Vitis 22, 363—374 (1983)

Department of Pomology and Viticulture, New York State Agricultural Experiment Station, Cornell University, Geneva, NY, USA

In vitro vegetative propagation of *Vitis*: Application of previously defined culture conditions to a selection of genotypes[1])

by

R. CHÉE and R. M. POOL

Multiplication végétative de la vigne *in vitro*: Application des conditions de culture définies auparavant pour une sélection de génotypes

R é s u m é . — La multiplication végétative *in vitro* a été réalisée pour une collection d'espèces, de cultivars et d'hybrides du genre *Vitis*.

21 génotypes furent mis en culture à partir d'apex de 2 à 4 ébauches foliaires, par une méthode que nous avions développé pour l'hybride Rougeon. Ces conditions de culture n'ont pas été favorables á la croissance de l'hybride Seyval.

La production de pousses herbacées fut obtenue pour ces 21 génotypes sur un milieu nutritif que nous avions défini pour l'hybride Remaily Seedless. Pour 4 génotypes les conditions de cultures n'ont pas été favorables. L'influence de la photopériode sur la production de pousses fut étudiée également. Les journées de 10 h bénéficièrent 3 génotypes et les journées de 16 h un seul. En général, la production en jours courts s'est avérée meilleur qu'en jours longs.

La multiplication des pousses fut éffectuée par repiquage de boutures terminales obtenues *in vitro* et comprenant 3 à 4 noeuds (1,5 cm). Pour les 5 premiers passages la multiplication de 17 génotypes fut continuée sur le milieu de culture utilisé pour la production des premières pousses. Les meilleurs résultats ont été obtenus pour Remaily Seedless. En 2 mois chaque bouture apicale de ce cultivar a produit en moyenne 13 pousses ayant au moins 3 noeuds. Le 6e repiquage a été fait sur un milieu, C_2D, développé auparavant pour améliorer la multiplication de Remaily Seedless. La récolte fut augmentée ainsi de 31 % à 350 % selon le génotype sauf pour *V. labruscana* Catawba qui s'est nécrosé.

Pour l'enracinement de boutures apicales obtenues en culture, un milieu défini à cet effet pour Remaily Seedless fut utilisé avec succès pour 15 génotypes.

Introduction

Previously, we reported a method for *in vitro* micropropagation of grapevines from shoot apices (CHÉE and POOL 1982). This method was developed for the *Vitis* hybrid Rougeon. The hybrid Remaily Seedless was used to further investigate effective techniques of micropropagation (CHÉE 1982). As a result, adequate incubation conditions and media have been defined for the establishment in culture of shoot apices for shoot production from the established cultures, for shoot multiplication from subcultured shoots and for rooting of subcultured shoots.

In this report we examined the behavior of various *Vitis* genotypes under the procedures which had previously been successful in the micropropagation of Rougeon and Remaily Seedless.

---

[1]) This manuscript has been approved by the Director of the New York State Agricultural Experiment Station for publication as journal paper No. 3523.

Materials and methods

Plant materials

Dormant canes of 21 *Vitis* species and cultivars (Table 1) growing in vineyards at the New York State Agricultural Experiment Station, Geneva, New York, were collected in January, 1981, and stored in plastic bags at 7 °C. In late March, 1-node cuttings were forced in water under the incubation conditions used for micropropagation and in 10-h days. Shoot tips, 2—3 cm long, were collected at the separated flower cluster stage. All flower clusters and expanded leaves were removed. Following surface sterilization, 0.5—1 mm shoot apices (containing apical dome and 2- to 4-leaf primordia) were excised and established in culture.

Incubation conditions

The light source used for the establishment in culture, shoot production and shoot multiplication was a 1 : 1 mixture of "Gro-lux" (F40GRO) and "Cool White" (F40CW) fluorescent tubes (Lifeline series, manufactured by Sylvania). The tubes were 14 cm apart and 30 cm above the cultures. Total radiant energy measured at the explant level was 1900 μW cm^{-2}. For root production only "Cool White" fluorescent tubes were used, total energy being 2200 μW cm^{-2}. Daylength was 10 h for all experiments and the temperature varied from 18 °C in the dark to 27 °C in light. Shoot production was also investigated with 16-h days.

