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,

there were many claims of isolation of the endosymbiont[4–6] which were disputed by others who could not culture the organism after isolation[7–9]. Recently, there have been reports on the successful isolation and culturing of the cyanobacterium using different techniques such as gentle roller method[10], enzymatic digestion[11,12], capillary pipetting technique[13] and blender method[14]. In all these methods, the fronds of *Azolla* have been used for the isolation of the organism from the leaf cavities. It results in the isolation of *A. azollae* contaminated heavily with certain green algae, a variety of bacteria and even certain other types of cyanobacteria, inspite of surface sterilization. Because of this, the authenticity of the culture is being questioned (Peters personal communication). In the present investigation, a simple technique has been developed which could result in the isolation of an authentic culture of *A. azollae* from the akinetes found inside the indusial cap of the megasporocarp of *Azolla*[15,16].

Megasporocarps from *Azolla mexicana* were obtained from R. W. Fisher's laboratory (USA) and were completely dried in shade for 6 months. These were surface-sterilized with chlorox and inoculated in agar slants containing BG11 medium. They were kept under flourescent illumination (1,500 lux) in a 14 + 10 L/D cycle at 27 ± 2°C. After twenty-five days, the indusium was separated from the sporocarp and the akinetes were found to have germinated in the form of long filaments (figure 1a) which were then isolated (figure 1b) and subcultured in a nitrogen-free BG11 medium resulting in a pure culture of *A. azollae*. Work on making the culture axenic and characterization is under way.

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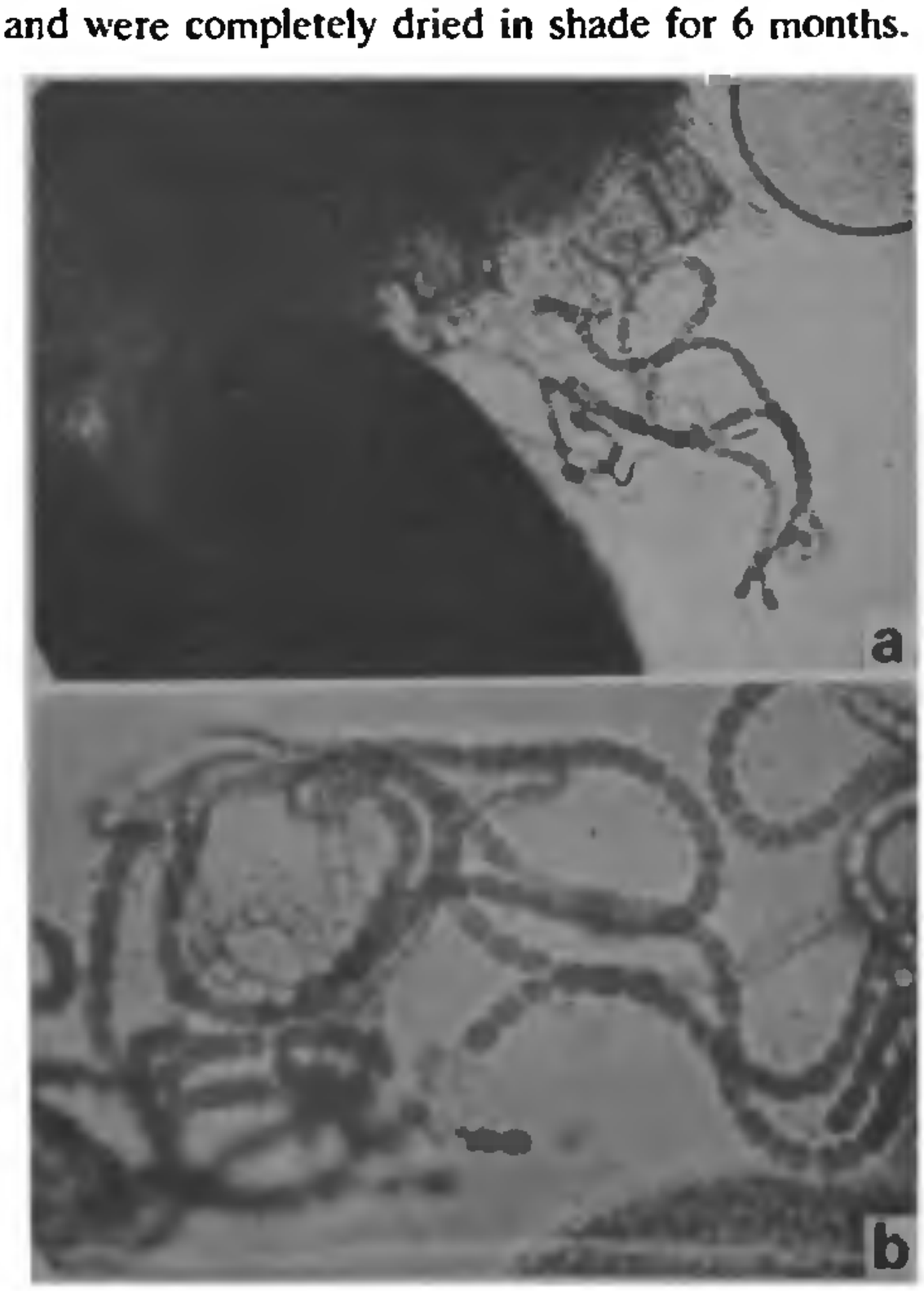

Figure 1a,b. **a.** Part of the megasporocarp and indusium showing the release of *A. azolla*, and **b.** *A. azollae* isolated from the indusium in BG11 medium.

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