es are hand washed then dipped in bleach solutions in the transfer chamber before pouring with fresh agar.

Conclusions. At Briggs Nursery we have worked out some of the fine points of getting started in tissue culture. There are several advantages to this method of propagation. From a single cutting there is potential for an infinite number of plants providing no mutations occur. Plants in tissue culture reproduce regardless of season. There is no watering requirement until plants are removed from sterile culture. Thousands of plants can be started in a comparatively small area. We believe we can produce rhododendrons at equal or less cost in the same time frame as by traditional means.

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VIRUS ELIMINATION AND RAPID PROPAGATION OF GRAPES *IN VITRO*

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Abstract. Heat treatment and *in vitro* culture of shoot tips were used to free *Vitis vinifera* 'Liemberger' of leafroll virus and 'Forta' and 'Auxerrois' ('Cl-21') of fanleaf virus. Rapid propagation of the French hybrid 'Baco' was obtained on full-strength MS medium plus adenine sulfate (80 mg/l), $NaH_2PO_4 \cdot H_2O$ (170 mg/l), i-inositol (100 mg/l), thiamine-HCl (0.4 mg/l), and BAP (3 to 4 mg/l). Rooting of proliferated shoots was most rapid on ¼-strength MS with 0.08 mg/l IBA. The method appears suitable for the rapid propagation of other cultivars.

INTRODUCTION

British Columbia grape growers and wineries are continually seeking new cultivars for increasing hardiness and wine

transferred to a 1:1 mixture of sterilized peat and vermiculite in high humidity for 3 to 7 days. When the plants from the virus eradication program were well established they were indexed by the PEQ Station.

Unless otherwise stated shoot-tips from the French hybrid Baco 22A were used.

Effect of Medium Strength and Addition of Adenine Sulfate ($AdSO_4$) and Monobasic Sodium Phosphate ($NaH_2PO_4 \cdot H_2O$): Shoot-tips were cultured on full-, ¾-, and ½-strength MS media with 3 mg/l 6-benzylaminopurine BAP, and with or without 80 mg/l $AdSO_4$ and 170 mg/l $NaH_2PO_4 \cdot H_20$. The explants were cultured on agar medium for 56 days followed by 16 days in liquid medium of the same composition. Shoots $\geq$ 2 cm long were removed and counted 82 and 102 days after the start of cultures and 6 shoots from each treatment placed on rooting medium. After 102 days shoots $<$ 2 cm were also counted.

Effect of BAP Concentration in Initial Culture Medium: Shoot-tips were cultured on agar with 0, 1, 2, 3 and 4 mg/l BAP for 27 days, recultured on agar for a further 17 days, and then transferred to liquid media. Sixty days after initial culture, 6 shoots $\geq$ 2 cm in length from each treatment were placed on rooting medium. Two cultures from each treatment were each divided into 3 parts and recultured for a further 45 days on media with the original BAP concentration as the parent cultures.

Effect of BAP Concentration for Continuous Culture: Shoots were removed from the 3 mg/l BAP treatment in the previous experiment. Six shoots 2 to 3 cm long were cultured on 5 ml of liquid medium in 50-ml erlenmeyer flasks with 0, 1, 2, 4 & 5 mg/l BAP. The following procedure was followed:

No. of days after initial culture	
70	Shoots $\geq$ 2 cm removed and counted. Two reps of each treatment were: 1) divided into 3 parts and recultured into 125-ml erlenmeyers. 2) recultured into 455-ml jars containing 30 ml of medium without cytokinin. 3) recultured into jars on media with the same BAP concentration as the parent culture (A).
90	Cultures in 125-ml flasks recultured as after 70 days; 2 each divided and cultured in 125-ml flasks, 2 recultured in jars with, and 2 in jars without BAP (B).
111	Two reps in 125-ml flasks divided and recultured as before, and 4 reps recultured to jars with the same BAP concentration (C). Shoots from A cultures counted in two sizes $\geq$ 2 cm and $<$ 2 cm and 6 shoots $\geq$ 2 cm of each treatment placed on rooting medium.
122	122 shoots from B culture counted as before.

Power Twist lamps providing 2000 lux for 16 hrs/day.

