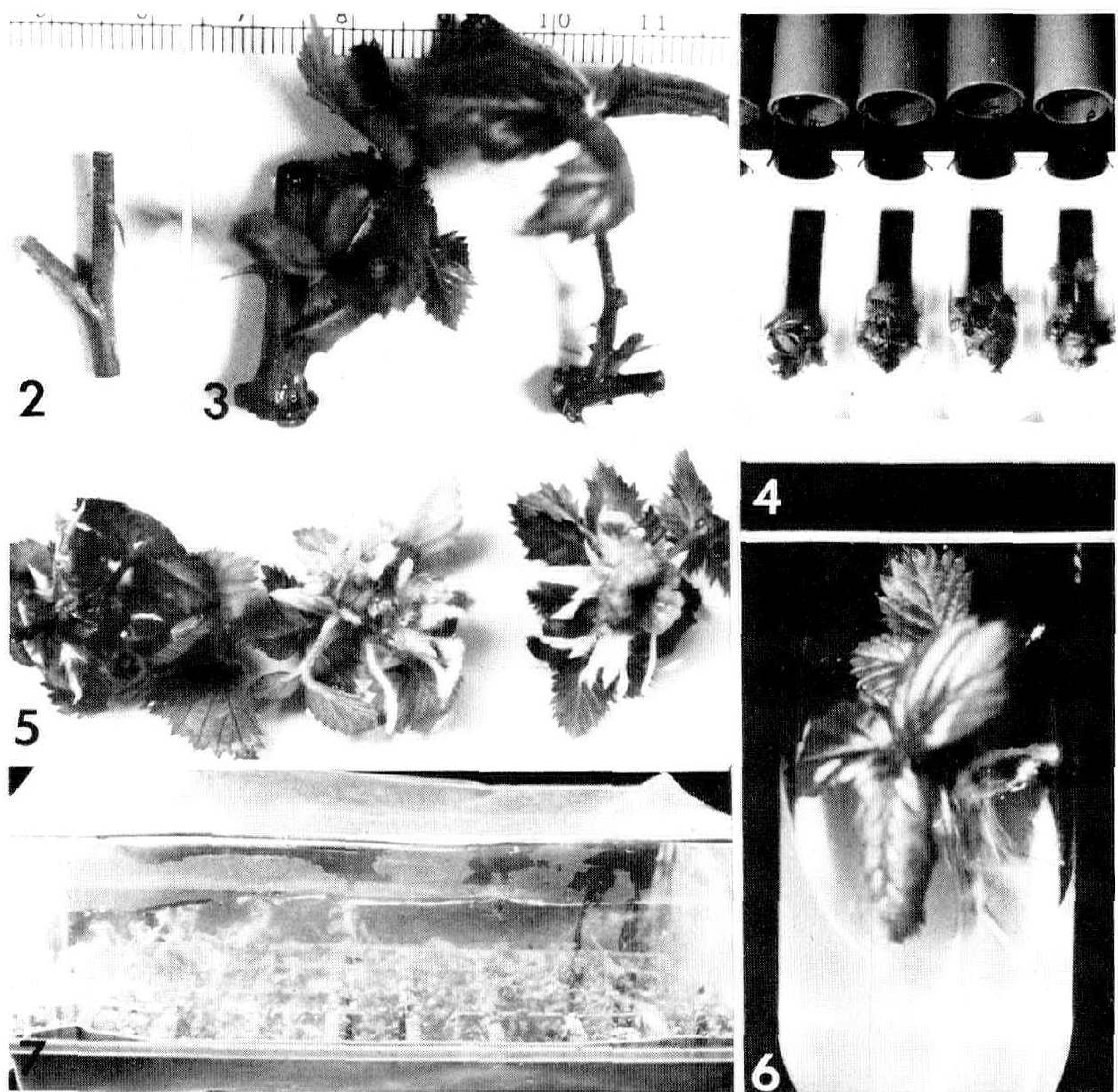
Contrasting with the large amount of information on herbaceous plant and greenhouse plant tissue culture, woody plant tissue culture has been progressing very slowly. The main problems encountered are common on plants of many species, and very often explants are collected from field-grown plants. The endogenous physiological condition of the plant changes according to season, and the juvenility or maturity of the plant will affect the proliferation of shoots and roots. Progress has been obtained by:

1) Growing stock plants indoors and maintaining healthy plants.
2) Using seedlings as stock plants; they are cleaner and respond more readily to culture.
3) Using hypocotyls or embryonic tissue.
4) Changing medium rapidly to avoid accumulation of any inhibitors leaching into the medium.
5) Reculturing already established plantlets.

