to seven cells in length (Figs. 2 and 7) but as their course is followed further in cross-sections, the inner cortical cells which are just adjacent to the trabeculæ also develop intercellular spaces and thus an irregular mass of tissue is formed between the outer cortex and the stele of the stem (Fig. 9). In older stems of this species they are completely missing or when present are multicellular (Fig. 9). In case of *S. remotifolia* they are multicellular in young as well as in mature stems (Figs. 4 and 7). In *S. monospora* in one trabecular cell two degenerated nuclei have been observed without any trace of partition wall. It, therefore, indicates that unicellular condition in some species might have been attained by the dissolution of the partition walls between two trabecular cells (Fig. 8). In *S. sp* they sometimes give the appearance of a single cell connecting the cortex with one or two cells on one side and the stelar tissue on the other (Fig. 6). It must be pointed out here that whenever a multicellular trabecula has been observed in mature stems, the casparian strip has been found only in one cell which is adjacent to the pericycle (Figs. 6-9).

From the preceding account, it becomes clear that a trabecula may be unicellular or multicellular in young as well as in mature stems. However, if this turns out to be a usual condition (other species being investigated), we have to broaden our notion about the morphology of the trabeculæ. The usual concept that they are endodermal in nature would have to be modified, as true endodermis is rarely more than one layer thick; *Equisetum* and *Lygodium* root being only exception where it is two layers thick. Thus on the basis of the present work, it can be said that trabecula in case of *Selaginella* is partly endodermal, as the casparian strip develops only in one cell whenever a multicellular trabecula has been observed, and partly cortical in origin at least in those species where a multicelled condition is found in young as well as in mature stems. The multicelled condition of trabeculæ appears to be correlated with the nature of the air space system in the stem of *Selaginella*. If the air space system is large, a trabecula is made up of several cortical cells in addition to an endodermal cell.

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IN VITRO CULTURE OF *UTRICULARIA*

THE insectivorous plants have curiously modified leaves to capture the prey. Whether the trapping of the prey is a mere consequence of the structural modifications or the plants depend on the prey for a part of their nutrition is a fundamental question. What morphogenetic factors control the development of the trapping structures is also worthy of inquiry. Such problems can best be studied by cultivating the plants on defined nutritive media under controlled environmental conditions.

The first report of the aseptic culture of an insectivorous plant concerns *Drosera* (Burger, 1961). Pringsheim and Pringsheim (1962) raised sterile cultures of *Utricularia exoleta* and got them to flower after the addition of peptone and beef extract to the medium. Harder (1963) obtained flowering in this species by including into the medium peptone extract from meat or infusions of *Daphnia*. Another recent study is that of Withner (1964) on *Darlingtonia*, *Dionœa* and two species of *Sarracenia*. Harder (1964) was able to make cultured plants of *Drosera pygmœa* flower, fruit and complete their life-cycle without the addition of animal proteins into the medium.

The present work consists of preliminary observations on the germination of the seed and the development of the adult plant of *Utricularia gibba* Linn. sub sp. *exoleta* (R.Br.) Taylor.* The formative effects of some growth-regulating substances have also been studied.

The fruits of *Utricularia*, collected from Bangalore in July 1964, were surface-sterilized with chlorine water. The seeds were dissected out aseptically and planted on White's basal medium (WB) with 2% sucrose. For later studies the medium was supplemented with one of the following: naphthaleneacetic acid (NAA, 1 and 5 ppm), 2, 3, 5-triodobenzoic acid (TIBA, 1 and 5 ppm); gibberellic acid (GA, 5 and 10 ppm); coconut milk (10% v/v), 2, 4-dichloro-

