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In vitro propagation of *Vitis*

by

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In-vitro-Vermehrung von *Vitis*

Zusammenfassung. — Es wird eine *in-vitro*-Methode beschrieben, mit deren Hilfe in 4 Monaten aus einem einzigen Sproßstückchen von 3—5 mm Länge über 12.000 Triebe gezogen werden können. Bei der französischen Rebkreuzung Baco, die auf organischem Minimalnährboden nach Murashige (MMO) mit Zusätzen von 80 mg Adeninsulfat/l, 170 mg einbasigem Natriumphosphat/l und 3—4 mg N^6-Benzylaminopurin/l (BAP) kultiviert wurde, war die Sproßproduktion auf das 7fache gesteigert, wenn zuerst eine 4wöchige Anzucht auf festem Nährboden (0,7 g Agar/l) und danach eine Übertragung in flüssiges Nährmedium erfolgte. Auf dem gleichen Substrat wurden weitere 21 Rebsorten vermehrt, wobei die BAP-Menge auf 2 mg/l und das Übertragungsintervall von 3 auf 2 Wochen herabgesetzt waren.

Über 90 % der Triebe bewurzelten sich im Verlauf von 1—2 Wochen ohne Schwierigkeiten, wenn sie auf Filterpapierbrücken gebracht wurden, die in flüssigem, auf 1/4 verdünntem MMO standen, das mit 150 mg einbasigem Natriumphosphat/l, 10 g Saccharose/l und 0,1 mg Indolyl-3-essigsäure/l angereichert war; innerhalb von 2—3 Wochen bewurzelten sie sich auch in grobem Perlit, das mit denselben Nährstoffen, aber ohne Saccharose, gesättigt war.

Introduction

Canadian grape growers and wineries are constantly looking for new hardy grape cultivars suitable for producing superior wines. However, according to regulations, all vines planted must come from a certified virus-free source. Since only one plant is usually certified virus-free, it takes several years to propagate enough vines by conventional methods. On the other hand, the development of *in vitro* methods for many plant species provides a promising method for the rapid propagation of grapes.

Previous work on callus and protoplast culture, embryogenesis and organogenesis in *Vitis* and virus eradication methods described by GIFFORD and HEWITT (1961), GALZY (1972) and BINI (1976) had little significance for developing propagation methods. Later, however, JONA and WEBB (1978) sucessfully propagated *V. vinifera* Sylvaner Riesling from lateral buds and BARLASS and SKENE (1978, 1980) propagated Cabernet Sauvignon from fragmented shoottips.

The Saanichton Research and Plant Quarantine Station has been developing *in vitro* methods for virus eradication, rapid propagation, and *Vitis* germ-plasm storage. The propagation methods are described in the present paper.

Materials and methods

Apical shoot-tips from virus-free plants of the French hybrid cv. Baco growing in a greenhouse during the winter and a shadehouse during the summer were used to develop the procedure. The method was later tested on 21 *V. vinifera* and hybrid genotypes.

Shoots 3 cm long were harvested as required, expanded leaves removed, and shoot-tips surface sterilized in either 70 % alcohol for 1 min or 7 % aqueous solution of commercial bleach (12 % hypochlorite) containing 0.1 % Tween 20 for 20 min with constant stirring. After surface sterilization, shoot-tips were rinsed 3 or 4 times in sterile distilled water, outer leaves of the apical buds removed in a laminar flow cabinet, and shoot-tips reduced to 3—8 mm in length. The tips were placed on 15 ml of nutrient medium solidified with 0.7 g Bacto agar/l in 25 × 150 mm culture tubes and covered with polypropylene caps (Kaput).

The pH of all media was adjusted to 5.7 for agar and 5.0 for liquid media. All constituents were added prior to autoclaving at 121 °C for 20 min at 1 atm.

Containers with liquid media were placed on Saanichton-designed devices which tilted the containers 30° in two opposite directions every 20 s. The tilting alternately exposed the explants to the air and submerged them in the medium and ensured that the media was thoroughly mixed at all times. One tilter was designed for 50 and 125 ml erlenmeyer flasks and a second one for 455 or 910 ml wide mouth jars. The mouths of the flasks and jars were covered with aluminum foil.

