

utilized by the fungi and part of this is converted into gaseous nitrogen also.

TABLE I

Changes in total nitrogen content of 'Arhar' seeds due to infestation with storage fungi

Fungi	Total nitrogen in mg/100 mg	Percentage of loss
<i>Aspergillus flavus</i>	2.05	43.33
<i>A. niger</i>	1.88	48.05
<i>Fusarium moniliforme</i>	2.65	25.66
<i>Curvularia lunata</i>	3.12	13.61
<i>Helminthosporium tetramera</i>	3.17	12.22
Control	3.61	No loss

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#### IN VITRO INDUCTION OF ADVENTITIOUS SHOOTS ON STEM EXPLANTS OF *BOERHAAVIA DIFFUSA* L.

PLANT tissue cultures are becoming increasingly important as an experimental system to study many fundamental problems in developmental morphogenesis and biosynthesis of natural products<sup>1,2</sup>. *Boerhaavia diffusa* Linn. is known to be a medicinal shrub and commonly used in jaundice, ascites, anisarcia and scanty urine diseases<sup>3</sup>. This communication describes a method for obtaining callus and adventitious shoots from the stem explants of *B. diffusa*.

Stem explants (2-3 cm) were placed horizontally on Murashige and Skoog's<sup>4</sup> medium supplemented with auxins (NAA and 2, 4-D) and cytokinins (Kn or BAP). The cultures were maintained in light grown chamber at  $28^{\circ} \pm 2^{\circ}$  C in 16 height (1,000 lux). Various concentrations of NAA, Kn and BAP (alone and combinations) were tried to study the callus growth and morphogenetic responses of explants.

The callus induction was observed after 7 days of transplant, on MS medium containing NAA (1.0 ppm), 2, 4-D and Kn (each in 0.25 ppm). The maximum callus growth was maintained on MS with NAA (0.5 ppm), 2, 4-D (0.25 ppm) and Kn (0.25 ppm). Callus obtained was soft, fragile and watery. Higher NAA (1.0-10.0 ppm) concentrations were found inhibitory to callus growth. The callus turned compact, hard and light brown with the increase in Kn (1.0-2.5 ppm) concentrations. Organogenesis occurred on auxin devoid medium, containing BAP (0.25 ppm) after 3-4 weeks (Fig. 1). No root was observed in



FIG. 1. Shoots of *B. diffusa* produced from stem explant on MS + BAP + Kn (each in 0.25 ppm) media, devoid of auxin.

shoot forming explants. A large number of adventitious shoots were formed on various concentrations of BAP in combination with Kn (0.25 ppm) and NAA (0.05 ppm) (Fig. 2). The maximum (20-25), well developed shoots were produced with BAP (1.0 ppm) after 6-8 weeks. Leaves produced on these shoots were small, curved and light green in colour. The stem was thick, soft, and yellowish green. These differentiating explants initiated only a small amount of callus which stopped further growing after 2 weeks.

It can, therefore, be concluded from above results that the formation of adventitious shoots and callus from explant requires a particular ratio of cytokinin and auxin. A parallel correlation has also been observed with *Citrus*<sup>5</sup> and *Narcissus*<sup>6</sup> tissues.



FIG. 2. 6 week old stem explant of *B. diffusa* showing large number of adventitious shoots on MS + BAP (1.0 ppm) + Kn (0.25 ppm) + (NAA 0.05 ppm).

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Abbreviation used: NAA:  $\alpha$ -naphthaleneacetic acid; 2, 4-D: 2, 4-dichlorophenoxyacetic acid; Kn: 6-furfurylaminopurine and BAP: 6-benzylaminopurine.

