plant cells to grow for approximately 3 months. Then the rhododendrons are ready to be planted into gallon cans.

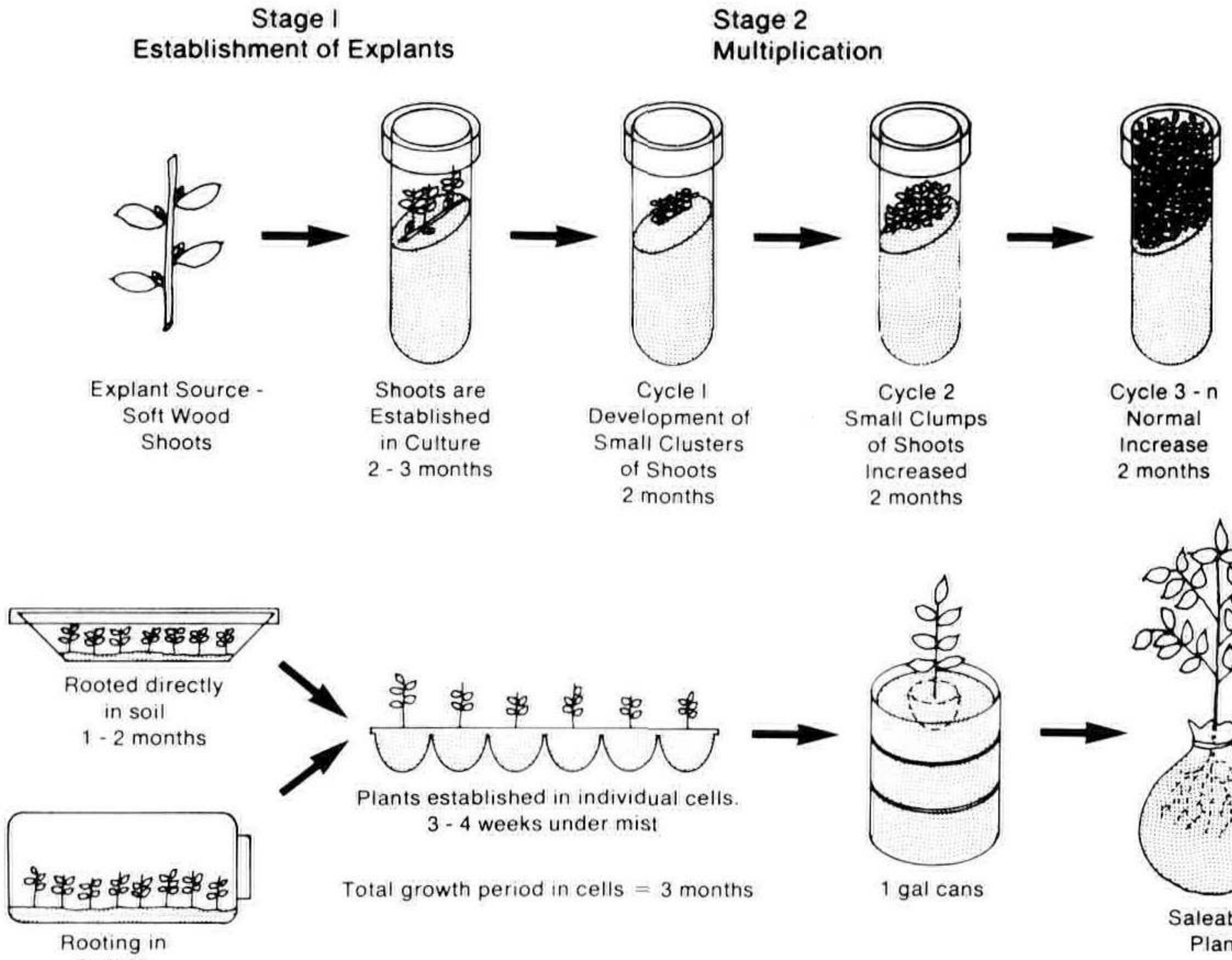

Figure 1. Steps in tissue culture propagation of rhododendron.

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CLONAL PROPAGATION OF WOODY PLANT SPECIES THROUGH TISSUE CULTURE TECHNIQUES

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Abstract. Significant progress has been made in development of tissue culture techniques to meet the requirements for mass clonal propagation of

selected woody species. Regeneration of plantlets from Douglas fir cotyledons has been accomplished under defined conditions. Differentiation originated from cells residing in the hypodermal layer. Biochemical studies have shown that newly synthesized proteins with molecular weights ranging from 16,000 to 20,000 daltons are associated with developmental events. A workable Douglas fir protoplast system has been established for obtaining calluses from protoplasts isolated from cotyledon. In studies with deciduous species, *in vitro* requirements for mass clonal propagation on a scale of more than one million plantlets per year has been accomplished for pear rootstock, 'Old Home × Farmingdale 51', plum rootstocks, 'Pixy' and 'St. Julien X' and ornamental *Prunus cerasifera* 'Newport.' Research with apples, cherries and other ornamentals is less advanced but is indicative that high frequency plantlet regeneration will be obtained.

REVIEW OF LITERATURE

The potential use of *in vitro* techniques in forest tree improvement programs has been described (4,5,12,20,31,34). Morphogenesis in culture of coniferous tree species has been reported for *Sequoia sempervirens* (3), *Platyeladus orientalis* (syn. *Biota orientalis*) (29,39), *Pinus gerardiana* (39), *Cryptomeria japonica* (24), *Picea glauca* (7,8), *Pinus palustris* (35), *Pseudotsuga menziesii* (Mirb.) Franco (11,14,15,36,41,42), *Tsuga heterophylla* (13), *Thuja plicata* (18) *Pinus taeda* (12,16,33), *Pinus contorta* and *Picea sitchensis* (40), and other species (38).

With regard to the development of tissue culture techniques for mass clonal propagation of deciduous woody species, various degrees of success has been reported for fruit trees — apples (1,16,21,25,26,27), almonds (32,37), almond-peach hybrid (37) and cherry (16) — and for the ornamental species *Ilex aquifolium* (23), forsythia (19), rhododendron (2) and bougainvillea (9).

In this article, we report the current status of our research toward development of tissue culture techniques for mass clonal propagation of selected woody species including conifers, fruit trees and ornamentals. We have made significant progress and our successes lead us to believe that economic, large scale propagation of woody species is not far away.

