Growth of mulberry synthetic seeds on vermiculite, sand and soil media

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Recently, in many crops attempts have been made to obtain synthetic seeds using somatic embryos produced by plant tissue culture. However, these attempts have been successful in only several annual crops, such as alfalfa (Redenbaugh et al., 1986, 1987), celery (Redenbaugh et al., 1986; Sakamoto, 1990), lettuce (Sakamoto, 1990) and carrot (Kitto and Janick, 1985a, 1985b).

On the other hand, in the case of mulberry tissue culture a method was developed to induce adventitious buds directly from leaf cultures (Oka and Ohyama, 1981; Katagiri and Nishiguchi, 1986). Moreover, it was demonstrated that a larger number of adventitious buds could be induced more efficiently from immature leaves isolated from winter buds than from mature leaves of shoots cultured in vitro (Saito and Katagiri, 1989; Machii, 1992a; Saito, 1992). In a previous paper we reported that such adventitious buds were useful materials for producing synthetic seeds of mulberry (Machii, 1992b).

In the present paper the growth and rooting of synthetic seeds of mulberry on three different growth media, vermiculite, sand and soil are reported.

Materials and Methods

As described previously (Machii, 1992b), adventitious buds were used as explants for the production of synthetic seeds. These adventitious buds were induced from immature leaves of the mulberry variety 'Shin-ichinose' cultured on MS medium (Murashige and Skoog, 1962) modified by the addition of 3% fructose and 5 μM benzyladenine, pH 5.8, 1.0% agar. About 30 days after the start of the culture, adventitious buds were excised from immature leaf cultures and transferred to a 3% sodium alginate solution. Then, the adventitious buds were dropped into a 100mM calcium chloride solution one by one for encapsulation, resulting in the production of synthetic seeds. Immediately after this procedure, half of the synthetic seeds were sown on growth media, the remaining half being stored in MS liquid medium containing 1.0mg/l BA, 0.1mg/l NAA and 30g/l sucrose at 4°C for 30 days, and then sown on the growth media. The growth media consisted of vermiculite, sand and soil (clay loam) added to 100-ml Erlenmeyer flasks, which were all autoclaved at 121°C for 15 min. No nutrients and plant growth regulators were added except for water. After sowing, the Erlenmeyer flasks were placed in a growth cabinet at 27°C with fluorescent lamps (60–80 μmol m−2·s−1). After 30 days of incubation, germination, shoot growth and rooting were observed. As described in the previous paper (Machii, 1992b), when the expanded leaves emerged from the capsules, the growth stage of the synthetic seeds corresponded to germination. When the shoot stem emerged from the capsules, it was assumed that shoot growth had occurred.

Results and Discussion

Table 1 shows the growth of stored and non-stored synthetic seeds on the different media.
Table 1. Growth of synthetic seeds on different growth media.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Growth medium</th>
<th>No. of synthetic seeds tested</th>
<th>Germination No.</th>
<th>Germination %</th>
<th>Shoot growth No.</th>
<th>Shoot growth %</th>
<th>Rooting No.</th>
<th>Rooting %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-stored</td>
<td>Vermiculite</td>
<td>16</td>
<td>3</td>
<td>18.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>16</td>
<td>11</td>
<td>68.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>soil</td>
<td>16</td>
<td>7</td>
<td>43.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stored*</td>
<td>Vermiculite</td>
<td>16</td>
<td>8</td>
<td>50.0</td>
<td>5</td>
<td>31.3</td>
<td>2</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>sand</td>
<td>16</td>
<td>10</td>
<td>62.5</td>
<td>8</td>
<td>50.0</td>
<td>2</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>soil</td>
<td>16</td>
<td>12</td>
<td>75.0</td>
<td>10</td>
<td>62.5</td>
<td>4</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Mulberry variety: 'Shin-ichinose'.

The data were collected 30 days after sowing.

* Stored in MS liquid medium containing 1.0mg/l BA, 0.1mg/l NAA and 30g/l sucrose at 4°C for 30 days before sowing on each growth medium.

Fig. 1. Synthetic seeds rooted on different growth media.

The growth media were 1; vermiculite, 2; sand and 3; soil. The synthetic seeds were stored in MS liquid medium containing 1.0mg/l BA, 0.1mg/l NAA and 30g/l sucrose at 4°C for 30 days. The photo was taken 30 days after sowing on the growth media.

The non-stored synthetic seeds germinated on all the three growth media, with a rate of 18.8% on vermiculite, 68.8% on sand and 43.8% on soil. However, neither shoots nor roots developed on any growth media. On the other hand, more than 50% of the synthetic seeds stored at 4°C for 30 days after the procedure were able to germinate and they developed shoots on all the growth media. The highest rates of germination, shoot growth and rooting (75.0%, 62.5% and 25.0%, respectively) were observed on the soil medium, followed by the sand and the vermiculite media. Fig. 1 shows the rooting of the synthetic seeds grown on the three growth media, vermiculite, sand and soil, respectively. Rooting was most vigorous on the soil, followed by the vermiculite and the sand media.

Thus, synthetic seeds which were sown on the growth media without storage did not root on any of the growth media, whereas synthetic seeds which were stored at 4°C for 30 days before sowing rooted on all the growth media. In the previous paper (Machii, 1992b), synthetic seeds did not root on the vermiculite medium, regardless of whether they were stored at 4°C or not stored for some time before sowing. In this experiment, synthetic seeds rooted on vermiculite at the low rate described above. The finer size of vermiculite medium used in this experiment than that described in the previous paper may account for the difference between the results in the previous and present experiments. It is interesting to note that 25% of the stored synthetic seeds rooted on soil without the addition of nu-
rients and plant growth regulators except for water.

If the synthetic seeds produced by using somatic embryos or adventitious buds could regenerate on soil, the impact on mulberry cultivation for sericulture would be significant. Somatic embryos are able to regenerate whole plants directly on some media at one time. However, no somatic embryos have been induced from mulberry tissue cultures so far, whereas whole plants are generally regenerated from adventitious buds through the formation of shoots followed by that of the rooting system on different media. It is thus interesting to note that 25% of the synthetic seeds produced displayed a rooting ability on soil in our material.

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References


