Leaf pieces are pressed slightly into the surface of a Linsmaier-Skoog medium (1) modified by adding 500 mg/liter casein hydrolysate, 500 mg/liter malt extract and 160 mg/liter adenine sulfate and using only 6 g/liter agar. The pieces are grown under cool white fluorescent lights ca. 3klx at 26°C. Maximum plant proliferation is stimulated by adding 1 mg/liter naphthylacetic acid (NAA) and 0.1-0.5 mg/liter kinetin to the medium. Subsequent studies have shown that adenine sulfate and malt extract are not necessary and probably even inhibit plantlet production from horseradish leaf pieces. Thiamine HCl and inositol were definitely needed for maximum plantlet production and casein hydrolysate has no effect on plant production.

The small plants proliferate from the leaf segments in one stage and are well rooted and easily transferred to a light weight potting mix in about 6 weeks. Rooted plantlets are watered with Hoagland's solution and shaded for a few days in the greenhouse. Plantlets forced in a greenhouse will produce 3-4 leaves 10 cm or longer in 4 months. One leaf can produce 60-100 pieces giving 8-10 plants on average. We calculate 1 plant could easily produce a million plants a year. Several hundred plants have been grown from a single leaf and all appear uniform and true-to-type.

Tissue culture propagation of horseradish from leaves has the potential to break the cycle of root borne diseases and to rapidly increase new cultivars and eliminate viruses from older cultivars.

Literature Cited


Shoot Culture for Almond and Almond-Peach Hybrid Clones in Vitro1

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Additional index words. benzyladenine, kinetin, micropropagation, shoot tip culture, Prunus amygdalus, Prunus sp.

Abstract. A method for growing almond (Prunus amygdalus Batsch) and almond-peach hybrid shoots in vitro is described using dormant shoot buds collected from December to February and stored at 3°C. Shoot tips can be cultured in 0.7 to 0.8% agar, modified Knop's macro-element mineral solution, 2% sucrose, FeEDTA, microelements and organic supplements of Murashige and Skoog medium, 6-benzyladenine (BA), and light. At 0.1 mg/liter, BA produced shoot elongation; at 1 mg/liter, lateral shoot proliferation. Limited rooting has been obtained.

The isolation of individual shoot tips and their propagation in aseptic culture has useful applications for various botanical and horticultural problems including, but not limited to, propagation.

Considerable success has been achieved in propagating herbaceous plant species, such as carnation (5), chrysanthemum (6), gerbera (13), strawberry (3, 11), asparagus (14), and others (12) in vitro. Less success has been had with shoot tips of mature woody plants, but some positive results have developed from apple (1, 8), rhododendron (2), and Prunus (4).

We are interested in developing procedures for shoot tip culture of clones of almond and hybrids of almond and peach [Prunus persica (L.) Batsch] hybrids as a tool for studying certain problems in somatic variation in almond, for vegetative propagation, for maintenance of germplasm, and for production of roots for isozyme studies.

'Nonpareil' almond is a standard commercial cultivar which is susceptible to noninfectious bud-failure (9). The almond is difficult-to-root vegetatively, but many of the clones of almond × peach root readily (10). The 0609 culture was derived from PA 2-16-8-63 hybrid clone which is a potential rootstock that roots readily by cuttings; 0607 is a hybrid seedling of 'Nonpareil' × peach.

We report here success in growing these materials through Stage I (explant establishment), Stage II (multiplication) and, to a limited extent, Stage III (rooting) (12).

The basic culture medium consisted of macroelements of a modified Knops (K) solution (7), plus the other ingredients found in Murashige and Skoog (MS) (15) (Table 1). The pH was adjusted to 5.9 before autoclaving. Twenty ml of the melted medium were dispensed into 25 x 150 mm culture tubes stoppered with propylene tube closures and autoclaved at 121°C at 6.8 kg pressure for 15 min. The cultures were maintained at 25°C on a 16 hr photoperiod with a light intensity of 2.2 to 4.3 klx on the cultures.

With K medium, the shoots developed normally but had a nodular, callus-like structure at the base. The latter appeared to function in absorption (Fig. 1).