MATERIALS AND METHODS

Sources of plant materials and methods for their preparation. For conifers, our principal experimental material has been cotyledons of Douglas fir [*Pseudotsuga menziesii*]. Other conifers included in this study are listed in Table 1. In addition to using cotyledons as initial explants, plant materials from different plant parts and different ages have also been used. Seeds were obtained from various sources: (a) Douglas fir (*Pseudotsuga menziesii)*, western hemlock (*Tsuga heterophylla*), loblolly pine (*Pinus taeda*), and Caribbean pine (*Pinus caribaea*) from

the Western Forestry Research Center, Weyerhaeuser Co., Centralia, Washington; (b) *Cryptomeria japonica* from the Government Forestry Experimental Station, Tokyo, Japan; and (c) other species from the commercial suppliers, Herbst Brothers (Brewster, N.Y.), F.W. Schumacher Co. (Sandwich, Mass.), and Roy Carter (Sylmar, Calif.). Juvenile Douglas fir of approximately 6 to 7 years of age was obtained from Alfred Teufel Nursery (Portland, Oregon). Plant materials from adult Douglas fir were collected from the vicinity of the Oregon Graduate Center.

Table 1. Conifer species for which the potential for clonal propagation through tissue culture techniques has been established.

Species	Adventitious Shoot Formation	Adventitious Root Formation	Plantlet Regeneration
1. *Cryptomeria japonica*	+	+	+
2. *Cupressus arizonica*	+	+	+
3. *Picea abies*	+	+	+
4. *Picea sitchensis*	+	+	+
5. *Picea caribaea*	+	+	+
6. *Pinus elliottii*	+		
7. *Pinus ponderosa*	+		
8. *Pinus taeda*	+	+	+
9. *Pseudotsuga menziesii*	+	+	+
10. *Tsuga Heterophylla*	+	+	+

Sources and ages of tissue explants used:
(a) Excised mature embryos: 5,7,8 and 9.
(b) Cotyledons (up to 3 months): 1,2,3,4,5,6,8,9 and 10.
(c) Hypocotyls (up to 3 months): 9.
(d) Needles (up to 2 years): 9.
(e) Stems (up to 2 years): 9 and 10.
(f) Stems (adult trees): 9.

For deciduous woody species, actively growing herbaceous shoots obtained from clonal stock plants maintained in a greenhouse were used as experimental materials. These stock plants, fruit tree rootstocks and ornamentals, were provided by A. McGill & Son Nursery (Fairview, Oregon), Oregon Rootstock, Inc. (Gervais, Oregon), Stark Bro's Nurseries and Orchards Co. (Louisiana, Mo.), and Willow Drive Nursery (Toledo, Wash.).

To obtain aseptic tissues to be used in culture establishment, plant materials, after washing thoroughly with running tap water, were sterilized by submerging with slight agitation in 6 to 20% Clorox (5.25% sodium hypochlorite, NaOCl, the Clorox Co., Oakland, Calif.) for a period of 8 to 20 min., depending on the condition and source of tissue, and then rinsed several times with sterile, deionized water until free of Clorox. Prior to the establishment of tissues in culture, a preconditioning treatment was applied to all Clorox sterilized plant materials by placing them for about a week on an agar solidified nut-

rient medium with no supplement of plant growth regulators. This preconditioning step enables the selection of vigorously growing tissues for use in culture establishment and allows the elimination of contaminated and injured tissues. The aseptic tissues were sliced into 3 to 10 mm pieces which were subsequently cultured on appropriate media.

Culture media and systems. The basal medium contains, per liter, (a) inorganic compounds: 825 mg NH_4NO_3, 950 mg KNO_3, 220 mg $CaCl_2 \cdot 2H_2O$, 185 mg $MgSO_4 \cdot 7H_2O$, 85 mg KH_2PO_4, 6 mg $FeSO_4 \cdot 7H_20$, 7.2 mg Na_2EDTA, 3.1 mg H_3BO_3, 11.2 mg $MnSO_4 \cdot 4H_2O$,. 5.3 mg $ZnSO_4 \cdot 7H_2O$, 0.4 mg KI, 0.2 Mg $NaMoO_4$, 0.01 mg $CuSO_4 \cdot 5H_2O$, and 0.01 mg $CoCl_2 \cdot 6H_2O$; and (b) organic substances: 250 mg myo-inositol, 2.5 mg thiamine·HCl, and 30 g sucrose. In general, the basal medium was used at full strength. However, if retardation of tissue growth was observed, 2- or 4- fold diluted basal medium was used. The plant growth regulators added to the basal medium were as follows, (a) auxins: indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2-naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D); and (b) cytokinins: N_6-benzylaminopurine (BAP), 6-(3-methyl-2-butenylamino) purine (2iP), and kinetin. The final pH of the nutrient medium was adjusted to 5.5. The heat labile compounds, after adjusting the pH to 5.5, were sterilized separately by filtering through 0.20μ Nalgene Filter Units (Nalgene Sybron Corp., Rochester, N.Y.), and then added to the autoclaved nutrient media.

Two types of culture systems, agar solidified solid system and fabric tissue support liquid system, were employed in culturing various tissues. For preparing solid medium, 0.6 to 1.0% Bacto-agar (Difco Lab., Detroit, Mich.) added to the nutrient medium was autoclaved and subsequently poured into either 60 × 15 mm or 100 × 20 mm plastic petri dishes (Falcon Plastics, Oxnard, Calif.). The fabric tissue support liquid system consisted of a culture vessel containing 100% polyester fleece saturated with an appropriate liquid nutrient medium. Replacing the solidified agar with a fabric material for tissue support allows the nutrient to flow in a liquid state and, thus, facilitate the process of supplying tissue explants with appropriate nutrients required for each developmental stage without transfer of cultured tissues. Owing to its flexibility and simplicity, this system exhibits the potential for industrial application in mass clonal propagation (15).

RESULTS AND DISCUSSION

CONIFEROUS SPECIES

In this laboratory, significant advances have been made in

development of tissue culture techniques to attain the goal of mass clonal propagation of forest tree species. Initiation of multiple shoots and roots, and regeneration of plantlets have been accomplished to various degrees for several species of gymnosperms (Table 1). Practically, all coniferous species studied, Japanese cedar, Arizona cypress, Norway spruce, Sitka spruce, Caribbean pine, slash pine, ponderosa pine, loblolly pine, Douglas fir, and western hemlock, have exhibited morphogenetic responses. Furthermore, tissues from different plant parts and from different ages of plants respond to tissue culture treatment. For example, various Douglas fir tissues, excised mature embryos, cotyledons, needles, and stems of juvenile and adult trees produced adventitious buds (Figure 1).