VIRUS ELIMINATION

The medium was made up of the macronutrients of Morel and Muller (8), 0.5 ml/l of Berthelot's (3) micronutrients, and organic supplements (Table 1). After about 14 days when the explants were approximately 1 cm long they were transferred to filter paper bridges in tubes containing 15 ml of liquid medium for rooting. The rooting medium had the same composition as the growth medium but minus potassium chloride (KCl), ammonium sulfate $(NH_4)_2SO_4$ and naphthalene acetic acid (NAA).

Table 1. Nutrient medium used for growing shoot-tips of grapes for virus eradication.

Macronutrients (Morel & Muller)	mg/l	Micronutrient Stock	mg/100 ml
$(NH_4)_2SO_4$	1000	$MnSO_4 \cdot 4H_2O$	161
KCl	1000	KI	50
$Ca(NO_3) \cdot 4H_2O$	500	$NiCl_2 \cdot 6H_2O$	5.6
$MgSO_4 \cdot 7H_2O$	125	$CoCl_2 \cdot H_2O$	6.0
KH_2PO_4	125	$ZnSO_4 \cdot 7H_2O$	10
		$BeSO_4 \cdot 4H_2O$	10
		$CuSO_4 \cdot 5H_2O$	5
		H_3BO_3	5
		$NaMoO_4 \cdot 2H_2O$	0.015
Organics etc.*	mg/100 ml	Organics etc.*	mg/100 ml
Thiamine-HCl	100	Calcium pantothenate	100
Pyrodoxine-HCl	100	Inositol	1000
Nicotinic acid	100	Biotin	1.0

* Add 0.5 ml of micronutrient and 1.0 ml of organic stock to the amount of macronutrients shown and dilute to 1000 ml.

RAPID PROPAGATION

The basic medium contained Murashige & Skoog (9) (MS) salts with 100 mg/l i-inositol, 0.4 mg/l thiamine-HCl, and 30 g/l sucrose. The pH was adjusted to 5.7 for agar and 5.0 for liquid media.

When the proliferating shoot-tips were 20 to 25 mm in diameter they were transferred to 15 ml of liquid medium of the same composition in 125-ml erlenmeyer flasks stoppered with aluminum foil. The flasks were placed upright on a device which tilted the flasks 35° in opposite directions 3 times per minute, thus the explants were alternately exposed and submerged. Agar cultures were recultured every 4 weeks and liquid cultures every 3 weeks.

Proliferated shoots were removed when they were 2 to 3 cm long and placed on filter paper (Whatman #5) bridges in culture tubes containing 15 ml of $^2/_5$-strength MS salts with 0.3 mg/l indoleacetic acid (IAA) and 20 g/l sucrose for rooting.

When the root system was well developed the plants were

transferred to a 1:1 mixture of sterilized peat and vermiculite in high humidity for 3 to 7 days. When the plants from the virus eradication program were well established they were indexed by the PEQ Station.

Unless otherwise stated shoot-tips from the French hybrid Baco 22A were used.

Effect of Medium Strength and Addition of Adenine Sulfate ($AdSO_4$) and Monobasic Sodium Phosphate ($NaH_2PO_4 \cdot H_2O$): Shoot-tips were cultured on full-, ¾-, and ½-strength MS media with 3 mg/l 6-benzylaminopurine BAP, and with or without 80 mg/l $AdSO_4$ and 170 mg/l $NaH_2PO_4 \cdot H_20$. The explants were cultured on agar medium for 56 days followed by 16 days in liquid medium of the same composition. Shoots $\geq$ 2 cm long were removed and counted 82 and 102 days after the start of cultures and 6 shoots from each treatment placed on rooting medium. After 102 days shoots $<$ 2 cm were also counted.

Effect of BAP Concentration in Initial Culture Medium: Shoot-tips were cultured on agar with 0, 1, 2, 3 and 4 mg/l BAP for 27 days, recultured on agar for a further 17 days, and then transferred to liquid media. Sixty days after initial culture, 6 shoots $\geq$ 2 cm in length from each treatment were placed on rooting medium. Two cultures from each treatment were each divided into 3 parts and recultured for a further 45 days on media with the original BAP concentration as the parent cultures.

