PROC. N.S. INST. SCI (1989)
Volume 38, pp. 83-89

SHOOT MULTIPLICATION, GROWTH AND ADVENTITIOUS ROOTING IN THREE CULTIVARS OF VITIS, IN VITRO

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An in vitro propagation scheme was established for three commercially attractive cultivars of *Vitis* spp.: "Marechal Foch", "Precose del Colmar" and "Siegfried". Terminal and axillary buds from growing vines were used to establish stock multiple shoot cultures on Murashige and Skoog medium (MS), supplemented with N^6-benzylamino-purine (BAP). In shoot multiplication trials, the highest shoot numbers were observed after 12 weeks using 5.0 μM BAP; shoot morphology was somewhat distorted at 5.0-10.0 μM BAP, but not at lower concentrations (2.5-3.75 μM BAP). In rooting trials, rapid rooting at high frequencies was observed on MS medium. Indole-3-butyric acid increased the number of roots in some trials, and rooted microcuttings could be grown on in soil.

Une méthode de propagation *in vitro* a été établie pour trois cultivars du genre *Vitis*: Maréchal Foch, Precose del Colmar, et Siegfried. On s'est servi de bourgeons terminaux et axillaires provenant de vignes en croissance pour établir des cultures de souches avec plusieurs scions sur un milieu Murashige et Skoog (MS), augmenté de 6-benzylaminopurine (BAP). Quant aux essais sur la multiplication des scions, les scions les plus nombreux ont été observés après 12 semaines dans un milieu de BAP à 5.0 μM; la morphologie des scions a été déformée aux concentrations de BAP entre 5.0 et 10.0 μM, mais non pas aux concentrations inférieures (2.5-3.75 μM). L'enracinement s'est démontré rapide et à haute fréquence dans le milieu MS. L'acide indole-3-butyrique a augmenté le nombre de racines dans certains essais, et les petites boutures enracinéssont poussé dans le sol.

Introduction

In vitro techniques for propagation of select grape cultivars are important in relation to plant germplasm preservation and rapid multiplication of both new plant introductions and of virus-indexed plants (Krul and Mowbray 1984). Although certain grape cultivars and other woody angiosperms are now commercially produced in tissue culture (Zimmerman 1985), optimum *in vitro* protocols are required to achieve efficient propagation (Reisch 1986).

Recently we reported the micropropagation of hardy "Michurinetz" grapes (Hicks, Dorey, Chiarot and Murray 1986). The present paper extends this work to three other cultivars of potential value to the Canadian wine industry which have performed well in field trials in Nova Scotia (Bishop 1984). Our objective was to devise micropropagation methods by manipulating the composition of culture media during the shoot multiplication and rooting stages.

Materials and Methods

Actively growing specimens of "Siegfried", "Marechal Foch" and "Precose del Colmar" were supplied by Sheffield Nurseries, Centreville, Nova Scotia. To establish sterile stock cultures, terminal and axilllary buds were stripped of expanding leaves and surface sterilised for 20 minutes in dilute sodium hypochlorite (3%) containing 0.1% Tween 20. Then they were rinsed three times in sterile, double distilled water and inoculated on to 20 ml semi-solid Murashige and Skoog (MS) medium (Carolina Biologicals), supplemented with inositol (1 g L^{-1}), sucrose (30 g L^{-1}), thiamine (0.1 g L^{-1}), agar (8 g L^{-1}), and 6-benzylaminopurine (BAP; 10 μM— at pH 5.8. Tubes were incubated under cool-white fluorescent lamps (75 μEinsteins $m^{-2} \cdot sec^{-1}$) at 24°C.

Explant axillary bud meristems grew, producing multiple shoot cultures. These were subcultured at 3-4 week intervals for over 20 months, in 100 mL jars on the medium described, but with 5 μM BAP.

For shoot multiplication and growth trials, microcuttings, each consisting of a shoot (1-2 cm long) bearing two expanded leaves, were harvested from the multiple shoot cultures. For each cultivar, BAP was tested at 0, 2.5 and 5 μM in the first trial (trial A), and at 0, 2.5, 3.75 and 5.0 μM in a second trial (trial B). Since 10 μM BAP produced extremely fasciated shoots in Trial A, this treatment was omitted in the second trial. For "Marechal Foch", there were three passages each of four weeks duration, while there were four passages of three weeks each, for the other cultivars. Explants of "Precose" deteriorated with passage times of more than three weeks. At each transfer, the whole explant was subcultured. After twelve weeks the total number of shoots greater than 0.5 cm in length, the fresh weight, and the dry weight of each explant were determined.

Table I Effect of BAP concentration on shoot number and fresh and dry weights of grape microcuttings,[1] at 12 weeks.

