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**Reviewed Research Paper**

# *In Vitro* Propagation of Peach: I. Propagation of 'Lovell' and 'Nemaguard' Peach Rootstocks.[1]

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## Abstract

An improved tissue culture propagation medium was developed for in vitro multiplication of peach (*Prunus persica* L.) rootstocks 'Lovell' and 'Nemaguard. A 144-fold increase in number of shoots was achieved after 10 weeks with 26.7$\mu$M (6.0 mg $l^{-1}$) BA and 0.04$\mu$M (0.01 mg $l^{-1}$) IBA. Shoots continued to regenerate with subsequent subcultures. 20% of the shots rooted in the new medium without growth regulators at one half the recommended concentrations of $KNO_3$ and $(NH_4)_2SO_4$ plus 44.3$\mu$M (9 mg $l^{-1}$) IBA. Complete plants were transferred to soil, and grown with 100% survival. Explants taken from juvenile and mature plants did not differ significantly in their growth in the new medium.

## Introduction

Seedling rootstocks have the potential disadvantage of genetic variation among them due to segregation that may lead to variability in the growth and performance of the scion cultivar. Improvement of rootstocks via incorporation of dominant genes through crossing, progeny testing and clonal rootstock propagation of selected progeny requires only one generation while rootstock propagation via seedling requires additional generations of breeding and selection to fix the genes and characters. Combinations of genes are also most conveniently maintained via vegetative propagation of clonal rootstocks, since recombination does not occur as would be the case for sexual reproduction. However, seedlings are presently used due to ease and economy of propagation.

Reports of *in vitro* peach propagation are numerous (1, 5, 9, 10, 11, 13, 15, 16). Media used in some studies (10, 12, 13, 15) have been limited to either MS or its close modifications. In addition, these media have not been demonstrated to be adequate for multiplication of more than few cultivars *in vitro*. Our study reports the development of a medium of *in vitro* multiplication of 'Lovell' and 'Nemaguard' and the continued regeneration of shoots without decline for up to 13 weeks. This study also reports the effects of 9 different media used on peach explants.

## Materials and Methods

Actively growing shoots from juvenile and 14 year old peach plants

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(Foundation Seed and Plant Materials Service, U.C. Davis) (ca 1.5 cm long) were surface sterilized by immersion for 30 min in 0.79% (v/v) NaOCl (15% Clorox) and rinsed 3 times with sterile distilled water. Shoot apices (0.5 cm long), consisting of the apical meristem and several leaf primordia, were excised for culture. Shoot apices used in all experiments were uniform in size. Shoot apices excised from greenhouse grown juvenile 'Lovell' peach plants were used for media selection and modification because they were available in large numbers.

Pyrex tubes (2.5 x 15 cm) were used for explant establishment and rooting. Polycarbonate containers (6.5 x 6.5 x 10 cm, Magenta Co., Chicago IL) were used for shoot multiplication. Gelrite (0.2%) from Kelco Co., San Diego, CA, was used for media gelation. Media were distributed in aliquots of 25 or 75 ml per tube or container, respectively. Media were sterilized by autoclaving under 1.1 kg $cm^{-2}$ pressure at 121°C for 15 min. Media pH was adjusted before autoclaving.

To test the hormonal requirements for shoot multiplication, 0 to 44.4$\mu$M (0 to 10 mg $l^{-1}$) $N^6$-benzyladenine (BA) and 0 to 24.6$\mu$M (0 to 5 mg $^{-1}$) $\alpha$-indolebutyric acid (IBA) were added to the medium singly and in combinations. For rooting, shoots multiplying *in vitro* (1 to 1.5 cm long) were excised. Since multiplying shoots were relatively small, a shoot elongation experiment was conducted where each shoot was placed in a culture tube containing 2 ml liquid basal medium rotated in a roller drum at 2 rpm for 2 weeks. The liquid basal medium used was AP medium at either 1x or 2x the recommended concentration. Shoots were then subjected to the following treatments for root induction: 1) cultured directly on AP medium containing 0 to 44$\mu$M (0 to 9 mg $^{-1}$) IBA; 2) basal ends immersed in AP liquid medium containing 0 to 44.3$\mu$ IBA then cultured on AP basal medium; 3) dipped in IBA plus talc powder at 0 to 3% (w/w) then cultured in AP basal medium. In all rooting experiments, the media contained one half the AP basal medium concentrations of potassium nitrate and ammonium sulfate (designated as ½N-AP)(6).