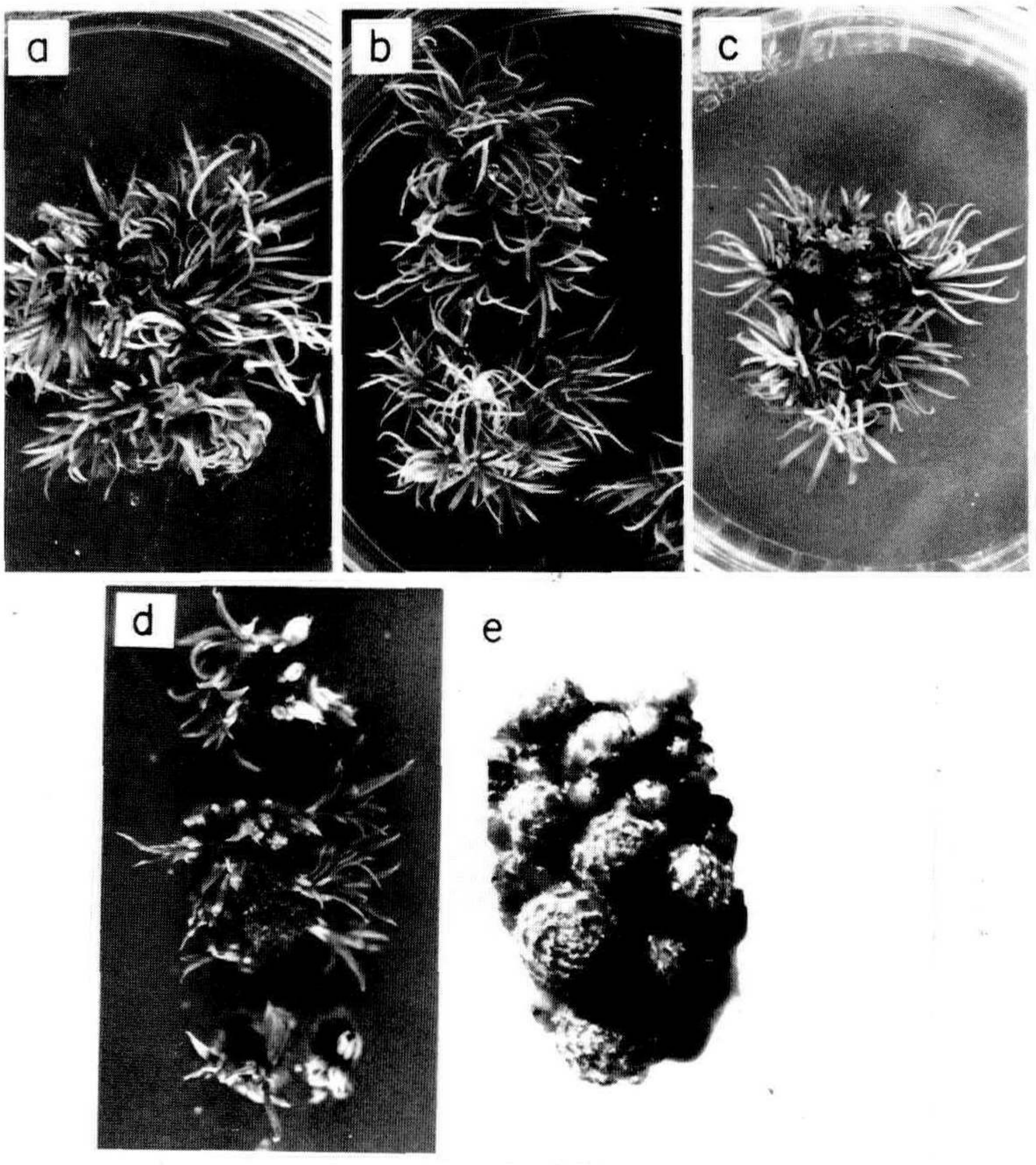

Figure 1. Multiple adventitious shoot formation in culture from various Douglas fir tissues. Plant materials used were: (a) excised mature embryo, (b) cotyledon explants from young seedlings, (c) needle explants from young seedlings, (d) stem explants from juvenile trees, and (e) stem explants from adult trees.

In order to better define various factors controlling differentiation *in vitro*, Douglas fir cotyledons were chosen as a model system for an in-depth study of (a) tissue culture requirements for each developmental stage leading to plantlet regeneration (11-17), (b) the histological sequence of adventitious bud development (10), (c) the elucidation of biochemical mechanisms controlling differentiation (22,43), and (d) requirements for a workable protoplast system (28). The progress we have made in each of these various areas of research are reported here.

Factors influencing adventitious bud formation. For stimulation of adventitious bud formation in culture, a relatively high concentration of cytokinin with respect to that of auxin is required (Figures 2a and b). The extent and nature of morphogenetic responses expressed by tissue explants depends on the genomic composition and physiological condition of the plant material. The diversity of morphogenetic responses was more apparent when natural auxins (i.e. IAA and IBA), instead of synthetic auxins (i.e. NAA and 2,4-D) were used. Using the identical nutrient medium supplemented with natural auxin, different morphogenetic responses occurred for Douglas fir cotyledon explants derived from different regions of the same seedlings or from the same regions of different seedlings. The most likely interpretation of these differences is due to the existence of different amounts of auxin-degrading enzymes among a heterogeneous seedling population. As a consequence of these differences, even applying the same amount of exogeneous natural auxin to all tissue samples, the actual functional auxin concentrations among these explants differed significantly.

Synthetic auxins have low susceptibility to enzymatic degradation, thus addition of low concentration of either NAA or 2,4-D to nutrient medium provided a uniform stimulation of adventitious bud formation. Among the most commonly used cytokinins (i.e. BAP, kinetin, and 2iP), BAP was most effective in stimulating adventitious bud formation; 2iP was the least effective. To date, the most effective concentrations of plant growth regulators for stimulating Douglas fir cotyledons to produce adventitious buds are 5μM BAP plus 0.5 to 5.0 nM NAA, or 5μM BAP plus 0.25 to 5.0 μM IAA or IBA,

A histological analysis of adventitious bud formation in culture of Douglas fir cotyledon showed that the organized structure originated from hypodermal cells of cotyledon and that its development sequence advanced through four anatomically distinguishable structures: (1) meristemoid, (2) bud primordia, (3) shoot apex with needle primordia, and (4) adventitious bud. Bud primordia were well-defined after 21 days in

culture. The anatomical structures of adventitious buds were similar to those formed on intact plants. Beneath the shoot apex, the stem consisted of a well-differentiated pith and vascular bundle. This vascular system extended from stem axis acropetally into the needles.

