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A DEMONSTRATION OF THE ECONOMIC FEASIBILITY OF
PLANT-TISSUE CULTURE FOR JOJOBA (SIMMONDSIA CHINENSIS)
AND EUPHORBIA SPP.

FINAL REPORT

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SUMMARY

The major objective of this contract was to demonstrate the economic feasibility of plant tissue culture as applied to two plants: jojoba (Simmondsia chinensis) and Euphorbia spp. The gopher weed (Euphorbia lathyris) was selected as the species of Euphorbia to research due to the interest in this plant as a potential source of hydrocarbon like compounds.

High yield female selections of jojoba were chosen from native stands and were researched to determine the economic feasibility of mass producing these plants via a tissue culture micropropagation program.

The length of the contract period was twelve months. The tasks proposed by Plant Resources Institute for each plant included a review of existing scientific information, and development of a tissue culture system aimed at plant production in volumes of at least 5000 plants. While in vitro culture volumes of over 5000 have been achieved for both species, problems with rooting have limited the number of plants transferred to soil. Additional research is needed to develop commercially feasible greenhouse technologies for these species.

The female jojoba selection was successfully mass produced through tissue culture. Modifications in initiation techniques, as well as in multiplication media and rooting parameters, were necessary to apply the tissue culture system, which had been developed for juvenile seedling tissue, to mature jojobas. Since prior attempts at transfer of tissue cultured plantlets were unsuccessful, transfer research was a major part of the project and has resulted in a system for transfer of rooted jojoba plantlets to soil. Due to

the time involved in development of this system, transfer from the sterile soil conditions to greenhouse conditions on a commercial scale was not accomplished. Additional research on soil mixes, humidity regimen, fertilization schedules, light and temperature requirements, and containers is necessary before tissue cultured jojobas can be field planted on a large scale.

Euphorbia lathyris was successfully cultured using shoot tip cultures. Media and procedures were established for culture initiation, multiplication of shoots, callus induction and growth, and root initiation. Well-developed root systems were not attained and root initiation percentages should be increased if the system is to become commercially feasible.

The system developed for mature jojoba tissues was analyzed for costs in terms of labor, materials, overhead, and lab equipment. A cost-per-unit of \$0.17 was calculated for a rooted plantlet prior to greenhouse transfer in volumes of 10,000 per month, over a base period of 24 months. Additional volume data is necessary before a cost analysis of containerized jojoba plants from tissue culture can be made with a degree of accuracy.

The system for tissue culture of Euphorbia lathyris was compared to the jojoba cost analysis for a relative determination of the cost effectiveness of using a tissue culture system for mass-propagation. For several reasons, it would appear that a tissue culture system for E. lathyris is not as economically feasible as one for jojoba in terms of mass propagation. The major potential application for tissue culture technology with E. lathyris is as a method of new selection development.

This report demonstrates the economic feasibility of using tissue culture systems for two purposes: 1) for mass propagation of special selections of jojoba (Simmondsia chinensis) and 2) as a supplementary technology in a breeding program for gopher weed (Euphorbia lathyris).

BACKGROUND

The current energy crisis has resulted in an acute awareness of the limitations of the world's fossil fuel resources. This has resulted in a re-examination of renewable resources to help meet energy demands. The world demand for plant by-products is expected to rise sharply over the next few decades. To meet this growing demand, there will be an increased need for a large number of species of improved quality, quicker growth rate and increased photosynthetic efficiency. Present day practices of plant improvement offer only limited possibilities to achieve these goals. New techniques, in addition to the older ones, are required. This is particularly true when considering the time lag experienced from typical theoretical research to field implementation. Plant cell tissue culture can offer the benefits of reduced time, costs, and the ability to produce superior selections to meet a variety of environmental, social, and economic goals.

In addition to increased demand for plant materials, the pressure of dependence on foreign sources of petrochemicals has severely affected the economy of the United States. As the current program of the Department of Energy indicates, the goal in all areas is to markedly decrease this dependence in the upcoming years. In this regard, plants will play a significant role.

Dependence of the United States on petrochemicals as a source of fuels has been a concern for nearly all Americans. When the United States was introducing Project Independence in 1973-74, this country's foreign oil imports equalled 12.9×10^{15} Btu of energy. In 1976, two years after Project Independence was instituted, our foreign energy imports had increased to 15 quads which is equivalent to an increase of 10^{30} Btus. (Reference Calvin, 1978, Inman, 1977).

From the period 1920-1950, the rate of oil discovery per unit well drilled averaged about 20 billion barrels of oil discovered for every 100 million feet of well drilled. By 1950, the rate of discovery per 100 million feet drilled was about 7 billion, or nearly a third less (Figure 1). The American Petroleum Institute reported that in 1977 we imported 100% more oil than we discovered. These facts emphasize the need for the United States to be engaged in a serious search for alternative or renewable sources of energy materials. Considerable attention has been given to plants which produce hydrocarbons in one form or another. These secondary plant products have considerable potential as feedstock materials for the chemical, pharmaceutical, and cosmetic industries. Two species which have received widespread attention as natural replacements, or renewable sources, of much needed hydrocarbon materials are jojoba (Simmondsia chinensis) and Euphorbia spp.

