

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.

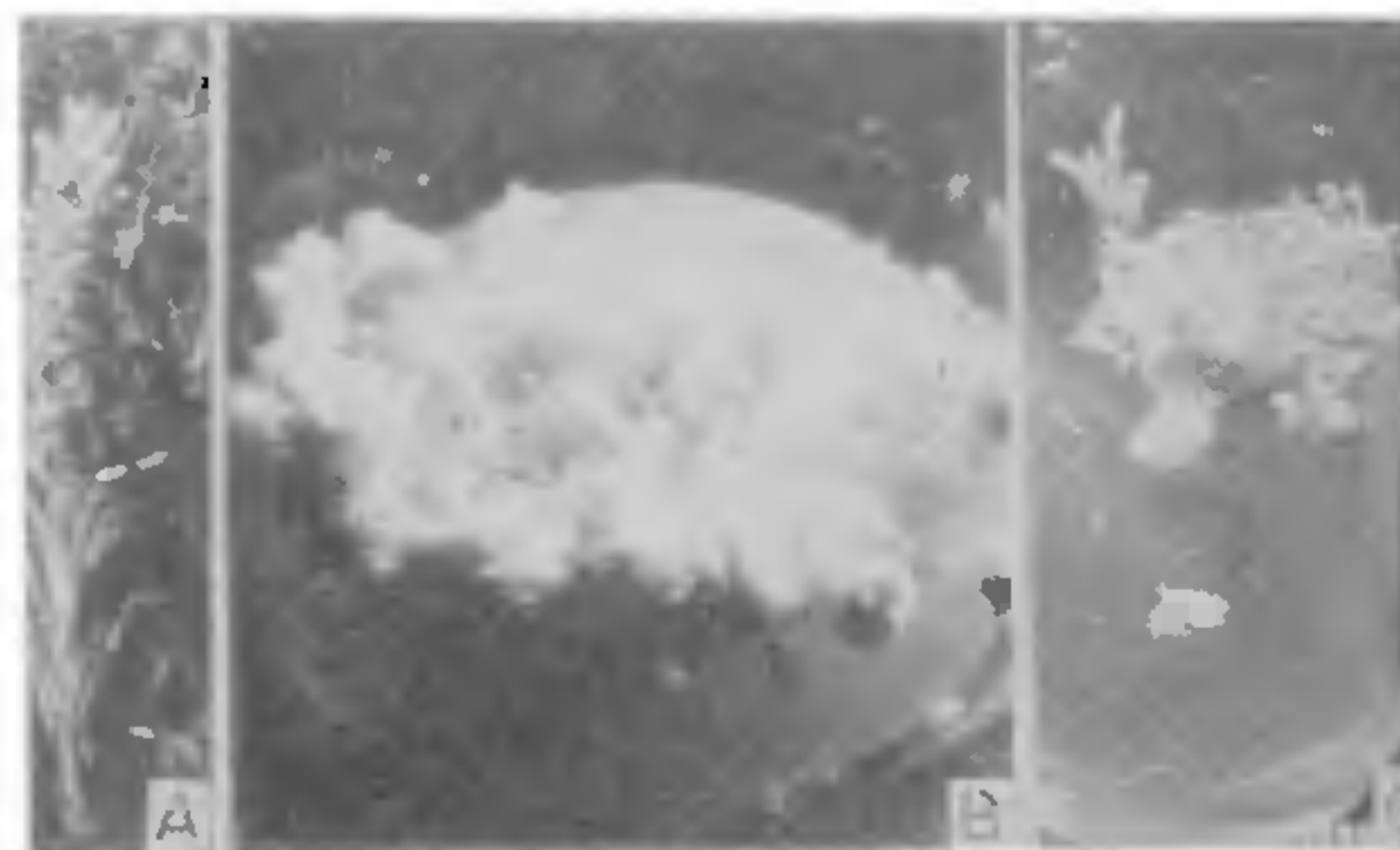
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### IN VITRO SEED GERMINATION OF ZEUXINE STRATEUMATICA SCHLR. (= Z. SULCATA LINDLEY), ORCHIDACEAE

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.