Explants were grown at 23 ± 2 °C under Vita-Lite Power Twist fluorescent tubes providing between 35 and 43 $\mu E\ m^{-2}s^{-1}$ for 16 h/d. 6 cultures (replicates) of all treatments were used. Unless indicated otherwise, a 3-wk reculture interval was maintained.

Establishing aseptic cultures (stage 1)

Treatments in the first two experiments were assessed by counting the numbers of shoot-tips alive and growing 14 d after initial culture, and by a visual evaluation of the amount and type of growth after 28 d.

E1: Media. — Murashige's minimal organic medium (MMO) at 3 concentrations and a modified Gamborg B5 medium were compared with and without 170 mg $NaH_2PO_4 \cdot H_2O$/l (PO_4) and 80 mg adenine sulfate/l (Ad) and were designated as follows:

Medium	Strength	Without PO_4	With PO_4
Murashige's minimal organic	Normal	MMO	MMO +
	3/4	3/4-MMO	3/4-MMO +
	1/2	1/2-MMO	1/2-MMO +
Modified Gamborg B5	-	MB5	MB5 +

All media contained 3 mg N^6-benzylaminopurine/l (BAP) and were solidified with agar.

The MMO medium consisted of MURASHIGE and SKOOG (1962) mineral salts, 0.4 mg thiamine-HCl/l, 100 mg i-inositol/l and 30 g sucrose/l. In the MB5 medium, the macro salts of GAMBORG, *et al.* (1968) were reduced to 60 % and the micronutrients to 80 % of normal strength but the normal levels of vitamins and sucrose were retained.

E2: BAP concentration. — 0, 1, 2, 3, 4 and 5 mg BAP/l were tested in 3/4-MMO + solidified with agar.

E3: Length of time on agar. — 36 shoot-tips were cultured on 3/4-MMO + with 3 mg BAP/l solidified with agar. After 4 wks the explants were arranged in order of size and the 6 poorest removed. The remaining 30 explants were divided into 6 groups according to size. 1 explant from each group was selected at random and recultured on MMO + liquid in 125 ml erlenmeyer flasks and the remainder recultured

on MMO+ agar. 2, 4 and 6 wks later the selection and subculture procedure was repeated. Every 3 wks shoots ≥ 2 cm were harvested and the explants subcultured on fresh media. After 63 d, explants in the 4-wk treatment were divided into 4 parts and 1 part from each explant subcultured to maintain the treatment. The other 3 parts were discarded.

Shoot multiplication (stage 2)

P1: Media. — Shoot-tips were cultured on 3/4-MMO+ with 3 mg BAP/l solidified with agar. After 21 d the explants were sized and selected as in experiment E3 and recultured on liquid MMO or MMO+, each at full-, 3/4-, and 1/2-strengths with 3 mg BAP/l. Explants were recultured and all shoots ≥ 2 cm harvested every 21 d. The experiment was terminated after 63 d when all shoots ≥ 0.3 cm were counted and added to the previous totals.

P2: BAP concentration. — Shoot-tips were cultured on 3/4-MMO+ agar with 1, 2, 3 or 4 mg BAP/l for 21 d and then recultured on normal-strength liquid MMO+ at the same BAP concentration. The explants were recultured every 21 d and divided into 3 parts 63 and 84 d after initial culture. 1 part of each explant was recultured on the same BAP concentration and the other 2 parts discarded. Shoots were harvested after 63, 84 and 105 d.

P3: Long term culture on different BAP concentrations. — 6 shoots, 1—1.5 cm long, from each of the BAP treatments except the 1 mg/l in experiment P2 were cultured in 5 ml of liquid MMO+ at the same BAP concentration. A 5 mg/BAP/l treatment was added using shoots from the 4 mg/l treatment.