One of the most obvious energy sources involves harvesting and converting the solar energy stored in Euphorbia spp. These plants naturally convert solar energy into chemical energy which in turn drives a variety of chemical processes that result in production of such varied products as sugars, proteins, oils, and complex hydrocarbons. Euphorbias produce latex, an emulsion of 2/3 water, 1/3 complex hydrocarbons, which, when separated, yields an oil capable of being processed in petroleum refineries. Scientists from the Stanford Research Institute have estimated that present biomass production could yield 10 tons (dry/acre) annually, giving an oil production of 7 barrels. With improved selection and culture, these yields can be expected to dramatically increase.

Euphorbia lathyris (gopher weed) is a latex-containing plant that attains a dry weight in excess of 200 grams in nine months when grown in climatic

Cumulative U.S. crude-oil discoveries as a function of cumulative depth of exploratory drilling. (King Hubbert, 1974)

XBL 776-4467

Fig. 1 Cumulative U.S. crude oil discoveries as a function of depth of drilling

zones comparable to California, Arizona, and Texas. The oil coagulates in the dried plant, and is readily extracted with organic solvents. The chemical composition is similar to fossil derived crude oil. The chemical aspects of Euphorbia oil have been well documented by Dr. Melvin Calvin and co-workers at the University of California, Berkeley.

Euphorbias contain many complex hydrocarbons which have promising potential as replacements for imported petroleum commodities. One of the many limiting factors to the "farming" of such energy plants, however, is the toxic or hazardous esters found within their latex. If demonstrated successfully on the genus Euphorbia spp., tissue culture could eventually select strains which lack the toxic chemicals but still produce the desired latex constituents. This ability to select for such characteristics will be absolutely essential if these plants are to become a viable cash crop.

Whenever vegetative propagation of plants is economically possible, it is generally preferred to sexual propagation because genetic characteristics are better maintained. At present, common methods of vegetative propagation are rooting of cuttings and grafting, the latter generally being used where rooting of cuttings has not proven successful. However, rooting of cuttings is far from universally applicable and the use of tissue culture as supplementary technique should be seriously considered.

For Euphorbia and other hydrocarbon producing plants to be developed agronomically, further research is required. One aspect of this research will be genetic improvement of these plants for crop development. Tissue culture technology affords a means of rapidly propagating plants selected for specific growth, form, or yield characteristics.

Simmondsia chinensis (jojoba) is another plant that has great value as a source of hydrocarbons. Native to the Sonoran region of California, this shrub has a lifespan of 200 years, persists in a dioecious form (separate male and female plants), and the females produce a nut that is about 40% oil per dry weight.

The oil from the jojoba bean can be used as a replacement for automobile motor oil, a substitute for important hard waxes, and converted into bases for the production of cosmetics. An additional benefit of mass economic production and availability of the oil would be a reduction in the pressure to hunt endangered species of whales. Jojoba oil is chemically similar to sperm whale oil and can be used by the same industries that have been historically dependent on sperm whale oil. Similarly, as the National Academy of Sciences has discussed, jojoba plantations will create increased employment opportunities on lands of marginal value. In this regard, the conversion of a vegetation resource into biomass of regional and national importance is obvious and direct.

At present all jojoba seed is collected from the wild or "native" stands of vegetation. Seed availability is highly limited since most of the seed collected from these native stands are processed into oil and wax.

Under cultivation, 90% of the seedlings are male, while a 4:1 ratio of female to male plants is necessary for a successful orchard. Methods must be found to asexually produce female jojoba if this species is to become economically viable and to significantly contribute to our efforts at energy independence. Cutting-grown material has thus far produced disappointing results due to the low rooting percentages of jojoba plant cuttings.

Plant tissue culture offers a method to solve this serious problem in the economic botany of jojoba. Jojoba has been successfully tissue cultured in Israel in the laboratory environment. This research is important to Israel because of their need to expand drought resistant crops on lands of marginal productivity. In fact, Israel has committed over 10,000 acres to jojoba oil production. Unfortunately, in the United States, the excellent research work of the Israelis has yet to be reproduced on a practical demonstration basis.

The methods of cell morphogenesis are well documented and follow the pathways of normal tissue culture. Cultures are formed from meristematic tissues of desired plants, in this instance, individuals of the female sex. Concentrations of inorganic salts, organic compounds, and plant growth regulators are varied in the culture medium, causing the tissues to undergo a morphogenetic response. Generally, shoot multiplication results from this procedure, and these shoots are then rooted via separate treatment. In some cases entire plantlets can be regenerated directly. Each species requires variations in the standard procedures.

The potential exists for plant tissue culture to overcome several of the major barriers currently preventing maximum commercial utilization of two important energy crops: jojoba (Simmondsia chinensis) and gopher weed (Euphorbia lathyris). This report discusses the major advances in the tissue culture of these species, which were made by Plant Resources Institute during the period of this contract.

