Number 109, 1984 ISSN 0362-0069

New York's Food and Life Sciences Bulletin

New York State Agricultural Experiment Station, Geneva, a Division of the New York State College of Agriculture and Life Sciences, a Statutory College of the State University, at Cornell University, Ithaca

A METHOD FOR LARGE SCALE *IN VITRO* PROPAGATION OF *VITIS*

Raymond Chee, Robert M. Pool, and Donald Bucher

INTRODUCTION

Commercial grapevine propagation has not radically changed for centuries. Recognition of critical environmental restrictions and phytosanitary requirements have lessened the chance for failure, but the basic methods used today are those of our ancestors. Own-rooted grapevines are produced by rooting 12-to 18-inch dormant hardwood cuttings. This technique has survived because it is economical and efficient. One basic drawback of the system, however, is that it does not allow a rapid buildup of grape material that is in limited supply. More and more often today as a result of clonal selection programs, as unique virus-free clones are identified by grape virologists, or as new varieties are produced by grape breeders, there is a need for the rapid buildup of unique specimens so that vines may be available in sufficient quantities for commercial production. One method that has evolved to meet this demand is the so called "rapid propagation" of vines, whereby softwood cuttings are rooted, forced into growth, and repropagated as soon as possible. This technique has enabled nurserymen to supply large numbers of vines in a short period of time.

This bulletin describes an alternative method, *in vitro* vegetative multiplication or micropropagation, which has grown out of laboratory studies on the growth of detached organs using tissue culture techniques.

It is important to understand what we mean by *in vitro* vegetative multiplication. Fundamentally, vegetative multiplication is the result of mitosis or division of cells without altering the genetic make up of the plant. This can be extended to the multiplication of plant organs. *In vitro* literally means in glass and refers to the use of tissue culture techniques under carefully controlled laboratory conditions. Therefore, *in vitro* vegetative multiplication refers to the rapid propagation of a specific clone so that the genetic makeup is unaltered and the basic biological, physiological, and horticultural characteristics are preserved.

Within the context of tissue culture, this technique would be referred to as organ culture. It excludes the use of substances or physical treatments that lead to a basic disruption of normal organogenesis and cellular differentiation. Undifferentiated tissue growth (callus) or adventitious shoot production (as obtained with fragmented leaf tissue) are avoided. The genetic variation obtainable via callus or protoplast culture is of interest to the breeder but a nuisance to the propagator.

Although tissue and organ culture have great potential practical applications, it has had only limited use in viticulture. The primary use has been the elimination of grapevine diseases via meristem culture (4,6). As with most woody perennials considerable difficulties were encountered and recovery percentages were low. By refining these techniques we have developed a method of *in vitro* vegetative propagation of grapevines. It has staggering potential; from a single vine a nursery might propagate 10 vines in a year using hardwood cuttings. With the method described here one person is able to propagate 2,000 vines or more a month.

PHASES OF MICROPROPAGATION OF GRAPEVINES

We distinguish six phases of micropropagation: (1) selection of explants, (2) establishment of the explants *in vitro,* (3) shoot production from these established explants, (4) shoot multiplication from thesubcultured shoots,(5) rooting of the subcultured shoots, and (6) transfer of propagules to soil (Fig. 1).

The experiments that were performed in order to

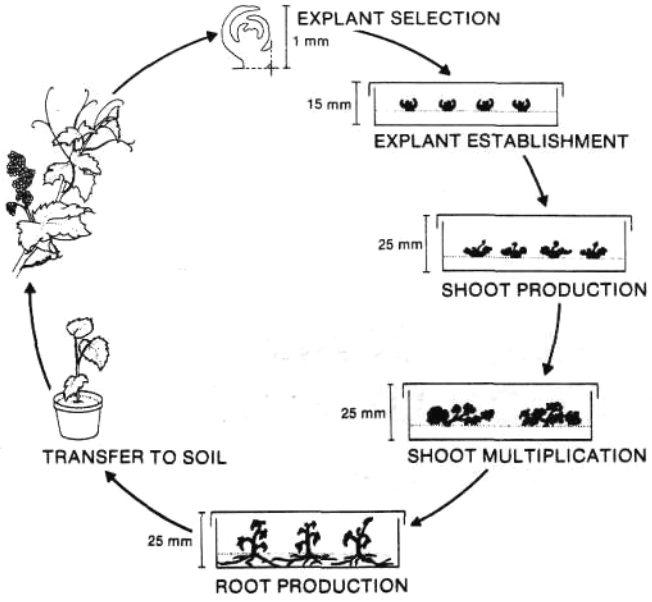

Figure 1. Phases of micropropagation of grapevines.

develop this technique are reported elsewhere. In them we investigated inorganic media constituents, organic media constituents, growth regulators, light conditions, and their interactions for the differing phases of *in vitro* vegetative propagation of the graps cultivars 'Remaily Seedless' (2) and 'Rougeon' (1,3). Selected media and incubation conditions were also used to establish in culture, multiply, and root 21 grape genotypes (2).