Experiments and culture media

The behavior of the different genotypes was surveyed for four steps in micrpropagation of grapevines. These are: 1) establishment in culture, 2) initial shoot production, 3) shoot multiplication, and 4) rooting.

Establishment in culture. — Shoot apices were plated on medium contained in sterile plastic petri dishes 100 mm in diameter and 15 mm high. The medium utilized was essentially the one devised for the establishment in culture of the *Vitis* hybrid Rougeon (CHÉE 1980, CHÉE and POOL 1982). It contained the salts of MURASHIGE and SKOOG (1962), 3 μM thiamine · HCl, 55.5 μM myo-inositol, 8 μM nicotinic acid, 5 μM pyridoxine · HCl, 5 μM benzylaminopurine, 0.5 μM naphthaleneacetic acid, 3 % sucrose, and 0.8 % "Bacto Agar" (DIFCO Laboratories). The pH was adjusted to 5.7—5.8 before autoclaving.

Data which were collected after 55 d included: 1) the percentage of surviving explants; 2) for live cultures, the area covered by each explant as a measure of vigor; 3) the percentage of explants that were caulescent; 4) the percentage of explants that produced shoots; and 5) the percentage of explants that produced callus. The general appearance of the culture was also recorded.

Shoot production. — 21 of the genotypes were tested under 10-h days and 18 genotypes under 16-h days. The explants established in culture, as described above, were subcultured "as is" at 55 d onto media contained in sterile plastic petri dishes 100 mm in diameter and 25 mm high. The medium used for shoot production differed from that used for establishment in that naphthaleneacetic acid was deleted.

After 57 d of subculture each genotype was evaluated for: 1) percentage of cultures that produced shoots; 2) mean number of shoots produced per culture; and 3) the percentage of shoots of at least 3 nodes (1 cm long or longer). Shoots of at least 3 nodes are adequate for further micropropagation.

Shoot multiplication. — Shoot multiplication was evaluated with two sets of inorganic media constituents. 17 genotypes were tested on the medium used for

shoot production which contained the salts of MURASHIGE and SKOOG. The results are given for the 5th subculture. 16 of the genotypes were then multiplied on the salts C_2D (CHÉE 1982).

The salts C_2D are: 1) macronutrients (mM; mg l^{-1}): NH_4NO_3 (20.6; 1650), KNO_3 (18.8; 1900), $MgSO_4 \cdot 7\ H_2O$ (1.5; 370), KH_2PO_4 (1.25; 170), $Ca(NO_3)_2 \cdot 4\ H_2O$ (3.0; 708.5), $FeSO_4 \cdot 7\ H_2O$ (0.1; 27.1) $Na_2 \cdot$EDTA (0.1; 37.3); 2) micronutrients (µM; mg l^{-1}): $MnSO_4 \cdot 4\ H_2O$ (5.0; 0.845), H_3BO_3 (100.0; 6.2), $ZnSO_4 \cdot 7\ H_2O$ (30.0; 8.6), $Na_2MoO_4 \cdot 2\ H_2O$ (1.0; 0.25), $CuSO_4 \cdot 5\ H_2O$ (0.1; 0.025), $CoCl_2 \cdot 6\ H_2O$ (0.1; 0.025).

The explants were 3- to 4-node shoots (1.5 cm) produced *in vitro*. They were placed horizontally and lightly pressed into the medium. The cut base was submerged into the medium. The cultures were in sterile plastic petri dishes 100 mm in diameter and 25 mm high.

Data recorded after 66 d for the 5th subculture and after 67 d for the 6th subculture were: 1) percentage of explant survival; 2) percentage of explants producing shoots; and 3) mean number of shoots of 3 or more nodes per explant.

Rooting of subcultured shoots. — 15 genotypes were tested. The explants were 3- or 4-node shoots (1.5 cm) produced in culture. They were placed vertically down to the basal node into medium contained in culture tubes 25 mm in diameter and 150 mm high.

