Germination and Regeneration of Plants from Old Cucumber Seed

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Abstract. Cucumber (Cucumis sativus L.) seeds of ‘Marketable’, ‘Marketmore’, ‘Wisconsin SMR-18’, ‘Tablegreen’, ‘Spotfree’, and ‘China’ were stored at 3°C and 38% relative humidity for up to 26 years. Seed older than 13 years did not germinate. Cultivars stored 10 years gave 80% germination, except Wisconsin SMR-18’ (40%). Ten-year-old seeds were separated from their seedcoats, and cotyledons were excised into six segments. Explants were placed on Murashige and Skoog medium with all combinations of BAP (0, 1, 2, and 3 mg·liter\(^{-1}\)) and NAA (0, 0.1, 0.2, and 0.3 mg·liter\(^{-1}\)). Plants were obtained from culture for all cultivars grown on medium containing NAA and 1 mg BAP/liter. No plants were regenerated when BAP or NAA was lacking. Chemical names used: benzylaminopurine (BAP), 1-naphthaleneacetic acid (NAA).

Seed storage is important for germplasm preservation, and most cucumber germplasm is stored as seed. Seed may be stored for decades, depending upon the storage conditions. No matter how good the seed storage is, seeds are likely to lose viability with age. The germplasm must be renewed regularly, requiring considerable land and labor (Roos, 1980). Irreversible degenerative changes during storage eventually result in death of the seeds (Roberts, 1972).

A tissue culture system that would enable successful regeneration from mature, excised dry seed pieces would offer a means to multiply plants rapidly when only a small amount of seed is available. Shoot regeneration and embryogenesis from cucumber cotyledons (Cade et al., 1987; Chee, 1990) and several Cucurbita species (Lange and Juvik, 1986a) has been reported. In this paper, we report the germination and regeneration in tissue culture of entire plants from cucumber cotyledons excised from aged seeds.

Seeds of ‘Market’, ‘Marketmore’, ‘Wisconsin SMR-18’, ‘Tablegreen’, ‘Spotfree’, and ‘China’, which were 26, 20, 18, 17, 16, 15, 13, and 10 years old, were stored at 3°C and 38% relative humidity (RH) from harvest until use. Seeds (five) of each cultivar and age were germinated in petri dishes with Whatman #1 filter paper. Water was added and the seeds incubated for 10 days at 20°C. Radicle emergence (5 mm long) was used as the indicator of germination. The size of these experiments was limited due to the few seeds available for the older lots.

For regeneration studies, the outer seedcoat was removed from five seeds each of 13- and 10-year-old lots, sterilized in 70% ethanol for 1 min, in 0.5% sodium hypochlorite for 10 min, and rinsed three times with sterilized distilled water. Cotyledons were separated aseptically and then divided into three segments such that each seed provided six cotyledon segments. Ali (1990) had shown that cotyledon size was not a factor in plant regeneration, and the six cotyledon segments were kept as a unit to provide repetition.

The 30 cotyledon segments from each cultivar were randomly explanted onto Murashige and Skoog (1962) medium (MS) with BAP (0, 1, 2, and 3 mg·liter\(^{-1}\)) and NAA (0, 0.1, 0.2, and 0.3 mg·liter\(^{-1}\)). Plants were obtained from culture for all cultivars grown on medium containing NAA and 1 mg BAP/liter. No plants were regenerated when BAP or NAA was lacking.

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Table 1. Plant regeneration from old cucumber cotyledons.*

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Seed age (year)</th>
<th>Survival of cotyledons (days)</th>
<th>Explants forming callus (%)</th>
<th>Plants regenerated (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Market</td>
<td>10</td>
<td>55</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Marketmore</td>
<td>10</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wisconsin SMR-18</td>
<td>13</td>
<td>65</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Tablegreen</td>
<td>10</td>
<td>29</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Spotfree</td>
<td>10</td>
<td>44</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>China</td>
<td>10</td>
<td>81</td>
<td>77</td>
<td>19</td>
</tr>
</tbody>
</table>

*Explants on MS media with 4 mg BAP/liter and 0.2 mg NAA/liter.