MATERIALS

The culture vessels for multiplication and rooting in the studies reported here were sterile plastic petri dishes 100 mm in diameter and 25 mm high. Deeper plates (15 mm high) were used to establish the plant material in culture. They contain 35 ml of medium and were closed with a 'Parafilm' plastic film overwrap. Recent experience demonstrated that screw top glass 60 x 90 cm jars closed with a deep plastic cap, available from Magenta Corporation (see appendix 2), give equivalent or superior results.

Illumination of the cultures is provided by a 1:1 mixture of "Gro-Lux" and "Cool White" fluorescent tubes (F40, Lifeline series, manufactured by Sylvania). The "Cool White" spectrum alone is satisfactory. The tubes are 14 cm apart and 30 cm above the cultures. This gives an irradiance of 1900 uWcm"^{2}at the explant level. Both 10 and 16 hour days were tested. The great majority of genotypes tested did as well or better under the short day regime. Thus, because of energy savings, 10 hour light periods are recommended. Temperatures range from 23 C in dark to 27 C in light.

The culture medium contains the salts, C2D, or those of Murashige and Skoog (Table 1) as indicated forihe different steps. Also included are the vitamins used by Chee(1) as described in Table 2. Needed growth regulator additions are benzylaminopurine and naphthaleneacetic acid in concentrations indicated for each phase (Table 2). Sucrose is added at 3 per cent for establishment and multiplication, and 1 per cent is used for the rooting phase. The medium is solidified with 0.7-0.8 per cent "Bacto- Agar" (DIFCO Laboratories). The pH should be adjusted to 5.7-5.8 before autoclaving.

Table 1. Inorganic media constituents used for *in vitro* vegetative propagation of *Vitis*.

Compound	C_2D, 1982		Murashige and Skoog, 1962	
Macronutrients	mM	mg l^{-1}	mM	mg l^{-1}
NH_4NO_3	20.6	1650	20.6	1650
KNO_3	18.8	1900	18.8	1900
$MgSO_4.7H_2O$	1.5	370	1.5	370
KH_2PO_4	1.25	170	1.25	170
$CaCl_2.2H_2O$	--	--	3.0	440
$Ca(NO_3)_2.4H_2O$	3.0	709	--	--
$FeSO_4.7H_2O$	0.1	27.8	0.1	28
$Na_2.EDTA$	0.1	37.3	0.1	37
Micronutrients	µM	mg l^{-1}	µM	mg l^{-1}
KI	--	--	5.0	0.83
$MnSO_4.4H_2O$	5.0	0.845	100.0	22.3
H_3BO_3	100.0	6.2	100.0	6.2
$ZnSO_4.7H_2O$	30.0	8.6	30.0	8.6
$Na_2MoO_4.2H_2O$	1.0	0.25	1.0	0.25
$CuSO_4.5H_2O$	0.1	0.025	0.1	0.025
$CoCl_2.6H_2O$	0.1	0.025	0.1	0.025

Table 2. Media constituents and incubation conditions used for *in vitro* vegetative propagation of *Vitis*.

Inorganic media constituents
salts, C_2D, 1982; also Murashige and Skoog's 1962.

Organic substance in µM
thiamine.HCl (3); myo-inositol (55.5); nicotinic acid (8); pyridoxine.HCl (5)

Growth regulators
establishment in culture :0.5 µM NAA and 5 uM BA
shoot production and multiplication:5 µM BA
root production :0.4 µM NAA

Other media factors
Sucrose: 3 % (1 % for rooting)
Bacto-agar (DIFCO):0.7-0.8 %
pH:5.7-5.8

Incubation conditions
temperatures: from 23 C in dark to 27 C in light
light source: mixture "Cool White":"Gro-Lux" (1 to 1); also "Cool White" alone is suitable
daylength: 10 hours
irradiance: 2000 µW cm^{-2}
40 W fluorescent tubes, 14 cm apart, 30 cm above cultures

SELECTION OF EXPLANTS

Shoots are obtained from one node cane cuttings forced in water under the incubation conditions described in the Materials section. Shoot tips (2 to 3 cm

long) are collected at the separate flower cluster stage (Fig. 2). Flower clusters and expanded leaves are removed. The shoots are surface sterilized in a solution of distilled water, 1.2 per cent sodium hypochlorite, and 0.1 per cent "Tween 20" for 10 minutes, and are then thoroughly rinsed in sterile water.

Explants can also be successfully obtained from vines growing in the vineyard or in the greenhouse. Collections made from the middle of June to the second half of July give best results. For field collection, about 10 4 to 6 cm long shoot tips, with leaves attached are placed into small (10 x 20 cm) plastic bags. These are sealed and kept on ice. The shoot tips should be processed as above within 4 hours of collection.

ESTABLISHMENT IN CULTURE

The medium used forthe results described here contains the salts of Murashigeand Skoog, 5JLJM benzylaminopurine, 0.5^iM naphthaleneacetic acid, and 3 per cent sucrose. We have also successfully used the salts of C2D with apparent success.