14 and 28 d after culturing, the explants were recultured in new media in 50 ml flasks for 42 d, then transferred to 125 ml erlenmeyer flasks with 15 ml of media. Every 21 d thereafter, shoots ≥ 2 cm long were removed and counted and 2 explants from each treatment divided into 3 parts and recultured. The remaining cultures were discarded. 150 d after the shoots were cultured (210 d from initial culture) the explants were transferred to 30 ml of liquid MMO+ in 455-ml wide mouth jars for 21 d and the number of shoots ≥ 0.3 cm long were determined.

Rooting (stage 3)

The effect of the shoot proliferation media on subsequent rooting was assessed by placing 6 shoots from each of the treatments in the previous experiments on Whatman No. 5 filter paper bridges in 25 × 150 ml culture tubes containing 1/3-MMO with 0.3 mg indole-3-acetic acid/l (IAA). The earliness of rooting and the number of shoots that rooted in 14 d was determined.

The following five experiments were conducted to determine the effect of different media and compounds on rooting. Shoots were excised from explants growing in liquid MMO+ with 3 mg BAP/l and placed on individual filter paper bridges in liquid media. 6 shoots were used per treatment except in the tests with "soil" mixes.

Media and sucrose concentrations. — Normal-, 3/4-, 1/2-, and 1/4-strength MMO with 10, 20, 30 or 40 g sucrose/l were evaluated in all combinations. Auxins. — In a series of experiments IAA, indolebutyric acid (IBA) and α-naphthalene acetic acid (NAA) were compared at 0—1.0 mg/l in 1/4-MMO with 10 g sucrose/l added (total sucrose 17.5 g/l).

PO_4. — The effect of added PO_4 on rooting was compared in two experiments. In the first 0, 50, 100, 150 and 200 mg/l and in the second 0, 125, 150 and 175 mg $NaH_2PO_4 \cdot H_2O$/l were added to 1/4-MMO with 0.1 mg IAA/l and sucrose increased to 17.5 g/l.

Temperature. — Rooting of shoots in MMOR (1/4-MMO plus 10 g sucrose/l, 0.1 mg IAA/l and 150 mg $NaH_2PO_4 \cdot H_2O$/l) were compared at 20, 25, 30 and 35 °C. Cul-

ture tubes containing the shoots were placed in water baths which maintained temperatures within ± 1 °C.

Direct rooting in "soil". — Rooting in perlite, 1 : 3, 1 : 1 and 3 : 1 mixtures of peat-perlite, peat-vermiculite, and perlite-coarse sand was compared in a series of experiments. The media was sterilized, placed in sterile 10 × 16 × 3 cm plastic trays, and dampened with MMOR, with and without sucrose. Shoots placed in media without IAA were pre-soaked in sterile distilled water containing 0.01 mg IAA/l for 1 h. High humidity was maintained by placing the trays under plastic covers for 2 to 3 wks and then transferring them to intermittent mist for a further 10 to 14 d. 15 shoots were used per treatment.

Culturing, proliferating and rooting of other genotypes

To determine whether the procedures developed for propagating Baco could be applied to other genotypes, 1—3 shoot-tips of 21 genotypes were cultured on MB5+ or 3/4-MMO+ with 3 mg BAP/l and solidified with agar. Any genotypes which failed to grow the first time were cultured from new shoot-tips. The basic proliferation procedure was later adjusted by reducing the culture interval to 2 wks and the BAP to 2 mg/l.

Shoot-tips were taken from plants of Okanagan Riesling, Seyval Blanc, Vidal 256, Concord Seedless, Oraniensteiner, Limberger (2 clones), Weisser Burgunder, GM 32258, GM 31657, GM 31158, Castel 19637, Kerner, and the rootstocks SO 4 (2 clones), 5 C (3 clones) which had indexed free of known viruses, and Verdelet, Muller Thurgau, Perle of Csaba, 125 AA, Rotberger, GM 32458 and Interlaken, which were free of known viruses except Fleck.

Over 100 shoots of each accession were rooted on MMOR and planted in the field to determine trueness to type.

Results and discussion

Propagation by protoplast, callus, embryogenesis or organogenesis is relatively slow in comparison to the adventitious shoot proliferation methods described by MURASHIGE (1974). Likewise, the in vitro shoot-tip culture methods used by GIFFORD and HEWITT (1961), GALZY (1972) and BINI (1976) for eradicating viruses produced only 1 shoot from each shoot-tip and the shoots were difficult to root. These methods were therefore of limited use in developing a rapid propagation method.