Table 2. Plant regeneration from cotyledons of 10-year-old cucumber seed in nine combinations of BAP and NAA.*

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Market</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marketmore</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wisconsin SMR-18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tablegreen</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Spotfree</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

*No plants were regenerated when the medium lacked BAP or NAA.
did not; recently, Chee (1990) reported that only 5% of the explants from ‘Poinsett 76’ cucumber cotyledons formed embryonic callus. In this study, shoots <10 mm long seldom survived ex vitro. In general, the number of shoots that survived ex vitro was directly proportional to the length of time the explant survived in vitro (Table 1). Cotyledons of ‘China’ survived the longest and regenerated the most plants; ‘Spotfree’ survived the shortest and produced only one plant. Cotyledons of other cultivars turned green and expanded but survived for 32 days or less and produced no plants. Cotyledons from nonviable seed lots did not develop chlorophyll or callus.

The seeds used here were about the same age, but there was a wide range of regeneration frequencies (Table 1). Variation in regenerability among cultivars has been reported for cucumber (Lange and Juvik, 1986b; Msikita et al., 1988) and other cucurbit (Lange and Juvik, 1986a). In this study, ‘Wisconsin SMR-18’, a monoecious line, did not regenerate shoots. Alvarez (1989) reported more ethylene was produced by ‘Wisconsin 98’, a gynoecious cucumber, compared to monoecious lines. Seed-generated ethylene may have restricted regeneration of ‘Wisconsin SMR-18’ in vitro.

Seed aging reduced in vitro regenerability more than viability. Thirteen-year-old ‘Marketmore’ seeds germinated 40%, and cotyledon explants turned green in vitro, but they failed to yield plants ex vitro. In contrast, 10-year-old ‘Marketmore’ seeds germinated 80% and yielded nine plants (Table 1). Regeneration of plants from cucumber cotyledon culture varies among genotypes (Maleszpy and Orczyk, 1983; Msikita et al., 1988).

Seeds older than 13 years did not germinate, but these seeds might possess islands of living cells that might regenerate in vitro with the proper stimulus of growth regulators. However, nonviable seeds failed to regenerate. Among viable ones, none regenerated on medium lacking in either BAP or NAA (data not presented). At the lowest level of BAP (1 mg·liter⁻¹), all cultivars produced some shoots that rooted and grew as plants ex vitro (Table 2). At 2 mg BAP/liter, ‘Market’ and ‘Spotfree’ did not regenerate, and at 3 mg BAP/liter, ‘Wisconsin SMR-18’ and ‘Spotfree’ did not regenerate (Table 2). ‘China’ and ‘Marketmore’ responded to a wide concentration of growth regulators (Table 2), and ‘China’ regenerated well even at 4 mg BAP/liter (Table 1).

‘China’ regenerated over a wide range of BAP (1 to 3 mg·liter⁻¹) and NAA (0.1 to 0.3 mg·liter⁻¹) combinations. With an increase from 1 to 3 mg BAP/liter, ‘Marketmore’ regenerated over the entire range of NAA concentrations (Table 2). Similar reports for various cucurbit species have been made by Dirks and Buggenum (1989), Msikita et al. (1988), and Pink and Walkey (1984). Mackay and Ng (1989) reported that shoot count in Cucumis melo decreased as NAA level increased, but in this study with cucumber, no such pattern of NAA inhibition was found. Chee (1990) recently reported somatic embryogenesis induced from cucumber cotyledons on MS medium with (2,4-dichlorophenoxy) acetic acid (2,4-D; 2.0 mg·liter⁻¹), N-(2-furanylmethyl)-1H-purin-6-amine (kinetin, 0.5 mg·liter⁻¹) and NAA (1.0 mg·liter⁻¹). In our study, we moved our regenerates to rooting medium supplemented with NAA only (1.5 mg·liter⁻¹).

**Literature Cited**