The requirements for root production have been investigated elsewhere with Remaily Seedless (CHÉE, 1982). The medium used for rooting in this study differed from the one used in establishment in culture in terms of sugar concentration and in phytohormone content. Sucrose was reduced from 3 % to 1 %. Naphthaleneactic acid was 0.4 µM and there was no cytokinin.

The data collected after 30 d of culture were: 1) the quality of the shoots; 2) the number of nodes expanded per explant; 3) the percentage of explants that formed roots; 4) the number of roots produced per explant; and 5) the total length of roots per explant.

The quality of the explant was reported as percentage of shoots rated "good" or better. Shoots rating "good", had only minor defects that in our experience would not affect their potential for growing roots. The defects were one or more of the following: yellow areas on leaves, pale color of shoots, and shoots that did not appear luxurious or vigorous when compared to those rating "excellent".

Statistical analyses

For the attribute data proportions were compared among cultivars using the significance test for multiple comparison of proportions after RYAN (1959). The level of significance was 0.05. The proportions were reported as percent of explants cultured. Continuous variates were analyzed by the component of variance method when the data were balanced and by multiple regression analysis when the data were unbalanced. The separation of the means among genotypes was by Duncan's Multiple Range Test at the 0.05 level of significance.

Results

Establishment in culture

The data recorded after 55 d for the 21 genotypes cultured and the statistical separation of the means are given in Table 1. The survival rate was least for Seyval (13 %)

Table 1

In vitro culture of *Vitis* · Establishment in culture from 0.5—1 mm shoot apices · Results after 55 d

Culture de la vigne *in vitro* · Mise en cultures à partir d'apex de pousses herbacées d'une longueur d'axe de 0,5—1 mm · Résultats à 55 d

	Number of explants plated	Explant survival[1]) (%)	Mean explant area[2]) (mm^2)	Explants cauliescent[1]) (%)	Explants with shoots[1]) (%)	Explants with callus[1]) (%)	Appearance
Vitis species[3])							
argentifolia	12	100 ab	158 b	42 b	33 abc	42 ab	Pale green
labruscana Concord	16	100 ab	247 b	19 b	19 abc	50 a	Pale green
labrusca Alba	19	100 a	79 b	5 b	5 abc	0 c	Light green
riparia Pulliat	19	95 abc	94 b	100 a	0 c	0 c	Green & brown
cinerea	17	71 abcde	27 b	0 b	0 bc	0 c	Yellow-green; minor leaf necrosis
riparia Quebec	20	60 bcde	70 b	0 b	0 c	0 c	Yellow-brown; major leaf and stem necrosis
Vitis vinifera							
Cabernet Sauvignon	10	100 ab	479 a	20 b	60 a	40 ab	Yellow-green
Gewurztraminer	10	80 abcde	102 b	100 ab	25 abc	0 abc	Pale yellow-green
Chardonnay	14	100 ab	214 b	71 ab	14 abc	0 abc	Green; leaf necrosis
White Riesling	14	79 abcde	52 b	27 b	0 abc	0 abc	Pale yellow-green
Pinot noir	11	36 cde	89 b	25 b	0 abc	0 abc	Yellow-green
Vitis hybrids							
Baco noir	18	100 a	144 b	50 b	39 ab	0 c	Pale yellow-green; leaf necrosis
Delaware	11	100 ab	207 b	91 ab	9 abc	0 abc	Yellow-green
Rougeon	19	95 abc	132 b	18 b	6 abc	18 c	Yellow, brown & green; leaf necrosis
Seibel 880	20	95 abc	92 b	5 b	11 abc	11 c	White brown; major necrosis
Kober 5BB	12	92 abcd	111 b	9 b	0 abc	18 abc	Pale yellow-green
Cayuga White	20	90 abcd	104 b	33 b	6 abc	5 abc	Yellow, brown; major necrosis
Couderc 3309	19	42 cde	58 b	0 b	0 c	0 c	Yellow, brown; major necrosis
DeChaunac	12	33 de	107 b	25 b	0 abc	0 abc	Yellow, green & yellow-brown
Aurore	17	18 e	147 b	100 a	0 bc	0 c	Green
Seyval	16	13 e	22 b	0 b	0 abc	0 abc	Yellow-green

[1]) Separation of proportions after RYAN (1959) (p = 0.05). Because separations are influenced by number of replications, equal percentage values may have different associated letters.