Cultivar	Trial	BAP (μM)	Mean Shoot No. ± C.I.[2]	Mean Fresh Wt.[3] (g) ± C.I.	Mean Dry Wt.[4] (g) ± C.I.
"Precose del Colmar"	A	0	1.5 ± 0.9	0.29 ± 0.14	0.15 ± 0.0
		2.5	22.3 ± 8.4	5.67 ± 2.55	0.62 ± 0.2
		5.0	59.3 ± 11.3	17.70 ± 5.10	1.31 ± 0.3
		10.0	39.4 ± 10.6	17.62 ± 5.20	1.18 ± 0.3
	B	0	3.3 ± 1.9	0.84 ± 0.52	0.09 ± 0.0
		2.5	19.5 ± 9.7	5.35 ± 2.55	0.49 ± 0.2
		3.75	39.0 ± 17.2	9.92 ± 5.70	0.74 ± 0.3
		5.0	55.0 ± 14.8	18.60 ± 5.30	1.19 ± 0.3
"Foch"	A	0	1.2 ± 0.4	0.23 ± 0.06	0.03 ± 0.0
		2.5	8.2 ± 3.8	2.06 ± 1.84	0.31 ± 0.2
		5.0	77.1 ± 16.3	20.49 ± 5.10	1.26 ± 0.3
		10.0	30.8 ± 11.9	21.94 ± 5.45	1.09 ± 0.3
	B	0	1.4 ± 0.5	0.33 ± 0.12	0.14 ± 0.0
		2.5	12.8 ± 5.2	2.94 ± 1.35	0.39 ± 0.1
		3.75	38.6 ± 12.3	7.47 ± 3.40	0.69 ± 0.2
		5	68.3 ± 10.2	15.4 ± 3.7	1.02 ± 0.2
"Siegfried"	A	0	1.2 ± 0.6	0.31 ± 0.15	0.03 ± 0.0
		2.5	69.0 ± 8.4	26.63 ± 4.60	1.66 ± 0.1
		5.0	98.1 ± 19.4	40.91 ± 4.80	1.72 ± 0.2
		10.0	20.9 ± 5.1	40.91 ± 3.10	1.72 ± 0.1
	B	0	1.5 ± 0.4	0.35 ± 0.10	0.05 ± 0.0
		2.5	28.7 ± 1.0	14.50 ± 5.40	0.96 ± 0.3
		3.75	71.0 ± 16.7	28.61 ± 5.30	1.37 ± 0.2
		5.0	51.8 ± 15.4	31.12 ± 5.20	1.49 ± 0.2

[1](9-14 replicates, per BAP treatment; except for controls (6-11)).

[2]95% Confidence Interval.

[3]Initial wet weight per explant (mg): Foch: (56.1); Siegfried (36.2); Precose (74.3)

[4]Initial dry weight per explant (mg): Fosh: (5.4); Siegfried (3.6); Precose (9.7)

For rooting trials, MS salts were reduced to half-strength (Novak and Juvova 1982) and BAP was replaced with 4-(indolyl-3) — butyric acid (IBA) at 0, 0.25, 2.5, and 5.0 μM, while other constituents remained as previously described. Media were dispensed in 50 ml aliquots in 225 ml glass jars. Rooting hormone (IBA) was added to the autoclaved media through a Millipore filter (0.22 μM pore size). Microcuttings for rooting

Table II Effects of IBA concentration on root numbers.[1]

Cultivar	Trial	IBA Concentration (μM)	mean number of roots per shoot (± C.I.)[2]
"Precose del Colmar"	A	0	2.8 ± 0.8
		0.25	3.3 ± 0.7
		2.5	8.9 ± 3.1
		5.0	11.7 ± 3.0
	B	0	3.1 ± 0.7
		0.25	5.5 ± 1.2
		2.5	10.5 ± 3.1
		5.0	12.8 ± 2.3
"Marechal Foch"	A	0	3.9 ± 1.0
		0.25	5.8 ± 1.0
		2.5	3.4 ± 1.6
		5.0	3.2 ± 1.9
	B	0	5.8 ± 1.1
		0.25	9.3 ± 2.8
		2.5	15.9 ± 2.9
		5.0	18.0 ± 3.4
"Siegfried"	A	0	5.4 ± 0.9
		0.25	5.9 ± 1.1
		2.5	9.9 ± 2.7
		5.0	3.9 ± 1.4
	B	0	3.8 ± 0.9
		0.25	4.4 ± 0.9
		2.5	7.2 ± 2.1
		5.0	12.3 ± 2.6

[1]Microcuttings grown for 12-13 days on MS medium; (35-40) replicates per treatment per experiment (except "Marechal Foch", trial A, IBA 2.5 μM N = 25).

[2]95% Confidence Interval.