MURASHIGE (1974) identified three stages for *in vitro* propagation, namely: establishment of aseptic cultures, multiplication, and preparation for establishment in soil. The requirements of all three stages for *Vitis* are described in this paper.

Establishing aseptic cultures (stage 1)

No difficulty was experienced in producing aseptic cultures by either of the two methods used for surface sterilization. In all experiments less than 1 % were contaminated.

The optimum conditions for establishing shoot-tips in culture were difficult to determine because of wide differences in the results obtained when experiments were repeated, and because there were no practical objective ways to measure establishment. Tissue weight was impractical, since the heaviest explants were compact masses of tissue which were slow to produce shoots while the lightest explants produced only 1 or 2 shoots. The most productive explants were intermediate in weight and compact-

ness. Similarly, shoot-tip survival was of limited value because of wide differences in the subsequent rate of growth. The number of shoot-tips which survived in 14 d and the type of growth after 28 d appeared to be the most promising method.

All shoot-tips on all media were alive after 14 d in 3 of the 7 times that the different media and media strengths were tested. On other occasions, in some treatments as few as 2 out of 6 shoot-tips survived. After 14 d out of a total of 84 shoot-tips cultured on each medium, 75 survived on MB5 and MB5+, 72 on 3/4-, 69 on 1/2-, and 67 on MMO and MMO+. The addition of PO_4 and Ad reduced the survival on MMO but had no apparent effect in other media.

Although many shoots were alive after 14 d the amount and type of growth varied considerably with different shoot-tips on the same medium. When survival was very high, growth was generally very rapid and there was little or no difference between treatments. At other times, however, many shoot-tips developed into a compact mass of tissue and buds which were slow to elongate into shoots. Fewer shoot-tips produced this type of growth on MB5 than on other media.

Survival and growth in the first 14 d was slightly better on MB5+ but by the 28th d, explants on 3/4-MMO+ were larger and had a better color than explants on any other medium. The 3/4-MMO+ medium was therefore adopted for culturing shoot-tips in future experiments.

Shoot-tips of many of the 21 genotypes tested developed into compact masses of tissue. At first this was thought to be a characteristic of the genotype. Eventually, however, a few long shoots were produced and if the shoot-tips of these were removed and cultured on fresh media usually proliferated normally. In addition, when new shoot-tips of difficult or easily proliferated genotypes were recultured the ease of proliferation was often reversed, with formerly difficult genotypes proliferating readily and formerly easy genotypes proliferating with difficulty. This suggested that at least part of the difference in the ease of proliferation was due to differences in the condition of the mother plants when the tips were collected.

A review of the results showed that shoot-tips taken from plants with a good green color survived better and proliferated more readily than shoot-tips from plants with a relatively poor color. This indicated that shoot-tip survival was reduced and more tissue masses produced if a mother plant had been subjected to periods of moisture or nutrient stress, or low light intensities.

Shoot-tips of Limberger, 5 C and SO 4 from plants growing in the greenhouse had a good green color and all survived and proliferated readily. All other 18 genotypes were cultured from plants growing in screenhouses under low-light intensity. Of these, Kerner, Muller Thurgau, Okanagan Riesling, GM 31657, and Interlaken survived and grew rapidly the first time they were cultured. 8 genotypes, Verdelet, Vidal 256, Rotberger, Weisser Burgunder, GM 32258, GM 32458, GM 31158 and Perle of Csaba did not survive the first culturing but new shoot-tips survived and proliferated readily. 5 genotypes, Oraniensteiner, Castel, 125 AA, and especially Concord Seedless and Seyval blanc were difficult to culture and slow to produce shoots. Some clones of SO 4, 5 C and Limberger were easy to culture and proliferated readily, while other clones were much more difficult, but eventually all clones of the 21 genotypes proliferated.