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

[3]) *V. argentifolia* = GBC 17 595/68; *V. cinerea* = ILL 194-1 62/69.

and Aurore (18 %). 13 of the 21 genotypes had at least 80 % successful establishment and 7 of these had 100 % establishment. Cabernet Sauvignon explants grew largest, producing the largest explant area. The remaining explant area means were not statistically separated, but *V. cinerea* and Seyval were smallest.

Shoot apices developed either into rosettes of leaves or expanded into caulescent shoots. The shoot apices of *V. cinerea*, *V. riparia* Quebec, Couderc 3309 and Seyval developed strictly as rosettes. Those of *V. riparia* Pulliat, Gewurztraminer and Aurore developed thin internodes. Other genotypes showed both types of development. Shoots were produced by 11 genotypes. For these genotypes, the percentage of explants with shoots varied from 5 % for *V. labrusca* Alba to 60 % for Cabernet Sauvignon.

Callus production occurred on *V. argentifolia*, *V. labruscana* Concord, Cabernet Sauvignon, Rougeon, Seibel 880, Kober 5BB, and Cayuga White explants. The percentage of explants producing callus varied from 5 % for Cayuga White to 50 % for Concord.

The color of the stem and leaves varied among genotypes. Excessive necrosis (at least 50 % of the explants) was observed for Quebec, Seibel 880, Cayuga White and Couderc 3309.

In conclusion, all genotypes except Seyval were successfully established in culture. Most luxurious and vigorous growth was with *V. argentifolia*, Concord, Cabernet Sauvignon, Gewurztraminer, Baco noir, and Delaware. Success was questionable for *V. cinerea*, Quebec, Pinot noir, DeChaunac and Aurore.

Shoot production

21 genotypes were cultured in 10-h days (Table 2). The genotypes with least shoot production were *V. cinerea* (28 %), Pinot noir (25 %), Seyval (33 %), and Aurore (50 %). *V. argentifolia* (60 %), *V. labrusca* Alba (71 %) and *V. riparia* Quebec (60 %) produced an adequate number of shoots. With the remaining 14 genotypes, shoot production occurred in at least 75 % of the cultures.

The number of shoots produced per explant was greatest with Cabernet Sauvignon (48 shoots), otherwise the number of shoots ranged from 1 and 2 for Seyval and *V. cinerea* to 16 for *V. riparia* Pulliat and 17 for Gewurztraminer and Kober 5BB.

With *V. cinerea*, Quebec, DeChaunac and Aurore no shoots of at least 3 nodes (the size considered adequate for micropropagation) were produced.

Generally, shoot production under 10-h days was not adequate with *V. cinerea*, Quebec, Pinot noir and Seyval. It was adequate for the other 15 genotypes.

18 genotypes were subcultured to test shoot production under 16-h days (Table 3). For 10 genotypes shoot production occurred on at least 75 % of the cultures. It was fair for *V. labruscana* Concord, Alba, and Delaware with at least 67 % of the cultures producing shoots. It was least for *V. cinerea* (17 %), Quebec (50 %), Chardonnay (50 %), Pinot noir (25 %) and Rougeon (50 %). Shoot size was not adequate for micropropagation in the case of Concord, *V. cinerea*, Quebec, White Riesling, Pinot noir, Rougeon, and Couderc 3309.

A comparison of the results for the 10-h days and the 16-h days showed that the percentage of explants that produced shoots was significantly higher in 10-h days for Rougeon ($p = 0.05$). The number of shoots produced per explant was significantly larger in 10-h days for Cabernet Sauvignon ($p = 0.05$). The percentage of shoots of at least 3 nodes was significantly higher in 10-h days for Concord ($p = 0.05$). The opposite trend was observed with *V. argentifolia* which produced larger shoots in 16-h days ($p = 0.05$).