Biochemical mechanisms involved in adventitious bud formation of the Douglas fir cotyledon system were studied by analyzing double labeled (^{14}C, ^{3}H) newly synthesized cytoplasmic soluble proteins by SDS polyacrylamide gel electrophoresis. Increase of a low molecular weight protein fraction ranging from 16,000 to 20,000 daltons (i.e. bud protein fraction) was observed for cotyledon culture capable of producing adventitious buds (i.e. bud culture). An increase of bud protein fraction was detected after 2 days in culture and reached a maximum level at day four. Association of the bud protein fraction with adventitious bud formation was supported by results obtained from comparing bud culture with that of other morphologically distinct types of cultures in that bud culture always contained a higher amount of this protein fraction. Furthermore, when bud culture was transferred from bud medium to callus medium, suppression of bud protein synthesis occurred. Partial purification of the bud protein fraction was achieved by solubilizing proteins with 60% ammonium sulfate solution. Preliminary results obtained from fractionation of proteins with DNA-cellulose column showed that a bud protein exhibited a strong binding affinity with DNA. Further elucidation of the bud protein fraction is necessary if the nature and function of these proteins are to be understood. Meanwhile, this characteristic protein marker can be used as a probe in design and evaluation of tissue culture experiments.

Regeneration of plantlets. Regeneration of Douglas fir plantlets under defined culture conditions has been accomplished (Figure 2c). Excised tissue-culture-produced shoots were subjected to root initiation by placing them on an agar solidified rooting medium. In addition to an appropriate concentration of auxin (NAA), a concentration of sucrose lower than that used for shoot initiation was beneficial for root initiation. For stimulation of root formation, concentrations of NAA and sucrose at 0.25 μM and 0.5% respectively were required; under these conditions about 80% of tissue culture-produced shoots formed roots. The incubation temperature also had a profound influence on the regeneration frequency of plantlets and on their anatomical structure. For example, at 24°C only a few plantlets were produced and friable callus which formed at the transition region between stem and root caused a discontinuity in the anatomical structure of the plantlets. In contrast, at 19°C

a high frequency regeneration of plantlets showing a normal morphological appearance was obtained.

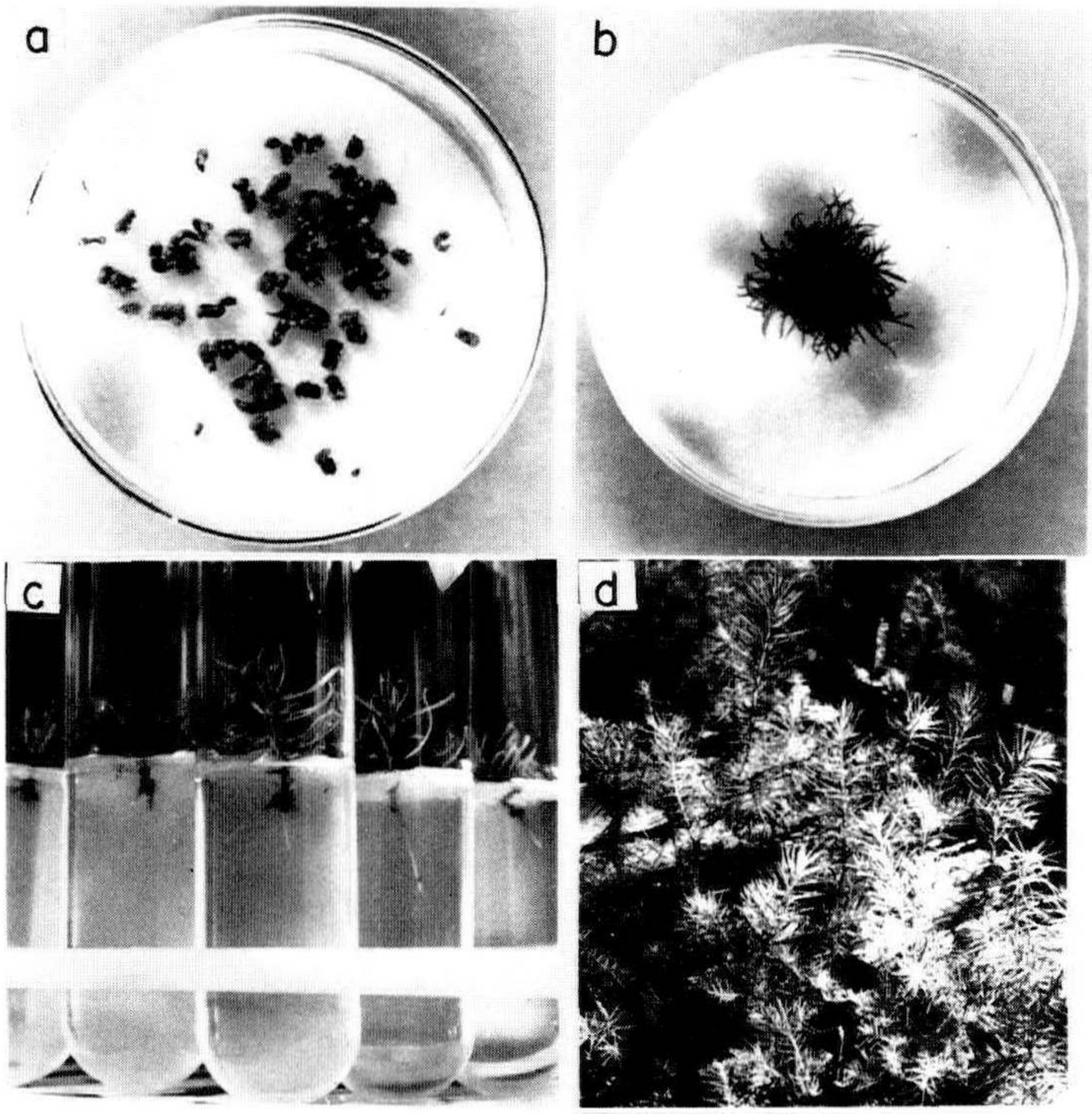

Figure 2. Sequential development in culture of plantlets from Douglas fir cotyledons. (a) Initiation of adventitious buds, (b) Development of multiple shoots, (c) Regeneration of plantlets, (d) Plantlets grown in greenhouse.

For establishing tissue culture produced plantlets in soil, it is essential that the humidity surrounding plantlets be reduced gradually from the high humidity condition of culture to the lower humidity of the greenhouse; a drastic change in humidity causes dehydration of plantlets and results in a reduction of their survival rate. Douglas fir plantlets grown in a greenhouse appear to be normal (Figure 2d). Long-term observations of the growth behavior of these plantlets are necessary to evaluate their performance.

Douglas fir protoplast system. A potential of in vitro techniques, other than that for mass clonal propagation, is to provide an effective method for introducing desirable new strains through genetic engineering at a cellular level. By using protop-

lasts or single cells asexual hybrids, mutants and cells containing foreign organells or molecules might be produced. A prerequisite for somatic cell genetic manipulation is the establishment of a workable protoplast system with respect to 1) methods of protoplast isolation, 2) culture conditions capable of supporting protoplasts to resynthesize new cell walls, and 3) culture conditions capable of supporting active proliferation of regenerated cells. In this laboratory, we have demonstrated, using the Douglas fir system, the first successful results in culturing protoplasts of Gymnosperms (28).