Effect of BAP Concentration for Continuous Culture: Shoots were removed from the 3 mg/l BAP treatment in the previous experiment. Six shoots 2 to 3 cm long were cultured on 5 ml of liquid medium in 50-ml erlenmeyer flasks with 0, 1, 2, 4 & 5 mg/l BAP. The following procedure was followed:

No. of days after initial culture	
70	Shoots $\geq$ 2 cm removed and counted. Two reps of each treatment were: 1) divided into 3 parts and recultured into 125-ml erlenmeyers. 2) recultured into 455-ml jars containing 30 ml of medium without cytokinin. 3) recultured into jars on media with the same BAP concentration as the parent culture (A).
90	Cultures in 125-ml flasks recultured as after 70 days; 2 each divided and cultured in 125-ml flasks, 2 recultured in jars with, and 2 in jars without BAP (B).
111	Two reps in 125-ml flasks divided and recultured as before, and 4 reps recultured to jars with the same BAP concentration (C). Shoots from A cultures counted in two sizes $\geq$ 2 cm and $<$ 2 cm and 6 shoots $\geq$ 2 cm of each treatment placed on rooting medium.
122	122 shoots from B culture counted as before.

ROOTING

Effect of Agar vs. Liquid Rooting Media: Six shoots from each of 6 treatments were placed either on agar or on bridges on liquid media; the number rooted were counted every 3 to 4 days.

Effect of Medium Strength and Sucrose Concentration for Rooting: Six proliferated shoots $\geq$ 2 cm were put on each of either normal-, ¾-, ½- or ¼-strength MS medium. The normal strength medium contained 100 mg/l i-inositol, 0.4 mg/l thiamine-HCl, and 0.3 mg/l IAA, and the ¾-, ½-, and ¼-strength a proportional amount of each of the above. Sucrose was added to provide 10, 20, 30 and 40 g/l at all media strengths.

PROLIFERATION OF OTHER GRAPE CULTIVARS

To determine the feasibility of propagating other grape cultivars *in vitro*, 12 virus-free and 2 virus-infected cultivars were established *in vitro* and 6 were included in rooting experiments on either full-, ¾-, ½- and ¼-strength MS with 0 to 2 mg/l IAA.

RESULTS

VIRUS ERADICATION

There was very little difficulty in growing shoot-tips to 1- or 2-cm in length, but rooting was very inconsistent and the ability to root varied considerably among accessions.

Sixty-three plants of 33 accessions were grown and rooted. Of the 63 plants, 28 were not indexed because other sources of virus-free material had become available and another 13 were discarded because the accession was no longer required by the growers.

Eleven plants were indexed, with 5 positive, 3 suspicious, and 3 testing negative. The 11 plants remaining were duplicates of the above and are now entering the indexing program.

The 3 accessions which have tested negative are all *Vitis vinifera*; cvs Liemberger, Forta and Auxerrois (Cl-21). 'Liemberger' was infected with both leafroll and fleck while the other two were infected with fanleaf virus.

RAPID PROPAGATION

Effect of Medium Concentration: Three-quarter strength medium produced about twice as many shoots as full- and ½-strength media when $AdSO_4$ and$NaH_2PO_4 \cdot H_2O$ were omitted (Table 2). With the addition of both compounds there was no difference in the number of shoots produced between full- and ¾-strength media, while shoot production on ½-strength medium was slightly lower.

Rooting of shoots from full-strength medium was more

rapid both with and without the addition of $AdSO_4$ and $NaH_2PO_4 \cdot H_2O$ than from ¾- or ½-strength (Table 3).

Table 2. Effect of strength of medium, with and without $AdSO_4$ and $NaH_2PO_4 \cdot H_2O$ on proliferation of 'Baco' grape *in vitro*.

	Mean No. of shoots $\geq$ 2 cm produced in 102 days[1]	
Medium strength	With	Without
Normal	17.3	6.3
¾	18.8	13.3
½	14.2	7.7

[1] Two cultures of each treatment were divided into 3 parts and recultured after 82 days. To determine total potential production multiply by 3.

Table 3. Effect of strength of medium with and without $AdSO_4$ and $NaH_2PO_4 \cdot H_2O$ in proliferation medium on subsequent rooting of 'Baco' grape.