Table 1. Basic medium for growing shoot tip explants and cultures of almond and almond-peach hybrids (see text).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concn (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>200</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>200</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>410</td>
</tr>
<tr>
<td>Ca(NO₃)₂·4H₂O</td>
<td>15.8</td>
</tr>
<tr>
<td>Na₂EDTA</td>
<td>37.3</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>27.8</td>
</tr>
<tr>
<td>MnSO₄·7H₂O</td>
<td>16.9</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>8.6</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.025</td>
</tr>
<tr>
<td>KI</td>
<td>0.83</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>0.025</td>
</tr>
<tr>
<td>H₂B₄O₇</td>
<td>6.2</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>0.25</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.0</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>100</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.5</td>
</tr>
<tr>
<td>Pyridoxin·HCl</td>
<td>0.5</td>
</tr>
<tr>
<td>Thiamine·HCl</td>
<td>0.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20,000</td>
</tr>
<tr>
<td>pH</td>
<td>5.9 before autoclaving</td>
</tr>
</tbody>
</table>

¹Received for publication August 12, 1977.
²Laboratory Assistant and Professor of Pomology, respectively.
MS medium at full strength and modified by diluting macroelements one-half produced generally unsatisfactory growth although in certain instances the total fresh wt was somewhat greater than with the K medium (Table 2). With MS medium, shoots were inhibited but leaves expanded, elongated, and thickened. The shoots tended to emerge from the medium, and many became chlorotic. The unsuitability of the MS medium for shoot tips of woody plants also has been reported by others (2, 4).

An agar concn of 0.7 to 0.8% was critical to allow the shoot tips to maintain proper contact with the medium. Growing shoots in liquid medium on rotating wheels or on paper wicks was tried with little success (Fig. 1).

Dormant scions for shoot tip explants (Stage I) were collected in Oct., Dec., and Jan. Some were used immediately while others were stored at 3°C for later use. Buds were removed from the shoots, surface sterilized for 15 to 20 min in Clorox (5.25% sodium hypochlorite) with 0.1% Tween 20 added. Fully dormant buds collected in early winter (before Jan.) were placed in 2 Clorox:3 water while those showing activity (non-resting) collected Jan. or later were placed in 1:5 solution. This avoided injury to the buds but contamination was sometimes greater. Buds were rinsed several times in sterile water. Scales were removed, and the entire shoot tip excised, and transplanted onto agar medium. Ten replicates were used for each test.

Shoot tips of ‘Nonpareil’ almond buds removed in late Oct. remained alive for only a short period, developed some callus, but became chlorotic and eventually died. These buds were evidently in a resting condition. Storing ‘Nonpareil’ 1 to 2 months at 3°C produced surviving shoot-tip explants. Buds collected in late Dec. and Jan., however, grew readily without chilling (70% or more good shoots) and the scions could be stored for as long as 9 months to provide a source of viable materials for culturing. Rate of shoot development appeared to be greater in material collected in Jan. than that collected earlier although quantitative data were not obtained. With 0609, bud explants could be taken successfully in Oct. Dormancy and rest patterns for this clone were different than for ‘Nonpareil’.

A cytokinin was required for shoot survival, for subsequent growth of the explants (Stage I) and for their continuation in subsequent transfers (Stage II). BA at 1.0 mg/liter consistently has been the most satisfactory for proliferation and multiplication (Fig. 2). At this concn, shoot elongation

<table>
<thead>
<tr>
<th>Stage</th>
<th>Medium</th>
<th>No. of tubes</th>
<th>Avg. fresh wt (g)</th>
<th>Shoot proliferation²</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Explant</td>
<td>K</td>
<td>7</td>
<td>0.162±0.103⁴</td>
<td>–</td>
<td>Excellent growth</td>
</tr>
<tr>
<td></td>
<td>½ MS</td>
<td>7</td>
<td>0.25±0.316</td>
<td>–</td>
<td>Chlorosis developing</td>
</tr>
<tr>
<td>II. Proliferation</td>
<td>K</td>
<td>7</td>
<td>0.885±0.330</td>
<td>2.5±0.7⁵</td>
<td>Excellent growth</td>
</tr>
<tr>
<td></td>
<td>½ MS</td>
<td>6</td>
<td>1.089±0.585</td>
<td>1.7±0.5</td>
<td>Chlorosis</td>
</tr>
</tbody>
</table>

²1 = 1-5 shoots; 2 = 5-10 shoots; and 3 = more than 10 shoots.
⁴Mean ±SD.