Slant cultures were maintained under continuous illumination (1:1 cool white fluorescent and Gro-lux tubes) at 55 $\mu$mole $sec^{-1}m^{-2}$ (200 ft. c.) for both explant establishment and shoot multiplication. Cultures were maintained under a 15 hr photoperiod of 30$\mu$mole $sec^{-1}m^{-2}$ (140 ft. c.) for rooting. A constant temperature of 26 ± 2°C was maintained.

*In vitro*-derived plants were acclimatized following their removal from the culture vessels and Gelrite media, and were transplanted to 7.5 cm plastic cups containing 1:1 volumes of perlite and peat moss. The potted plants were grown at 26 ± 2°C with illumination of 2300 $\mu$mole $sec^{-1}m^{-2}$ (1000 ft. c.). Each established plant was covered with an inverted beaker that was removed gradually over a period of 10 days. Plants were then established in a greenhouse.

One explant was used per culture vessel in all of the experiments conducted, and unless stated otherwise, 8 replications comprised each treatment. Growth measurements consisted of fresh weight, number of leaves, length of shoots, and number of axillary shoots.

## Results and Discussion

Nine media were tested, including Murashige and Skoog (MS), Linsmaier and Skoog (LS), Wolter and Skoog (WS), Rangan *et al* (RA), Driver and Kuniyuki (DKW), Gamborg, *et al* (B5), White (WT), Lloyd and McCown (WPM) and Cheng (CH); references 12, 7, 18, 14, 3, 4, 17, 8, and 2; respectively. The pH of these media were adjusted to 5.7. Table 1, Expt. 1, shows that B5, DKW, and RA media

**Table 1. Effect of nutrient media on fresh weight, number of leaves, and length of 'Lovell' peach shoots after 3 weeks in culture.**

| Media[z] | Fresh weight (mg)[y] | No. of leaves[y] | Shoot length (mm)[y] |
|---|---|---|---|
| Expt. 1: | | | |
| B5 | 441a | 12.6a | 13.6bc |
| DKW | 423a | 7.9b | 22.1a |
| RA | 414a | 6.9bc | 16.0b |
| MS | 338b | 6.6bc | 19.6a |
| LS | 308b | 5.9bc | 12.9bc |
| WPM | 188c | 5.4bc | 13.5bc |
| CH | 171c | 3.4d | 11.1c |
| WS | 130c | 2.4d | 6.5d |
| WT | 65d | 2.1d | 8.5d |
| Expt. 2[x]: | | | |
| AP | 741a | 18.3a | 30.9a |
| B5 | 419b | 11.5b | 25.4b |

[z]B5 = Gamborg (4), DKW = Driver and Kuniyuki (3), RA = Rangan *et al* (16), MS = Murashige and Skoog (13), LS = Linsmaier and Skoog (7), WPM = Lloyd and McCown (8), CH = Cheng (2), WS = Wolter and Skoog (21), WT = White (20).

[y]Mean separation in columns by Duncan's Multiple Range Test (P = 0.05).

[x]Based on 10 replications per treatment.

produced the highest fresh weight of tissue, B5 medium produced the highest number of leaves while DKW and MS media induced long shoots. Shoots growing on B5 medium appeared healthier than shoots growing on the rest of the tested media. Therefore, B5 medium, plus 30 g $l^{-1}$ sucrose was tested at 7 pH levels from 4.2 to 7.2. B5 medium with pH of 5.2 was selected for modification since it produced the largest increase in fresh weight.

