Tissue culture and plant regeneration from immature embryo explants of Barley, *Hordeum vulgare*

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**Summary.** Immature embryo explants taken 8 days after anthesis were used to establish callus cultures of spring barley. Two types of calli were observed. A soft, watery callus produced a limited number of shoots and a harder, more compact, yellowish callus gave rise to numerous green primordia and shoots. Gamborg's B5 basal medium supplemented with either 2,4-D (2,4-dichlorophenoxyacetic acid) or Cl₅ POP (2,4,5-trichlorophenoxypropionic acid) was found to give good callus growth and shoot initiation. Media containing 2,4-D at 1.0 mg L⁻¹ or Cl₅ POP at 5.0 mg L⁻¹ produced numerous cultures resulting in regeneration of plants. Plantlets developed roots on basal medium with Cl₅ POP at 1.0 mg L⁻¹ or on auxin-free medium. Twenty genetically diverse genotypes were screened to determine if these techniques were suitable for a wide range of spring barley cultivars. Regeneration of plantlets was obtained for 19 of the 20 genotypes approximately 4 months after culture initiation. Lines differed in the ability to develop vigorously growing calli and in the ability of calli to develop large numbers of shoots and regenerated plantlets.

**Key words:** Immature embryos – Spring barley – Plantlet regeneration – Auxin – Genotypes

**Introduction**

Reproducible regeneration of plants has recently been reported for a number of cereal species including wheat (Sears and Deckard 1982; Ahloowalia 1982; Eapen and Rao 1982a), rye and triticale (Eapen and Rao 1982b), proso millet (Heyser and Nabors 1982a), oats (Heyser and Nabors 1982b), and barley (Deambrogio and Dale 1979; Bayliss and Dunn 1979). A high rate of regeneration from callus cultures is a prerequisite for the use of tissue culture as a tool in crop improvement.

Specific differences exist in the in vitro response of genetically diverse genotypes. Distinctions can be made among cultivars for their callus growth response, ability to initiate shoots and roots, and regeneration potential. Varieties and breeding lines have been identified for their ability to yield numerous regenerated plantlets. The identification of specific genotypes that are capable of rapid callus production and high rates of plantlet regeneration is an important step toward the application of tissue culture techniques to agriculture.

Results will be presented to substantiate the successful regeneration of a genetically diverse range of spring barley cultivars. Experiments were conducted to determine optimal explant stage of maturity, growth regulator requirements, and distinct genotype responses. Detailed explanations of culture responses at various stages of callus growth and plantlet development are provided.

**Materials and methods**

Plants used in these experiments were grown under greenhouse conditions. Measures were taken to avoid environmental stress. Pots containing a greenhouse soil mixture of peat, leaf mulch, and soil were watered daily. A 15-15-15 fertilizer was incorporated into the soil mixture at the time of planting and a one-tenth strength Hoagland's fertilizer solution was applied one week prior to anthesis. Plants for hormone studies were kept at constant temperatures of 35 to
38°C with a 14 h day-length. Plants for genotype studies were held at temperatures ranging from 26 to 38°C and approximately 14 h daylight. The seed sources for all plants were acquired from breeders seed maintained as pure lines.

Spikes were bagged a few days before anthesis. Immature spikes were collected 8 days after anthesis. The caryopses were surface-sterilized with 20-fold diluted Clorox (5.25% sodium hypochlorite) for 5 min and rinsed 5 times with sterile distilled water. Embryos were excised using a dissecting microscope under aseptic airflow conditions of a laminar flow hood. Three immature embryo explants were taken from individual spikes and placed in the same petri dish.

Immature embryo explants were placed in 15 by 60 mm pre-sterilized petri dishes. Cultures were established and maintained on Gamborg’s B5 basal medium with the addition of the following components (concentrations are mg L^-1): NaH₂PO₄·H₂O, 150; KNO₃, 2,500; (NH₄)₂SO₄, 134; MgSO₄·7H₂O, 250; Ferric EDTA, 40; Gamborg and Wetter (1975) supplemented with 2% sucrose, and 0.7% to 0.8% Sigma agar-agar. The pH of the medium was adjusted to 5.5 with either NaOH or HCl before the addition of agar.