Shoot apices0.5 to 1 mm long (containing the apical dome and 2 to 4 leaf primordia) are exised from sterilized tips. Four or more explants are plated per dish. The cut surface is pressed lightly into the medium. The apices should be cultured for 4 to 6 weeks. Shoot tips . of 1 cm length (comprising the apical bud and one ' visible node with its leaf removed) can also be established in culture. In this case naphthaleneacetic acid is not required in the medium.

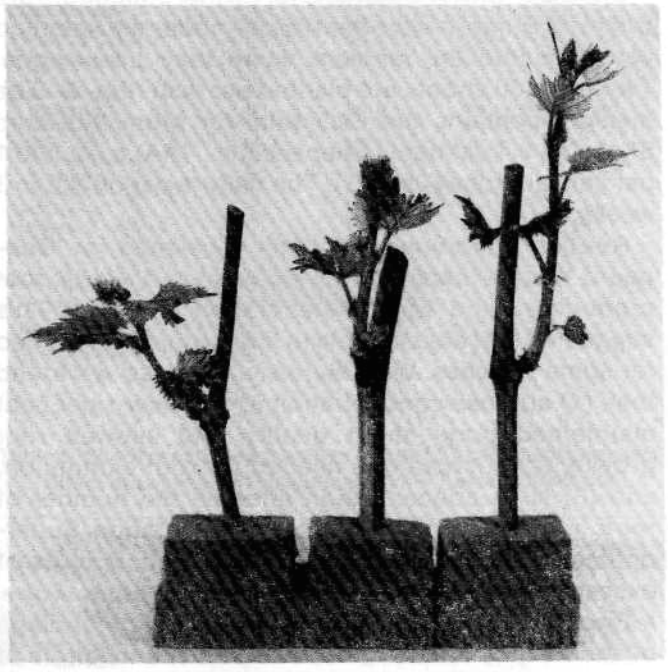

Figure 2. Forcing cane cuttings for explant selection.

Most often, the 0.5 to 1 mm explants grow into rosettes of leaves (Fig. 3), although sometimes shoots are produced depending on the genotype. If the explants are 1 cm shoot tips, shoots of variable size can be expected. Their number is dependent on the genotype. With 'Remaily Seedless' we averaged 10 shoots per explant.

We tested this technique with 30 grape genotypes, and all were successfully established in culture except 'Seyval'.

FIRST SHOOT PRODUCTION

This step is not necessary when shoots are obtained during the establishment step or when using larger shoot tip explants.

The cultures obtained in the first step are transferred "as is" to sterile dishes containing the medium of Murashige and Skoog (or C2D), 5juM benzylaminopurine, and 3 per cent sucrose. Three to four explants should be subcultured in a single plate. They are cultured for 6 to 8 weeks. From 1 to 48 shoots were obtained per explant in our experiments.

SHOOT MULTIPLICATION

Ideally shoots that are 1.5 cm long with three to four visible nodes are selected for further culture. They should be trimmed 2 mm below the basal node and placed horizontally and lightly pressed into the medium taking care that their cut ends are submerged. The medium in the plates contains C2D salts, 5juM benzylaminopurine, and 3 per cent sucrose. The culture period is 3 to 6 weeks (Fig. 4).

Ideally, the incubation conditions and media should be defined for each *in vitro* phase and each genotype

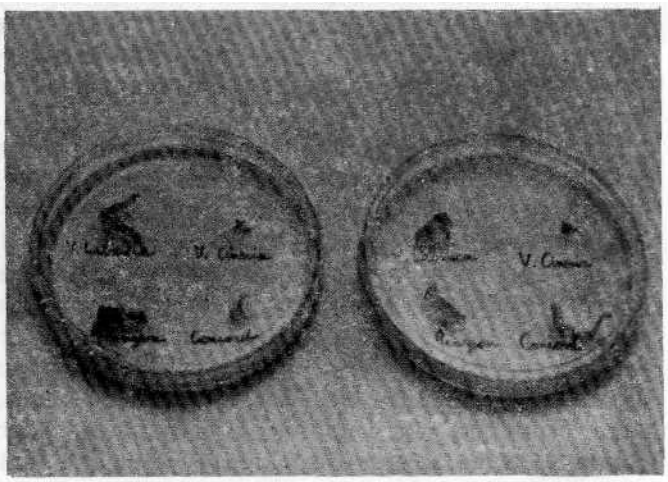

Figure 3. Vitis shoot apices after 30 days in the establishment in culture phase. Note variation of growth among cultivars.

Figure 4. Shoot multiplication from 3 to 4 node (1.5 cm) subcultured shoots of Vitis 'Remaily Seedless' after 4 weeks. Three shoots were cultured per plate.