Table 2

In vitro culture of *Vitis* · Shoot production in 10-h days · Explants subcultured following establishment in culture · Results after 57 d[1])

Culture de la vigne *in vitro* · Production de pousses herbacées en jours avec 10 h de lumière · Les explantats furent repiqués du passage de mise en culture · Résultats à 57 d[1])

	Number of explants subcultured	Explants with shoots %	Mean number of shoots/explant	Shoots with ≧3 nodes %
Vitis species[2])				
argentifolia	5	60 a	1 b	30 ab
labruscana Concord	7	100 a	10 b	44 ab
labrusca Alba	7	71 a	7 b	18 b
riparia Pulliat	6	100 a	16 b	18 b
cinerea	7	28 a	2 b	0 b
riparia Quebec	5	60 a	4 b	0 b
Vitis vinifera				
Cabernet Sauvignon	5	100 a	48 a	72 a
Gewurztraminer	4	100 a	17 b	75 a
Chardonnay	6	100 a	4 b	6 b
White Riesling	4	75 a	4 b	22 b
Pinot noir	4	25 a	4 b	30 ab
Vitis hybrids				
Baco noir	6	83 a	7 b	51 ab
Delaware	6	100 a	3 b	58 ab
Rougeon	6	100 a	16 b	14 b
Seibel 880	7	86 a	14 b	21 b
Kober 5BB	4	100 a	17 b	24 ab
Cayuga White	7	100 a	13 b	59 ab
Couderc 3309	4	100 a	11 b	7 b
DeChaunac	5	100 a	10 b	0 b
Aurore	4	50 a	9 b	0 b
Seyval	3	33 a	1 b	0 b

[1]) Separation of means within columns by Duncan's Multiple Range Test ($p = 0.05$); separation of proportions after RYAN (1959) ($p = 0.05$).
[2]) *V. argentifolia* = GBC 17 595/68; *V. cinerea* = ILL 194-1 62/69.

To conclude, only *V. argentifolia* might fare better in long days, but shoot production for Concord, Cabernet Sauvignon and Rougeon was significantly better with short days. The other genotypes had similar shoot production in 10- or 16-h days.

Shoot multiplication

The data for shoot multiplication in the 5th subculture are presented for 17 genotypes in Table 4. With Seibel 880 there was 70 % survival, and all other genotypes had at least 95 % survival. The percentage of cultures with shoots was least for Gewurztraminer (33 %), Chardonnay (47 %), and Delaware (42 %). Almost 75 % of the genotypes surveyed had at least 75 % of their cultures producing shoots.

Table 3

In vitro culture of *Vitis* · Shoot production in 16-h days · Explants subcultured following establishment in culture · Results after 57 d

Culture de la vigne *in vitro* · Production de pousses herbacées en jours avec 16 h de lumiere · Les explantats furent repiqués du passage de mise en culture · Résultats à 57 d

	Number of explants subcultured	Explants with shoots[1]) %	Mean number of shoots/explant[2])	Shoots with ≧ 3 nodes %
Vitis species[3])				
argentifolia	5	80 a	9 a	67 ab
labruscana Concord	6	67 a	10 a	0 cd
labrusca Alba	6	67 a	3 a	18 bc
riparia Pulliat	4	75 a	7 a	15 bc
cinerea	6	17 a	1 a	0 cd
riparia Quebec	4	50 a	5 a	0 bc
Vitis vinifera				
Cabernet Sauvignon	5	100 a	27 a	72 a
Gewurztraminer	4	100 a	10 a	45 abc
Chardonnay	4	50 a	8 a	42 abc
White Riesling	4	75 a	1 a	0 bc
Pinot noir	4	25 a	4 a	30 abc
Vitis hybrids				
Baco noir	3	100 a	3 a	77 a
Delaware	3	67 a	2 a	45 abc
Rougeon	6	50 a	10 a	0 cd
Seibel 880	4	100 a	15 a	25 bc
Kober 5BB	5	80 a	23 a	13 bc
Cayuga White	4	100 a	7 a	47 ab
Couderc 3309	3	100 a	4 a	0 bc

[1]) Separation of proportions after RYAN (1959) ($p = 0.05$). Because separations are influenced by number of replications, equal percentage values may have different associated letters.
[2]) Separation of means within columns by Duncan's Multiple Range Test ($p = 0.05$).
[3]) *V. argentifolia* = GBC 17 595/68; *V. cinerea* = ILL 194-1 62/69.