The current main use of tissue culture for woody plants is in clonal propagation of new cultivars, selections, or rootstocks, and the production of disease-free plants.

THORNY BLACKBERRY TISSUE CULTURE

Stem cuttings were used for clonally propagating thorny



Figures 2 to 7. Thorny blackberry tissue culture. **Fig. 2,** stem cuttings; **Fig. 3,** *left* lateral bud; *right* lateral shoot development; **Fig. 4,** lateral shoots in rooting medium; **Fig. 5,** IBA rooting treatment: a. *left* 1 mg/l, b. *center* 3 mg/l, c. *right* 6 mg/l; **Fig. 6,** plantlet; **Fig. 7,** acclimatization of plantlets.

blackberries (Figure 2). The medium used was that of Murashige and Skoog at pH 5.7. All cultures were placed on growth shelves at 26 ± 1°C, 16h photoperiod and a light intensity of 15 to 30 lux (provided by Sylvania Grow-Lux bulbs).

Blackberry stem cuttings were disinfected by a 10 to 15 sec dip in 30% EtOH. They were then rinsed in double distilled water, surface sterilized in 0.25% NaOCl, plus Tween-20, and rinsed three times in sterile water. Stem cuttings developed axillary shoots in MS basic salts, supplemented with 0.4 mg/liter myo-inositol, 0.1 mg/liter (GA_3) gibberellic acid, 30 g/liter sucrose, 8 g/liter agar, 0.1 mg/liter benzylaminopurino (BA) and 0.1 mg/liter indolebutyric acid (IBA) (Fig. 3). Once shoots reached 1 cm in length they were excised and transferred to a rooting medium (Fig. 4) where optimum rooting was accomplished using 3 mg/liter IBA (Fig. 5). Immediately after root initiation, plantlets were transferred to a medium without growth regulators (Fig. 6). Plantlets were finally transplanted into 1 peat.1 perlite (v/v). Acclimatization was accomplished by a weekly exchange of a series of polyethylene covers with 0, 14, 40 and 90 1-cm perforations. Plantlet survival was 100% (Fig. 7).

Other woody plant species have been tissue cultured, and procedures have been published for the following species.

Acacia koa	koa
Aesculus hippocastanum	horsechestnut
Coryllus avellana	filbert
Eucalyptus spp	eucalyptus
Hevea brasiliensis	rubber plant
Liquidambar styraciflua	sweetgum
Paulownia spp	
Santalum album	sandalwood
Tectonia grandis	teak
Ulmus americana	elm
Betula spp	birch
Populus tremuloides	aspen
Kalmia latifolia	mountain laurel
Coffea arabica	coffee
Vitis vinifera	grapes
Vaccinium spp	blueberries
Rubus	blackberries
Malus domestica	apple
Prunus persica	peach
Prunus amygdalus	almond
Prunus insititia	plum
Prunus avium	cherries
Rubus idaeus	red raspberry
Ribes inebrians	currants
Rosa hybrida	roses
R chinensis	roses
Fuchia hybrida	fuchia
Castanea sativa	chestnut
Theobroma cacao	cacao

Hydrangela macrophylla	hydrangea
Rhododendrons	azaleas
Morus alba	mulberry
Ficus spp	ficus
Simondsia chinensis	jojoba
Citrus	
Panax gingseng	gingseng
Araucaria cunninghamii	hoop pine
Cryptomeria japonica	japanese cedar
Picea excelsa	spruce
Pseudotsuga menziesii	douglas fir
Sequoia sempervirens	redwood
Thuja plicata	western red cedar
Tsuga heterophyla	hemlock
Pinus spp	pine

PECAN TISSUE CULTURE

Stem cuttings from pecan seedlings are presently being cultured in modified WPM liquid media. Shoot development has been very uniform and some cultures with multiple shoots have been obtained.

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DEVELOPMENTS IN DIRECT ROOTING

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For generations nurserymen have rooted cuttings in beds. When the cuttings rooted, they were uprooted and planted in soil beds. When these plants were large enough to transplant they were uprooted again. All of this uprooting put growth on hold and took time and effort.

In the last decade direct rooting has become standard procedure with many nurseries throughout the century. No particular nursery or nurseryman could claim the distinction of originating the system because a considerable number of nurserymen embraced the concept at the same time. Evidently the time for this significant development had arrived and many saw fit to give it a try. There is no particular time when one could say direct rooting was born because there have been isolated instances of the practice going on for some time. In a meaningful way the system was basically born during the seventies.

From the beginning there was a considerable saving of