Fig. 1. Shoot tip cultures of 0609 hybrid. Above. Knop’s (K) medium, left liquid, right 0.75% agar. Below. Murashige and Skoog (MS) medium, left liquid, right 0.75% agar.

Fig. 2. Effect of BA on shoot development in ‘Nonpareil’ almond shoot tip cultures. Stage I. 60 days in culture.

Fig. 3. Effect of BA on shoot development in 0607 hybrid shoot tip cultures. Stage II. 60 days in culture.
is inhibited and growth of axillary shoots promoted. Reducing the BA 
concn to 0.1 mg/liter allows predomin­
antly shoot elongation and produces 
less axillary shoot proliferation. Kinetin was 
not satisfactory at the concn used, 
0.01 to 5 mg/liter, producing chlorosis 
and growth failure. Best results for 
kinetin were at 5 mg/liter and it is 
possible our concn range was too low. 
Hybrid 0607 responded to BA similarly 
to almond. Hybrid 0609 responded 
somewhat differently than almond 
primarily in relation to cytokinin 
requirements. In some experiments 
shoots grew better in kinetin than in 
BA, indicating differences in physi­
ological requirements of the two 
clones.

Application of auxins as low as 
0.01 mg/liter shifted development from 
shoot growth to callus production; 
the higher the concn, the greater this 
shift. 2,4-Dichlorophenoxyacetic acid 
was the most effective callus-producing 
material and naphthaleneacetic acid 
(NAA) was nearly as good. Indoleacetic 
acid caused less callus formation and 
allowed more shoot growth.

Several rooting experiments were 
conducted with limited success. Small 
roots were produced from callus in 5 
out of 10 tubes where indolebutyric 
acid was used in the dark. In another, 
NAA at 1.0 mg/liter induced rooting 
from callus in 2 out of 5 cultures in 
light (Fig. 4). In third, where elongated 
shoots were placed in vermiculite 
inside a test tube, a strong growing 
root was produced in 1 out of 10 
where no auxin was applied.

A supply of stock cultures of 'Non­
pareil' almond, Hybrid 0607 and 
Hybrid 0609 material has been pro­
duced through Stage I and II using 
this procedure. Although we do not 
have long-term experience, we believe 
this procedure has direct potential for 
maintaining germplasm and stock plants.

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Development of a culture medium for 

Tissue Culture of Salpiglossis sinuata L. 
from Leaf Discs1

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Additional index words. salpiglossis, in vitro propagation

In vitro propagation from leaf 
tissues has been reported in various 
solanaceous plants including Peumnia 
hybrida, P. inflata (6), Lycopersicon 
esculentum (5), Nicotiana tabacum 
(3), Solanum nigrum, S. dulcamara, 
and Physalis peruviana (7). Tissue 
culture of Salpiglossis sinuata was 
first reported by Hughes et al. (1) 
who found that the plantlets generated 
from anther culture were normal 
diploids derived from sporophytic tissue. 
We have since found that adventitious 
shoots of salpiglossis can be readily 
generated from leaf discs in vitro 
using a modified Murashige and Skoog 
(MS) medium (4). Salpiglossis leaf 
discs respond dramatically to variation 
in auxin and cytokinin concentration 
(2) and are excellent material for 
classroom demonstration of tissue-
culture techniques.

Leaf discs, 4 mm in diam, are punched 
out with #1 corkborer (Fig. 1). It is 
esential that only young leaves be 
used; leaves older than about 2 months 
seldom undergo organogenesis. Sterili­
zation is achieved by soaking the 
leaf discs in 5% Clorox (active ingre­
dient sodium hypochlorite, 5.25%) for 
10 minutes followed by three 3-minute 
washes in sterile water. We place leaf 
discs on media (using culture tubes 
25 mm diam x 150 mm length con­
taining 10 ml of media) and then 
incubate in a growth chamber with 
16-hr day (320 lux, 26°C) and 8-hr 
night (21°C).

Inclusion of the midvein has little 
effect on subsequent callus and organ 
formation from leaf discs. Naphthale­
necetic acid (NAA) and kinetin 
interact to affect organogenesis. When 
combinations of kinetin and NAA each