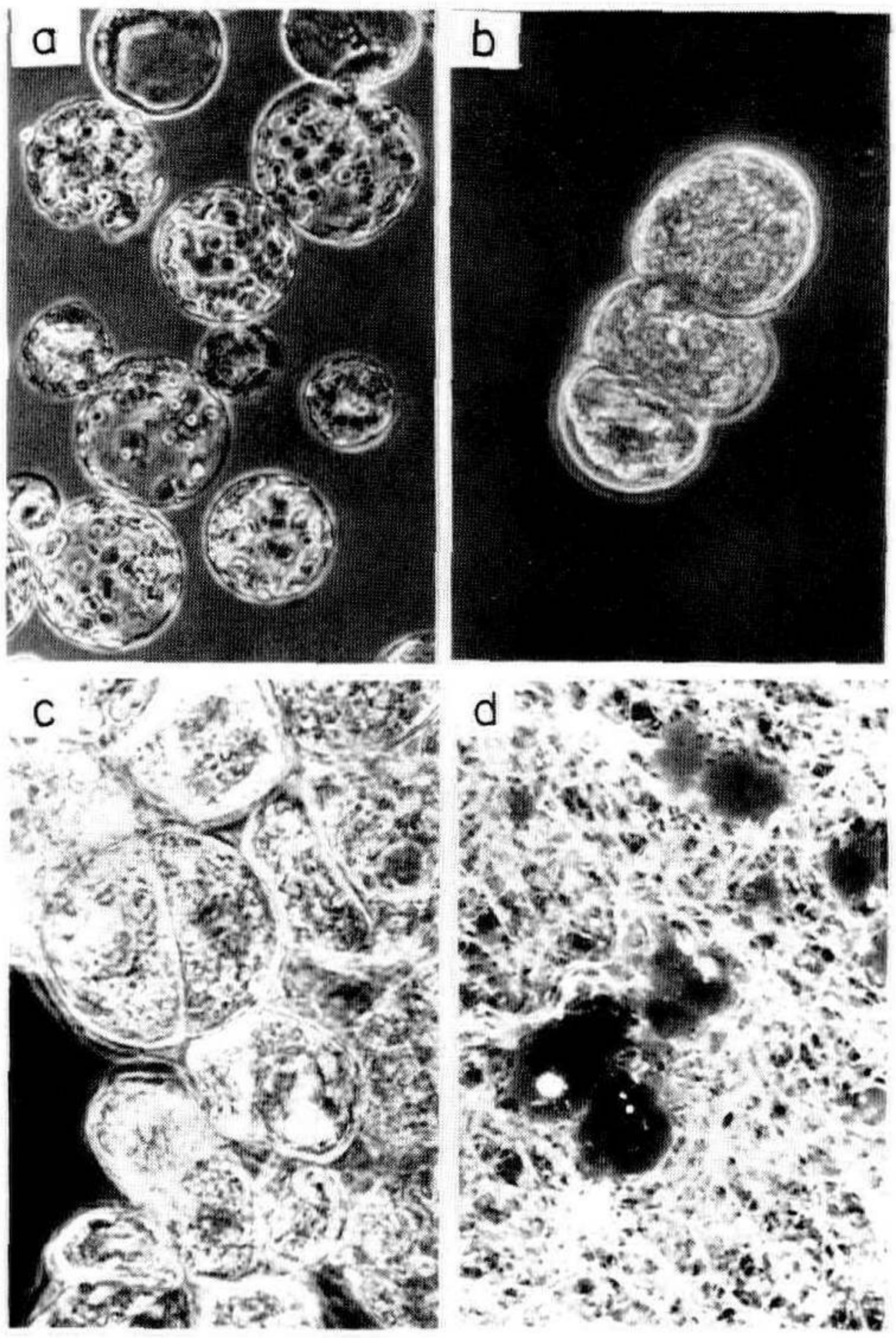

Figure 3. Callus formation from protoplasts isolated from Douglas fir cotyledons. (a) Freshly isolated protoplasts. Protoplasts, after resynthesizing new cell walls, underwent cell division showing (b) 4-cell stage, (c) colony formation, and (d) callus formation.

Douglas fir cotyledons obtained from 2-4 week old seedlings were used for protoplast isolation. The yield of protoplasts was significantly increased by subjecting cotyledons, prior to protoplast isolation, to a preconditioning treatment for 8 days in the presence of 15μM BAP and 0.5μM NAA. The most effective protoplast isolation mixtures consisted of Cellulysin, Macerase and sorbitol at concentrations of a) 4%, 1% and 0.4M and b)

2%, 0.5% and 0.6M, respectively; in both cases, isolation of approximately 5.5 × 10^4 protoplasts from 100 mg of tissue was achieved at the end of 3 hours incubation. Freshly prepared protoplasts were relatively free of cell debris and were extremely viable (Figure 3a). For culturing protoplasts, a fabric tissue support saturated with an appropriate liquid nutrient medium was used. Most of the protoplasts were capable of resynthesizing new cell walls after culturing for 72 hours in a nutrient medium containing 5μM BAP and 15μM NAA. Addition of a high concentration of glutamine (10 mM) to the culture medium was required for proliferation of regenerated cells derived from protoplasts. However, in addition to an appropriate culture medium, the method of protoplast culture was also important. For example, if the droplet method was used, regenerated cells failed to proliferate beyond the 20 cell stage. However, when a fabric tissue support, saturated with an appropriate liquid medium was used, regenerated cells proliferated actively (Figures 3b and c) leading to callus formation (Figure 3d). This combination of a fabric tissue support and culture medium supplemented with a higher glutamine concentration provides an effective way for culturing Douglas fir protoplasts.

DECIDUOUS SPECIES

Significant progress has been made during the past eight months toward development of tissue culture techniques for mass clonal propagation of selected deciduous species including apple rootstocks, cherry rootstocks, pear rootstocks, plum rootstocks, apple cultivars, and ornamentals. Results obtained from evaluation of tissue culture responsiveness of these deciduous species with respect to multiple shoot formation, root formation and plantlet regeneration are summarized in Table 2. Plantlet regeneration in culture is accomplished by a two step method: 1) stimulation of stem explants to produce multiple shoots, and 2) root initiation of tissue-culture-produced shoots. A reproducible, high frequency regeneration of plantlets which meets the specifications for commercial scale production has been obtained for four clonal plants: pear rootstock 'Old Home × Farmingdale 51,' plum rootstocks 'Pixy' and 'St. Julien X,' and ornamental *Prunus cerasifera* 'Newport.'

