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Somatic embryogenesis in *Triticum aestivum* L. Morphological observations on germination

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Somatic embryos were induced from scutellar callus of immature zygotic embryos of *T. aestivum* cv. Chinese Spring. Observations on precociously germinating somatic embryos revealed that: (i) In the initial stages the coleoptile is split, exposes the shoot apex and forms a green trichomatous leafy structure. In the germinating zygotic embryo, the coleoptile is tubular, (ii) Unlike what has been inferred earlier the leafy structure is the coleoptile and not the scutellum, (iii) Bipolarity of the embryoid is established later when root develops at the basal end.

For a long time cereals were considered recalciterant to culture. The discovery of potent auxins, such as 2,4-D and 2,4,5-T, has allowed induction of regenerable callus from a variety of plant parts[1]. Somatic embryogenesis seems the preferred pathway of plant regeneration in cereals since most reports of high frequency regeneration involve embryogenic cultures induced from immature explants such as embryos, inflorescences and leaves[2-5]. The degree of normalcy and the efficiency of embryogenesis, however varies between species[1]. In wheat, atypical embryoids characterized by leafy structures subtending multiple shoots and typical embryoids possessing normal scutellum, coleoptile, coleorhiza, epiblast, shoot and root in callus induced from the scutellum or epiblast of immature embryo explants have been reported[3,6,7]. Magnusson and Bornman[8] studied the ontogeny of somatic embryos and concluded that the embryoids could arise through any of three different pathways from the dermal, ground or vascular tissue systems of the scutellum of immature embryo explants. In this report we describe the morphological changes associated with the germination of somatic embryos.

Materials and Methods

Immature embryos of *Triticum aestivum* cv 'Chinese Spring' were used as explants. Caryopses in the late-milk stage (about 19 days after anthesis) were removed from the spike, surface sterilised with 0.1% (w/v) mercuric chloride for 10 min and washed in several changes of sterile distilled water. Embryos were dissected from the caryopses and inoculated with scutellum oriented away from the medium. Callus initiation medium comprised of the inorganic and organic constituents of Murashige and Skoog's[9] medium (MS), 3% sucrose and 1 mg/l 2,4-D. Agar (0.8%) was added to gel the medium after adjusting the *p*H to 5.8.

Cultures were initially incubated in dark at 25° ± 2°C, and were transferred to light a week after inoculation, when signs of plant regeneration became apparent. About 2 weeks after callus initiation, clusters of somatic embryos arising from the callus were transferred for germination to hormone free MS containing 1% sucrose and 0.8% Agar.

Calli were dissected under a stereomicroscope and whole mounts of the somatic embryos were prepared for studying the stages in the germination of the embryoids.

Results and Discussion

Callus initiation was observed by 3rd day after inoculation. While callus arising from the coleoptile, coleorhiza and mesocotyl region of the embryo explant was translucent and loose in texture, that arising from the scutellum was white, compact and became increasingly nodulated and friable. Clusters of globular somatic embryos, each delimited by a distinct epithelium, were visible on the surface of the scutellar callus by the seventh day after inoculation. Upon transferring the cultures to light, the embryoids rapidly germinated into green, trichomatous leafy structures each subtending a shoot.