Attempts to overcome the compact growth habit met with inconsistent results. Reducing BAP, omitting Ad and/or PO_4, and reducing the light intensity appeared to help on some occasions.

The amount of BAP had no effect on the number of shoot-tips that survived after 14 d, or on the number of shoot-tips which formed compact masses. However, the type of growth was significantly altered. The 0 and 1 mg/l treatments produced 1—3 long green shoots with well developed leaves whereas 4 and 5 mg/l produced compact

explants having many short, red shoots with abnormally shaped leaves. The 2 and 3 mg/l treatments produced numerous reddish shoots and a less compact type of growth but the reddish color disappeared as the stems elongated, and normal leaves were produced. The 3 mg/l treatment was selected for all subsequent experiments.

JONA and WEBB (1978) cultured lateral shoots of *V. vinifera* Sylvaner Riesling solely on medium solidified with agar. BARLASS and SKENE (1978, 1980) found that fragmented shoot-tips of Cabernet Sauvignon grew better on liquid media but found it was necessary to solidify the media with agar for adventitious shoot production. In the work reported here the opposite results were obtained. Preliminary tests had produced a consistently higher survival on agar and more rapid proliferation using liquid media.

The earliest shoot production and greatest yield of shoots was obtained from explants which had been on agar for the shortest time (4 wks). In this treatment, the first shoots were harvested 63 d after the shoot-tips were cultured compared to 77, 84, 90 and 98 d from the 6-, 8-, 10- and 14-wks-on-agar treatments, respectively (Table 1). After 105 d the maximum yield of shoots was obtained from the 4-wks-on-agar treatment but the optimum time on agar may be less than 4 wks.

After 63 d the explants from the 4-wk-on-agar treatment were 6 to 8 cm in diameter and tips of some shoots were necrotic. The explants were divided into 4 parts but it was not feasible to reculture all 24 divisions. Consequently, only 1 part of each explant was recultured and the other 3 parts discarded. This restored growth and eliminated the tip necrosis but reduced the potential yield by 75 %. Since the necrosis indicated a growth deficiency it was decided to reduce the reculture interval from 4 to 3 wks in future experiments.

On the basis of the above experiments a standard of 3 wks on 3/4-MMO+ with 3 mg BAP/l and 0.7 g agar/l was adopted for the initial culture of shoot tips.

Shoot multiplication (stage 2)

The strength of the medium had no effect on the number of shoots ≥ 0.3 cm produced in 63 d (21 d on 3/4-MMO+ and 42 d on stage 2 media). The mean number of shoots produced were 10.9, 11.8 and 16.1 on 1/2-, full-, and 3/4-strength media, respectively (sample size 12, standard error 5.91). The addition of PO_4 and Ad increased the

Table 1

Effect of length of time in agar medium before reculturing to liquid medium on the earliness of shoot production and the number of shoots produced by Baco grape

Die Abhängigkeit der Wachstumsgeschwindigkeit und der Anzahl der Triebe von der Kulturdauer auf Agarmedium vor der Übertragung auf Flüssigmedium bei der Rebsorte Baco

No. of wks on		1st harvest		105 d harvest
Agar	Liquid	No. of d	No. of shoots ≥ 2 cm	No. of shoots ≥ 0.3 cm
4	10	63	5.2	39.2[1])
6	8	77	1.9	20.3
8	6	84	2.2	16.7
10	4	90	1.8	8.3
14	0	98	2.2	9.7

[1]) Explants from this treatment were divided after 63 d and only ¼ of each explant was recultured. The number of shoots ≥ 0.3 cm is, therefore, only ¼ of the potential.
Std. error: 3.53..
Sample size: 6.

Table 2

Effect of BAP concentration on number of shoots of Baco grape produced *in vitro*

Der Einfluß der BAP-Konzentration auf die Anzahl der *in vitro* gebildeten Triebe bei der Rebsorte Baco

Culture Interval (d)	Shoot length (cm)	BAP (mg/l) 1	2	3	4	5	Sample size	Std. error
0— 63 d	≥ 2 cm	1.00	2.4	4.8	6.6	—	5	1.03
63—105 d[1])	≥ 2 cm	1.80	11.2	17.4	8.2	—	5	1.01
63—105 d[1])	≥ 0.3 cm	4.60	20.8	31.4	17.4	—	5	2.35
105—231 d[2])	≥ 0.3 cm	—	59.6	54.2	76.2	55.2	5	5.21

[1]) Experiment P1. — All explants were divided after 63 and 84 d. and only 1/3 of each explants was recultured. The numbers of shoots shown are therefore, only 1/9 the potential which could have been obtained if all divisions had been recultured.