Seshagiriah<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, X 0.5. Fig. B. Protocorms in the culture flask, X 1.5. Fig. C. Branched shoots and protocorms in the culture tube, X 1.5.

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

Gangotri, Mysore) were surface sterilised using saturated chlorine water. They were opened under aseptic conditions and the seeds inoculated on to the experimental media (pH 5.2). Three kinds of media, viz., Murashige and Skoog's<sup>7</sup>, White's<sup>8</sup> and Knudson's<sup>9</sup> with IAA (1 ppm) + kinetin (1 ppm) + coconut milk (20% vol./vol), and without the supplements were used. The cultures were maintained under the laboratory conditions of light, temperature and humidity. The experiments were repeated twice with five replicates for each medium.

The seeds did not germinate during the first 50–60 days in any of the media. But later they did respond to the modified Knudson's medium<sup>9</sup> supplemented with growth substances, only after 90 days of sowing. The seeds remained as such on Murashige and Skoog's<sup>7</sup> or on White's<sup>8</sup> media, alone or with growth substances, although sometimes with a little shrinkage. As a first step in germination the seeds swelled considerably. Then gradually the cylindrical and cone-shaped protocorms appeared (Fig. B), as the seed coats bursted. Numerous absorbing hairs developed on the protocorms. The first scale leaf was visible as a bulge at the terminal part of the protocorms 120 days after sowing. This was followed by the organization of vegetative shoot apex and more scale leaves. The cylindrical stem then branched (Fig. C). when roots appeared at its base. It is obvious, therefore, that *in vitro* seedlings of *Z. strateumatica* could be raised. However, unlike those of most other orchids these seedlings remained cream coloured (non-green) throughout the experiment.

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## EPIDERMAL STRUCTURE AND DISTRIBUTION OF STOMATA IN *SAGITTARIA GUAYANENSIS* H.B. & K.

*Sagittaria guayanensis* H.B. & K. are aquatic, anchored floating herbs belonging to the family Alismataceae. Two distinct forms of this species are found: an adult with well developed vegetative one organs and a juvenile form. In the latter there are mostly present flattened, ribbon-like, submerged axes, comparable with the petioles of adult forms, which may end distally either with small floating leaves (juvenile leaves) or without them. Besides, in a recent collection trip we also observed adult plants growing in aerial condition in a rice field in which water had receded from the soil surface. Juvenile plants were not found growing under such conditions. Govindarajulu<sup>1</sup>, who has studied the systematic anatomy of this taxon, does not report stomata on the undersurface of either the adult or juvenile floating leaves. His report is in conformity with the usual condition in floating leaves where the stomata are restricted to the upper epidermis. However, this was found to be at variance with the studies of Shinobu on *Potamogeton fryeri* and *P. gramineus*, Gupta *et al.*<sup>2</sup> on *Nelumbo nucifera*, Kaul<sup>4</sup> on *Sparganium fluctuans* and *S. minimum*, and Paliwal on *Aponogeton natans* which have revealed that on the floating leaves of these taxa the stomata may even be found on the lower surface. The present study was, therefore, undertaken to confirm the report of absence of stomata from the lower epidermis of floating leaves and also the stomatal distribution along the two surfaces of the aerial leaves of *S. guayanensis*.

The material was collected from the village Kusiari in District Mainpuri, U.P. Leaves were fixed in FAA and subsequently stored in 70% alcohol. Epidermal strips from the upper as well as the lower surface of leaves were peeled away, stained with Delafield's hematoxylin and mounted in glycerine jelly.

*Adult floating plants*: These measure up to 34 cms in length. The plants growing along the periphery of the pond or a ditch (shallow water forms) and possess comparatively small leaves when compared with those growing in deep water (deep water forms). The average size of the lamina in the former is 1,937 mm<sup>2</sup> whereas in the latter it is 4,285 mm<sup>2</sup>.

The cells composing the upper epidermis in both deep and shallow water forms are smaller, isodiametric and possess highly undulated walls whereas those of the lower epidermis are larger with relatively less undulated walls (Figs. 1, 3, 4). However, the lower epidermal cells in deep water forms are larger than those of shallow water forms (Table I). On the lower epidermis there are present groups of cells which take a darker stain than the other epidermal cells (Fig. 2). These have been termed 'hydropoten'