The number of shoots with 3 or more nodes was highest for Remaily Seedless (13 shoots). Otherwise it varied from 7 for *V. argentifolia* and *V. labruscana* Concord to 2 for Gewurztraminer.

The data for shoot multiplication in the 6th subculture on the C_2D salts are presented in Table 5. All 16 genotypes had 100 % explant survival except *V. labruscana* Catawba which died. For 12 genotypes all explants produced shoots. For 3 genotypes at least 82 % of the explants produced shoots. The number of shoots of at least 3 nodes was highest for Concord, Kober 5BB, *V. riparia* Pulliat, and Remaily Seedless with 17—20 shoots produced. Otherwise 5—15 shoots were produced per explant.

Rooting of subcultured shoots

The data recorded was a measure of the quality of the shoots subcultured and the extent of their root production (Table 6). The percentage of shoots rating "good" or bet-

Table 4

In vitro culture of *Vitis* · Shoot multiplication from 3- to 4- node (1.5 cm) shoot explants (5th subculture) · Results after 66 d · Salts of MURASHIGE and SKOOG (1962)

Multiplication végétative de la vigne *in vitro* à partir de boutures terminales obtenues en culture et ayant 3 à 4 noeuds (1,5 cm) · Résultats à 66 d pour le 5e passage · Les sels minéraux sont d'après MURASHIGE et SKOOG (1962)

	Number of explants plated	Explants alive[1]) (%)	Explants with shoots[1]) (%)	Number of shoots/ explant[2,3])
Vitis species[4])				
argentifolia	23	100 a	92 abc	7 b
labruscana Concord	25	96 ab	92 abc	7 bc
labrusca Alba	18	100 a	100 a	6 bcd
riparia Pulliat	4	100 ab	75 abcde	4 bcd
labruscana Catawba	23	100 a	96 a	3 bcd
Vitis vinifera				
Cabernet Sauvignon	18	100 a	83 abcde	3 cd
Gewurztraminer	15	100 a	33 e	2 d
Chardonnay	20	95 ab	47 cde	3 cd
Vitis hybrids				
Delaware	19	100 a	42 de	3 cd
Rougeon	12	100 a	83 abcde	3 cd
Seibel 880	10	70 b	71 abcde	6 bcd
Kober 5BB	22	100 a	59 bcde	4 cd
Cayuga White	29	100 a	93 ab	4 bcd
DeChaunac	26	96 ab	88 abcd	5 bcd
Aurore	9	100 a	78 abcde	5 bcd
Glenora	34	100 a	100 a	6 bcd
Remaily Seedless	18	100 a	100 a	13 a

[1]) Separation of proportions after RYAN (1959) ($p = 0.05$). Because separations are influenced by number of replications, equal percentage values may have different associated letters.
[2]) Separation of means within columns by Duncan's Multiple Range Test ($p = 0.05$); separation of proportions after RYAN (1959) ($p = 0.05$).
[3]) Shoots of at least 3 nodes.
[4]) *V. argentifolia* = GBC 17 595/68.

ter was best for *V. labruscana* Concord (100 %) and Gewurztraminer (100 %), least for Seibel 880 and Chardonnay (30 %). For the others it varied from 40 % to 70 %. Similarly, the mean number of nodes expanded was least for Seibel 880 (4.7), and largest for Concord (7.9). For other genotypes it varied from 5 to 6.9 nodes. The percentage of explants rooted was highest for Concord (100 %) and Gewurztraminer (100 %) and least for Delaware (20 %) and DeChaunac (20 %). Otherwise it varied from 30 % (*V. argentifolia*, Seibel 880) to 89 % (Kober 5BB, Cayuga White, and Remaily Seedless).