[2]) Experiment P2. — At the end of the 105 d interval 1—1.5 cm long shoots from all BAP treatments were placed on the same BAP concentration. The explants were divided after 63, 84 and 105 d and only 1/3 of each recultured. The number of shoots shown is, therefore, only 1/27 of the potential if all divisions had been recultured.

mean yield of all media from 9.1 to 16.8 shoots per plant (sample size 18, standard error 3.94). Since PO_4 and Ad markedly increased shoot production and there was no difference between media strength, the full strength medium with added PO_4 and Ad (MMO+) was used in future experiments on shoot multiplication.

On 1 mg BAP/l, each explant produced 1 or 2 long, green shoots with well developed leaves. However, the lower leaves died progressively as the shoots increased in length. With increasing amounts of BAP, more but shorter shoots were produced, the explants were more reddish in color, and at 4 and 5 mg BAP/l the leaves were deformed. However, if the explants were not recultured to fresh media, the reddening gradually disappeared, the shoots elongated and newly formed leaves had a normal shape.

The number of shoots ≥ 2 cm produced in the first 63 d increased linearly with BAP concentration up to a maximum at 4 mg/l, but between 63 and 105 d more shoots were produced with 3 mg/l BAP (Table 2). There was no apparent relationship between BAP concentration and the number of shoots ≥ 2 cm produced between 105 and 147, or 147 and 231 d. However, the total number of shoots ≥ 0.3 cm in length produced between 147 and 231 d was greatest at 4 mg/l (Table 2).

These differences appear to be due to an interrelationship between the size and rate of growth of the explants, and the length of the interval between dividing and reculturing. At low BAP concentrations a few buds are produced which rapidly develop into shoots and in the short term produce relatively more shoots than explants on higher BAP concentrations. On the other hand, explants on high BAP concentrations continue to form buds until the BAP is reduced sufficiently to allow the shoots to elongate and in the long run produce more shoots.

Optimum BAP concentrations for shoot production on explants of Baco on a 3-wk reculture interval was, therefore, between 3 and 4 mg/l. However, during the propagation of the 21 genotypes it was found that as good or better shoot production could be obtained by reducing the BAP to 2 mg/l and the reculture interval to 2 wks. In addition, the explants had a better color and the shoots rooted readily. The optimum BAP con-

centration for propagation of grapes is, therefore, about 2 mg/l which is in agreement with JONA and WEBB (1978) and BARLASS and SKENE (1978).

A single 3 mm long shoot-tip of Limberger cultured on 3/4-MMO+ agar and 3 mg BAP/l for 2 wks and then recultured on MMO+ liquid and 3 mg BAP/l had after 3 months produced 10 cultures each about 6 cm in diameter with 80 to 100 shoots. Subsequent tests showed that dividing each of the 10 cultures into 4 would provide 3,000 shoots within 2 wks. If these 40 cultures were again divided the 160 cultures would produce 12,000 shoots within 2 wks. In other words, the system described in this study would produce over 12,000 shoots in 4 months compared to 8,000 by the BARLASS and SKENE (1978) method.

Rooting of proliferated shoots (stage 3)

Inconsistent rooting of shoots produced *in vitro* appears to be a problem in many species. In *Vitis*, GIFFORD and HEWITT (1961) rooted only 2 % of the shoots; GALZY (1972) rooted 40 % but only succeeded in producing normal plantlets from 21 %; BINI (1976) and JONA and WEBB (1978) each rooted "some"; CHEE (1980) rooted 24 from an uspecified number; and BARLASS and SKENE (1978, 1980) found that shoots of Cabernet Sauvignon and Cabernet Franc rooted readily on a hormone free medium but an auxin was required for all other cultivars.

