Research Notes: United States: In vitro fertilization of perennial soybean species

John J. Silvoy
Clemson University

Earlene A. Rupert
Clemson University

Emerson R. Shipe
Clemson University

Follow this and additional works at: http://lib.dr.iastate.edu/soybeangenetics

Part of the Agriculture Commons, Agronomy and Crop Sciences Commons, and the Plant Breeding and Genetics Commons

Recommended Citation

Available at: http://lib.dr.iastate.edu/soybeangenetics/vol11/iss1/29

This Article is brought to you for free and open access by the Journals at Iowa State University Digital Repository. It has been accepted for inclusion in Soybean Genetics Newsletter by an authorized editor of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
1) **In vitro** fertilization of perennial soybean species.

Interspecific hybridization in the genus *Glycine* is difficult and few successes have resulted from a multitude of attempts (Newell and Hymowitz, 1982, 1983). Because flowers of the perennial species are especially difficult to manipulate in the field or greenhouse, a study was undertaken to examine the potential of fertilizing these species *in vitro*, thereby partially alleviating mechanical damage to the ovulary and environmental stress. Initial studies were intended to develop *in vitro* procedures under which ovularies would survive and form viable embryos. Therefore, all flowers were self-fertilized.

Seeds of perennial species *G. clandestina*, *G. falcata*, *G. latifolia*, and *G. tabacina* were obtained from Dr. R. L. Bernard and planted in the field and greenhouse. Flowers from greenhouse plants were used exclusively as explant material because those from field plots could not be disinfested adequately. Flowers were collected immediately before dehiscence and disinfested with 33% commercial chlorox solution. The corolla and androecium were removed. Pollen was applied to the stigma from a more mature flower of the same plant and the pistil with intact calyx implanted on agar-based medium in a 10 mm glass vial.

The most successful medium for pistil development consisted of an equal volume mixture of Murashige-Skoog and Gamborg B-5 mg salt formulations supplemented with 0.05 mg BA (benzylladenine), 0.5 mg GA₃ (gibberellin), 2.0 mg thiamine-HCl, 0.5 mg pyridoxine-HCl, 250 mg myo-inositol, 100 mg casein hydrolysate and 5.5 g Difco Bacto-agar per liter of solution. Sugar concentrations of 3% and 5% were more supportive of pod and embryo development than 10% (Table 1). Greater concentrations of the cytokinin BA caused extensive callus formation at the base of the pistil.

Embryos that matured to the stage at which they could be easily excised, usually after about four weeks, were subcultured on the medium described by Cutter and Bingham (1975) where shoots and roots developed. The plants were then easily transferred to potting mix.

Viable embryos were recovered from all species (Table 1). All but *G. falcata* were successfully cultivated into mature, fertile plants. Although the recovery percentage was not high, averaging only 9% overall, we expect improvement with increased experience.

Additional *in vitro* studies may be useful in facilitating wide hybridizations, thus expanding the soybean germplasm base.
Table 1. Influence of sucrose concentration on the incidence of pod and embryo development *in vitro*.

<table>
<thead>
<tr>
<th>Species</th>
<th>30</th>
<th>Embryos recovered</th>
<th>50</th>
<th>Embryos recovered</th>
<th>100</th>
<th>Embryos recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pistils implanted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. clandestina</td>
<td>30</td>
<td>7</td>
<td>17</td>
<td>5</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>G. falcata</td>
<td>6</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>80</td>
<td>3</td>
</tr>
<tr>
<td>G. latifolia</td>
<td>--</td>
<td>--</td>
<td>40</td>
<td>4</td>
<td>58</td>
<td>3</td>
</tr>
<tr>
<td>G. tabacina</td>
<td>100</td>
<td>10</td>
<td>14</td>
<td>1</td>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td>All species</td>
<td>136</td>
<td>17</td>
<td>71</td>
<td>10</td>
<td>204</td>
<td>10</td>
</tr>
<tr>
<td>Percent recovery</td>
<td>12.5</td>
<td></td>
<td>14.1</td>
<td></td>
<td>4.9</td>
<td></td>
</tr>
</tbody>
</table>

*No pod contained more than one embryo.*
References


John J. Silvoy
Earlene A. Rupert
Emerson R. Shipe