The mean number of roots per rooted explant was largest for Concord (5.7) and Cayuga White (6.3). Otherwise it varied from 1.5 roots (DeChaunac) to 5.2 roots (Gewurztraminer). Root production was also estimated by the total length of root produced per rooted explant. Aurore produced the greatest length of root (122 mm), *V. labrusca*

Table 5

In vitro culture of *Vitis* · Shoot multiplication from 3- to 4-node (1.5 cm) shoot explants (6th subculture) · Results after 67 d[1]) · Salts, C_2D, of CHÉE (1982)

Multiplication végétative de la vigne *in vitro* à partir de boutures terminales obtenues en culture et ayant 3 à 4 noeuds (1,5 cm) · Résultats à 67 d pour le 6e passage[1]) · Les sels minéraux, C_2D, sont d'après CHÉE (1982)

	Number of explants plated	Explants alive (%)	Explants with shoots (%)	Number of shoots/ explant[2])
Vitis species[3])				
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				
Carbernet 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

[1]) Separation of means within columns by Duncan's Multiple Range Test ($p = 0.05$); separation of proportions after RYAN (1959) ($p = 0.05$).
[2]) Shoots of at least 3 nodes.
[3]) *V. argentifolia* = GBC 17 595/68.

Alba (6 mm) and DeChaunac (9 mm) the least. It otherwise varied from 17 mm (Catawba) to 100 mm (Kober 5BB).

All things considered, rooting was best for Concord and Gewurztraminer, good for Kober 5BB, Cayuga White, Aurore, Glenora and Remaily Seedless. It was poor for *V. argentifolia*, Chardonnay, Delaware, Seibel 880, and DeChaunac.

Discussion

Establishment in culture

The medium developed for the establishment of the *Vitis* hybrid Rougeon in culture (CHÉE 1980, CHÉE and POOL 1982) was adequate for all *Vitis* genotypes surveyed except Seyval. The relative success varied among species and among cultivars within a

Table 6

In vitro culture of *Vitis* · Rooting of 3- to 4-node (1.5 cm) shoot explants obtained *in vitro* · Results after 30 d[1])

Culture de la vigne *in vitro* · Enracinement de boutures terminales obtenues en culture et ayant 3 à 4 noeuds (1.5 cm) · Résultats à 30 d[1])

	Shoots ≧ good[2]) (%)	Mean number of nodes/ explant	Explants rooted[2]) (%)	Mean number of roots/root-ed explant	Total root length/root-ed explant (mm)
Vitis species[3])					
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
DeChaunac	70 a	5.0 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

[1]) Separation of means within columns by Duncan's Multiple Range Test ($p = 0.05$); separation of proportions after RRYAN (1959) ($p = 0.05$).
[2]) 10 explants per variety, except 9 explants for Remaily Seedless.
[3]) *V. argentifolia* = GBC 17 595/68.

given species. Among the hybrids, the least successful genotypes shared a relatively significant portion of their ancestry as being *V. lincecumii* (DeChaunac, Aurore, Seyval).

A high survival rate was not always associated with a high (*V. cinerea*) or a low (Aurore) vigor in culture. While caulescent explants did not always produce shoots (*V. riparia* Pulliat, Aurore), rosetted explants never did (White Riesling, Kober 5BB). Finally, some callus production was sometimes associated with shoot production. For Kober 5BB callus grew in the absence of shoots.

Shoot production

Shoot production occurred for all genotypes on the medium developed for the *Vitis* hybrid Remaily Seedless (CHÉE 1982). However, it was not adequate for *V. cinerea*, Pinot noir and Seyval. Those genotypes were also the ones found to be difficult to establish in culture.

Differences in shoot production were observed among species and among cultivars within a species. No particular relationship between species and shoot production was observed.

There was no relationship between percentage of explants producing shoots, number of shoots per explant and size of the shoots produced. Genotypes with a high percentage of cultures producing shoots, also grew more shoots with the exception of Chardonnay and Delaware.

Previously we reported on the beneficial effect of short days on shoot production for Rougeon (CHÉE and POOL 1982). This was confirmed here. Short days (10 h) were also beneficial to shoot production of *V. labruscana* Concord and Cabernet Sauvignon, while long days (16 h) were beneficial to shoot production of *V. argentifolia.* For the other genotypes, the effect of the photoperiod could not be assessed, as experiments with more replicates are necessary. However, it would appear that shoot production of grapevines *in vitro* in short days was usually better or equal to that in long days.