SIMMONDSIA CHINENSIS

Research Emphasis

The emphasis of the research with jojoba (Simmondsia chinensis) has been to develop and maximize an efficient and economic production technology for female strains of jojoba through tissue culture. While Plant Resources Institute had previously reported successful culture of seedling materials on a limited scale, this has now been applied to mature selections on a large scale. The main selection chosen for propagation was a female bush, the 'Paul Thomson' (patent pending) which has a reportedly higher yield of seeds and a larger than average nut size. Additional varieties, both male and female, were also cultured from several locations in California and Arizona. For the volume production research, however, the emphasis was placed on the 'Paul Thomson' variety.

Initial Cultures

The use of fully mature plant tissues present some problems when working with woody species such as jojobas. Modifications were necessary to successfully culture mature tissues from selections growing in the wild. It was found that shoot tips collected in late March, when a flush growth of at least 2 nodes was evident, could be cultured by the same procedure as that for six-month old seedling tips. The tips were taken from the parent plant in San Ysidro and cultured within 12 hours to give the greatest success. Tips from rooted cuttings could also be used.

Multiplication

Once cultures had been established and were growing vigorously, multiplication rates and rooting were found to be much the same as for the wild-type seedling cultures used previously. Currently, over 42,000 cultures of

the 'Paul Thomson' variety are producing shoots for rooting, which gives a production capacity of over 250,000 shoots per six to eight weeks. This clearly demonstrates the production volumes which can be attained using mature female jojobas through tissue culture. All five of the strains tested were successfully micropropagated using the medias given in Table 1. Slight modifications in the hormone levels of the multiplication medias could be made to affect the general multiplication rate, which varies between 7 and 10 fold increases per six to eight weeks, depending on the strain.

Culture Environment

Jojoba cultures were found to be temperature responsive. The greatest overall multiplication rates of 9 to 10 fold increases could only be maintained at temperatures of over $\pm 27^{\circ}\text{C}$ (80°F). At $\pm 24^{\circ}\text{C}$ (75°F) or below, multiplication rates declined with successive subcultures to below 5 fold per 8 weeks. All cultures are currently being maintained and rooted at $\pm 28^{\circ}\text{C}$ ($82-84^{\circ}\text{F}$). Cultures were initially kept on a night temperature of $\pm 22^{\circ}\text{C}$ (70°F) but it was found that a constant temperature produced better results.

Lighting, in terms of quality, intensity, and photoperiod, was not found to be as critical to production of jojoba plantlets as temperature. No appreciable differences were observed between photoperiods of 16, 18, or 24 hour days, nor were differences noted between light intensities of 200, 400, or 600 foot-candles. Cultures were maintained on both multiplication and rooting media under various lights (GE Chroma 50, GE Cool White, and GE Plant Light) without noticeable differences.

Rooting

The problem most frequently encountered with the micropropagation of jojoba is obtaining a healthy plant in the greenhouse from rooted plantlets

Table 1. Compositions of media used for jojoba.

A. Composition of Initiation Medium

Component	Concentration (mg/L)
MS Salts	(Table 2)
Myo-Inositol	100
Thiamine hydrochloride	0.4
Sucrose	30,000
N ₆ - benzyladenine (BA)	1
Naphthalene Acetic Acid (NAA)	0.001
Phytagar	7,000

B. Composition of Multiplication Medium

Component	Concentration (mg/L)
MS Salts	(Table 2)
Myo-Inositol	100
Thiamine hydrochloride	0.4
Sucrose	30,000
N ₆ - benzyladenine (BA)	1.50
Naphthalene Acetic Acid (NAA)	0.5
Phytagar	7,000

C. Composition of Rooting Medium

Component	Concentration (mg/L)
White's basal salts	(Table 2)
Myo-Inositol	100
Thiamine hydrochloride	.40
Sucrose	10,000
Dimethylamino purine (DMAP)	0.25
Indolebutyric acid (IBA)	2.0
Bacto agar	8,000

Table 2. Components of basal salts (in mg/L).

	<u>Murashige and Skoog (1962)</u>	<u>White (1963)</u>
KNO_3	1900	80
$\text{Ca}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$	---	300
NH_4NO_3	1650	---
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	370	720
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	---	16.5
KH_2PO_4	170	---
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	440	---
KCl	---	65
Na_2SO_4	---	200
KI	0.83	0.75
H_3BO_3	6.2	1.5
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	22.3	7.0
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	8.6	3.0
$\text{Na}_2\text{MoSO}_4 \cdot 2\text{H}_2\text{O}$	0.25	---
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	27.8	---
$\text{Fe}_2(\text{SO}_4)_3$	---	2.5
Na_2EDTA	37.3	---
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.025	---
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.025	---

in culture. This problem is centered on two aspects of the plantlet prior to its transfer to soil:

1. the lack of truly functional roots;
2. the lack of a properly formed plant cuticle.

Research has, therefore, concentrated on improving the nature of the plantlet in culture as well as improving techniques for the successful transfer of those plantlets into soil.

We have found that many factors influence both the quality of roots formed and the percentage of shoots which form roots. As with most tissue culture systems, the interaction of plant hormones plays an important role in the initiation of roots; with jojoba, however, the nature of the roots formed and the frequency of root initiation in culture is also closely related to the salt composition of the rooting medium and the gas exchange capacity of the culture vessel (Table 3). Thus, a wide variety of rooting responses can be obtained using a single medium composition.

