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SOMATIC EMBRYOGENESIS AND PLANT REGENERATION FROM SEED CALLUS OF BARLEY (*HORDEUM VULGARE* L.)

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In barley, regeneration has been reported from many explants including the immature embryos[1], apical meristems[2–4], anthers[5], mesocotyl[6], mature embryos[7] and seedling tissues[8]. Regeneration from young leaves of barley has also been reported[9]. By choosing the appropriate explants at a defined stage of development, it has now become possible to regenerate plants from a number of species belonging to the family Gramineae[10]. Immature embryos and young inflorescence segments are most preferred explants for inducing embryogenesis in grasses and cereals[10] including barley[1]. However, it must be pointed out that immature explants are available only in restricted periods of the year. Therefore, use of mature tissues such as seeds as a source of explant is preferred for the better organization of experiments. In the present report regeneration has been obtained from mature seed derived callus.

Seeds of barley (*Hordeum vulgare* L. genotype RD 387) were obtained from the Agriculture Research Station, Durgapura. After surface sterilization for 5 min in 0.1% $HgCl_2$ solution, the seeds were aseptically cultured on MS[11] basal medium, supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D). The pH of the medium was adjusted to 5.8 before autoclaving at 1.06 kg/cm^2 for 20 min. The cultures were incubated at 26 ± 2°C under 24 h illumination from white fluorescent tubes and incandescent bulbs.

Callus proliferated in 2–3 weeks from the cultured seeds in the embryonal root portion on media, with 2,4-D concentrations ranging from 1 to 5 mg/l (figure 1a). 2,4-D at higher concentrations (5 mg/l) gave better callus proliferation. Approximately 400 mg callus was formed from a single cultured seed on MS medium with 5 mg/l of 2,4-D. The callus was soft, watery and pale with green patches. Three to four weeks after inoculation, the seed callus was subcultured on a medium with a lower concentration of 2,4-D (0.5 mg/l) + kinetin (0.2 mg/l). In the first subculture, a white, hard, opaque and nodulated callus was formed along with the proliferation of soft and watery callus mass. On microscopic examination this white nodulated callus was found to be embryogenic. In the subsequent subcultures only the embryogenic callus was subcultured on the fresh medium for further proliferation of embryogenic callus and embryoids. In every subculture some nonembryogenic callus were also produced which were excluded from being subcultured in the next