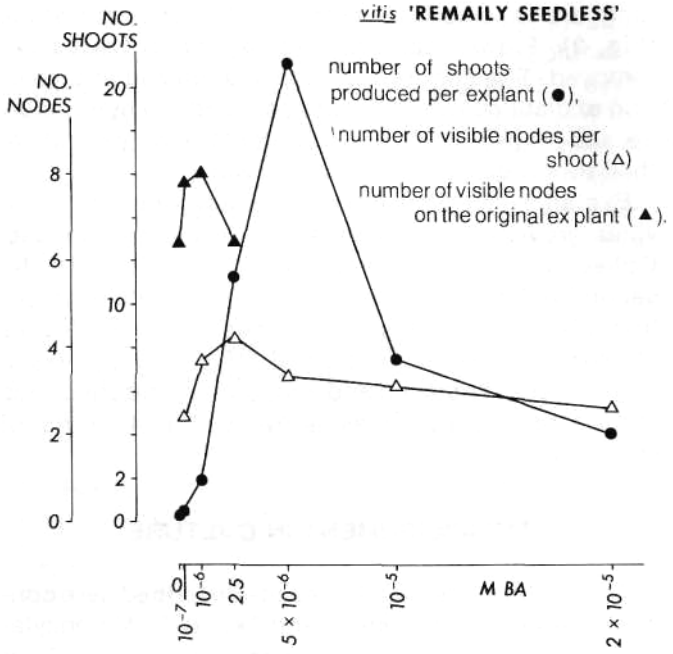

Figure 5. Effects of benzylaminopurine on number of shoots produced per explant, number of visible nodes per shoot and number of visible nodes on the original explant.

grown. This is most important for the shoot multiplication phase, the core of a rapid propagation method. Our research demonstrates the benefits of the additions of the vitamins and inositol (Table 2) (2). Experiments with benzylaminopurine (BA) concentration show that there is a very narrow concentration peak for maximum shoot production of'Remaily Seedless'(Fig. 5). Ourexperience with other genotypes indicatesthat the specific concentration of cytokinin may need to be readjusted in some cases.

We also compared basal salts of well known media used by others for culture of woody plants and investigated specific inorganic nutrients. This led to the development of the C2D salts. Using these salts we increased the rate of shoot multiplication in comparison with salts of Murashige and Skoog that gave us best results in previous experiments. The increase was from 40 per cent to 350 per cent depending on the variety. With 'De Chaunac' we obtained no improvement. Shoot production on the C2D salts is given in Table 3 for various genotypes.

Because continuous shoot production is needed in a rapid propagation program, shoot multiplication was investigated for 'Remaily Seedless' through six subsequent subcultures. The results are given in Table 4. More shoots were produced in the last three cultures than in the preceding ones. This probably reflected the result of more care in selecting high quality explants in the later stages when the profusion of available shoots let us be more selective. On average, 14 suitable shoots were produced per explant per subculture. With 'Remaily Seedless', once the material was established, we were abletocontinously obtain subcultures for 43 weeks.

The potential for vegetative micropropagation is indicated in Table 5. If we had subcultured every 3 node shoot produced, starting from a 1 cm shoot tip established in culture, the crop at 330 days would have been 75,000,000 shoots of adequate size.

ROOT PRODUCTION IN VITRO

Shoots of the type suitable for further subculture are trimmed 2 mm below their basal node, and planted vertically into the medium up to their basal node. Twenty to 30 shoots are planted per dish (Fig. 6). The medium contains the C2D salts (or Murashige and Skoog's), 0.4 wM naphthaleneacetic (NAA) acid, and 1 per cent sucrose (Table 1). The culture period is 2 to 3 weeks.

Rooting conditions were first developed for 'Rougeon'. When 'Remaily Seedless' was used, erratic root production, and shoot deterioration occurred. This suggested that adjustments to the medium are needed for some varieties. With 'Remaily Seedless' we found that: (1) sucrose concentration at or below 1 per cent maintained good shoot quality and allowed root growth, (2) sucrose was needed for root elongation, and (3) NAA concentration affected the number of roots initiated at a given sucrose concentration. Superoptimal NAA concentrations result in short roots of excessive girth.

Root initiation starts within 2 weeks. The percentage of shoots that initiate roots, the number and length of roots produced, and the appearance of the shoots varies among genotypes (Table6). In general, good shoot

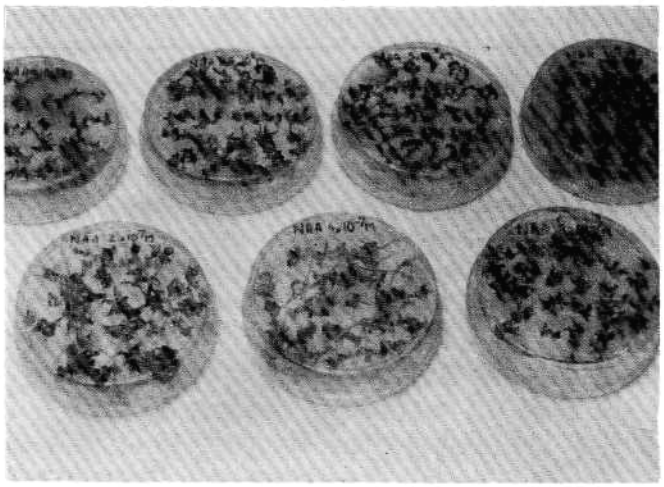

Figure 6. Rooting of subcultured 1.5 cm grapevine shoots in vitro. Best rooting is with 0.4 µM naphalenacetic acid (NAA). Plates are seen from underneath.