On high salt mixes, especially those high in phosphates, the roots which are formed are typically thick and spongy in nature and covered with root hairs. These roots often originate from callus formed at the base of the shoot and are easily broken off when the shoot is removed from culture. Upon transfer to soil, these types of roots collapse and disintegrate, resulting in a plantlet with a non-functional root system.

Low salt formulations, such as Whites (Table 2), with the proper hormone levels, produce roots directly off of shoot stems which are thinner, stronger, and have no root hairs. These roots don't break off the shoot when it is removed from agar, and, when transferred to soil, these roots form root hairs within three days of transfer with visible root growth occurring within seven days.

Table 3. Rooting percentages for jojoba using various containers and the rooting medium shown on Table 1.

Pint jars with parafilm seal and petri dish, 100 ml/pint jar	32%
Pint jars in Ziplock plastic bag, 100 ml/pint jar	12%
Pint jars in Kingston plastic bag, 100 ml/pint jar	47%
Pint jars in Gladwrap, 100 ml/pint jar	0%
Baby food jars (lg) with parafilm seal and small petri dish lid	0%
Baby food jars (sm) with parafilm seal and small petri dish lid	0%
Baby food jars (lg) with magenta top	17%
Baby food jars (sm) with magenta top	43%
Test tubes with Kimble tops, 25 ml/H	0%
Test tubes with Kimble tops, 10 ml/H	14%
Test tubes with Bellco tops, 25 ml/H	0%
Test tubes with Bellco tops, 10 ml/H	10%
Magenta boxes, 350 ml/box, 51 divisions/box	21%
Magenta boxes, 300 ml/box, 51 divisions/box	43%
Magenta boxes, 200 ml/box, 51 divisions/box	65%

n=300

Another factor found to significantly affect the rooting of jojoba shoots is the gas exchange capacity of the culture vessel. As shown in Table 3, the same medium under the same environmental conditions will produce a wide range of rooting responses dependent upon the type of culture vessel used. In addition to the effect on rooting percentages, the quality of roots is also affected by gas exchange. Roots are formed singly or in multiples, depending on the vessels and seals; roots formed are brownish in color under conditions of less gas exchange.

The most likely explanation for the effect of gas exchange on root initiation and development is that ethylene produced by the shoots is building up inside the culture vessel to levels inhibiting root formation. Conceivably, culture vessels with an active form of gas exchange could further increase rooting of jojoba shoots in culture.

An alternate approach to rooting in vitro is to subject shoots to various treatments and place them directly into a soil mix for rooting. This has not been successful so far, although a system of this sort may still be developed using advanced culture vessels.

The culture vessel found most satisfactory for rooting jojobas was the box developed by Magenta Corporation for plant tissue culture. The amount of media is a factor which affects rooting percentages; lower amounts of media (200 ml/box or 3.9 ml/shoot) give consistently higher rooting percentages and the best quality roots (Table 3). Although these vessels are more expensive, initially, than the glass vessels, they are less expensive in the long term analysis due to the reduction in labor costs and the increased efficiency of space usage in the culture rooms.

Transfer to Soil

Rooted jojoba plantlets have been successfully transferred to soil under carefully controlled conditions. Of the soil mixes on Table 4 only the Perlite:peat mix was found effective in the promotion of functional root systems and vigorous shoot growth. The mix was watered with a solution containing MS salts (Table 1) and 1 ppm BA (N_6 -Benzyladenine) in order to overcome the transfer shock, which had previously resulted in dormancy or cessation of shoot growth. Plantlets must be maintained at high humidity during the initial period of root establishment and the soil mix must be well aerated. This can be accomplished by transferring well-rooted plantlets into dry, sterile soil mix, and watering sparingly (40-50 ml per 200 ml volume of dry mix). Keeping the plantlets in sealed containers (such as Flow Lab Plant-Con boxes) for the first seven days allows the development of root hairs and root growth through a relatively dry mix while maintaining the humidity required for the shoots.

Since a shoot in tissue culture is in a 100% humidity environment, the shock of transfer to a lower humidity -- especially if in conjunction with changes in temperature, nutrients, and lighting -- may result in death of the plantlet. Transfer must be extremely gradual with jojoba plantlets.

The maintenance of transferred plants at a constant temperature and the use of antitranspirants on the shoots when they are first exposed to outside air have also been found helpful in the successful transfer of rooted plantlets to soil.

Table 4. Variables tested for transfer of jojobas to soil.