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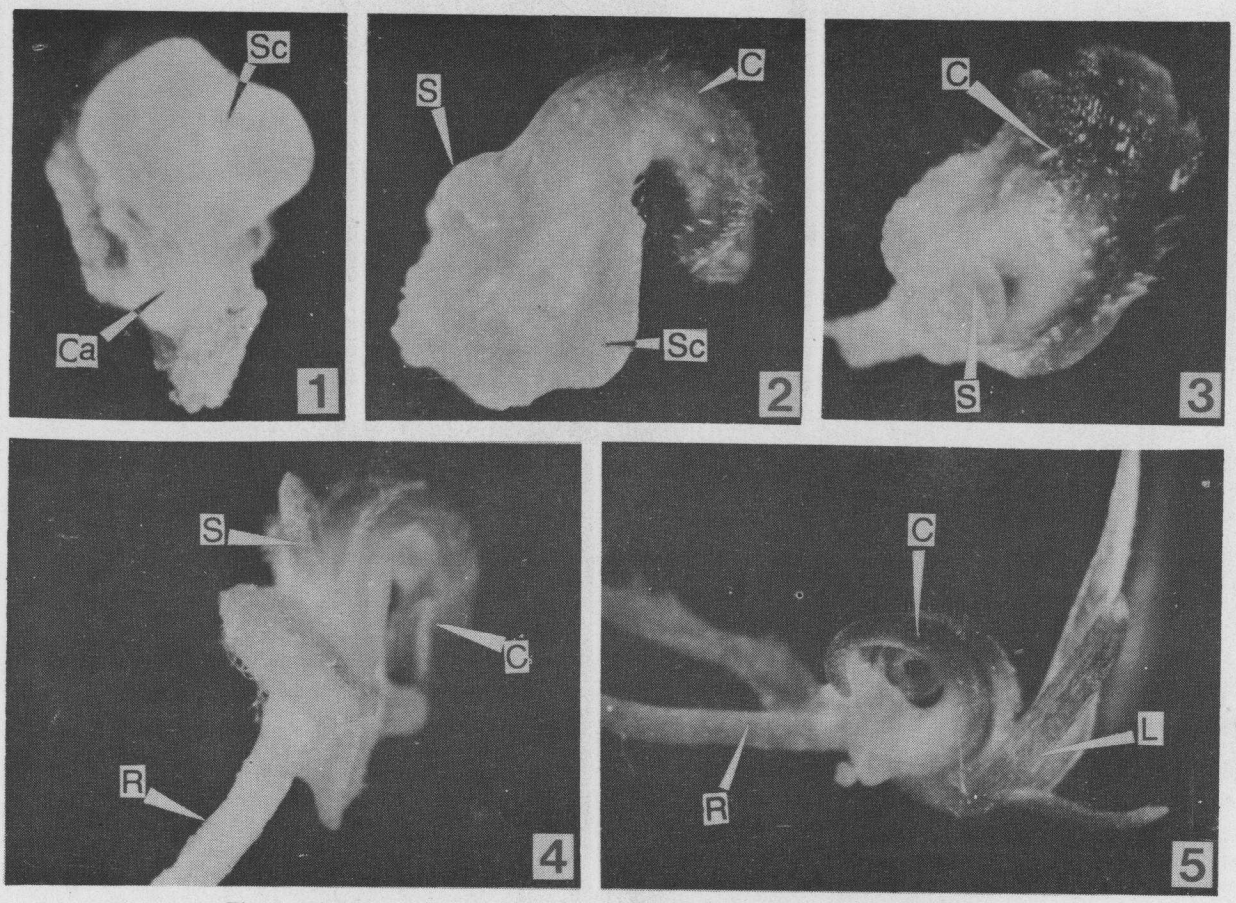
The response of explants with respect to callus formation is summarized in Table 1. The callusing response was 100% and 83.28% of the cultures regenerated plants. While 61.09% of the cultures were embryogenic, in 11.94% cultures, green shoots were produced from relatively loose, translucent callus, presumably through the organogenic pathway. In 6.82% cultures, plants were regenerated through both pathways.

Stages of germination of somatic embryos could be identified by examining whole mounts of embryogenic cultures under a stereomicroscope. The somatic embryos, which arose mostly in clusters from the callus surface, each had a flat, white scutellum with a distinct epithelial covering (Fig. 1). Due to precocious germination on the callus initiation medium itself, the scutellum did not always develop completely. Instead, the coleoptile grew and split to form a leafy structure which was green and trichomatous (Fig. 2), unlike the tubular coleoptile of germinating zygotic embryos. This coleoptile subtended the shoot apex (Fig. 3), which soon developed the first foliar structures (Fig. 4). Simultaneously, the seminal roots developed, thereby establishing the bipolar nature of the embryo (Fig. 5).