In preliminary tests it was found that on media solidified with agar the percentage of shoots that rooted was very low, and those that did root usually produced a single thick "rat-tail" root and were difficult to establish in soil. Several methods of rooting proliferated shoots were examined but filter paper bridges in liquid media were the most consistent. However, it was not unusual to obtain 100 % rooting in 8 d in some tests but little or no rooting when the test was repeated.

Because of rooting inconsistencies and paucity of information on the factors that affect rooting of proliferated shoots, the observations made during the progress of this work are given in more detail than is normal for unreplicated data. However, these observations are justified since they resulted in a procedure in which nearly 100 % of the shoots root consistently in 8—14 d.

The proliferation medium had only a marginal effect on the subsequent rooting of the shoots. Shoots produced on MMO with PO_4 and Ad rooted 1—2 d earlier and provided 1 or 2 more rooted plantlets than shoots produced on weaker MMO or media without PO_4 and Ad. The amount of BAP in the multiplication medium had no consistent effect on the total number that rooted but rooting was slightly earlier with lower amounts. Increasing the length of time on agar decreased the number of rooted shoots from 4 out of 6 after 4 wks-on-agar to 2 or 3 in the 6-, 8- and 10-wk, and 1 in the 14-wk agar treatments. Number and quality of the roots also decreased with increasing length of time on agar.

Differences in rooting of shoots from the various multiplication media are believed to be due to the condition of the shoots rather than a direct effect of the media. Reddish shoots with poorly shaped stems or leaves caused by BAP in the tissue rooted poorly. Shoots from explants in which some of the shoots were necrotic also rooted poorly. The necrosis appears to be caused by a deficiency of one or more compounds which results in a hardening of the tissue. This suggests that in order to determine when to remove shoots for rooting more attention should be given to the appearance of the tissues. If there are indications of necrosis of the shoot-tips the reculture interval should be shortened and explants divided more often while if the explants are red with poorly shaped leaves, the reculture interval should be increased. In the propagation of the 21 genotypes it was found that a 2-wk divide and reculture interval was nearly ideal when the BAP was reduced to 2 mg/l.

After 8 d on rooting media the average number of rooted shoots decreased with increasing media strength from 4.5 shoots (out of 6) on 1/4-MMO to 3.5, 3.0 and 2.2 on 1/2-, 3/4 and full-strength MMO, respectively. At the same time an average of 4.2 shoots had rooted with 20 g sucrose/l compared to between 3.0 to 3.2 on the other sucrose concentrations. After 14 d the difference between treatments had narrowed, ranging from 4.2 to 4.8 on the different media strengths and 4.0 to 4.9 on the sucrose concentration. There was no interaction between media and sucrose concentrations.

In the series of experiments with different auxins, shoots on media with IBA produced very few roots. Shoots on NAA produced numerous short, thin roots and considerable callus but were difficult to transplant successfully in soil. Rooting on IAA was generally better and the rooted shoots established in soil quite readily. However, the optimum level of IAA fluctuated between 0 and 0.3 mg/l in identical tests repeated several times. This suggested that either different amounts of IAA decomposed during autoclaving or there were differences in the amount of endogenous auxins in the different tests. With 0.3 mg/l or less, practically no callus was formed while with over 0.3 mg/l, heavy callus was formed in every test. The fluctuations are, therefore, more likely to be caused by differences in the endogenous auxin in the tissue. A concentration of 0.1 mg IAA/l was adopted and produced good rooting on shoots of all the genotypes subsequently tested.

With 150 mg $NaH_2PO_4 \cdot H_2O$/l in the rooting medium 5 of 6 shoots rooted in 9 d compared to 3, 3, 2, and 1 with 100, 200, 50 and 0 mg/l, respectively. Similarly, in the second test 5 of 6 shoots rooted in 10 d with 150 mg/l compared to 4, 3 and 2 in media with 125, 175 and 0 mg $NaH_2PO_4 \cdot H_2O$/l, respectively. In the later test, after 16 d all 6 shoots had rooted on media containing 150 and 175 mg/l compared to 5 on 0 and 125 mg/l. The mean number of primary roots per shoot after 16 d was not affected but secondary branching of the roots decreased with increasing PO_4. While rooting the 21 genotypes it was found that 100 % rooting can be obtained without added PO_4 which suggests that additional PO_4 is only needed to improve rooting of more mature shoots.