Table III Survivorship of rooted grape microcuttings *ex vitro* (%).[1]

Cultivar	Previous IBA dose (ppm)			
	0	0.05	0.5	1.0
"Foch"	94	82	62	74
"Siegfried"	68	65	68	74
"Precose del Colmar"	61	65	82	67

[1]Rooted microcuttings were transferred to soil after 12-13 days *in vitro* on the rooting media. Survivorship was recorded as the percentage of healthy plants days after lid removal from germination trays at 24°C and 18 h photoperiod. N = 32-36.

came from the stock multiple shoot cultures and consisted of a stem (ca. 2 cm long), bearing the terminal bud and three expanded leaves. For each IBA concentration, there were 36 to 40 replicate microcuttings in each of two trials, lasting 12-13 days. A microcutting was recorded as having rooted if it bore at least one emergent adventitious root. Statistical analysis of the rooting data was performed using the programme GLIM (Baker and Nelder 1978). The ability of rooted microcuttings to establish *ex vitro* was tested by transferring rooted plantlets from the IBA experiments to potting soil in plastic pots seated in covered germination trays. Trays were kept at 24°C with an 18 h photoperiod, using mixed incandescent and fluorescent lamps. After two weeks, the tray lids were removed and 5 days later, the percentage of healthy surviving plants was recorded.

Results

BAP stimulated development of the axillary buds to produce multiple shoot clusters accompanied, in most instances, by roots (Fig 1). On 0, 2.5, and 3.75 μM BAP, shoot morphology was essentially normal, but at 10 μM BAP, stems were fasciated and short. They bore distorted leaves and a mass of compact green tissue at the base of the shoots. For all cultivars, 5 μM BAP produced the highest shoort numbers in trial "A" (Table I), while 10 μM depressed shoot numbers. This pattern was not reflected in the fresh and dry weight values, where there was little difference between the 5.0 and 10.0 μM treatments. In the "B" trials, 5.0 μM BAP yielded the highest numbers of shoots, except in the case of Foch and Siegfried. Fresh and dry weights were also highest using 5.0 μM BAP, with exception of Foch and Siegfried.

After an initial lag of 4-5 days, rooting percentages increased sharply, reaching 85% or greater, even without IBA treatment (Fig 2,3). There was no significant effect of either IBA concentration or cultivar on rooting frequency, no significant interaction between IBA concentration and cultivar, nor any significant difference between trials. There was, however, a significant effect of IBA concentration on the mean

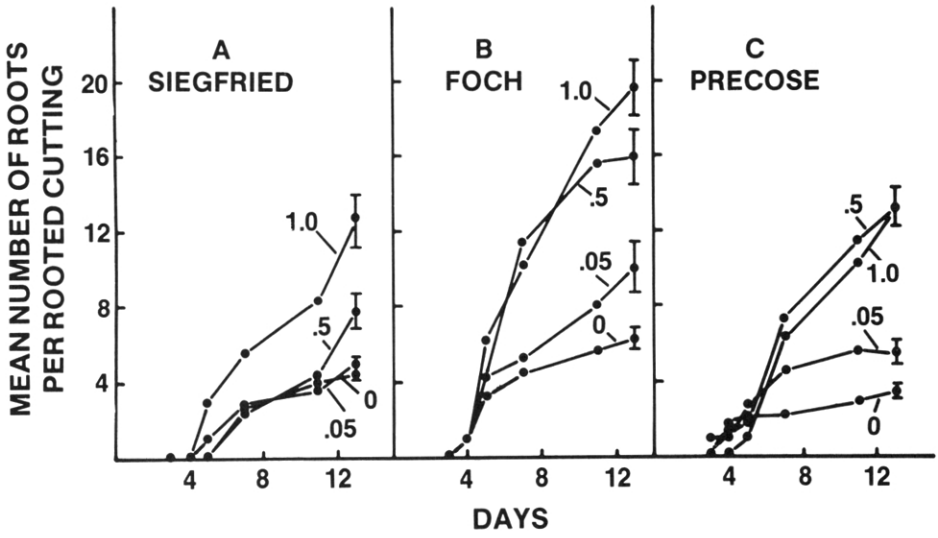

Fig 3 Rooting frequencies plotted against time.

Fig 1 Multiple shoot culture of 'Precose' after 12 weeks on MS medium with 3.75 μM BAP. Bar = 2 cm.

number of roots per shoot at 2.5 and 5.0 μM IBA (Table II), with the exception of Trial A for Siegfried and Trial B for Marechal Foch.

After transfer to soil, the rate of survival was in the range of 60-95%, but this did not depend on prior auxin treatment (Table III).