Individual compounds in 4 groups of nutrients were tested, each at 6 concentrations (0, 0.25, 0.5, 1, 2, and 4 times the recommended concentration in B5 medium). All groups of nutrients were held constant except the group being tested. The composition of the medium was progressively altered as new information was obtained. The improved nutrient medium will be referred to as Almehdi and Parfitt (AP) medium. The composition of AP medium is shown in Table 2. Number of leaves and fresh weight of explants increased significantly after 4 weeks, when cultured on AP medium vs. B5 medium (Table 1 Expt. 2). Since growth regulator concentrations were the same, the differences were due to an improved nutrient balance.

Shoot apices excised from greenhouse-grown juvenile and field-grown mature 'Lovell' peach explants were cultured on AP medium. Explants taken from juvenile and mature plants did not differ significantly ($t_{0.05}$) for fresh weight (821 vs 720 mg), no. of leaves (17.5 vs 17.4), or shoot length (25.1 vs 24.9 mm).

Shoot multiplication was enhanced by the addition of 22.2 to 31.1$\mu$M (5 to 7 mg $l^{-1}$) BA and 0 to 4.9$\mu$M (0 to $l^{-1}$) IBA with higher concentrations of BA and IBA being inhibitory (Figures 1 and 2). The highest observed mean value for number of shoots, 12 ± 5 per 5 weeks, was obtained when 26.7$\mu$M (6 mg $l^{-1}$) BA and 0.04$\mu$M (0.01 mg $l^{-1}$) IBA were added to the medium.

**Table 2. Basal nutrient medium composition of B5 and AP media.**

| Ingredient | B5 | | AP multiplication medium | |
|---|---|---|---|---|
| | $\mu$M | mg/l | $\mu$M | mg/l |
| A. Inorganic macronutrients (x $10^3$) | | | | |
| $KNO_3$ | 24.8 | 2.5 | 24.8 | 2.5 |
| $MgSO_4.7H_2O$ | 1.01 | 0.25 | 0.76 | 0.19 |
| $(NH_4)_2SO_4$ | 1.02 | 0.13 | 2.03 | 0.27 |
| $CaCl_2.2H_2O$ | 1.02 | 0.15 | 1.02 | 0.15 |
| $NaH_2PO_4.H_2O$ | 1.09 | 0.15 | 1.09 | 0.15 |
| B. Inorganic micronutrients | | | | |
| $MnSO_4.H_2O$ | 59.2 | 10.0 | 118.3 | 20.0 |
| $ZnSO_4.7H_2O$ | 6.9 | 2.0 | 6.9 | 2.0 |
| $CuSO_4.5H_2O$ | 0.2 | 0.05 | 0.2 | 0.05 |
| KI | 4.5 | 0.75 | 4.5 | 0.75 |
| $CoCl_2.6H_2O$ | 0.1 | 0.03 | 0.1 | 0.03 |
| $H_3BO_3$ | 48.4 | 3.0 | 72.6 | 4.5 |
| $Na_2Mo0_4.2H_2O$ | 1.0 | 0.25 | 0.2 | 0.06 |
| $FeSO_4.7H_2O$ | 100.7 | 28.0 | 100.7 | 28.0 |
| $Na_2EDTA$ | 110.7 | 37.2 | 110.7 | 37.2 |
| C. Organic constituents | | | | |
| Thiamine HCl | 29.7 | 10.0 | 0.0 | 0.0 |
| Nicotinic acid | 8.1 | 1.0 | 0.0 | 0.0 |
| Pyridoxine HCl | 4.9 | 1.0 | 9.8 | 2.0 |
| Myo-Inositol | 555.6 | 100.0 | 138.9 | 25.0 |