		No. shoots which rooted[1]			
Medium strength		With		Without	
	No. of days →	11	16	11	16
Full		6	6	4	6
¾		5	5	0	2
½		5	6	0	2

[1] Maximum 6

Effect of the Addition of $AdSO_4$ and $NaH_2PO_4 \cdot H_2O$. The addition of 80 mg/l $AdSO_4$ and 170 m g/l $NaH_2PO_4 \cdot H_2O$ increased shoot production approximately 180% on full strength medium, and 40 and 50% on ¾- and ½-strength media (Table 2). In addition, these compounds either decreased the time to root and/or the percentage of rooted shoots on all media strengths (Table 3). On full strength media 100% rooting was achieved in 11 days with $AdSO_4$ and $NaH_2PO_4 \cdot H_2O$ as compared to 16 days without these compounds.

The Effect of BAP Concentration for Initial Culture of Shoot-tips. The O BAP treatment grew poorly and was discarded after 28 days. After 67 days in culture, explants on media with 4 mg/l BAP had produced an average of 5.5 shoots over 2 cm long as compared to 4.8, 3.2 and 1 for 3, 2 and 1 mg/l BAP respectively (Table 4).

After 11 days, more shoots had rooted from BAP cultures with 2 and 4 mg/l than from the 3 mg/l treatment, but after 17 days the number of rooted shoots were similar to all BAP concentrations (Table 5). Concentration of BAP had no consistent effect on the number or branching of primary roots that developed.

The Effect of BAP Concentration for Continuous Culture. Growth was very slow in the 0 BAP treatment and little or no proliferation of shoots occurred in the 1 mg/l BAP treatment.

Table 4. Effect of BAP concentration in initial culture medium on shoot production of 'Baco' grape.

mg/l BAP	Length of shoots No. of days →	Mean number of shoots produced[1] ≥ 2 cm 60	≥ 2 cm 105	< 2 cm Total 105	Total —
1		1.0	2.5	2.5	6.0
2		3.2	9.3	6.0	17.2
3		4.8	14.5	9.3	27.8
4		5.5	6.8	7.7	20.0

[1] Cultures were divided into 3 parts after 60 days. To obtain an estimate of potential production after 105 days multiply by 3.

Table 5. Effect of BAP concentration in proliferating medium on subsequent rooting of proliferated 'Baco' grape shoots.

mg/l BAP in proliferation medium	No. of shoots rooted[1] No. of days on rooting medium 11	18
1	1	3
2	3	5
3	0	6
4	3	5

[1] Maximum 6

These treatments were discontinued after 31 and 60 days, respectively. At all harvest intervals up to 122 days, 4 mg/l BAP cultures produced more shoots than other concentrations (Table 6). Explants recultured on a medium without BAP failed to develop shoots.

Shoots from 2 mg/l BAP cultures usually rooted more rapidly than those from other treatments but the concentration had no significant effect on the total number of shoots that rooted (Table 7).

Table 6. Effect of continuous culturing on medium with different amounts of BAP on shoot production of 'Baco' grape.

No. of days cultured	Length of shoots (cm)	No. of shoots produced BAP concentration (mg/l) 2	3	4	5	Multiplication factor[1]
90	≥ 2	8.4	6.6	9.4	7.2	3
111	≥ 2	40	25	60	28	9
	< 2	36	27	40	30	9
	Total	76	52	100	58	9
122	≤ 2	22	19	43	28	27
	≥ 2	30	52	93	38	27
	Total	52	71	136	66	27

[1] Cultures were divided into 3 parts at intervals. To obtain an estimate of potential production if all cultures had been retained multiply by the factor shown.

Table 7. Effect of BAP concentration in proliferation medium on subsequent rooting of 'Baco' grape shoots.

Experiment	No. of days to rooting	No. of shoots rooted[1] BAP concentration (mg/l)			
		2	3	4	5
A	12	3	2	2	1
	18	4	5	4	5
B	12	3	1	1	0
	18	3	3	4	1

[1] Maximum 6

ROOTING PROLIFERATED SHOOTS

Agar vs. Bridges on Liquid Medium. After 8 days on rooting medium, 9 out of 18 shoots had rooted on bridges as compared to 2 out of 18 on agar. After 18 days 16 shoots on bridges and only 3 on agar had rooted.

Effect of Medium Strength and Sucrose Concentration: Eight days after culturing on rooting medium more shoots had rooted on all sucrose concentrations on ¼-strength MS than all other medium strengths. Similarly, at all medium concentrations more shoots rooted on 20 g/l sucrose than at all other concentrations (Table 8).

