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PROPAGATION IN VITRO OF 'MICHURINETZ' GRAPES

There has been substantial interest recently in the potential for a wine industry in Nova Scotia (Dial 1979; Cattell 1984; Schreiner 1984/1985). The hardy Russian cultivar *Vitis amurensis* "Michurinetz" has, along with others, been grown for use as a red wine grape at Grand Pré, N.S. It was of considerable interest to begin to develop *in vitro* propagation strategies for such grapes, strategies which might afford a year-round means of true-to-type cloning and of virus eradiction (Barlass, Skene, Woodham and Krake 1982; Mantell, Matthews and Mckee 1985). In this note we report successful maintenance of stock multiple shoot cultures and also high-efficiency rooting *in vitro*.

Dormant budwood of the Michurinetz cultivar was flushed while standing in tapwater at 24-26° C in an 18 h photoperiod for 8-12 days. The resulting expanded leaves were carefully dissected away and surface sterilization of the remaining buds was effected by immersion in sodium hypochlorite (2% w/v), for 6 minutes, followed by 3 rinses of sterile double-distilled water. Buds (3-6 mm long) were inoculated on a standard plant tissue culture medium, consisting of Murashige/Skoog (MS) salt base (Carolina Biologicals) with sucrose (30 $g.L^{-1}$), agar (8 $g.L^{-1}$), thiamine (10 $mg.L^{-1}$), inositol (1 $g.L^{-1}$) and the cytokinin N^6-benzylaminopurine (BAP; Goussard 1982; Chee and Pool 1982; Monette 1983). The pH of media was adjusted to 5.8 prior to autoclaving. Medium (30 mL) was dispensed in 100 ml screw-top glass jars. The culture environment was at 26-28° C with a photoperiod of 18 h, under cool-white fluorescent lamps (9000 lux). The passage time was 4 weeks. Single bud explants formed multiple shoot cultures (Fig. 1) through the development of axillary buds. Such stock cultures have been routinely subcultured for over 2 years.

Table I Shoot multiplication and wet weight in relation to cytokinin dose for shoots of 'Michurinetz' grape.[1] *in vitro*.

BAP (μM)[2]	Mean No. shoots per culture[3]	C.I.[4]	Mean fresh wt. per culture[5] (gm)	C.I.[4]	Callus Index[6]	Frequency of cultures with roots (%)
0	1.79 a	± 0.054	0.540 a	± 0.172	0.14	57
0.01	3.52 b	± 0.800	0.940 b	± 0.250	0.13	61
0.10	2.30 a	± 0.610	0.637 ab	± 0.156	0.26	61
1.00	2.00 a	± 0.030	0.580 ab	± 0.210	1.00	21
2.50	8.61 c	± 2.190	1.860 c	± 0.470	0.88	18
5.00	27.76 d	± 3.940	7.100 d	± 1.420	1.80	56

[1] Standard shoots cultured on MS media + BAP at 26-28° C, photoperiod 18 hours; 9000 lux.
[2] 23-28 replicate shoot per treatment; pooled data from 2 experiments; exception: 14 shoots only survived on 0 BAP.
[3] Similar letters in a vertical column indicate two means are not significantly different (Student t-test $P > 0.05$).
[4] 95% confidence interval.
[5] Initial wet weight = 0.0441 g per shoot (N = 15).
[6] Calculated as follows: a semi-quantitative ranking of the amount of callus on each stem base was used after visual inspection: 0, 1, 2, 3 denoted increasing amounts of callus; for each treatment, these values were summed and divided by the number of replicates to give a number for callus quantity.

For measurement of shoot multiplication, the above medium was used with BAP at 0, 0.01, 0.1, 1.0, 2.5 and 5.0 μM. From the stock cultures, 'standard' microcuttings were harvested, each consisting of a terminal bud, 1-2 cm of stem and 2 expanded leaves. There were 11-18 replicate microcuttings per treatment in each of 2 experiments; not all these survived (Table I, footnote[2]).

After 3 passages measurements were recorded, for each microcutting, of a) total number of shoots greater than 0.5 cm; b) length of the longest shoot; c) fresh weight; d) presence of callus; e) presence of roots.

For rooting trials, the MS salts were reduced to half-strength and BAP was replaced by 4-(indolyl-3) butyric acid (IBA; George and Sherrington 1984; Krul and Mowbray 1984), at 0, 0.25, 2.5 and 5 μM. All other media components were as above. Stock IBA was added to the cooling autoclaved medium through a 0.22 μ Millipore filter system. Rooting medium (40 mL) was dispensed into 100 mL glass jars with plastic screw-top closures. Standard explants for rooting were single shoots, 1-2 cm on length, bearing the terminal bud and 2 expanded leaves. Stems were inserted in the medium near the vessel wall, for periodic observation of emerging root primordia, using a dissecting microscope. Twenty-four shoots were tested on each dose of IBA and the experiment was repeated once.

In shoot multiplication trials, BAP did not significantly promote shoot production at doses less than 2.50 μM, or else gave a weak promotive effect (Table I). A better than four-fold increase in shoot production was observed with 2.50 μM BAP. While very high shoot numbers were produced on 5.0 μM BAP, their stems were short (mean 2.8 cm vs 4.9 cm on 2.5 μM BAP), fasciated and bore distorted leaves. BAP significantly increased the mean fresh weight per culture at 0.01 μM and especially at 2.50 and 5.0 μM (Table I). Callus, at the base of shoots, was produced on all treatments, particularly in the range 1.0-5.0 μM BAP. Rooting was observed on all treatments, but the frequency of rooting bore no clear relationship to cytokinin dose (Table I).

