Lectin levels in tissues of cultured immature wheat embryos

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Abstract
Levels of wheat germ agglutinin have been determined by radioimmunoassay in tissues of immature wheat embryos cultured under different conditions in order to determine the suitability of the lectin as a marker for somatic embryogenesis. Embryos cultured on media favouring continued embryo development accumulated lectin in a similar manner to zygotic embryos in planta unless precocious germination occurred. Embryos cultured on media containing 2,4-D produced callus, and some of this developed somatic embryos. Both embryogenic and non-embryogenic callus contained WGA, that in non-embryogenic callus possibly arising from developmentally arrested root primordia.

Abbreviations
ABA abscisic acid
dpa days post anthesis
PBS phosphate buffered saline, (10 mM KH2PO4, K2HPO4, 145 mM NaCl, pH 7.4)
RIA radioimmunoassay
WGA wheat germ agglutinin
2,4-D 2,4-dichlorophenoxyacetic acid

Introduction
The lectin wheat germ agglutinin [WGA] characteristically accumulates in embryos of Triticum aestivum during mid-stages of grain development, reaches maximal levels by maturity, and decreases rapidly in amount as embryos germinate. The rate of lectin accumulation in excised immature embryos cultured in vitro can be modified by inclusion of ABA or mannitol in the growth medium as these agents inhibit precocious germination [Morris et al. 1986]. The specific location of WGA in zygotic embryogenic structures and the persistence of lectin synthesis in cultured embryos suggested that WGA might be a suitable marker for embryogenesis in vitro. In many instances of plant regeneration from cultured immature cereal tissues, the plantlets may arise by a process of somatic embryogenesis (Bright and Jones, 1985). This involves the initial differentiation of structures resembling zygotic embryos which are variously known as somatic embryos, embryoids or secondary embryos and which later 'germinate' in vitro to form shoots and/or roots. Often, however, the pattern of development may be somewhat abnormal and precocious germination may occur before a clear embryo develops. This is usually the case in regenerating cultures of wheat [Ozias-Akins & Vasil, 1983; Maddock et al., 1983; Bright and Jones, 1985]. In such instances it is not always possible to identify callus as embryogenic, nor to determine the origin of the shoots and roots. This study was carried out to determine whether WGA is formed in somatic as well as zygotic wheat embryos, and whether its presence could be used as a marker for somatic embryogenesis in cultured tissues of wheat.

Materials and Methods
Culture of wheat embryos.
Developing wheat grains (cv. Timmo) were collected at 7-12 dpa from glasshouse-grown plants during summer 1984. The embryos were 0.1 mm long and translucent in appearance, and the endosperm was still fluid. The grains were surface sterilized [7% w/v calcium hypochlorite followed by washing in sterile deionised water] and the embryos excised and cultured scutellum upwards on agar plates containing five different culture media [table I, 250 embryos per medium]. Eight embryos were plated out per 9 cm diameter petri dish containing 20 ml medium. In all media, pH was adjusted to 5.8 before solidification with 0.6% (w/v) agar-agar (Fisons Ltd.) and sterilisation by autoclaving. ABA, when added, was filter-sterilised and added to media immediately before pouring.

Table 1. Media additives for the culture of immature wheat embryos

<table>
<thead>
<tr>
<th>Medium</th>
<th>Addition</th>
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<tbody>
<tr>
<td>A</td>
<td>Murashige + Skoog's medium</td>
</tr>
<tr>
<td>B</td>
<td>Medium A + 10% (v/v) coconut milk</td>
</tr>
<tr>
<td>C</td>
<td>Medium A + 10% (v/v) coconut milk + 0.1 mg l⁻¹ ABA</td>
</tr>
<tr>
<td>D</td>
<td>Medium A + 10% (v/v) coconut milk + 1 mg l⁻¹ 2,4-D</td>
</tr>
<tr>
<td>E</td>
<td>Medium A + 10% (v/v) coconut milk + 1 mg l⁻¹ 2,4-D + 0.1 mg l⁻¹ ABA</td>
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Cultured embryo samples were taken for assay at intervals up to 49 days after plating out. At each time point 15 embryo-derived cultures were selected at random; those cultures showing different morphologies (germinated embryos, undifferentiated callus, embryogenic callus, etc.) were collected separately in replicates of 15. Samples were rapidly frozen and stored at -20°C prior to analysis.

Cell suspension cultures

An established cell suspension culture of wheat (c.v. Maris Butler, initiated from embryogenic callus) was grown in the presence or absence of ABA. This culture (Naddock, 1983) was normally maintained in liquid medium (pH 5.8) based on Murashige and Skoog salts (1962) and 2,4-D at 2.5 mg 1\(^{-1}\), and sub-cultured at 1:5 dilution every 7 days. Material was sampled for analysis after 6 days in the standard medium containing 0, 1.0 and 10.0 mg 1\(^{-1}\) ABA. A cell pellet was obtained by centrifugation and washed with 3% sucrose, and the cell supernatant was also retained. Samples were frozen and stored at -20°C before analysis.

Analysis of WGA

The material was assayed for WGA using radioimmunoassay [RIA] as described in Morris et al (1985). Essentially this involved extraction of tissues by homogenization and sonication in RIA buffer [PBS pH 7.4, 0.1% w/v Triton X-100, 100 mM N-acetylglucosamine]. The homogenate was centrifuged at 30 k g for 30 min at 4°C and the supernatant assayed for protein according to the method of Lowry (Lowry et al 1951) and for WGA by competitive RIA using a pre-formed goat anti-rabbit/rabbit anti-WGA IgG complex and \(^{125}\)I-WGA and analysed using the spline function programme of an LKB RackGamma. For assay of suspension culture supernatant, the medium was made to a fixed concentration with RIA buffer and after sonication and centrifugation, was processed as above.

Results and discussion

Embryogenesis in planta generally follows a predefined pattern of morphological and biochemical development, which, if analysed, can be applied to determine the nature and progress of embryogenesis in vitro.

Generally, cereal species are relatively unresponsive when cultured in vitro, and plant regeneration is limited in terms of both the range of tissues which are capable of in vitro morphogenesis and the culture conditions under which this is possible (Bright and Jones, 1985). However, immature embryos at the stage of development used in this study are capable of successful regeneration (Ozias-Akins & Vasil, 1982; Maddock et al., 1983). The presence of the auxin 2,4-D (1 mg 1\(^{-1}\)) is necessary for the initiation of secondary embryogenesis and shoot formation, with coconut milk stimulating the outgrowth of shoots and leaves after they have been initiated. The formation of shoots from somatic tissues is favoured if immature embryos are cultured with the scutellum uppermost; it is from this tissue that proliferation occurs.

The percentage of embryos that germinated on media A, B (no 2,4-D or ABA) and C (no 2,4-D, with ABA) is given in Fig. 1(i). The cultured embryos were separated into those in which precocious germination was prevented (Fig. 1(ii)) and those exhibiting precocious germination (Fig. 1(iii)). From a morphological and biochemical aspect, arrested embryos followed a developmental pattern very similar to that found in planta. The embryos increased in size, weight and protein content, and WGA accumulated throughout development (Fig. 1(ii)). No WGA was detected in freshly isolated

![Figure 1](https://example.com/figure1.png)

**Figure 1** Effect of in vitro culture of immature wheat embryos on media lacking 2,4-D. (i), Percentage germination with time. (ii), Lectin content and protein content of developmentally arrested embryos. (iii), Lectin content and protein content of precociously germinated embryos. See Table 1 for media ingredients (A, B, C)