Roots were visible after 5 d at 30 °C and in 7 d, 5 of the 6 shoots had rooted compared to 6 and 7 d for roots to appear at 35 and 25 °C, respectively. After 16 d, 5 of 6 shoots had rooted at 25, 30 and 35 °C, 4 at 20 °C and none at 40 °C. While the optimum rooting temperature is about 30 °C rooting of shoots of all 21 genotypes was accomplished at 22 °C without much trouble. Increasing the temperature for rooting would probably have produced earlier rooting but since this temperature is too high for multiplication it would have necessitated a separate growth room.

Rooting on filter paper bridges is a labour intensive, costly procedure but in the early stages gave the fastest and most reliable rooting. The rooted shoots established in soil with few losses if the humidity was kept high for about 14 d. First attempts to root directly in soil, peat, perlite and vermiculite and mixtures of the four gave very poor results. Algae and fungal contamination was a frequent problem in media containing sugar, peat or soil. With both perlite and vermiculite rooting occurred on the surface of the media indicating a possible lack of aeration. In later tests coarse perlite saturated with MMOR without sucrose gave equal or better rooting than bridges provided the shoots were retained in a humidity chamber for 3 wks. Mist appeared to leach the nutrients and decrease rooting.

Conclusions

Results obtained in this study have identified the requirements for *in vitro* multiplication of *Vitis*. The following are the procedures adopted for each stage.

Stage 1: Culture shoot-tips on MB5+ for 14 d, or, if the shoot-tips have a dark green color on 3/4-MMO+ for 14 to 21 d. Add 3 mg BAP/l and 0.7 g agar/l to both media.

Stage 2: Transfer from stage 1 medium to 5 ml of 3/4-MMO+ liquid with 3 mg BAP/l in 50-ml erlenmeyer flask. After 14—21 d, and every 14—21 d thereafter, reculture to MMO+ with 3 mg BAP/l. Move to 125 ml erlenmeyer flasks and 455 ml wide mouth jars before explants are too large to be removed without damage. When shoots start to elongate, reduce BAP to 2 mg/l and the reculture interval to 2 wks. When the explants reach about 6 cm in diameter divide into 4 pieces and reculture each piece separately.

Stage 3: Remove only soft, green shoots with well developed leaves and roots by one of the following methods:

a) Support shoots on filter paper bridges in 1/4-MMO plus 10 g sucrose/l and 0.1 mg IAA/l. After rooting pot in a sterile 1:4 peat-vermiculite mix and maintain a high humidity for 2 wks.

b) Place shoots in sterile coarse perlite saturated with 1/4-MMO with 0.1 mg IAA/l but no sucrose. Maintain a high humidity in a chamber for 3 wks and then place in intermittent mist for 1 wk.

Summary

An *in vitro* method capable of producing over 12,000 shoots in 4 months from a single 3—5 mm long grape shoot is described. Shoot production in the French hybrid cv. Baco grown on Murashige's minimal organic medium (MMO) supplemented with 80 mg adenine sulfate/l, 170 mg sodium phosphate/l (monobasic), and 3—4 mg N^6-benzylaminopurine/l (BAP) was increased 7fold by starting on media solidified with 0.7 g agar/l for 4 wks and then reculturing to liquid media. Subsequently, 21 grape genotypes were propagated on the same medium with the BAP reduced to 2 mg/l and the reculture interval from 3 to 2 wks.

Over 90 % of the shoots rooted readily in 7—14 d when supported on filter paper bridges in 1/4-strength MMO liquid supplemented with 150 mg sodium phosphate/l (monobasic), 10 g sucrose/l and 0.1 mg indole-3-acetic acid/l, or in 14—21 d in coarse perlite saturated with the same medium without sucrose.

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