Multiple shoot formation. The basal nutrient medium used for culturing deciduous tissues was similar to that used for conifer tissues. A nutrient medium supplemented with 5μM BAP plus 0.5 to 5.0 μM IBA stimulated a variety of deciduous species to produce multiple shoots (see Table 2). After approximately six weeks in culture, formation of 10-100 shoots per tissue explant was observed. Variations observed in shoot multiplication rate and shoot development (stem elongation and leaf

Table 2. Status in development of tissue culture techniques for clonal propagation of selected deciduous cultivars

Cultivars	Multiple Shoot Formation	Adventitious Root Formation	Plantlet Formation (%)
I. Apple Rootstocks			
1. Antonovka KA313	+	+	+ (>50%)
2. EMLA-7	+	+	+ (>70%)
3. EMLA-9	+	+	+ (>60%)
4. EMLA-27	+		
5. MAC-9	+	+	+ (>70%)
6. Seedlings	+	+	+
II. Cherry Rootstocks			
1. Colt	+	+	+ (>80%)
2. Mahaleb X Mazzard 14	+	+	+ (>80%)
III. Pear Rootstocks			
1. Old Home × Farmingdale 51	+	+	+ (>90%)
IV. Plum Rootstocks			
1. Pixy	+	+	+ (>85%)
2. St. Julien X	+	+	+ (>85%)
V. Apple Scion Cultivars			
1. Stark Jumbo	+	+	+
VI. Ornamentals			
1. *Acer platanoides* 'Crimson Sentry'	+	+	+
2. *Prunus cerasifera* 'Newport'	+	+	+ (>78%)
3. *Pyrus calleryana* 'Bradford'	+		
4. *Pyrus faurei*	+		

expansion) seem to be caused by differences in the physiological conditions and genomic characteristics of particular plant species. The morphological appearance of multiple shoots produced in culture of some fruit tree rootstocks (shown in Figure 4) is normal. Based on the shoot multiplication rate obtained to date for various deciduous species, three groups can be identified: 1) those producing more than 30 shoots per explant, pear rootstock 'Old Home × Farmingdale 51,' plum rootstocks 'Pixy' and 'St. Julien X', and ornamental *Prunus cerasifera* 'Newport'; 2) those producing about 10 shoots per explant: apple rootstocks 'Antonovka KA 313', 'EMLA-7', 'EMLA-9' and 'MAC-9', and ornamental *Pyrus calleryana* 'Bradford'; and 3) those producing less than 10 shoots per explant: apple rootstock 'EMLA-27', apple scion cultivar 'Stark Jumbo,' and ornamentals *Acer plantanoides* 'Crimson Sentry' and *Pyrus fauriei*. These results indicate that the shoot-forming medium for species in the second two groups is currently suboptimal and continued experimentation in optimization of medium is required. Judging from the *in vitro* growth behavior of these species, high shoot multiplication rates probably will require only a slight adjust-

ment of the existing shoot-forming medium. In some cases, such as some apple rootstocks and cherry rootstock 'Mahaleb × Mazzard 14,' tissue-culture-produced shoots are quite succulent and the medium composition needs to be modified further in order to produce shoots with better characteristics.

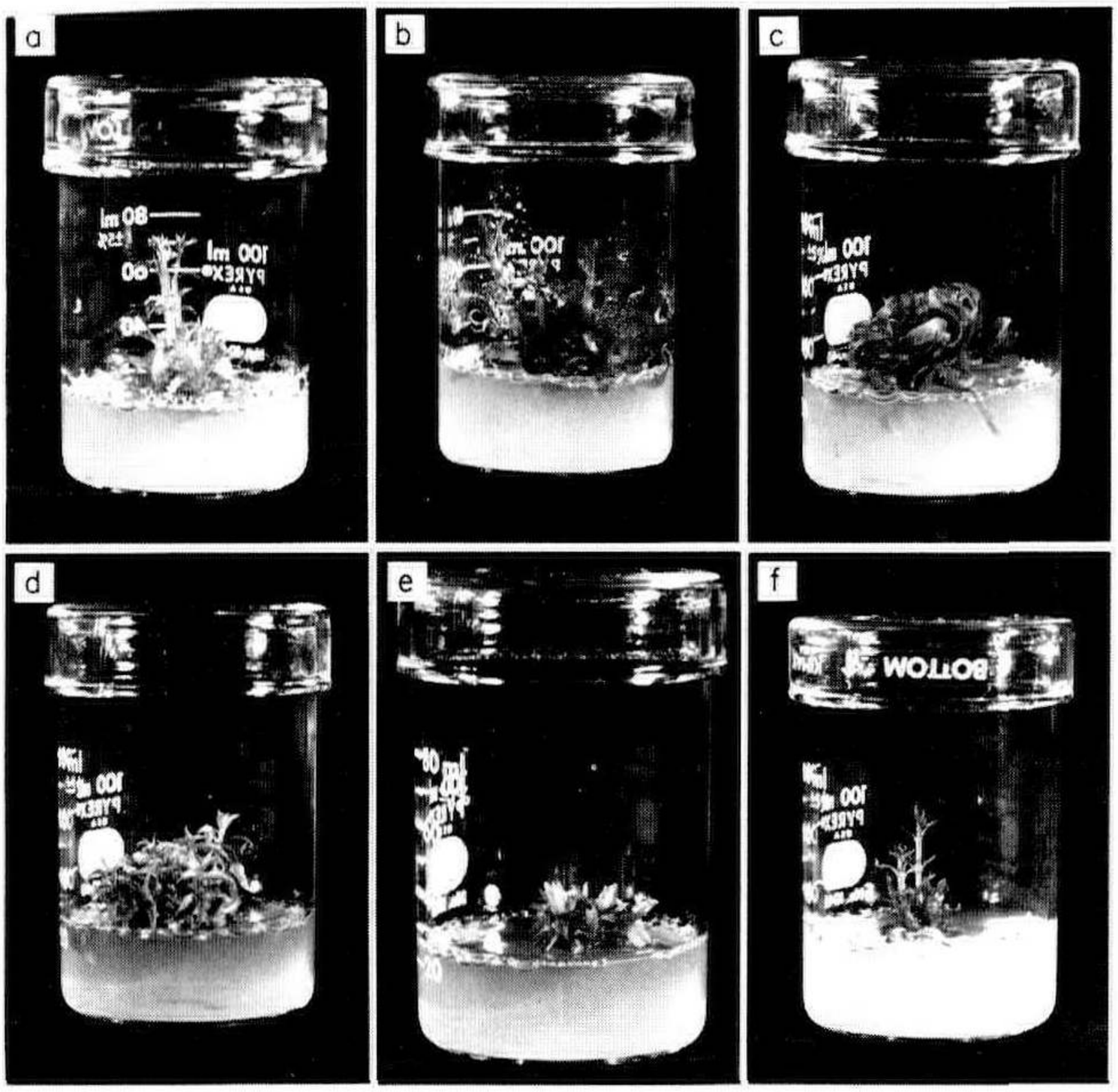

Figure 4. Formation of multiple shoots in culture from selected clonal fruit tree rootstocks. Apple rootstocks: (a) Antonovka KA 313 and (b) MAC-9. Cherry rootstock: (c) Mahaleb × Mazzard 14. Plum rootstocks: (d) St. Julien X and (e) Pixy. Pear rootstock: (f) Old Home × Farmingdale 51.