Discussion

Shoot formation using BAP has been reported for other cultivars of grape (Chee and Pool 1982; Harris and Stevenson 1982; Reisch 1986). The formation of roots on the multiple shoot cultures may be unusual in that other studies do not cite the phenomenon. Furthermore, it has been pointed out that cytokinins generally inhibit rooting in multiple shoot cultures, especially at high doses (George & Sherrington, 1984). Possibly, in our cultures, roots contributed to the development of multiple shoots, through root-derived cytokinins (Pool and Powell, 1975). The nature of the tissue mass in multiple shoot cultures has not been explored in detail, but it appears to be a compact callus. The inferior shoot quality noted above, with high levels of cytokinin, has been reported elsewhere for grapes (Goussard, 1981, 1982), but may not jeopardise propagation schemes seriously, since recovery on prolonged culture has been noted (Harris and Stevenson, 1982).

As in our study, microcuttings of 'Cabernet Sauvignon' and 'Cabernet Franc' grapes rooted readily on an auxin-free medium (Skene and Barlass, 1980). However, other cultivars in the latter study required naphthalene acetic acid (NAA) for rooting. Similar genotype-related responses to NAA have been noted (Chee and Pool, 1984).

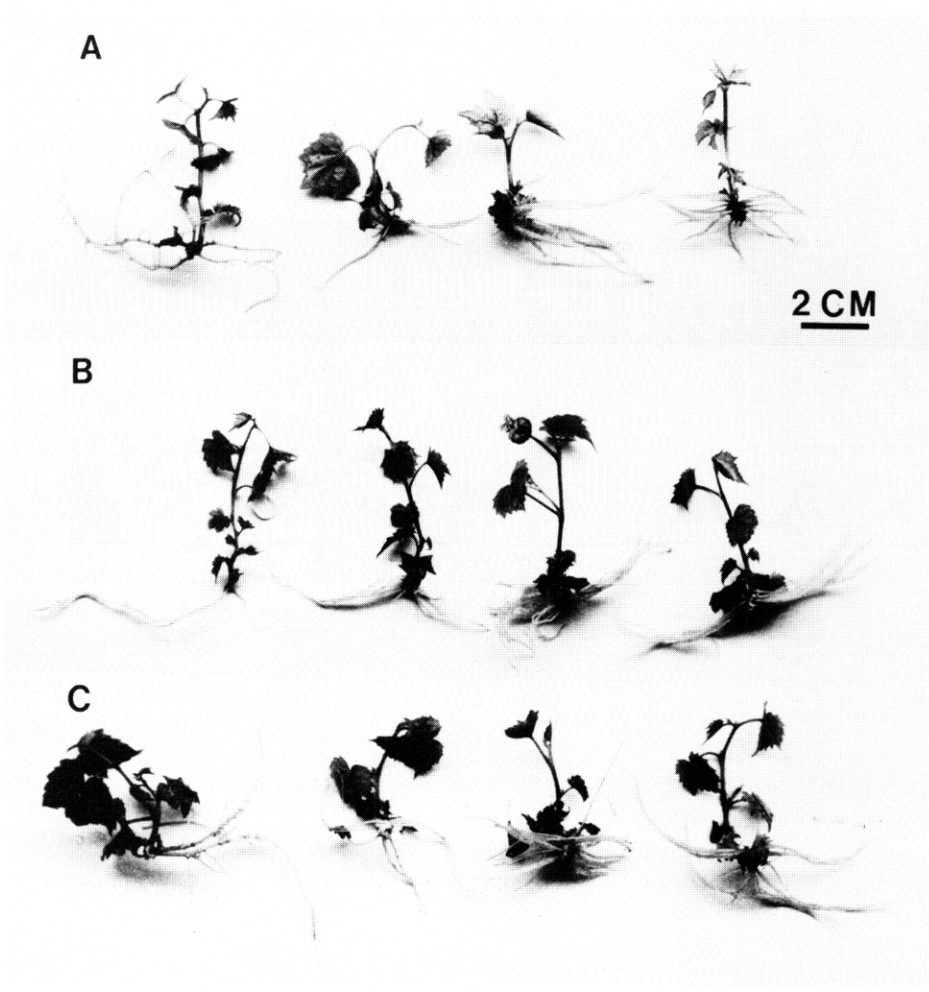

Fig 2 Rooted microcuttings of A - "Siegfried"
B - "Precose del Colmar"
C - "Marachal Foch"

In terms of the number of roots per shoot, we observed a promotive effect of high auxin concentrations, but the increase may have little significance for the develoment of micropropagation schemes. Our root numbers were generally higher than those reported (Li and Eaton 1984; Chee and Pool 1983). Overall, the present study has established the basis for a micropropagation scheme for these cultivars.

Acknowledgements

This work was partly supported by funds from a PILP subcontract and from the Natural Sciences and Engineering Research Council of Canada.

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(Received 29 March 1989)