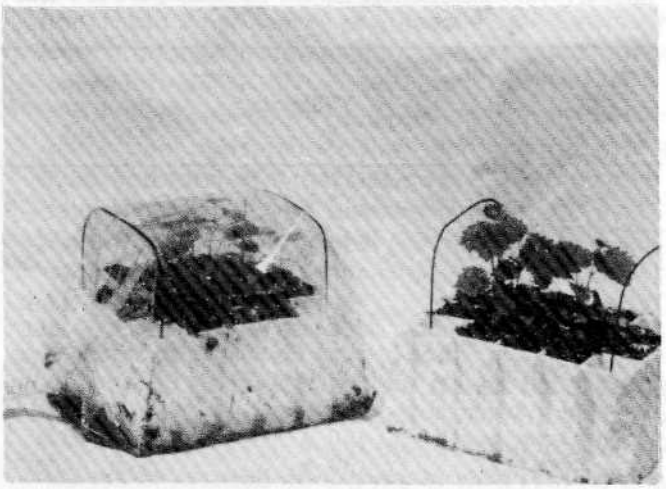

Figure 7. Transfer of Vitis 'Remaily Seedless' propagules from in vitro conditions to soil.

Table 3. *In vitro* **culture of** *Vitis*. **Shoot multiplication from 3 to 4 node (1.5 cm) shoot explants (6th subculture). Results at 67 days. Salts, C_2D of Chee, 1982.**

	Number of explants planted	Explants alive (%)	Explants with shoots (%)	Number of shoots/[y] explant
Vitis species[x]				
argentifolia	9	100 a	100 a	15 abc
labruscana 'Concord'	18	100 a	100 a	20 a
labrusca 'Alba'	18	100 a	100 a	10 cd
riparia 'Pulliat'	12	100 a	100 a	18 ab
labruscana 'Catawba'	14	0 b	0 b	0 f
Vitis vinifera				
'Cabernet Sauvignon'	15	100 a	100 a	5 def
'Gewurztraminer'	15	100 a	100 a	5 def
'Chardonnay'	15	100 a	87 a	11 cd
Vitis hybrids				
'Delaware'	12	100 a	100 a	5 def
Seibel 880	12	100 a	100 a	14 bc
Kober 5BB	18	100 a	89 a	19 a
'Cayuga White'	15	100 a	100 a	6 de
'DeChaunac'	17	100 a	82 a	5 def
'Aurore'	12	100 a	100 a	7 de
'Glenora'	14	100 a	100 a	9 cd
'Remaily Seedless'	18	100 a	100 a	17 ab

[z] Separation of means within columns by Duncan's Multiple Range Test (p=0.05); separation of proportions after Ryan, 1959 (p=0.05).

[y] Shoots of at least 3 nodes.

[x] V. argentifolia = GBC 17 595/68.

Table 4. Shoot production from 3 to 4 node (1.5 cm) shoot explants. Effect of subsequent subcultures (*Vitis* 'Remaily Seedless').

	No. days in culture	No. of shoots per explant	% Shoots w/ nodes >3	Shoots w/ nodes >3
First multiplication	53	20 b	60 a	13 a
1st subculture	52	21 b	58 a	14 a
2nd subculture	52	23 b	56 a	12 a
3rd subculture	45	39 a	42 b	17 a
4th subculture	43	40 a	40 b	16 a
5th subculture	58	28 a	44 b	13 a

[z] Separation of means within columns by Duncan's Multiple Range Test (p=0.05).

quality and root production are related. However, good shoot quality does not always assure good root production (as for 'De Chaunac').

For 'Remaily Seedless', 788 shoots were transferred into rooting plates. Sixty-two per cent of the shoots were rooted at 20 days; another 32 per cent were in good condition but without roots (Table 7).

TRANSFER TO SOIL

"Mini-greenhouses" were fabricated using plastic bags, wire supports, and styrofoam "Speedling trays" (Todd Planter, model 100, manufactured by Speedling Manufacturing, Inc.) (Fig. 7). The "Speedling trays" have 3 x 3 cm cells. Larger cells can accomodate the vines for a longer time. The trays should be cut to a convenient size for manipulation and so that they occupy at least one half of the volume of the plastic bag.

The soil is a 3:1 mixture by volume of peat moss and perlite. It is sifted through a 7 x 7 mm mesh. Two

Table 5. Potential of micropropagation. Multiplication of *Vitis* 'Remaily Seedless' *in vitro*.

	No. days in culture	No. days accumulated	No. shoots w/nodes >3
Establishment in culture[z]	30	30	10
Shoot multiplication[y]	53	83	140
Subculture 1	52	135	1,960
2	52	187	27,440
3	45	232	384,160
4	43	275	5,378,240
5	58	333	75,295,360

[z] For one explant.

[y] Mean number of shoots obtained previously over 6 subcultures = 14.21, stand. dev. = 0.9 (Table VIII.1).