Regeneration of plantlets. Plantlets were regenerated by subjecting tissue-culture-produced shoots to a rooting medium. Rooting responses varied using rooting medium supplemented with auxin at concentrations of 0.5 to 5.0 μM IBA (Table 3 and Figure 5). For those deciduous species exhibiting a high shoot multiplication rate, in the presence of 2.5 and 5.0 μM IBA, roots started to appear after one week in culture from more than 60% of shoots and from more than 80% at the end of 3 weeks. Shoots of plum rootstocks 'Pixy' and 'St. Julien X,' produced with different types of auxin (either IBA or 2,4-D), required different rooting conditions. Shoots produced with 2,4-D rooted readily on basal medium without supplement of IBA because the residual 2,4-D existing in shoots was more than sufficient to stimulate root formation; however, shoots produced with IBA required additional IBA for root initiation. For *Prunus cerasifera* 'Newport,' rooting efficiency is controlled by the anthocyanin

content of shoots; a high concentration inhibited root formation whereas a low concentration did not. Plantlets regenerated from some fruit tree rootstocks are shown in Figure 5. These plantlets grew vigorously in the rooting medium showing both rapid shoot and root elongation.

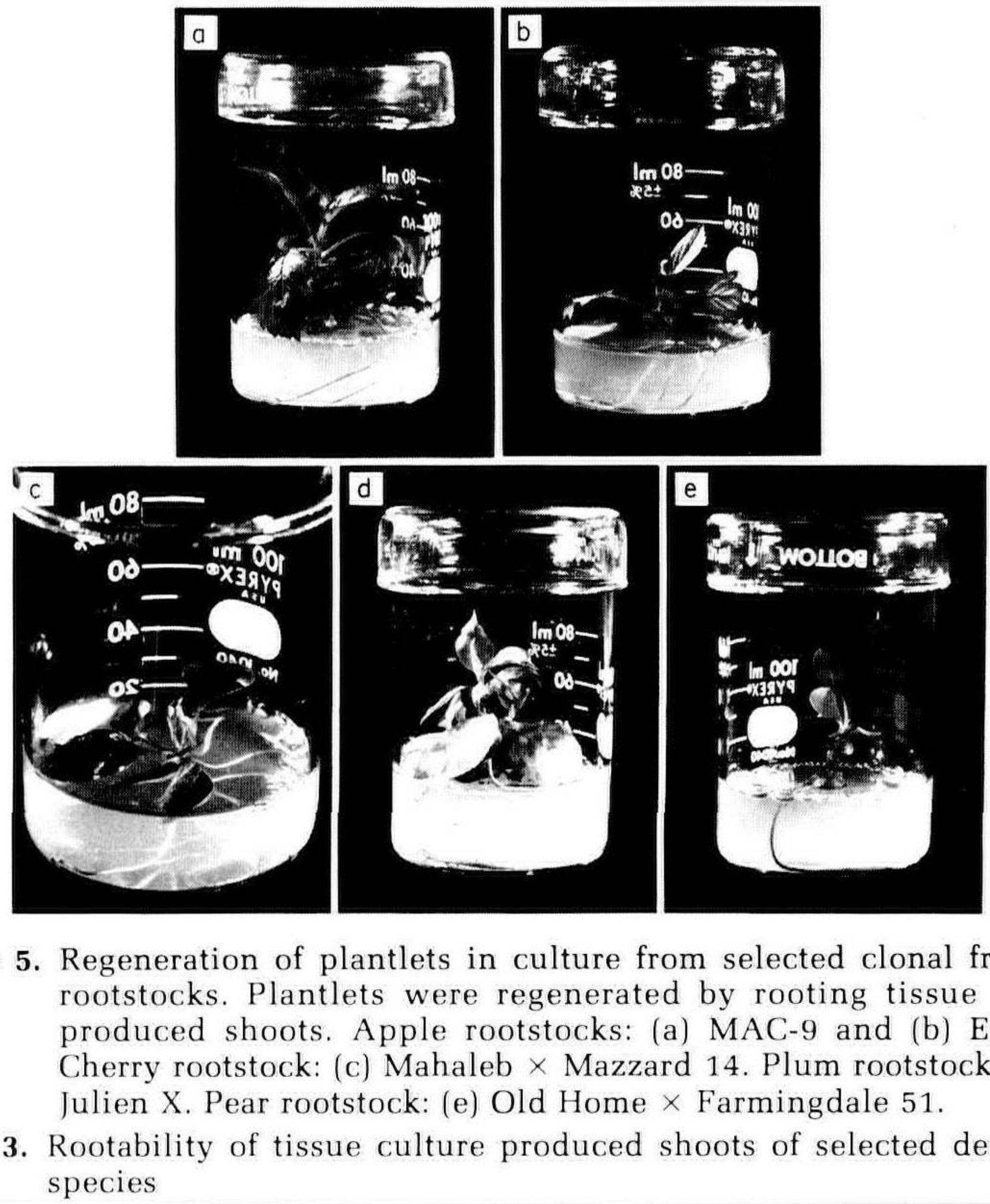

Figure 5. Regeneration of plantlets in culture from selected clonal fruit tree rootstocks. Plantlets were regenerated by rooting tissue culture produced shoots. Apple rootstocks: (a) MAC-9 and (b) EMLA-7. Cherry rootstock: (c) Mahaleb × Mazzard 14. Plum rootstock: (d) St. Julien X. Pear rootstock: (e) Old Home × Farmingdale 51.

Table 3. Rootability of tissue culture produced shoots of selected deciduous species

Cultivars	IBA Concentration μM	Total No. of Shoots	No. of Rooted Shoots	[1]Frequency of Rooting (%)
I. Pear Rootstocks				
1. Old Home ×	5.0	70	64	91
Farmingdale 51	2.5	30	28	93
II. Plum Rootstocks				
1. Pixy	5.0	52	46	89
	2.5	48	47	98
2. St. Julien X	5.0	84	73	87
	2.5	82	77	94
III. Ornamentals				
1. Prunus cerasifera	5.0	25	20	80
'Newport'	2.5	51	43	84

[1] Rooting frequency was estimated after subjecting tissue culture produced shoots to rooting medium for 3 weeks.

