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Morphoanatomy of rice embryoid development

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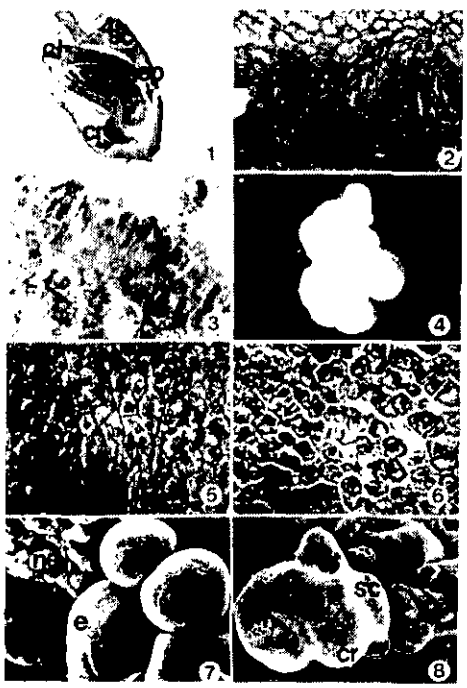
Histological studies of cultured embryos during the early stages of cell proliferation provide information about the initial sites and patterns of cell division activity and subsequent differentiation. Morphological observations through the scanning electron microscope (SEM) provide a clear picture of the natural concave-convex appearance of the various stages of embryoid development.

Dehulled mature seeds of rice variety Tetep were sterilized and inoculated in Linsmaier and Skoog's (LS) medium, each liter containing 1 mg 2, 4 - dichlorophenoxyacetic acid (2, 4-D) gelled with 1% agar. Cultures were maintained in darkness at 26-27 0C.

The scutella were isolated from embryos still embedded in the seed after 1 wk in culture. Samples for histological and SEM analysis were taken 2, 4, 6, 6, 8, 10, and 12 d after seed inoculation; subsequent samples from subcultures were taken every 4 weeks.

For plant regeneration, 3-mo-old calli were transferred to Murashige and Skoog's (MS) medium, each liter containing 4 mg benzylaminopurine (BAP), 0.5 mg indole-3-acetic acid (IAA), 0.5 mg naphthalene acetic acid (NAA), 500 mg casein hydrolysate, and 3% sucrose gelled with 0.8% agar. The calli were incubated at 26-27 O C in continuous 3000 lux light intensity.



Rice embryoid development: 1 = longitudinal section of a seed embryo showing the coleorhiza (cr), coleoptile (cl), scutellum (sc), and subepidermal layer (sep); 2 = callus formation at the subepidermal layer of the sc; 3 = thallus structure proliferating from the surface of the sc; 4 = 3-mo-old smooth, compact, round, and whitish embryoids; 5 = an embryogenic callus; 6 = a nonembryogenic callus; 7 = scanning electron micrograph of embryogenic calli (e) proliferating at the surface of nonembryogenic (ne) calli; 8 = scanning electron micrograph of an embryoid with sc, cr, and a developing cl. IRRI, 1987.

Active cell division took place in the scutellum, especially on the subepidermal (sep) portion (see figure 1-2). Calli formed on day 6 of culture. Elongated structures were visible at the periphery of the scutellum on day 10 (3). Cells pushed out the epithelial layer, giving the scutellum a wavy appearance. Active cell division was visible on day 12. Smooth, compact, round and whitish embryoids were obtained after 3 wk in culture (4).

Anatomical observations showed embryogenic calli with densely packed cells, prominent nucleus, and thin cell walls (5). Nonembryogenic calli had loosely packed cells, hardly visible nuclei, and thick cell walls (6).

A SEM study (7) showed globular and organized structures (e) proliferating at the surface of the nonembryogenic calli (ne). Embryoids containing the scutellar portion, coleorhiza, and a developing coleoptic (8) were exactly like the zygotic embryo, but sometimes did not have synchronized development.

Embryogenic calli transferred to regeneration medium produced prolific shoots and plantlets from embryoids after 3 wk in culture.

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