A. A list of the soil mixtures used to test the transfer of jojoba plantlets from sterile culture.

Components	Ratio
1. Peat:Vermiculite:Sand	1:1:1
2. 1/2 of 1 + 1/2 Sand	
3. 1/3 of 1 + 2/3 Sand	
4. 2/3 of 1 + 1/3 Sand	
5. Sand:Perlite:Vermiculite:	1:1:1
6. Sand	
7. Pumice	
8. Peat:Perlite	1:10

B. List of environmental parameters tested for the transfer of jojoba plantlets from sterile culture.

Greenhouse:

1. Mist with shading
2. Mist without shading
3. Saran covered flats with shading
4. Saran covered flats without shading
5. Hand mist 4 times daily with shading

Laboratory Growth Room:

1. Tubepak containers with various soil mixes
2. Jars with petri dish covers with pasteurized and sterilized mixes shown in Table 4A.
3. Flow Lab Plant-Con containers with sterile mixes

EUPHORBIA LATHYRIS

Research Emphasis

The primary emphasis of the *Euphorbia lathyris* program was to develop a micropropagation technology and, secondly, to develop that technology along economically feasible lines. Very little has been published regarding the tissue culture of plants in the genus *Euphorbia*; and, for the species, lathyris, no publications exist to this date reporting anything other than callus tissue induction. One other researcher, Dr. Keith Redenbaugh (unpublished results) is working with development of tissue culture system for Euphorbia lathyris.

Initial Cultures

Cultures of Euphorbia lathyris were initiated from 7-9mm shoot tips of six to 12 month old seedlings kept under greenhouse conditions. The shoot tips were sterilized by immersion in a 20% Clorox (.01% NaOCl) solution containing .01% Tween 20 for 17 minutes under constant vacuum and stirring. The tips were then rinsed three times in sterile deionized water and trimmed to a final length of 4 mm. Explants were placed on the initiation medium shown on Table 5 for the first eight weeks.

Several 100-media grids were used to screen appropriate hormone combinations for initiation media. The 50 media hormone grid shown on Table 6 was repeated twice ($n=20 \times 2$) and shows the effect of different cytokinins on initial bud cultures. The 1-5 ppm range of 2iP (6-gamma-gamma-Dimethylallyl Amino Purine) is clearly better than the other hormone concentrations tested and appears to be independent of the auxins used in this experiment. The media containing kinetin gave consistently poor results, the buds becoming deformed and necrotic.

Table 6. Percentage of apical explants of Euphorbia lathyris forming multiple buds after 8 weeks in culture (n=20).

	Auxin Concentrations (mg/L)				
	0	NAA 0.01	NAA 0.1	IAA 0.01	IAA 0.1
0	0	0	0	0	0
BA 0.5	20	20	15	10	10
BA 1.0	25	10	10	5	0
BA 5.0	10	15	5	10	5
K 0.5	0	0	0	0	0
K 1.0	0	0	0	0	0
K 5.0	0	0	0	0	0
2iP 0.5	10	10	15	20	40
2iP 1.0	90	85	75	95	95
2iP 5.0	95	95	85	50	5

Cytokinin Concentrations (mg/L)

Multiplication

The BA (N_6 -Benzyladenine) media did not produce as many multiple buds on the initial cultures, but there was much better shoot development. Repeated subcultures resulted in the choice of a BA-containing medium as the best long term multiplication media (Table 5). Repeated subcultures on the 2iP media showed an overall higher production of buds but these did not develop into healthy shoots and often the cultures turned black.

Cultures which were established on the multiplication medium (Table 5) grew vigorously and had an average multiplication rate of 5.3 fold increases per six weeks.

Callus Cultures

Callus cultures of E. lathyris could be initiated on a medium containing 1 ppm BA and 0.1 ppm NAA from leaf tissues and bud tissues taken from 12 month old greenhouse grown seedlings. Callus was grown on the multiplication medium for shoots (Table 5B) in light. Regeneration studies have not been initiated.

Rooting

Several approaches were used for the induction and development of roots on shoots obtained from the multiplication media. The simplest approach, and the most cost effective, is to root shoots directly in soil mixes. The two mixes used were sterile peat:vermiculite:sand (1:1:1) and peat:perlite (1:10) mixes. Shoots were excised and either treated with commercial rooting powders (Hormodin & Rootone F) or left untreated. These shoots were planted in the sterile mixes and kept under conditions of high humidity for 12 weeks without showing any signs of root initiation. Experiences with Euphorbia

Table 5. Compositions of media used for Euphorbia lathyris.

A. Composition of Initiation Media

Component	Concentration (mg/L)
MS Salts	(Table 2)
Myo-Inositol	100
Thiamine hydrochloride	0.4
Sucrose	30,000
6-gamma-gamma Dimethylallyl Amino Purine (2iP)	5.0
Indoleacetic acid (IAA)	0.1
Nutrient broth	1000.0
Phytagar	7000

B. Composition of Multiplication Media

Component	Concentration (mg/L)
MS Salts	(Table 2)
Myo-Inositol	100
Thiamine hydrochloride	0.4
Sucrose	30,000
N ₆ -Benzyladenine (BA)	0.5
Indoleacetic acid (IAA)	0.1
Phytagar	7,000

C. Composition of Rooting Media

Component	Concentration (mg/L)
MS Salts	(Table 2)
Vitamins	(Table 6)
Sucrose	2,000
6-gamma-gamma Dimethylallyl Amino Purine (2iP)	2.0
Indolebutyric Acid (IBA)	5.0
Bacto Agar	8,000

lathyris shoot cuttings under greenhouse conditions have shown that the stem ends have to be dried for $\pm$ one hour for treatment with a rooting powder to be effective; and since tissue cultured shoots cannot withstand drying, this method of direct in-soil rooting does not appear feasible.