The formation of somatic embryos, from scutellar or immature embryo explants of wheat, with well defined scutellum, coleoptile, one or more shoot primordia and a root primordium has been reported earlier by several workers. Based on histological and scanning electron microscopic observations, Ozias-Akins and Vasil[3] proposed that the embryoids germinate precociously into green 'leafy structures', due to which at later stages of development, the somatic embryos do not always resemble the zygotic embryos in morphology. Ozias-Akins and Vasil[3] considered the 'leafy structures' as equivalent to the scutellum because it is known that the scutellum, considered by some to be a leafy homologue, is capable of acquiring leafy characteristics under certain conditions. For instance, in barley, Norstog[10] found that Kinetin could induce the formation of trichomes and greening of the scutellum.



Figs 1 to 5—Stages of germination of somatic embryos from two-week old cultures
(1) Somatic embryo from callus. (2, 3) Germinating somatic embryo with split, green, trichomatous coleoptile subtending a shoot primordium, (4) Later stage in germination of somatic embryo showing shoot and root emergence. (5) Fully developed seedling with seminal roots and leaves. C—coleoptile; Ca—callus; L—leaf; R—root; S—shoot primordium; Sc—scutellum.

Table 1—Callusing and regeneration response of immature embryo explants of *T. aestivum*

	Type of callus				
	Nonmorphogenic	Organogenic	Embryogenic	Heterogenic (Organogenic + embryogenic)	Total
	Number (Freq. %)	Number (Freq. %)	Number (Freq. %)	Number (Freq. %)	Number (Freq. %)
Callusing response	49 (16.72)	37 (12.62)	187 (63.87)	20 (6.82)	293/293 (100)
Regeneration response		35 (11.92)	179 (61.09)	20 (6.82)	234/293 (83.28)

It is believed that in wheat, the scutellum can grow only to a limited extent and is incapable of developing chlorophyll *in vitro* as it does *in vivo*. Unlike rice and barley, the scutellum of mature embryos of wheat does not callus in culture, thus indicating that it is devoid of meristemoids or cells capable of reverting to meristematic state. Our observations indicate that the 'leafy structure' of a precociously germinating embryoid is not the scutellum as proposed by Ozias-Akins and Vasil[3] but is the coleoptile.

The coleoptile is often interpreted as a leaf homologue; it is an open leaf with a median vascular bundle in the primitive grass *Streptochaeta*[11]. However, this foliar interpretation is not universally accepted and some regard it as an outgrowth of the scutellum, the scutellar sheath, rather than as a product of apical meristem[12]. As may be seen in Fig. 2, the 'leafy structure' of a germinating embryoid subtends the shoot apex and does not appear to be a product of the apical meristem. We therefore interpret it to be the coleoptile or the scutellar sheath. The open structure of the coleoptile in germinating somatic embryos, as opposed to the tubular coleoptile seen in zygotic embryos, may be attributed to the presence of 2,4-D in the callus initiation medium. Ferguson *et al.*[13], studied polyembryony induced by spraying flowering wheat plants with 2,4-D and found that the coleoptile was often split and the epiblast was divided to expose the shoot apex.

The present study thus indicates that the process of germination of somatic embryos does not differ significantly from that of zygotic embryos and that minor morphological differences observed can be attributed to the cultural conditions. In both somatic and zygotic embryos of wheat, the scutellum is capable only of limited growth and does not acquire a 'leafy' character. The first morphological change associated with germination is the formation of a leafy coleoptilar extension. This, in the case of somatic embryos is an open structure with a split epiblast exposing the shoot primordium. Root development occurs subsequently and establishes the bipolarity of the embryoids.

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