Fourteen days after culturing on rooting media the number of shoots that rooted on ½-strength and ¼-strength media were similar and concentrations of sucrose had no consistent effect. At normal- and ¾-strength media, more shoots rooted with 30 g/l sucrose, at ½-strength medium with 20 and 40 g/l, and at ¼-strength with 20 g/l.

Table 8. Effect of rooting medium strength and sucrose combination on rooting of proliferated shoots of 'Baco' grape.

Medium Strength	Days cultured →	No. of shoots rooted[1]									
		8					14				
	g/l sucrose →	10	20	30	40	Mean	10	20	30	40	Mean
Normal		2	3	2	2	2.2	3	4	5	5	4.2
¾		3	4	3	2	3.0	4	4	5	4	4.2
½		3	5	3	5	4.0	4	5	4	5	4.5
¼		5	5	4	4	4.5	5	6	4	4	4.8
Mean		3.2	4.2	3.0	3.2		4.0	4.8	4.5	4.5	

[1] Maximum 6

PROLIFERATION OF OTHER CULTIVARS

All 14 cultivars were eventually established on proliferating medium. Those that failed to establish on the first attempt established readily on later attempts. 'Foch,' 'Schonberger,' 'Ortega,' 'Reichensteiner,' 'Leon Millot' and 'Oraniensteiner' proliferated readily with 2 to 3 mg/l of BAP, and rooted fairly readily on ¼- and ½-strength MS medium with 0 to 1 mg/l IAA.

The 3 rootstocks ('5BB,' '5C' and 'SO_4') and 'Pinot Noir,' 'Okanagan Riesling' and 'Rotberger' were slow to establish and proliferate. The two virus-infected accessions established, proliferated, and rooted readily.

DISCUSSION

VIRUS ERADICATION

Gifford and Hewitt (7) report that shoot-tips from heat-treated plants grew rapidly *in vitro* but only 2% of them rooted. Galzy & Compan (5) also indicate poor rooting if shoot-tips are taken from the initial culture but report that shoot-tips taken from explants in culture rooted more readily. Many variations of the proliferating and rooting media were tried but with little or no improvement on rooting.

The variation in rooting ability among accessions may have been due to a difference in photoperiod requirements as found by Alleweldt and Radler (1). They found short-day types of grapes in which the shoot-tips growing *in vitro* only rooted if the donor plants had been growing in 13 to 17 hour photoperiods, and long-day types which only rooted profusely if donor plants were grown in 10 hour photoperiods. Determining the photoperiod requirements of each cultivar would be a lengthy process. A better approach might be to grow the shoot-tips of heat-treated plants in a proliferating medium, and maintain them until rooted plants from proliferated shoots are well established in soil. This would reduce the danger of losing the heat-treated shoot-tip if rooting does not take place.

Three virus-free plants out of the 11 indexed is not a high percentage but is higher than that obtained by the shoot grafting method. Also, one of the accessions ('Liemberger') has proven extremely difficult to clean up by other methods and the eradication of leafroll virus by heat treatment and *in vitro* culture is the first reported. Both other accessions were infected with fanleaf virus which had been eliminated in other cultivars by both Gifford and Hewitt (7) and Galzy and Compan (6).

RAPID PROPAGATION

Several preliminary experiments showed that more consistent results were obtained by starting the shoot-tips on agar medium than on liquid medium. However, these tests also showed that after an initial 4 to 6 weeks on agar much more rapid shoot proliferation and growth could be obtained by transferring to liquid medium. Consequently, this procedure was followed.

Results confirmed preliminary experiments that ¾-strength MS salts produced more shoots than full- or ½-strength. How-

ever, the addition of $AdSO_4$ and $NaH_2PO_4 \cdot H_2O$ increased shoot production more on full-strength medium than on either ¾- or ½-strength so that with the additions there was little or no difference between full and ¾-strength. The addition of $AdSO_4$ and $NaH_2PO_4 \cdot H_2O$ also increased rooting, and on full-strength medium initiated the earliest rooting. In future experiments full-strength MS salts with $AdSO_4$ and $NaH_2PO_4 \cdot H_2O$ were used.