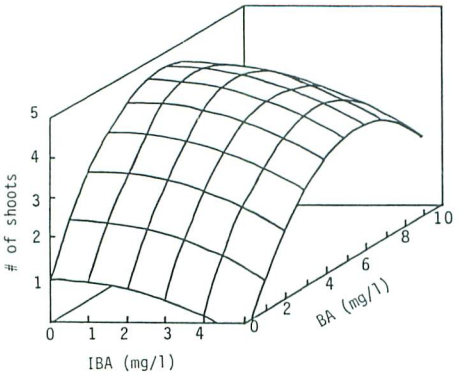


**Figure 1. Regression curve for number of shoots, after 5 weeks in culture, as affected by BA and IBA concentrations. Regression equation for predicted curve is $Y = 1.028 = 1.66x - 0.111x^2 - 0.57z^2$ where Y = number of shoots, x = BA and z = IBA.**

*In vitro* derived shoots continued to multiply without decline for up to 13 weeks with regular transfer to the same fresh AP medium. At the end of the 13th week a few shoots browned, but the remaining healthy shoots continued to multiply once they were subdivided and cultured on the same fresh AP medium.

Gelrite was substituted for agar for 3 reasons. First, its gel strength is highly dependent on the type and amount of ions added. Therefore, when nutrient exhaustion by cultured explants occurs, the gel loses its gelling strength and turns into liquid. This helps determine when explants need to be transferred into fresh medium. Second, since Gelrite is extremely clear once it is dissolved by heating, detection of media contamination at early stages is facilitated. Third, it is less expensive than agar. Table 3 lists the concentrations of inorganic ions present in both Gelrite and Bacto-agar (Difco Laboratories, Detroit, MI). These data indicate that except for Cl, Gelrite contains higher concentrations of P, K, Ca, Mg, Zn, Mn, Cu, and Fe than Bacto-agar. Therefore, some plant requirements for these elements may be satisfied by the Gelrite matrix.

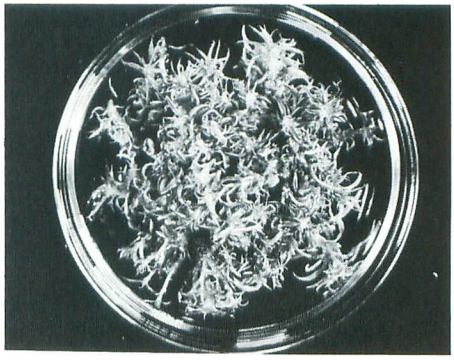

**Figure 2. Shoot multiplication from a cultured shoot tip after 11 weeks in culture.**

Shoot apices excised from field-grown trees of 'Lovell' and 'Nemaguard' peach were cultured on AP medium plus 0.89$\mu$M (0.2 mg $l^{-1}$) BA and 0.04$\mu$M (0.01 mg $l^{-1}$) IBA and on the medium developed for the *in vitro* propagation of 'Nemaguard' peach (10), referred to as Nemaguard medium. Both rootstocks differed significantly only for shoot length, on either media (Table 4). Significant differences were observed for all parameters due to treatment (media) effect. AP media gave significantly better performance ($t_{0.05}$) than Nemaguard medium for fresh weight (726 vs

**Table 3. Concentrations of inorganic ions present in Gelrite and Bacto-agar.[z].**

| Element | Concentration mg/g[y] | |
|---|---|---|
| | Gelrite | Bacto-agar |
| P | 3.5 | 0.1 |
| K | 14.5 | 0.8 |
| Ca | 5.2 | 2.6 |
| Mg | 2.6 | 0.8 |
| Na | 10.4 | 8.2 |
| Cl | 0.2 | 1.5 |
| Zn | 0.011 | 0.005 |
| Mn | 0.0025 | 0.0005 |
| Cu | 0.013 | <0.0005 |
| Fe | 0.023 | 0.007 |

[z] We acknowledge the assistance of the Cooperative Extension Laboratory, University of California, Davis for performing the analyses of Gelrite and Bacto-agar.