Table 6. *In vitro* **micropropagation of** *Vitis*. **Rooting of 3 to 4 node (1.5 cm) shoot explants obtained** *in vitro*. **Results at 30 days.**

	% Shoots[y] ≥ good	Mean nodes/ explant	% Explants[y] rooted	Mean roots/ rooted explant	Total root length/ rooted explant (mm
Vitis species[x]					
argentifolia	40 a	5.0 ef	30 ab	4.7 abc	19 bc
labruscana 'Concord'	100 a	7.9 a	100 a	5.7 a	93 ab
labrusca 'Alba'	50 a	5.5 cdef	60 ab	2.5 c	6 c
labruscana 'Catawba'	50 a	5.3 def	50 ab	2.2 c	17 bc
Vitis vinifera					
'Cabernet Sauvignon'	70 a	6.9 ab	60 ab	2.5 c	29 bc
'Gewurztraminer'	100 a	6.1 bcde	100 a	5.2 ab	99 ab
'Chardonnay'	30 a	5.7 cdef	50 ab	3.8 abc	18 bc
Vitis hybrids					
'Delaware'	40 a	6.2 bcde	20 b	3.5 abc	14 bc
'Seibel 880'	30 a	4.7 f	30 ab	2.7 bc	21 bc
'Kober 5BB'	60 a	6.3 bcd	90 ab	2.4 c	64 abc
'Cayuga White'	70 a	6.6 bc	90 ab	6.3 a	100 ab
'De Chaunac'	70 a	5 ef	20 b	1.5 c	9 bc
'Aurore'	80 a	6.1 bcde	80 ab	4.8 abc	122 a
'Glenora'	50 a	5.6 cdef	70 ab	4.1 abc	36 bc
'Remaily Seedless'	50 a	6.4 bcd	89 ab	2.0 c	38 bc

[z] Separation of means within column by Duncan's Multiple Range Test (p=0.05); separation of proportions after Ryan, 1959 (p=0.05).

[y] 10 explants per variety.

[x] *V. argentifolia* = GBC 17 595/68.

Table 7. Removal of propagules from *in vitro* **conditions (***Vitis* **'Remaily Seedless').**

Stage	Propagule	No. days in stage	No. units transferred	% with roots[z]	% Survival in stage	Overall recovery (%)
Transfer to[y] rooting medium	shoots (3 nodes)	20±5	788	62	94	94
Transfer to[x] soil (high R.H.)	plantlets, shoots	15±5	740	77	82	77
Transfer to pots	plants	20±5	610	64	82	64

[z] % of original 788 shoots.

[y] From 01/12 to 01/19/82.

[x] The relative humidity was decreased from 100 % to ambient in greenhouse.

volumes of soil are moistened with one volume of 1/10 Hoagland's nutrient solution (Table 8) and is autoclaved for 20 minutes.

Once planted, the trays were placed in a temperature- and light-controlled environment. Ambient temperature should not exceed 27 C. When we used growth chambers, the irradiance was 2500 |iW crrr^2 with a 16 hour day length. In greenhouses, the trays should be shaded in summer. In winter, the natural photoperiod should be supplemented with artificial light.

Transfer of propagules from the in vitro rooting phase

Plantlets obtained from the *in vitro* rooting phase are used. Shoots with many short roots (5 to 10 mm) are preferred. This avoids breakage and difficulties in replanting. To avoid dessication, the tray must be planted and the plastic bag closed within 10 to 15 minutes. Free sterile water is added to the bag to reduce the time needed to reach 100 per cent relative humidity, and the plants and tray surface are sprayed with a mixture of benomyl and captan to retard fungal growth. After 10 days, bags are opened to allow acclimation to ambient atmosphere. Vines can be transferred to single small pots after 15 to 20 days depending on their development. From then on normal greenhouse procedures for seedlings are used.

The results obtained with 'Remaily Seedless' are given inTable7. Fifteen per cent of the original propagules which had not rooted in *the in vitro* rooting phase produced roots after20 days in soil. Overall 82 percent of the propagules transferred to soil grew.

Table 8. Preparation of Hoagland's nutrient solution after Hoagland and Arnon, 1950.

Stock solutions	Compounds	Grams per liter
1 M Potassium phosphate	KH_2PO_4	
1 M Potassium nitrate	KNO_3	
1 M Calcium nitrate	$Ca(NO_3)_2$	
1 M Magnesium sulfate	$MgSO_4 \cdot 7H_2O$	
Solution a		
	H_3BO_3	2.86
	$MnCl_2 \cdot 4H_2O$	1.81
	$ZnSO_4 \cdot 7H_2O$	0.22
	$CuSO_4 \cdot 5H_2O$	0.08
	$H_2MoO_4 \cdot H_2O$	0.02
Solution b		
	Ferric tartrate	5.00

Hoagland's solution	Stock solutions	Milliliters
	H_2O	987
	1 M KH_2PO_4	1
	1 M KNO_3	5
	1 M $Ca(NO_3)_2$	5
	1 M $MgSO_4 \cdot 7H_2O$	2
	Solution a	1
	Solution b	1
Total volume		1002

The rooted plantlets were 4 to 6 cm tall, had 6 to 9, nodes and 2 to 3 leaves of at least 4 cm^2. Leaves that were produced *in vitro* eventually dessicated, but new leaves formed.

Surviving vines were transplanted to individual pots in the greenhouse. Vines with adequate root systems lived, and survival at that stage was 82 per cent after 20 days. They displayed juvenile morphology with no tendrils and spiral phyllotaxy. Overall recovery was 64 per cent.

