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Liquid Medium Overlays Enhance Growth and Multiplication of *In Vitro* Grapes and Apples

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Abstract

Liquid medium overlays of established cultures were compared to transfers to fresh medium. The number of micropropagated grape (*Vitis* sp.) shoots increased 67%, shoot length 73%, and fresh weight 125% with liquid medium overlays as compared to transfer to fresh medium. Callus growth at the base of shoots was 64% less. In apples (*Malus pumila*), the number of micropropagated shoots increased 35%, shoot length 69%, and fresh weight 67% with liquid medium overlays. Callus fresh weight decreased by 52% when compared to cultures transferred to fresh solid media.

Introduction

The quality of micropropagated shoots is an important factor for rooting either *in vitro* or *in vivo*. Maene and Debergh (2, 3) found that an additional stage of micropropagation before transplanting to a rooting medium improved shoot quality. However, this adds time and labor to the micropropagation procedure. An addition of fresh liquid medium over the exhausted multiplication medium enhanced shoot elongation, increased the number of microcuttings, and reduced production costs of micropropagated *Cordyline, Philodendron, Magnolia* and *Sapthiphyllum* (2). Similarly, this double phase technique used for four pear cultivars improved their quality and yield but reduced vitrification (6). Rodriguez et al. (5) reported continuous and greater proliferation of *in vitro* pears for more than three months using the double phase culture system. This study compared liquid medium addition to established cultures with transfer to fresh medium for grapes (*Vitis* sp. 'Valiant') relative to multiplication and growth of shoots and apples (*Malus pumila* Mill. 'Golden Delicious').

Materials and Methods

Grape (*Vitis* sp. 'Valiant') cultures were established using surface sterilized nodal sections of plants grown from rooted cuttings and multiplied on MS medium (Murashige and Skoog, 1962) supplemented with 5μM BAP, 3% sucrose and 0.8% agar with pH adjusted to 5.7 (1). Cultures of apple (*Malus pumila* Mill. 'Golden Delicious') were obtained from the National Seed Storage Laboratory, USDA, ARS, Fort Collins, CO. Stem sections, 2cm long, with one or two nodes were cultured on MS medium supplemented with 4.4μM benzylamionpurine (BAP), 1.4μM gibberellic acid (GA_3) 4.9μM indolebutyric acid (IBA), 0.65% agar (Sigma Co.) and 3% sucrose (7). Cultures of both species were incubated in the growth room under the following conditions; light intensity of 90-100 μmole $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD) for 16:8 hours light/dark period, temperature at 25 $\pm$3°C, and relative humidity of 30-50%.

Two treatments were compared using stage 2 cultures: 1) *Fresh solid medium:* Shoot clumps of both grapes and apples were transferred into appropriate fresh solid multiplication media after 6 weeks of culture and 2) *Liquid medium overlay:* 10ml of liquid multiplication medium was overlayed onto the solid medium of 6 week old cultures. Number of shoots, mean plant height, fresh weight of shoots and callus fresh weight of apples and

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Table 1. Mean number of shoots per explant, height of shoots, tissue fresh weight, and callus fresh weight in micropropagated grape cultures multiplied on solid medium as compared to cultures with liquid multiplication medium overlays.

Transfer	No. of shoots/ explant	Height[z] (cm)	Shoot fresh wt. (g)	Callus fresh wt. (g)
To Solid Medium	2.6 a[y]	2.5 a	1.3 a	1.5 b
Liquid Medium Overlays	4.3 b	4.4 b	2.9 b	0.4 a

z. Height of shoots was taken as the mean length of 10 shoots per clump.
y. Each number is an average of 40 observations. Means are separated using Tukey's Multiple Range Test. Means within columns not followed by a common letter differ significantly from one another at 0.05 level of significance.

grapes were measured for both treatments after 6 weeks. A total of 40 replicates were used for each treatment. Data were analyzed statistically using a one way analysis of variance with the means compared by Tukey's Multiple Range Test at 0.05% level of significance.

Results and Discussion

In grapes, the addition of liquid MS medium over the nutrient depleted solid medium increased the number of shoots from 2.6 in transfer to fresh medium to 4.3 shoots per explant (Table 1). Similarly, the average shoot length increased from 2.5cm to 4.4cm and their fresh weight increased from 1.3g to 2.9g. Callus growth at shoot bases was reduced significantly from 1.5g fresh weight per explant on solid medium to 0.4g in those grown in double phase cultures (Table 1).

Table 2. Mean number of shoots per explant, height of shoots, tissue fresh weight and callus fresh weight in micropropagated apple cultures multiplied on solid medium as compared to cultures with liquid multiplication medium overlays.

Transfer	No. of shoots/ explant	Height[z] (cm)	Shoot fresh wt. (g)	Callus fresh wt. (g)
To Solid Medium	2.4 a[y]	1.7 a	3.2 a	1.9 b
Liquid Medium Overlays	3.2 b	2.9 b	5.0 b	0.9 a

z. Height of shoots was taken as the mean length of 10 shoots per clump.
y. Each number is an average of 40 observations. Means are separated using Tukey's Multiple Range Test. Means within columns not followed by a common letter differ significantly from one another at 0.05 level of significance.

Similar results were obtained in apples where mean number of shoots per explant was increased significantly from 2.4 to 3.2 (Table 2) with the liquid medium addition. Average shoot length was also increased from 1.7cm to 2.9cm and their shoot fresh weight increased from 3.2g to 5.0g and basal callus was reduced from 1.9g to 0.90g per explant (Table 2). Callus formation at the base of shoots was reduced in both grape and apple cultures. This confirms and extends the value of the addition of liquid medium to established cultures for the improvement of proliferation and growth of in vitro grapes and apples. This will reduce the need for transplanting cultures with each multiplication phase onto fresh medium. These effects also contribute to the quality of in vitro shoots and potentially reduce labor costs.

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