Shoots cultured on the multiplication medium were also transferred to media grids for rooting evaluation. The first such grid consisted of 30 different media varying the IBA (Indolebutyric Acid) concentrations from 0 to 10 ppm and the BA concentrations from 0 to 0.3 ppm, using a basal composition of White's salts (Table 2), Sucrose (20 g/l), and Bacto Agar (8 g/l). None of these shoots developed roots and very little growth of the original shoot was observed. From this it was concluded that a low salt medium was not optimal for growth and rooting induction. Cultures were maintained for 12 weeks on the grid with no visible changes, except basal swelling occurred on shoots cultured on the 10 ppm IBA media. These shoots ultimately became necrotic and died.

Shoots were also cultured on a high salt base media grid with a complex of vitamins (Table 7) added to the basal formula. The hormone concentrations were again varied: between 0 and 5 ppm for the IBA and 0 to 2 ppm for the 2iP resulting in a grid of 18 media (Table 8). A basal sucrose concentration of 2 g/l and a 8 g/l concentration of Bacto Agar were used with all media on this grid. Only the medium containing 2 ppm 2iP and 5 ppm IBA produced any primordia (Table 8). These primordia developed root hairs but did not elongate into normal roots. Additional research into media variations is needed to determine an optimum media composition for the production of rooted Euphorbia plantlets. Transfer to soil of the shoots having visible roots of the type discussed above may result in elongation and development of the roots into a functional root system.

Table 7. Concentrations of vitamins used.

Component	Concentration (mg/L)
Myo-Inositol	18.00
Thiamine hydrochloride	0.04
Nicotinic acid	0.50
Pyridoxine hydrochloride	0.20
Biotin	0.01
Riboflavin	0.04
Ascorbic acid	0.02
D-Ca-Pantothenate	0.10
L-Cysteine hydrochloride	1.75
Glycine	0.04
Folic acid	0.50

Table 8. Percentage of shoots of Euphorbia lathyris forming root primordia after four weeks (n=10).

Cytokinin Concentrations (2iP)(mg/L)

Auxin Concentrations (IBA)(mg/L)						
	0	.001	.01	0.1	1.0	5.0
0	0	0	0	0	0	0
0.2	0	0	0	0	0	0
2.0	0	0	0	0	0	60%

Transfer

Transfer to soil of E. lathyris plantlets on a large scale has not been attempted due to difficulties encountered in development of a rooting medium. Preliminary results indicate that E. lathyris shoots in vitro have a better cuticle covering and transfer to lower humidities does not appear to be as difficult as with jojobas.

ECONOMIC FEASIBILITY

The commercial applicability of any tissue culture system depends on the economic competitiveness of the system in comparison to alternate systems already in production. The use of tissue culture in a production system becomes increasingly cost efficient when production volumes become high enough to amortize the fixed costs of the laboratory to a level where they become insignificant by comparison to the direct labor and materials costs. In evaluating the cost effectiveness of a tissue culture system, additional factors besides simply cost-per-unit-product may be considered, such as: limitations on the volumes produceable via alternate methods, the ability to produce the product on a predictable schedule, or significant improvements in quality of the product.

In this report, tissue culture has been applied to determine the feasibility, both technical and economic, of mass producing jojoba and Euphorbia plants. The immediate factors which are critical to the economic aspects of this technology are:

- What is the cost-per-plant of a tissue cultured plant versus a seedling or cutting?
- What is the availability of seedlings and cuttings versus the production capacities via tissue culture?
- What immediate economic benefits can be realized using this technology?

If the technology is being evaluated from a long term approach, additional factors also become relevant, such as:

- Is the market for these plants, especially for special selections of these plants, expected to expand with time?
- Are the costs of competitive products increasing at a more rapid pace than products derived from these plants?

Simmondsia chinensis

On a simple cost-per-plant basis, tissue cultured jojobas are currently economically feasible as an alternative to cuttings. Cuttings are currently selling for \$0.75 (American Jojoba Industries, Inc., personal communication). Jojobas from tissue culture cost \$0.17 (Table 9) to produce to greenhouse size, resulting in a selling price of \$0.80 when transfer and greenhouse costs are calculated in. This indicates tissue culture can become a viable alternative to cuttings.

In the jojoba market, there are several factors limiting the implementation of tissue cultured plants into field plantings. The most critical is the lack of well-established superior strains of plants. Since jojoba plants do not bear fruit for several years, it is time consuming to establish that a new variety has a consistently higher yield per acre or is more drought or frost resistant. To avoid the potential problems of monoculture plantations it would be necessary to have several high yield varieties for each of the major planting locations. This will require years to accomplish via conventional plant breeding programs.

The economics of a tissue culture system for mass propagation are enhanced when the plant in question is significantly unique when compared to other individuals within the same species. In the case of jojoba, two major considerations favor production of plants via vegetative methods over production using seed. The first consideration is that vegetative propagation produces known-sex plants, whereas seedlings can be expected to be over 50% male. The nut of the jojoba plant is only produced on plants having female parts and so, from a plantation point of view, seedling material is less desirable because multiple-seeded planting and subsequent thinning are currently required. Since

Table 9. Estimated costs of tissue cultured jojoba plantlets (prior to greenhouse transfer) in production quantities of 10,000/month.