Optimum concentration of BAP was 3 mg/l for initial culture of shoot-tips and 4 mg/l for continuous culture. However, the intervals between counts can affect the number of shoots obtained at different levels. At low BAP concentrations relatively few shoots are produced and these grow rapidly, thus enhancing counts taken at short intervals. If longer intervals are used between counts, the larger number of shoots produced by higher concentrations of BAP have a chance to grow and be counted.

Shoots produced with low (2 mg/l) BAP concentrations tended to root slightly earlier than higher concentrations, but transferring proliferating cultures from a medium with BAP to the same medium without any BAP resulted in a nearly complete inhibition of shoot growth. It remains to be determined whether reducing the BAP concentration from 3 or 5 mg/l to 2 mg/l or less on the last reculture will result in equal or better shoot growth and rooting.

It was not practical to continue growing all cultures indefinitely, so after counting the number of shoots produced, 4 of the 6 reps from each treatment were discarded at each reculture interval. The 2 remaining cultures were divided into 3 and recultured. This effectively reduced the potential shoot production by ⅔ each time this was done. The multiplication factor in Table 6 is the amount that each figure should be multiplied by to arrive at the potential yield of each treatment at the date given. The potential yield is, however, even greater since by placing each shoot on proliferating instead of rooting medium, each shoot should produce 540 more shoots in 111 days with 4 mg/l BAP, as can be determined from Table 6.

ROOTING

Throughout the preliminary experiments rooting was very inconsistent. Sometimes 100% rooting could be obtained in 8 days while at other times 100% rooting could not be obtained in 30 days. The results reported here indicate that more rapid rooting occurs in full-strength MS with $AdSO_4$, $NaH_2PO_4 \cdot H_2O$, and 2 mg/l BAP in proliferating medium. Reducing the rooting medium to ¼-strength also increased rooting but it needs to be ascertained whether this is due to the decreased salt

concentration or lower auxin.

Galzy (5) obtained a higher emergence and growth of roots at 35°C than at 20°C, and a higher emergence of roots if $(NH_4)_2SO_4$ was added to the medium and the KNO_3 replaced by KCl. This change in salt composition, however, reduced root growth. Barlass & Skene (2) rooted <3 mm long proliferated shoots of *V. vinifera* L., cv Cabernet Sauvignon on White's (10) medium.

Further work is needed to improve the consistency of rooting.

SUMMARY

The work reported above has shown that both fanleaf and leafroll viruses can be eradicated from grape plants by *in vitro* culture of heat-treated shoot-tips. It has also been shown that grape cultures can be rapidly proliferated *in vitro* on full-strength MS salts with 30 mg/l sucrose, 100 mg/l i-inositol, 0.4 mg/l thiamine-HCl, 80 mg/l $AdSO_4$, 170 mg/l $NaH_2PO_4 \cdot H_2O$ and 3 mg/l BAP, and that proliferated shoots can be rooted on ¼-strength MS salts with 20 g/l sucrose and 0.1 mg/l IAA. Some details of the proliferation procedure merit further study to improve efficiency and better rooting methods need to be developed.

Acknowledgements. The authors would like to acknowledge the assistance of Mr. D. Bertoia, Mrs. C. Winter, and the staff of the Agriculture Canada, Post Entry Quarantine, Sidney, B.C. for providing the heat-treated shoot-tips and indexing the plants in the virus eradication program.

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HOWARD BROWN: Let us take a few minutes now for questions.

VOICE: What is the significance of tilting the tissue cultures in the tubes?

R.E. HARRIS: I don't really know. All we know is that if you don't tilt them they just drown. The alternate exposure from the air and mixing the air with it is beneficial. You can seal the jars up tight and they will still grow well.

JOHN IRELAND: Dr. Cheng, what is the best time of year for the taking of the tissue of the apple plant?

DR. TSAI CHENG: Well, I maintain most of my stock plants in the greenhouse and I take a lush type growth, a very succulent type of new shoots. Another way of doing it; you can place them in tissue culture and force them to produce shoots. Therefore, you will have material from there, too. So this way you don't worry about dormancy if you take materials only in winter time.

VOICE: What happens to the variability in the offspring plants? When you start propagating through tissue culture, you get complete uniformity, and get away from the variation that has given us the kinds of plants that we have today.

DR. TSAI CHENG: We have come long ago to the stage where we must have a custom orchard. We cannot use seedlings or the orchard is not uniform. A uniform orchard is the way we have to go.