Transfer of shoots directly to soil

In the previous section we mentioned that some shoots did not root in the rooting media but only later after transfer to soil. It was thought that root initiation had been induced during the *in vitro* rooting phase, and the roots developed later. This was verified in a series of experiments, and the following procedure was developed.

Shoots suitable for further subculture were exised and placed in 10 ml of rooting solution contained in sterile plastic petri dishes 100 mm in diameter and 25 mm high. The shoots are, therefore, not submerged. The rooting solution contains the salts of Murashige and Skoog and 0.4 JJM NAA. The pH should be adjusted to 5.7-5.8 before autoclaving the solution. These shoots are normally soaked for 20 minutes but may be held for a few days without harm. After soaking, the shoots are planted directly into speedling trays, sprayed with fungicide, and incubated as described in the preceding section.

Using this system three roots of approximately 50 mm are produced per explant after 20 days in soil. With 'Remaily Seedless' the root system, developed after 20 days in soil, was larger both in number of roots and in root length than when the *in vitro* rooting phase was used. Auxins are known to induce root initiation but also to inhibit further root growth. Therefore, time was gained with regard to root development. In addition we did not incur the 18 per cent propagule loss due to transfer from rooting medium to soil (Table 7). We also saved the labor associated with the rooting phase which was conservatively estimated to be 30 per cent of the total labor involved in micropropagation after culture establishment.

Although this technique offers significant advantages, in some instances it has lead to an increased plant loss. The time between removal from culture and transfer to ambient air is doubled, and the vines are very vulnerable to fungal infection while in the "mini-greenhouses". The skill and experience of the operator will determine whether the losses suffered by transfer of rooted propagules exceeds those from subsequent fungal contamination during the extended acclimation phase.

VITIS GENOTYPES THAT WERE MICROPROPAGATED

In addition to the genotypes listed in Tables 3 and 6, we have successfully used the techniques to propagate an additional five named and numbered cultivars from the experiment station grape breeding program. Genotypes that had excellent ***in*** *vitro* shoot multiplication were 'Concord', *Vitis labrusca,* 'Alba', 'Catawba', 'Cabernet Sauvignon', 'Cayuga White', 'DeChaunac', 'Glenora', and 'Vanessa'.

CONCLUSION

Throughout our research, the potential commercial application of the micropropagation technique was kept in mind. Costs were considered. All chemicals used are readily available and not particularly costly. One hundred-twenty shoots were produced on a 100 cm^2 shelf space in less than 8 weeks. The most time consuming phase, rooting, was studied and bypassed. A method to transfer vines from culture to soil which is suitable for large quantities of plants was designed.

With this system, one person delivered 2,000 'Remaily Seedless' vines of 6 cm high and with 6 to 9 nodes, in separate pots to the greenhouse in 1 month (Fig. 8). The variable production cost per vine was crudely estimated at 60 cents when the *in vitro* rooting phase route was used. Ninety percent of this cost was for labor. Labor cost was taken at $7.00 an hour. Once material is established in culture (Steps 1 to 3), the *in vitro* rooting phase can be conservatively estimated at 30 per cent of the total cost. We demonstrated that the *in vitro* rooting phase can be bypassed. This lowers the variable production cost to 40 cents. This cost can be expected to be further lowered as worker expertise is gained. The yield during the multiplication phase was also doubled with our new salt formula (C2D).

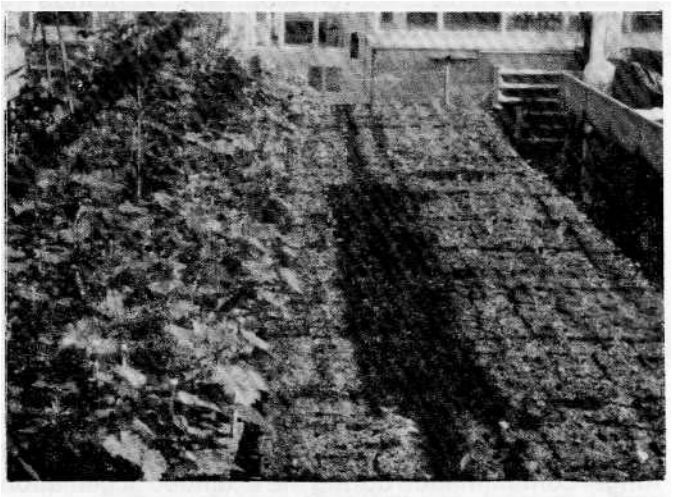

Figure 8. 'Remaily Seedless' grapevines obtained through micropropagation.

Plants can be sold in pots as are other green seedlings, or matured, they can be stored, and subsequently sold as dormant rooted grapevines. The standard nursery procedure is to ship bundles of rooted cuttings. Variable production cost per cutting is 20 cents (personal communication from New York State Fruit Testing Cooperative Association). Consequently, micropropagation for grapevine is seemingly restricted to one of a kind vines that have an urgent market demand.

APPENDICES

Appendix 1. Equipment required for grapevine micropropagation.