	<u>Cost/10,000</u>	<u>Unit Cost</u>
<u>A. Wage and Salary Cost</u>		
1. Start-up labor (amortized over 24 months)	\$ 2.57	0.03¢
2. Explanting labor	480.00	4.80
3. Media preparation	80.00	0.80
4. Production supervision	<u>352.00</u>	<u>3.52</u>
Total Wage and Salary Cost	\$ 914.57	9.15¢
<u>B. Media Cost and Plant Materials</u>		
1. Start-up media and plant materials (amortized over 24 months)	\$ 8.33	0.08¢
2. Production media	<u>60.00</u>	<u>0.60</u>
Total Media Cost	\$ 68.33	0.68¢
<u>C. Culture Vessel Cost</u>		
1. Start-up culture jars (amortized over 24 months)	\$ 0.21	0.00¢
2. Petri dishes	78.00	0.78
3. Culture jars (amortized over eight 8-week cycles)	<u>43.70</u>	<u>0.44</u>
Total Culture Vessel Cost	\$ 121.70	1.22¢
<u>D. Equipment and Laboratory Cost</u>		
1. Equipment (Table 10) (amortized over 60 months at 3/4 lab use)	\$ 360.00	3.60¢
2. Laboratory facility (amortized @ 12% - 5-year lease)	<u>87.50</u>	<u>0.87</u>
Total Equipment and Laboratory Cost	\$ 447.50	4.47¢
<u>E. Overhead Costs</u>		
1. Office, expenses	\$ 51.69	0.52¢
2. Utilities (electricity, water, gas, telephone)	62.80	0.63
3. Other indirect costs	<u>22.19</u>	<u>0.22</u>
Total Overhead Costs	\$ 136.68	1.37¢
TOTAL COSTS	<u>\$1688.78</u>	<u>16.89¢</u>

Table 10. Tissue culture equipment.

Steam Generator and Autoclave	\$14,700
Laminar Flow Hood	4,000
Water Purification/Distillation Apparatus	2,100
Analytical Balances	4,700
pH Meter	600
Growth Room Equipment	1,700
Dishwasher	200
Refrigerator	<u>800</u>
Total Tissue Culture Equipment	\$28,800

jojobas do not flower until they are $\pm$ three years old, the sex of the seedling cannot be determined until some time after planting, and a labor-intense thinning process adds considerably to the costs of production via seed.

The second basis for vegetative production techniques is the current lack of genetically stable superior seed. Single plant selections are being made and propagated by cuttings, but this is not always successful. In addition, production volumes of over 10,000 per month are not easily attainable.

The jojoba is a plant whose economic potential lies in maximizing productivity on marginal lands of the semi-arid Southwest. Crop plantations would require different selections of jojobas, depending on the special requirements of the specific area. If the ideal of maximum productivity with minimum energy input is to be approached, special selections will have to be developed for areas of drought or severe frosts. Development of selections having higher oil productivity per acre will remain a high priority for all areas.

Although breeding programs are underway, it is expected that selections will not be available as quickly as is possible with vegetatively propagated strains. Since jojoba seedlings require several years to flower and produce seed, a conventional breeding program is much more time consuming than it is with plants which flower in the first year. Even when a jojoba plant has reached the point of flowering and nut production, it does not attain full volume production for several additional years. Consequently, seed would not be available from a genetically stable seedling stand in plantation volumes for the first five to ten years, depending on variety; and strains being developed for high productivity could not be analyzed until volume production levels were attained.

Tissue cultured jojoba can only become cost effective over seedlings if proven superior strains, which are not available from seed, can be developed.

Tissue cultured plants can become cost effective over cuttings when insufficient cutting stock is available or when the selection is difficult to root from cuttings. The discovery of a monoescious jojoba plant by Dr. Yermanos of the University of California, Riverside, points out that breeding may result in superior seedling selections which would not require multiple-seeding or thinning. Tissue culture could provide several thousands of these monoe-sciuous plants for use in field studies and seed production.

On the long term evaluation, the tissue culture production of jojoba plants can only become economically feasible if the demand for a selection becomes greater than the volume production capabilities via cuttings or if the costs of tissue cultured plants become less than those for cuttings. Although lab production costs in terms of materials and labor can be expected to increase, the actual cost of tissue cultured plants may decrease as improved transfer and greenhouse procedures are developed. The dependence of cutting operations on time of year and the difficulties in obtaining commercially acceptable rooting percentages for many strains of jojoba may contribute to the commercial feasibility of a tissue culture propagation industry.

The jojoba industry as a whole is still in its early stages of development. As the market develops and expands, as new ways are found to reduce the costs of the oil (through mechanized planting and harvesting, superior strains, and byproduct utilization) and as the costs of competitive oils increase, the mass cultivation of jojoba for its oil can become an economically viable proposal and the use of tissue culture technologies to produce the new strains will become increasingly cost effective.