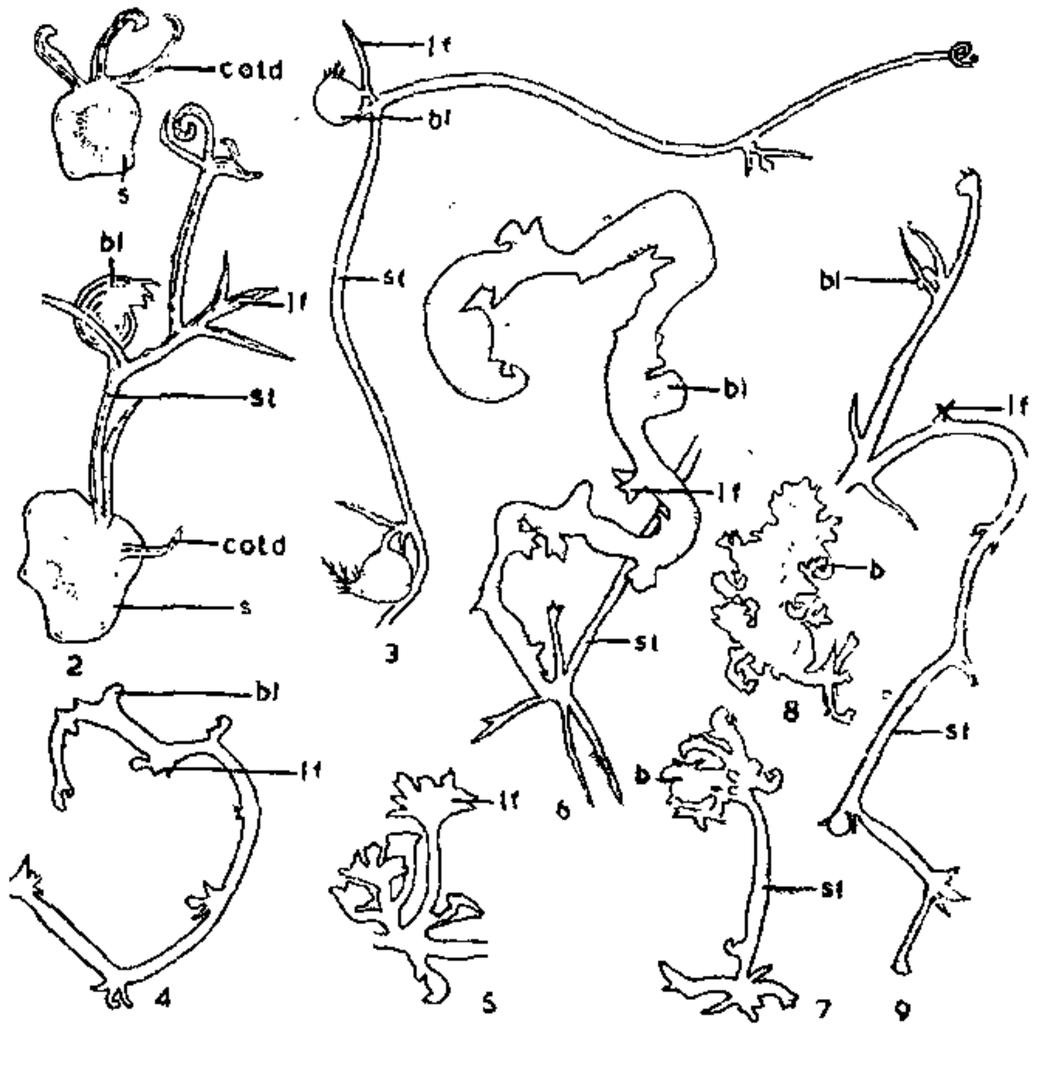
phenoxyacetic acid (2, 4-D, 5 ppm), indoleacetic acid (IAA, 1 ppm), kinetin (1 ppm), beef extract (400 ppm), tryptone (400 ppm) and casein hydrolysate (400 ppm). In a mature seed the embryo is totally unorganized; it has neither root and shoot primordia nor cotyledons. However, it has a growth pole from which the primary organs arise during germination.

Seventy per cent. of the seeds germinated on WB after 24 days. During germination three or four 'cotyledonoids' (Llyod, 1942) made their appearance at the growth pole. Of these one (sometimes more) developed into a stolon (Figs. 1, 2). The latter produced a series of alternately arranged dissected leaves. The first bladder made its appearance 8-10 days after germination. A semi-solid medium proved better for germination but the rate of growth of the seedlings was faster in liquid medium. The seedlings were transferred to a fresh liquid medium and later propagated by cuttings.

After the plants filled out the liquid medium, filamentous growth occurred at the surface or along the walls of the container (Fig. 3). Aerial branches grew above the surface and bore bladders which were free of hairs. Bits of the stolon were transferred to tubes containing various growth-regulating substances to study their morphogenetic effects. NAA was found to be toxic at 5 ppm. At a concentration of 1 ppm, the development of the bladders became arrested and the leaves remained stunted (Fig. 4). The anatomy of the stolon showed a marked reduction in the number of air chambers and the development of a few thick-walled cells in the central cylinder. On TIBA (1 and 5 ppm) the leaves and stems became fasciated (Fig. 5). GA (1 and 5 ppm) was markedly inhibitory to bladder development. The freshly grown parts showed elongated leaves which were free of bladders. The internodal length, however, remained unchanged. On 2, 4-D (5 ppm) the apex of the stolon became highly flattened and the bladders were inhibited. The addition of coconut milk to the 2, 4-D medium increased the effects of the latter (Fig. 6).