[y] Based on actual concentrations when 0.2% Gelrite and 0.9% Bacto-agar are used in the culture media.

**Table 4. Performance of the rootstocks 'Lovell' and 'Nemaguard' after 4 weeks Nemaguard and AP media.**

| Parameter | Source of variation | df | Mean square | F |
|---|---|---|---|---|
| Fresh weight | Rootstock | 1 | 141,778 | 4.1[ns] |
| | Treatment[z] | 1 | 196,878 | 5.7* |
| | Rootstock x treatment | 1 | 22,578 | 0.7[ns] |
| | Replicate (rootstock x treatment) | 28 | 34,331 | |
| No. of leaves | Rootstock | 1 | 11.3 | 1.4[ns] |
| | Treatment[z] | 1 | 552.8 | 66.6* |
| | Rootstock x treatment | 1 | 16.5 | 2.0[ns] |
| | Replicate (rootstock x treatment) | 28 | 8.3 | |
| Shoot length | Rootstock | 1 | 81.3 | 9.8* |
| | Treatment[z] | 1 | 132.0 | 15.9* |
| | Rootstock x treatment | 1 | 0.0 | 0.0[ns] |
| | Replicate (rootstock x treatment) | 28 | 8.4 | |

[ns]not significant.
[z]treatments are AP and Nemaguard media.
*significant at 5% level.

488 mg), no. of leaves (13.8 vs 5.1), and shoot length (19.7 vs 14.5 mm).

No significant effect ($t_{0.05}$) of AP medium strength on shoot length was obtained when proliferating shoots, 10 to 15 mm long, were excised and rotated at 2 rpm for 2 weeks in either 1x or 2x concentrations of liquid AP medium. Shoot length ranged between 23 mm (1x) and 28 mm (2x) with an $LSD_{0.05}$ value of 5.6. Shoot fresh weight increased significantly with 2x AP liquid medium (1x = 1934, 2x = 2875 mg). The $LSD_{0.05}$ value for shoot weight was 799.

After 2 weeks of suspension in 2x liquid AP medium, shoots were subjected to rooting treatments. Highest percentage (70%) of rooting was obtained after 3 to 6 weeks when shoots were cultured in ½N-AP medium supplemented with 44.3$\mu$M (9 mg $l^{-1}$) IBA. Roots were small, however, and plants did not survive transplantation to soil. Although fewer shoots rooted (20%) when shoots were transferred to ½N-AP medium without added growth regulators, the resulting roots were longer (Figure 3) and the plants were successfully established in soil with 100% survival.

The AP medium presented in this paper produced significantly better growth and multiplication of both 'Lovell' and 'Nemaguard' peach shoot tips than other reported media. The shoot tips could be rooted and grown into plants, providing an alternative to seed propagation. Shoots from juvenile and mature plants may be grown with equal success. In addition, AP medium has shown good potential for propagating other peach genotypes as reported in the companion paper, in the April 1986 FVJ.

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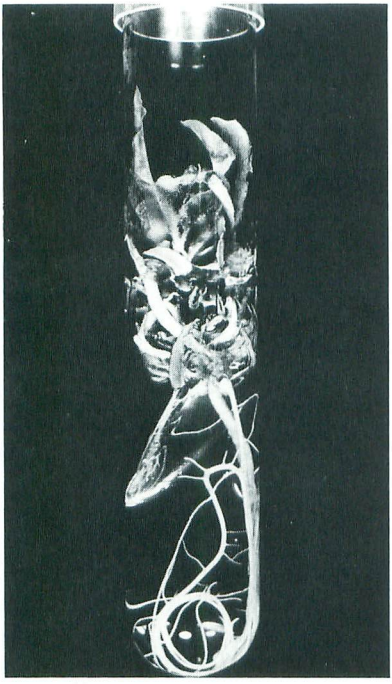

**Figure 3. Rooted peach shoot after 4 weeks in culture.**


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