Shoot multiplication

The medium used in the shoot production phase based on the salts of MURASHIGE and SKOOG was adequate to maintain 17 genotypes *in vitro* through the 5th subculture. ¾ of the genotypes had an adequate number of cultures producing shoots. The number of shoots of adequate size produced per culture was, however, not large except for Remaily Seedless. The large number of shoots produced by Remaily Seedless is consistent with its performance in the same conditions in other experiments (CHÉE 1982).

The medium C_2D used for the 6th subculture was developed to increase shoot multiplication of Remaily Seedless (CHÉE 1982). The medium used in subculture 5, based on the salts of MURASHIGE and SKOOG was our former best medium. While keeping in mind that this is not a formal comparison, we can compare shoot multiplication between subculture 5 and 6. Shoot production was from 31 % to 350 % higher on the C_2D salts except for *V. labruscana* Catawba which died.

Rooting of subcultured shoots

All 15 genotypes surveyed rooted on the medium which had been devised for rooting of subcultured shoots of Remaily Seedless (CHÉE 1982).

Differences in rooting occurred among species and among cultivars within species. However, no specific relationship could be recognized. Root production was not adequate for micropropagation of *V. argentifolia,* Delaware, Seibel 880 and DeChaunac. In general, genotypes with high percentages of rooted shoots produced more roots per explant and/or had a higher total root length.

Root production, shoot quality, and vigor were generally related. Genotypes with a low percentage of shoots rated "good" also had a low percentage of plantlets produced. However, good shoot quality did not assure root production (e.g. DeChaunac).

Conclusion

The culture conditions previously developed for micropropagation of Rougeon and Remaily Seedless were applicable to other *Vitis* genotypes. In particular, 20 out of 21 genotypes were successfully established in culture in conditions developed for Rou-

geon and 17 out of 21 genotypes produced shoots that were multiplied through the 6th subculture on media developed for Remaily Seedless. A rooting medium developed for Remaily Seedless was used to successfully root 15 genotypes.

Summary

A range of grapevine species, cultivars and hybrids was surveyed for their responses to *in vitro* micropropagation.

21 genotypes were established in culture from shoot apices (apical dome plus 2- to 4-leaf primordia) with a method previously developed for the *Vitis* hybrid Rougeon. However, this method proved to be inadequate for the establishment of the hybrid Seyval.

Shoots grew on for 21 genotypes using a medium previously devised for the *Vitis* hybrid Remaily Seedless. Cultural conditions were inadequate for 4 of these genotypes. 10-h days resulted in best shoot production for 3 genotypes and 16-h days for 1. In general, shoot production was better or equal with short days than it was with long days.

Shoots were multiplied from 3- to 4-node shoots (1.5 cm long) obtained *in vitro*. For the first 5 subcultures, 17 genotypes were multiplied on the medium used for first shoot production. Best results were obtained with Remaily Seedless which produced 13 shoots of at least 3 nodes, per subculture shoot after 2 months in culture. The 6th subculture employed the C_2D salts which had previously been devised to improve shoot multiplication of Remaily Seedless. Yields were increased from 31 % to 350 % for all genotypes except *V. labruscana* Catawba which died in culture.

For the rooting of subcultured shoots, a medium which had previously been developed for that purpose on Remaily Seedless was used successfully on 15 genotypes.

References

CHÉE, R., 1980: The effects of growth substances and photoperiod on shoot apices of *Vitis* cultured *in vitro* and their effects on subcultured shoot tip. M. S. Thesis, Cornell University, Ithaca, NY, USA.

— — 1982: *In vitro* micropropagation of *Vitis*. Ph. D. Thesis, Cornell University, Ithaca, NY, USA.

— — 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.

MURASHIGE, T. and SKOOG, F., 1962: A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum **15**, 473—497.

RYAN, T. A., 1959: Significance tests for multiple comparisons of proportions, variances and other statistics. Psychol. Bull. **57**, 318—328.

Eingegangen am 23. 6. 1983

R. CHÉE
Dept. of Pomology and Viticulture
NYS Agricultural Experiment Station
Geneva, NY 14456
USA