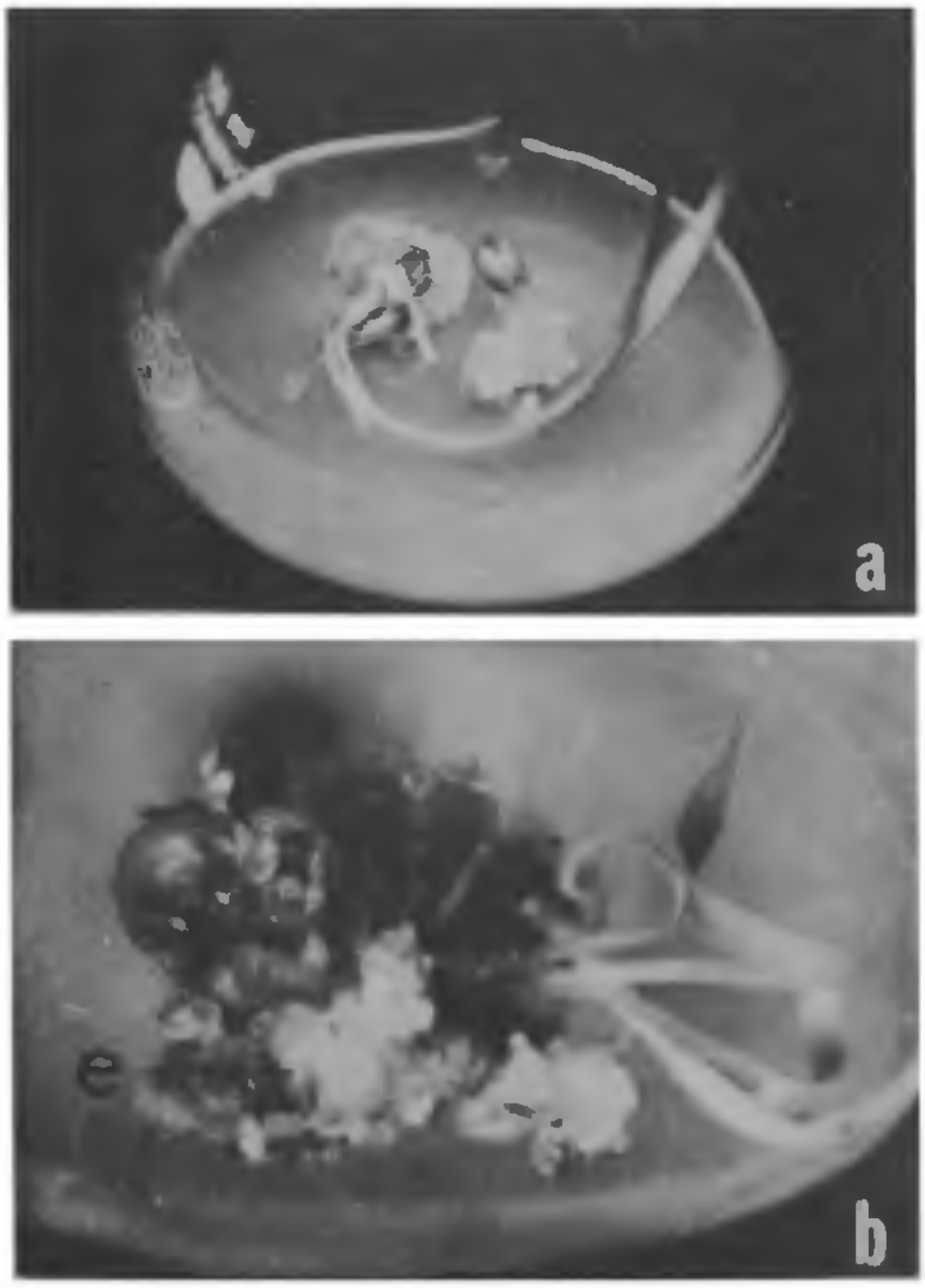

Figure 1a, b. **a.** Callus formation from embryonal root portion of the cultured seeds on MS medium with 5 mg/l of 2,4-D, and **b.** Embryogenic callus with embryoids and shoots on MS medium with kinetin (0.2 mg/l) + 2,4-D (0.5 mg/l); (e, embryoids).

passage. Embryoids differentiated in the callus in 3–4 weeks and if allowed to remain in the same medium, some of them germinated to develop into shoots while their root poles remained quiescent (figure 1b). Roots also developed from some embryoids during their germination.

When 2,4-D was excluded from the medium containing kinetin (0.2 mg/l) alone or with 5% coconut water (CW) the embryoids germinated to develop into well-developed shoots (figure 2). Prolific tillering from the base of the shoots was conspicuous on the medium with 5% CW. Sufficiently developed 2–4 cm long shoots were rooted on MS medium with 1. naphthaleneacetic acid (NAA) (1–3 mg/l) or indolebutyric acid (IBA) (1 mg/l). For this purpose shoots together with callus nodules were placed on filter paper bridges in culture tubes having liquid medium. Rooting was initiated after 5–7 days and a healthy plant with vigorous root system developed in 2–3 weeks.

The establishment of stable embryogenic callus cultures has been considered necessary for obtaining embryogenic suspension and protoplast cultures[10]. Such a type of embryogenic callus has been obtained with immature tissues of cereals[10]. We induced embryogenic callus formation from the callus derived from cultured seeds. The callus was formed through proliferation of the embryonal root. 2,4-D at all the concentrations (1–5 mg/l) was effective in inducing callus formation. Higher concentration of 2,4-D (5 mg/l) produced more callus which in subsequent subcultures on media with lower levels of 2,4-D became embryogenic. Lu *et al*[12], showed that higher concentration of 2,4-D (5 mg/l) was inefficient for embryogenic callus formation in maize. Addition of kinetin alone or CW increased the embryogenic callus formation as reported earlier[8,13].

Figure 2. Elongated shoots developed from embryoids on MS medium with 0.2 mg/l kinetin + 5% CW. The root poles of the embryoids remained undeveloped

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ISOLATION OF *ANABAENA AZOLLAE* FROM THE MEGASPOROCARP OF *AZOLLA*

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In view of the importance of *Azolla* as a biofertilizer, attention has been focussed on its nitrogen fixing endosymbiont *Anabaena azollae*. For long, it was thought that the cyanobacterium cannot be grown in isolation from the host[1–3]. However,