Euphorbia lathyris

A tissue culture system for mass production of Euphorbia lathyris can become economically feasible only if a rapidly growing demand for a highly superior selection results in a shortage of available plants. Although not currently commercially produced, E. lathyris roots well from cuttings (unpublished data) and sets seed in its first year. An established market does not exist for this plant as yet and breeding programs might yield positive results before the market demands have risen to a point where mass production becomes economically viable. Since the cost of seed would be much less than the cost of a tissue cultured plantlet, once an acceptable quality of seed is available, economic feasibility of a tissue culture propagation system will become possible only on a limited scale.

If an acceptable quality seed is not developed, the market demand for a specific selection has to be quite high for tissue cultured E. lathyris plants to become cost effective as compared to plants made from cuttings.

The cost-per-plant of an E. lathyris plant from tissue culture is comparable to that of a jojoba (Table 9), with a slightly higher cost of labor and materials due to the lower multiplication rates achieved in the multiplication phase. This could be offset by reductions in all of these costs should a higher multiplication rate or a high rooting percentage and transfer efficiency be attained. Additionally, reductions in greenhouse costs may result in more economically competitive plant costs.

The long-term mass-production of E. lathyris seems economically unfeasible given the ease of rooting cuttings and the potential of producing superior seed through concentrated breeding programs. The major economic potential of a tissue culture system for Euphorbia lies in its application to the development of superior strains. This can be accomplished in several ways:

- development of new strains using such technologies as genetic engineering, protoplast fusion, etc.;
- screening of plant tissues in culture for selection of high-producing strains;
- mass production of a newly selected strain to establish stock fields for seed or cutting production.

In the development of new plant strains, tissue culture is currently limited to research involving only a few species of plants. The technologies of genetic engineering, protoplast fusion, in vitro mutagenesis, etc., have not yet reached the point of widespread applications for breeding purposes; but rapid advances indicate that these technologies will be perfected for practical applications much sooner than expected only a few years ago. The basis for practical application of these technologies is a sound tissue culture system including all the steps for regeneration of a plant from a single cell. The logical progression for a system being developed for protoplast manipulation is:

- establish a system for in vitro shoot development, rooting and transfer to greenhouse;
- establish a system for callus induction, growth and maintenance; regenerate plants from callus;
- establish cell suspension cultures and regenerate plants from cell suspensions;
- establish protoplast isolation techniques and regenerate plants from protoplasts.

The development of a basic tissue culture system provides the framework for application of the new technologies aimed at increasing actual field

productivity. The expense of this type of research will be feasible from an economic standpoint if the potential advances will produce significant increases in productivity. In the case of E. lathyris, or any wild-type plant, the increases in productivity resulting from an intensified breeding program, such as is offered with tissue culture technologies, are potentially of the same order as the advances in corn productivity from the original wild species.

For plants which do not have sufficient natural genetic variability needed to increase the productivity of a specific compound to economically acceptable levels, these new technologies offer a wide range of possibilities for genetic modification. Tissue culture technologies are the logical supplements to conventional breeding programs for a plant of potential economic significance which is to be taken from a wild state and domesticated for crop productivity in the shortest possible time. The tissue culture system for E. lathyris presented in this report represents an important step towards the utilization of tissue culture technologies for development of superior strains of this plant.

DISCUSSION

Several major accomplishments have been reported for this project. Fully mature tissues of female jojoba plants have been successfully cultured in volumes designed for commercial operations. Euphorbia lathyris plants have been established and multiplied in vitro and the culture conditions, medias, and procedures reported.

The parameters for successful rooting of jojoba plantlets as well as a defined culture medium have been established. A method for successful transfer of rooted plantlets to soil has been reported.

Additional research is still required, however, if either of these plants are to be economically propagated through tissue culture. For the jojoba, the main points which must be addressed are:

- economic greenhouse transfer technology
- paired clones of both male and female strains
- field planting.

Economic transfer methods must be developed using commercial volume batches of rooted plantlets in order for accurate cost-per-plant estimates to be made. The technique of tissue culture must be demonstrated for a number of commercially attractive male and female plants. Selections of male plants which flower complementary to the female selections could result in plantations requiring a ratio of 8:1 (females per male) instead of 4:1 as is currently being used. Field plantings must be accomplished to demonstrate the survival of tissue cultured plants during the first year, to establish the hardiness of these plants when compared to seedlings and cuttings. Mechanical planting systems should also be tested to determine whether they can be applied to large field planting of tissue cultured plants.

For E. lathyris, basic techniques of the tissue culture system remain to be expanded and commercially developed. High rooting and transfer efficiencies in conjunction with improved multiplication rates must be developed. Organogenesis and plant regeneration from callus cultures should be demonstrated. A system of cell suspension cultures and protoplasts is necessary to establish a base for genetic manipulation of this species. Other species of Euphorbia which also have potential use as crops or for breeding studies with E. lathyris should be established in vitro.

For both jojoba and Euphorbia strains, there are several avenues which can be pursued with the goal of new crop development in mind. Tissue culture is one such avenue which offers great potential in terms of both the development and the mass production of new strains of these hydrocarbon and oil producing plants.

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