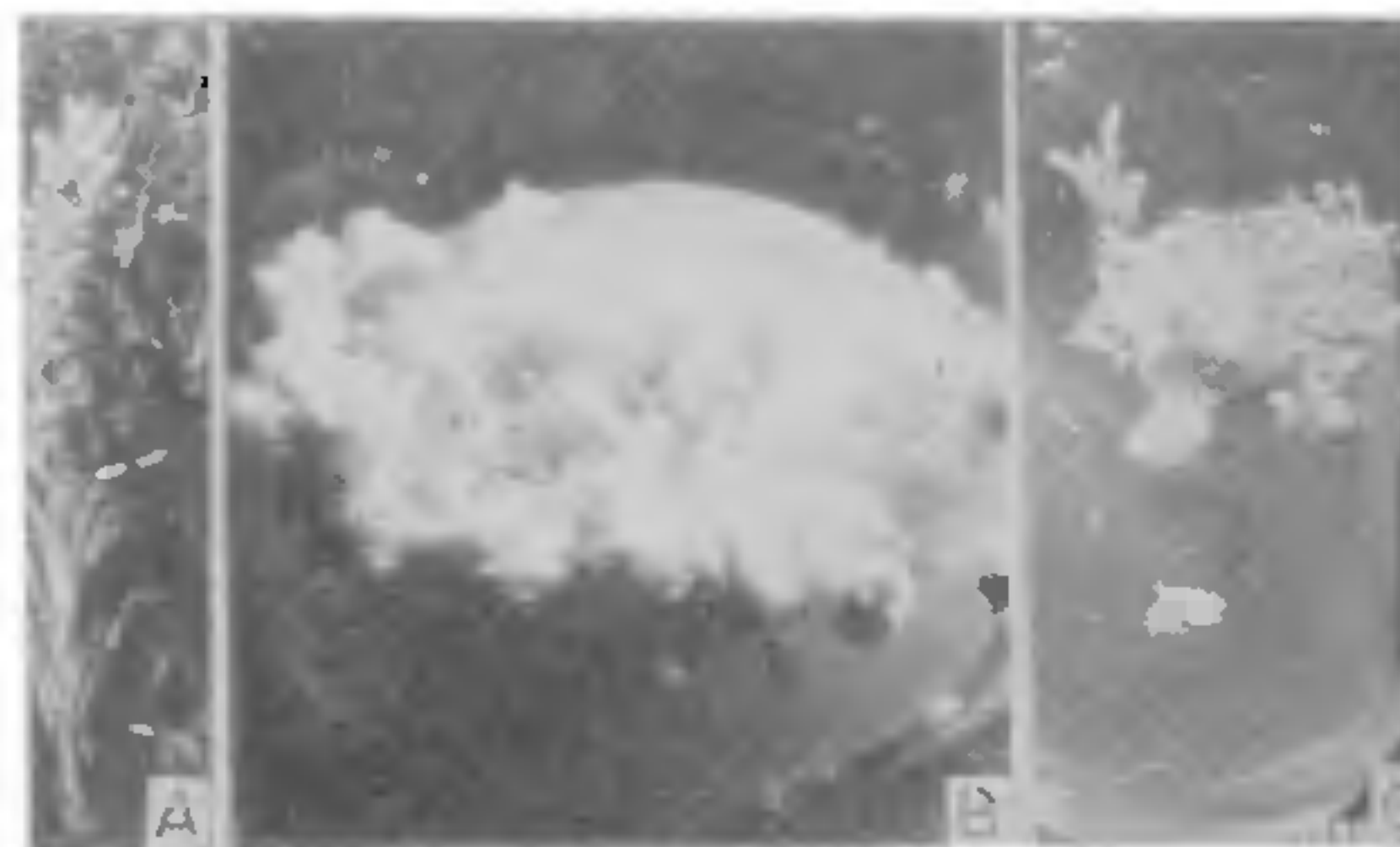
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# IN VITRO SEED GERMINATION OF ZEUXINE STRATEUMATICA SCHLR. (= Z. SULCATA LINDLEY), ORCHIDACEAE

A CONSIDERABLE amount of work has been done on the culture of orchid seeds in raising seedlings for horticulture. The utility of various artificial media has enabled the investigator to produce seedlings of different orchids *in vitro*. Nevertheless, there are some orchids which have eluded the worker despite the advancement of techniques and refinement of the culture media.

Seshagiriiah<sup>1</sup> who studied *Zeuxine sulcata* stated that "Germination experiments of seeds in *Zeuxine* were not successful in spite of providing the seeds with the necessary symbiotic fungus which was isolated, by means of pure culture, from the roots of the adult plants of *Zeuxine*" (p. 362). With regard to artificial germination of seeds of *Z. strateumatica* and *Listera ovata*, Stoutmaire<sup>2</sup> pointed out that, "there has been no successful germination of either, in this laboratory" (p. 103).

Joshi<sup>3</sup> reported the mode of perennation in *Zeuxine sulcata*, indicating that it is a perennial. However, Vickers<sup>4</sup> demonstrated that *Z. strateumatica* is an annual. He raised plants from seeds sown in orchid pots of other species under green house conditions. According to Porter<sup>5</sup> *Zeuxine* is essentially tropical and its seeds germinate only during hot summer months. But so far all attempts to raise *in vitro* seedlings from seeds of *Z. strateumatica* have never met with success. In our laboratory we have been trying to understand the growth requirements of Karnataka orchids of which the apomictic taxon<sup>6</sup> *Z. strateumatica* is one (Fig. A).



FIGS. A-C. Fig. A. Habit of the taxon, X0.5. Fig. B. Protocorms in the culture flask, X1.5. Fig. C. Branched shoots and protocorms in the culture tube, X1.5.

Brown pods of *Z. strateumatica* collected from Cauvery basin near Mysore city during the months January-February 1975 (Voucher specimen of taxon bearing No. 89 AKK has been deposited in the herbarium of the University of Mysore, Manasa