Regeneration of Arabidopsis callus in vitro

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Abstract. This paper reports on an easy and reproducible method of regenerating Arabidopsis plants from callus culture. A combination of 6-benzylaminopurine (BAP) and α-naphthalene acetic acid (NAA) in a Murashige and Skoog's (MS) based medium gives a high percentage of shoot formation in several genotypes.

Introduction

The merits of Arabidopsis as a research tool include the facts that it has a low chromosome number \((n = 5)\), it takes only about 30 days to complete its life cycle, about 50,000 seeds can be obtained per plant, and can be grown aseptically in test tubes [6]. There are a number of mutants available for genetic, biochemical and physiological studies, the best known among them is the thiamine auxotroph [7].

In this age of biotechnology, Arabidopsis should be a very useful tool in genetic engineering studies. Arabidopsis is an excellent host to \textit{Agrobacterium tumefaciens} and the cauliflower mosaic virus, which are considered the two most promising vectors for molecular genetic engineering. Recently, Leutweiler et al. (1984) reported that Arabidopsis has a low level of cytosine methylation and little repetitive DNA in its nuclear genome in contrast to other higher plants.

The use of Arabidopsis in genetic engineering studies has been hampered by the difficulty of regenerating plants in tissue culture. Previous reports of regenerating plants from calli are not easily reproducible. Arabidopsis is routinely regenerated from callus cultures in our laboratory and in the classroom [1].

Materials and methods

The genotypes of \textit{Arabidopsis thaliana} (L.) Heynh tested were the 1) Columbia wild type, 2) mutant A-154, 3) race Langridge, 4) chml mutant, and 5) thiamine mutant B-135 (Available at 117 Curtis Hall, University of Missouri, Columbia, MO. 65211).
Table 1. Composition of the different media used to induce callus formation and shoot differentiation in Arabidopsis

<table>
<thead>
<tr>
<th>Components</th>
<th>B5a</th>
<th>ARb</th>
<th>MSc</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4NO3</td>
<td>-</td>
<td>-</td>
<td>1650 mg/L</td>
</tr>
<tr>
<td>KNO3</td>
<td>2500 mg/L</td>
<td>500 mg/L</td>
<td>1900</td>
</tr>
<tr>
<td>CaCl2 • 2H2O</td>
<td>150</td>
<td>0.05</td>
<td>440</td>
</tr>
<tr>
<td>MgSO4 • 7H2O</td>
<td>250</td>
<td>50</td>
<td>370</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>-</td>
<td>-</td>
<td>170</td>
</tr>
<tr>
<td>(NH4)2SO4</td>
<td>134</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>NaH2PO4 • H2O</td>
<td>150</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>H3BO3</td>
<td>3</td>
<td>1</td>
<td>6.2</td>
</tr>
<tr>
<td>MnSO4 • H2O</td>
<td>10</td>
<td>3</td>
<td>22.3</td>
</tr>
<tr>
<td>ZnSO4 • 4H2O</td>
<td>2</td>
<td>0.5</td>
<td>8.6</td>
</tr>
<tr>
<td>NaMoO4 • 2H2O</td>
<td>0.25</td>
<td>0.1</td>
<td>0.25</td>
</tr>
<tr>
<td>CuSO4 • 5H2O</td>
<td>0.025</td>
<td>0.01</td>
<td>0.025</td>
</tr>
<tr>
<td>CoCl2 • 6H2O</td>
<td>0.025</td>
<td>0.01</td>
<td>0.025</td>
</tr>
<tr>
<td>Na2EDTA</td>
<td>37.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ferric EDTA (Calbioch)</td>
<td>-</td>
<td>37</td>
<td>36.7</td>
</tr>
<tr>
<td>KI</td>
<td>0.75</td>
<td>0.25</td>
<td>0.83</td>
</tr>
<tr>
<td>Inositol</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>1</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Thiamine • HCl</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>pH</td>
<td>5.5</td>
<td>6.0</td>
<td>5.6</td>
</tr>
</tbody>
</table>

a Gamborg’s B5  
Arabidopsis seeds were disinfected for 8 min in 5 percent calcium hypochlorite and rinsed in several changes of sterile distilled water. The disinfected seeds were dispersed in sterile viscous agar solution or distilled water, and with the aid of a Pasteur pipette they were easily spread over a thin layer of mineral medium solidified with agar [7]. The B5, MS and AR media described below may also be used for seed germination, but the hormones should be omitted. The seeds were allowed to germinate for approximately 3 days. The germinating seeds were picked as a whole or the cotyledons, hypocotyl, root or shoot apices cut separately and placed on the surface of a solidified medium supplemented with 4.5 μM (1.0 mg 1⁻¹) 2,4-dichlorophenoxyacetic acid (2,4-D).

Among the media tested were Gamborg’s B5 (B5) [2], Murashige and Skoog’s (MS) [5], and our own formulation of a tissue culture medium for Arabidopsis (AR) (Table 1). All media were solidified with 0.6 percent agar (Granulated, ICN Pharmaceuticals, Cleveland, Ohio), autoclaved at 121 °C for 7 min except IAA which was filter sterilized (Millipore, 0.22 μM). Each test tube (150 x 16 mm) contained 5 ml medium.

After 3 weeks, the calli formed from the explants or calli subcultured 2–3