I. **Facilities**
- -laboratory space for media preparation and glassware cleaning equiped with: refrigerator, cleaning oven, autoclave, transfer hood, balance, pH meter, magnetic stirrer, dissecting microscope, water distillation unit
- -incubation room equipped with lighted shelves, timers and air conditioning
- -greenhouse space

II. **Utensils**
- -glassware for stock solution storage, media preparation, plant material sterilization (Erlenmeyer flasks, beakers)
- -measuring glassware (volumetric flasks, graduated cylinders, pipets)
- -vessels for tissue culture (sterilized disposable plastic petri dishes, etc.)
- -culturing instruments (forceps, exacto knives, razor blades)

III. **Supplies**
- -"chlorox" or other hypochlorite bleach, ethanol, distilled water, "Tween 20" or other surfactant
- -prepared media or chemicals with which to prepare media
- -"Parafilm"

IV. **Others**
- -flame source (bunsen burner, alcohol lamp), timer, plastic basins, paper bags, paper towels, glass markers, detergent

Appendix 2. List of some suppliers.

1. Carolina Biological Supply Company
   2700 York Road Burlington, NC 27215

2. Cole-Parmer
   7425 North Oak Park Avenue
   Chicago, IL 60648
3. Fisher Scientific Company
   PO Box 379
   Medford, MA 02155
4. GIBCO Laboratories
   3175 Staley Road
   Grand Island, NY 14072
5. KC Biological Inc.
   PO Box 14848
   Lenexa, KS 66215
6. Magenta Corporation
   4149 W. Montrose Avenue
   Chicago, IL 60641
7. SIGMA Chemical Company
   PO Box 14508
   Saint Louis, MO 63172
8. VWR Scientific, Inc.
   PO Box 1050
   Rochester, NY 14603

Appendix 3. Sources of Additional Information

Books:

1. Conger, B.V., ed. *Cloning Agricultural Plants via In Vitro Techniques.* CRC Press, Boca Raton, FL (1981) 273 pages.
   Contains 90 pages on fruit crops in chapter by R.M. Skirven.
2. *Proceedings of the Conference on Nursery Production of Fruit Plants Through Tissue Culture - Application and Feasibility.* Apr. 21-22, 1980. Science and Education Administration, Agricultural Reserach Results, Northeastern Series #11, Dec. 1980. Science and Education Administration, USDA Beltsville, MD 20705. 106 p.
   Discusses all phases of micropropagation of fruit crops, industry planning and building facilities.
3. Thorpe, T.A., ed. *Plant Tissue Culture:Methods and Applications in Agriculture.* Academic Press, NY 1981. 379 p. General Tissue Culture Text.

Journals:

1. Journal of the American Society for Horticultural Science. Alexandria, VA.
2. HortScience. Am. Soc. Hort. Sci.; Alexandria, VA.
3. Plant Cell, Tissue and Organ Culture. Nijheff Junk, The Hogue, Netherlands.
4. Scientia Horticulturae. Elsevier Sci. Pub. Co., Amsterdam.
5. Journal of Horticultural Science. Invita Press, Ashford, Kent, England.
6. Physiologia Plantarum. Societors Physiologiae Plantarum Scandinavica, Coppenhagen, Denmark.
7. Science. American Associate for the Advancement of Science. Washington, D.C.
8. Plant Science Letter. Elsevier Sci. Pub. Co., Shanna, Ireland.
9. Vitis. Bundesfurschunganstaltfur Rebenzuchtung, Geiweilerhof, D6741 Siebeldingen, West Germany.

REFERENCES

1. Chee, R., 1980. The effects of growth substances and photoperiod on shoot apices of *Vitis* cultured *in vitro* and their effects on subcultured shoot tips. M.S. Thesis, Cornell University, Ithaca, New York, U.S.A. pp. 1-76.
2. Chee, R., 1982. *In vitro* micropropagation of *Vitis.* Ph.D. Thesis, Cornell University, Ithaca, New York, U.S.A. pp. 1-155.
3. Chee, R. and Pool, R.M., 1982. The effects of growth substances and photoperiod on the development of shoot apices of ***Vitis*** cultured *in vitro.* Scientia Horticulturae, 16:17-27.
4. Galzy, R., 1964. Technique de thermotherapie des viroses de la vigne. Annales der Epiphyties, 15:245-256.
5. Hoagland, D.R. and Arnon, D.I., 1950. The water-culture method for growing plants without soil. Circular347 of the California Agricultural Experiment Station, Berkeley, California, U.S.A. pp. 32.
6. Iri Masahiko, Shimura Tomio, Togawa Hideo and Ueno Katsuyasu, 1982. Elimination of grapevine viruses by meristem tip culture. In: Proceedings of the 5th international congress of plant tissue and cell culture. Yamanakako, Japan.
7. Murashige, T. andSkoog, F., 1962. A revised media for rapid growth and bioassays with Tobacco tissue culture. Physiologia Plantarum 15:473-497.

ACKNOWLEDGEMENT

We would like to acknowledge the contribution of John Kasper and the New York State Fruit Testing Cooperative who partially funded the research described herein.