Any embryo explants that showed signs of precocious germination were discarded. Cultures were transferred every 3 to 6 weeks. Once shoots began to develop cultures were placed in autoclaved glass test tubes or pre-sterilized baby bottle liners. All cultures were maintained in the same growth chamber. The controlled environment chamber was kept at 24 to 26°C, with a 12 h day-length.

Hormone experiments

A randomized complete block design consisting of six replicates, four treatment levels and three subsamples was used. Six plants of the cultivar ‘Klages’ were established in the greenhouse. Four randomly selected spikes were collected from each plant and labelled according to source for testing the four hormone treatment levels. The spikes were from both primary and secondary tillers.

Three immature embryo explants were taken from a single spike and placed in a petri dish supplemented with a specific hormone concentration. These three embryos were subsamples of a replicate. The hormone treatments were 2,4-D (2,4-dichlorophenoxyacetic acid) at 1.0 mg L^-1 and Cl₃ POP (2,4,5-trichlorophenoxypropionic acid) at concentrations of 1.0, 2.5, and 5.0 mg L^-1. Measurements were taken for callus gain in fresh weight, appearance of green primordia, shoot and root initiation, and plantlet regeneration.

Genotype experiments

A randomized complete block design consisting of five blocks and 20 genotypes was used. One spike was randomly selected from each of the 100 plants in the investigation. Three immature embryo explants were excised from the spike and placed in a culture dish. These three embryos were subsamples of the blocks.

Cultures of 20 genetically diverse genotypes were initiated on Gamborg’s B5 medium supplemented with 1.0 mg L^-1 2,4-D. Cultures were maintained on this same medium from initiation of callus through plantlet regeneration stages. Regenerated plantlets were transferred to hormone-free media to stimulate root development. Evaluations of successful growth and regeneration were based on measurements of callus fresh weight gain, shoot and root initiation, and plantlet regeneration.

Results

No differences were detected in callus growth, whether the scutellum was placed downward in contact with the medium or facing up. There were obvious differences in the response of embryos taken at various developmental stages. Embryos taken 7 to 8 days after anthesis developed into vigorously growing calli. This is the equivalent of stage II in wheat caryopsis development as described by Rogers and Quatrano (1983), when the caryopsis is at the milky stage. Not only were the number of days after anthesis important, but the size of the explant had a distinct effect on the success of establishing cultures. Embryo lengths of 0.5 to 1.0 mm were best for eliciting rapid callus development while avoiding the precocious germination of the embryo.

Hormonal responses

All auxin concentrations tested were suitable for the initiation of vigorously growing callus cultures. Significant differences existed in the effect of the growth regulators on callus fresh weight gains at both 4 weeks (Table 1) and 9 weeks (Table 2) after explants were established. Supplements of either 2,4-D at 1.0 mg L^-1 or Cl₃ POP at 1.0 mg L^-1 caused the greatest callus production by explanted embryos.

Table 1. Hormone treatment means for fresh weight of callus cultures 4 weeks after initiation for the cultivar ‘Klages’

<table>
<thead>
<tr>
<th>Treatment (mg L^-1)</th>
<th>Fresh wt (g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D 1.0</td>
<td>0.289 a*</td>
</tr>
<tr>
<td>Cl₃ POP 1.0</td>
<td>0.228 a</td>
</tr>
<tr>
<td>Cl₃ POP 2.5</td>
<td>0.149 b</td>
</tr>
<tr>
<td>Cl₃ POP 5.0</td>
<td>0.118 b</td>
</tr>
</tbody>
</table>

* Mean of six replications

Table 2. Hormone treatment means for fresh weight of callus cultures 9 weeks after initiation for the cultivar ‘Klages’

<table>
<thead>
<tr>
<th>Treatment (mg L^-1)</th>
<th>Fresh wt (g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl₃ POP 1.0</td>
<td>0.956 a*</td>
</tr>
<tr>
<td>Cl₃ POP 2.5</td>
<td>0.724 b</td>
</tr>
<tr>
<td>2,4-D 1.0</td>
<td>0.633 bc</td>
</tr>
<tr>
<td>Cl₃ POP 5.0</td>
<td>0.483 c</td>
</tr>
</tbody>
</table>

* Mean of six replications

**Means with a letter in common are not significantly different. LSD0.05 for comparison among these means = 0.078**