CHARLES HESS: Any form of vegetative propagation would do the same thing: cuttings, or grafts on a rootstock, giving a uniform total propulation, so you have the same circumstances with tissue culture. It has not really changed, it is just made more efficient by tissue culture methods.

Your question, though, is a good one; one thing that is being done is to establish a number of germplasm repositories. The University of California at Davis is going to be a center for germplasm repository for a number of fruit and nut species. There is one at Oregon State in Corvallis and another repository is at Geneva, New York. The idea is that there will be places where the genetic material will be preserved. The need for this is, for example, that we might get an outbreak of a particular

disease in a crop and the plant breeders need then to have genetic material to go back to try to get some resistant characteristics bred into commercial cultivars to counteract the disease.

J. MATSUYAMA: In tissue culture propagation you might get good results in a laboratory situation but when you go commercial, when you are talking about a thousand, ten thousand, one hundred thousand plants — are these methods feasible?

DR. TSAI CHENG: As I told you, when we started this project last year, we were commercially oriented. We don't want just to develop the techniques for the laboratory. We want to really use this technique for commercial production. We have been transplanting these materials over and over again in my laboratory and in the nurseries of my sponsors and also by my colleagues at Oregon State University. We all get similar conclusions — it is very easy to transplant the new tissue culture propagated plants. Some of the plants will have a lag period of one week or so and then they will start off and grow rapidly. Now, we are aware of the problems and we are paying attention to conditioning tissue culture plants in such a way that perhaps a less skilled type of person can handle them and know how to transplant them into a soil condition without special skill. We are also working on optimizing growth. We think that under our greenhouse conditions we can produce liners, or saleable ornamental trees in about three months with the right growth conditions and fertilizers, and so forth. Perhaps we can even shorten the growth into less than 3 months. Another step, we are working on acclimitization because we eventually must grow these plants under field conditions. We hope that we can come up with a simple type of cookbook recipes for the nurseries and growers.

J. MATSUYAMA: With a given species, how can you increase the survival rate?

DR. TSAI CHENG: We handle all those species simultaneously. Our commercial laboratory is not yet in production, but by next year they will have experience. Some of my sponsors will perhaps answer your questions; perhaps they will be at next year's IPPS meeting. They will know then more about the commercial end of handling these plants. At this point, I can tell you from my experience and from that of my colleagues' that we speculate that we are not going to have any problems. For actual commercial practice, by next year, when the nursery people have had experience, they will give you a better answer.

CHARLES HESS: What is the number of plants propagated by tissue culture in one particular group? Is it 100 plants, for

example, or more, that you did at one time?

DR. TSAI CHENG: No, we did thousands, tens of thousands.

SOME ASPECTS OF NURSERY PRODUCTION IN QUEENSLAND

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Queensland is a very large state of Australia, stretching from New Guinea in the north to approximately 1,500 miles south. So we have very tropical areas in the northern half and sub-tropical in the south. There are, of course, some areas with a temperate climate because of altitude.

We have a dividing range of mountains running north to south. On the eastern side of this range we have a very fertile coastal strip, with rainfalls ranging from 50 inches in the south to over 200 inches in the tropical coastal zone in the north. To the west of the range the rainfall decreases inland and a large area of the western region has less than 10 inches of rain per year and is subject to very bad drought periods at times.

The major part of our population of 2½ million lives along the coastal region, with about one million of these living in the state capitol, Brisbane, which is in the southeast corner of the state. Most of the nursery production takes place in this area. Nurseries in the north of the state produce a wide range of tropical fruit trees, some exotics, bedding plants and house plants, all without any heating costs.

As one moves to the southern part of the state a little heating becomes necessary during a period of about three months during mid-winter, but glasshouses are not used extensively unless for special crops such as ferns, crotons, dieffenbachia and other house plants. Sarlon shade houses are used to a very large extent because of the rather intense sunlight that we experience for most of the year. Most of the better nurseries use the most modern methods of production and disease control available and mechanize operations as much as possible to reduce labor costs, which are quite high in our country.

A Method of Propagating *Lagerstroemia indica* 'Mathewsii.' Traditionally this cultivar and, indeed, all *Lagerstroemia* cultivars have been propagated by hardwood cuttings taken during the dormant period of growth at the end of winter in Queensland. Results were always rather variable and rooting