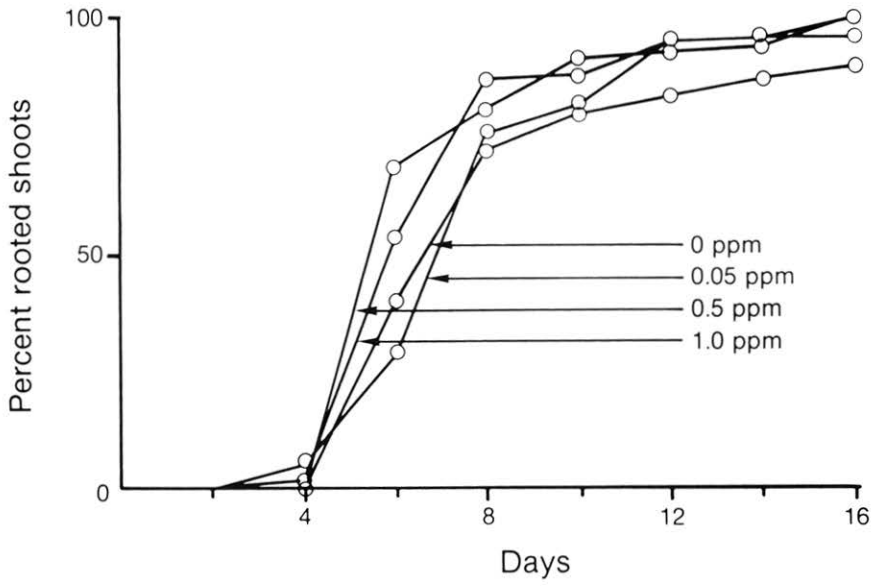

Fig. 3 The time course of rooting of microcuttings in relation to auxin dose.

Table II Rooting of single microcuttings of 'Michurinetz' in relation to auxin dose.[1]

IBA (μM)[2]	Mean number of roots per rooted shoot[3]	C.I.[4]
0	3.41 a	± 0.41
0.05	4.12 a	± 0.61
0.50	7.36 b	± 0.99
1.00	7.72 b	± 1.18

[1] Standard microcuttings grown for 16 days on MS medium supplemented with IBA; at 26-28° C; photoperiod 18 hours. 9000 lux.
[2] 41-48 replicates per treatment; pooled data from 2 experiments.
[3] Similar letters in a vertical column indicate two means are not significantly different (Student t-test at $P > 0.05$).
[4] 95% Confidence Interval.

Root primordia first appeared at very low frequencies by day 4 (Fig. 3). Over the next 4 days, rooting frequencies increased rapidly on all treatments, with a tendency toward higher frequencies on 2.5 and 5 μM IBA. Final rooting frequencies were high and rather similar, irrespective of IBA dose. Auxin, however, did significantly increase the mean number of roots per shoot at 2.5 and 5.0 μM (Table II). Roots were relatively thick, especially on the auxin treatments (Fig. 2). IBA regularly produced a basal stem callus (in 81-98% of cuttings vs 49% in controls); lateral roots formed in 31-43% of the auxin-treated cultures (vs 4% in controls).

From this work it is clear that 'Michurinetz' can be sustained readily through many passages *in vitro*. Importantly, it is an 'easy-to-root' cultivar. We propose that these data can provide a basis for further testing of this interesting cultivar.

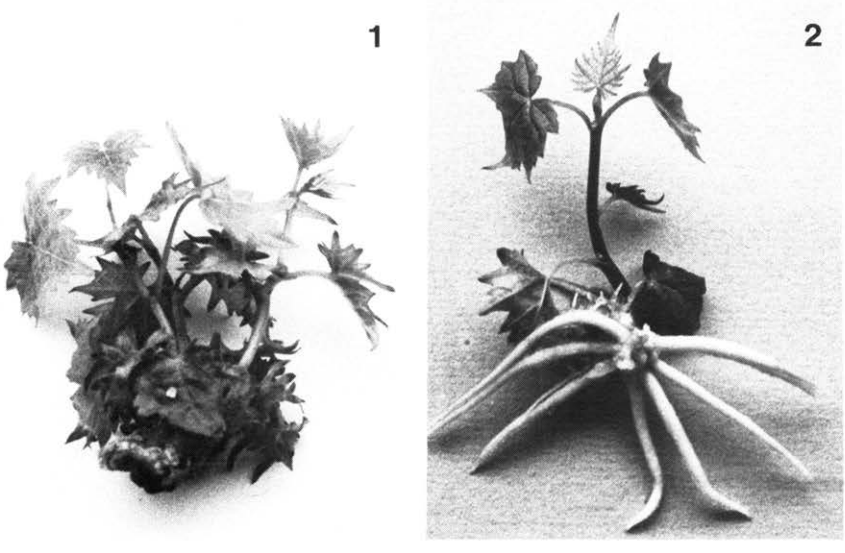

Fig. 1 Stock multiple shoot cultures from 2.5 μM BAP medium.

Fig. 2 Rooted microcutting after treatment with 2.5 μM IBA.

Acknowledgement

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