Establishment of regenerated plantlets in soil. The potting mixture used consists of soil: peat moss: perlite: sand (1:1:1:1) and mist propagation techniques were used in transferring sterile plantlets to soil. Potted plantlets were immediately placed in a mist chamber equipped with an intermittent-mist water spray and an electric bottom-heat cable. The temperature of the chamber was maintained at 25°C. The frequency of water spraying was adjusted such that plantlets were always covered with a thin layer of water. After one week, the frequency of water spraying was reduced to allow plantlets to harden-off for placement on a greenhouse bench. Hoagland nutrient solution was administered to plantlets at 2 to 4 week intervals. Plantlets grew vigorously under greenhouse conditions; apple plantlets grew to about 6 feet in 6 months and plum rootstock 'St. Julien X' and *Prunus cerasifera* 'Newport' plantlets grew about 2 feet in 3 months. We have tested various systems for transplanting deciduous plantlets to soil and find this described system to be the most effective way (Table 4).

Table 4. Survival rate of plantlets of selected deciduous cultivars grown under greenhouse conditions[1]

Cultivars	Experiment	Total No. Plantlets	No. of Plantlets Survived	Survival (%)
I. Pear Rootstocks				
1. Old Home ×	1	46	43	93
Farmingdale 51	2	51	43	84
II. Plum Rootstocks				
1. Pixy	1	49	41	84
2. St. Julien X	1	47	43	92
	2	45	44	98
	3	52	44	85
	4	91	74	81
III. Ornamentals				
1. *Prunus cerasifera*	1	45	41	91
'Newport'	2	62	55	89

[1] Plantlets, after transplanting from aseptic conditions into potted soil, were immediately placed inside a high humidity moist chamber for approximately one week followed by gradually decreasing the humidity by prolonging the water spray intervals. At the end of 2 weeks, potted plantlets were placed on greenhouse bench.

At present, we have approximately 400 plantlets of 'St. Julien X,' 200 each of 'Pixy' and *Prunus cerasifera* 'Newport', and 100 of 'Old Home × Farmingdale 51' growing in the greenhouse. The morphological appearance of these plantlets is uniform (Figure 6). We intend to outplant these plantlets for subsequent use as understock for appropriate fruit tree cultivars so that we can evaluate their performance. The anatomical structures and chromosomal complements of these plantlets

will also be analyzed in order to assure that tissue culture techniques we have developed produced true-to-type plantlets.

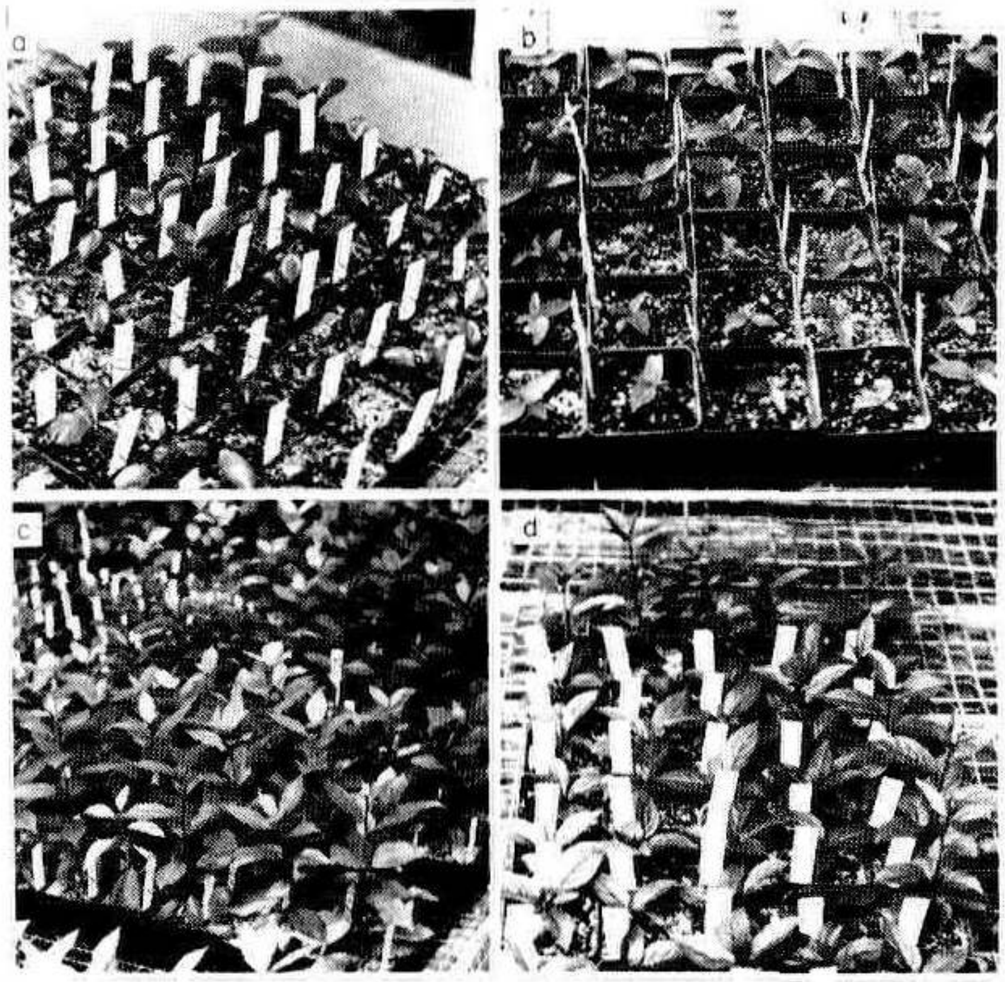

Figure 6. Fruit tree plantlets grown under greenhouse conditions. Pear rootstock: (a) Old Home × Farmingdale 51. Plum rootstocks: (b) Pixy and (c) St. Julien X. Ornamental: (d) *Prunus cerasifera* 'Newport.'

Mass clonal propagation of plantlets on a scale of one million plants annually can be accomplished for pear rootstock 'Old Home × Farmingdale 51,' plum rootstocks 'Pixy' and 'St. Julien ×,' and ornamental *Prunus cerasifera* 'Newport' if appropriate tissue culture facilities and personnel are provided. A significant contribution of this work is that it provides methods which allow the rapid introduction of new rootstocks such as 'Pixy' to the market and for highly reliable rooting of plants such as 'Old Home × Farmingdale', 'St. Julien' and *Prunus cerasifera* 'Newport' which are relatively hard to root by conventional cutting techniques. Our results show that woody species respond as readily as herbaceous species to tissue culture treatment for mass plantlet production.

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