Kintein (1 ppm) stimulated a proliferation of the cells at the nodes and internodes and the production of buds at the nodes' (Figs. 7, 8). The addition of IAA (1 ppm) to kinetin had no particularly stimulative effect. IAA (1 ppm), when used alone, brought about a shortening of the internodes and leaves and the bladders did not develop to their full size (Fig. 9).

Tryptone and beef extract accelerated the rate of vegetative growth and bladder development. On WB alone, the bladders tended to abscise after a few days growth. Tryptone and beef extract favoured their retention. What role proteins have in abscission is not clearly understood.



FIGS. 1–9. Figs. 1–2. Germination of the seed on WB (agar). FIGS 3–9. Formative changes induced by growth-regulating substances. Fig. 1. Germinating seed with three 'cotyledonoids'. Fig. 2. Young seedling with leaves and a bladder. Fig. 3. Portion of a mature plant cultured in liquid medium (WB). Fig. 4. Effect of NAA (1 ppm) on the growth of the shoot. The leaves are stunted and the bladders are arrested in development. Fig. 5. Highly expanded fan-shaped leaves in WB+TIBA (5 ppm). Fig. 6. Portion of shoot showing abnormal modification on WB+2, 4-D (5 ppm) + CM (10% v/v). Figs. 7–8. Kinetin-induced effects. Note callusing of the nodes and emergence of buds. Fig. 9. Growth on WB+ IAA (1 ppm). The leaves are short and the bladders are poorly developed. (All figs., × 6.) (*b*, bud; *bl*, bladder; *cold*, cotyledonoid; *lf*, leaf; *s*, seed; *st*, stolon.)

The cultures have been maintained in an active state of growth by repeated subculture for the past eight months. In spite of the addition of animal proteins like tryptone, beef extract and casein hydrolysate to the medium and exposure of cultures to varying daylight conditions, flowering has not been induced so far.

Further work on the nutrition and morphogenesis in the cultures of *Utricularia* is in progress.

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• This plant has been referred to in literature as *Utricularia exoleta* R.Br. but has been changed to *U. gibba* Linn, sub sp. *exoleta* (R.Br.) Taylor (Taylor, 1963).

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THE RICE COLEOPTILE STRAIGHT GROWTH TEST FOR AUXIN BIOASSAY

BIOASSAY of auxin using oat coleoptile straight growth test introduced by Bonner[1] has since been worked out by several authors[2,3,4] under different conditions and using different oat varieties. A few investigations[3,5] have also shown that wheat coleoptile could successfully be employed for the biological assay of auxin. However, it is desirable to investigate the suitability of other plants for auxin bioassay not only from the standpoint of finding more sensitive material but also in view of their ready availability. The present work therefore, attempts to find out whether the locally readily accessible rice plant could be adopted for the coleoptile straight growth assay of auxin.

A cultivated variety of rice (*Oryza sativa*, L. var. MTU-20) was selected for the investigation. The seeds were washed under the tap followed by distilled water and were surface sterilized by a rapid rinsing in rectified spirit. They were then washed with glass distilled water and soaked in the same overnight in darkness. The soaked seeds were sown on wet filter paper in 6" petri dishes in a dark room maintained at 24° to 26° C. and growth was allowed for 60 hours. The average length of the coleoptiles by then was about 1·5 to 2·0 cm.

After 60 hours germination the coleoptiles were decapitated in diffuse light by discarding the 3·0 mm. portions from the tops. The next 4·0 mm. sections were used for the bioassay. The range of concentration of Indole-3-acetic acid (IAA) tried was from 10^{-9} M to 10^{-3} M made up in 2·0% unbuffered sucrose. Ten coleoptile segments each were floated on 2·0 ml solution of 2·0% unbuffered sucrose for control and 2·0 ml of IAA solutions in watchglasses and were incubated in darkness at 24°–26° C. After 18 hours incubation the length attained by the coleoptile segments was measured under a binocular microscope using mm. graph paper. The experiment was repeated 4 times. The result is shown in Fig. 1.

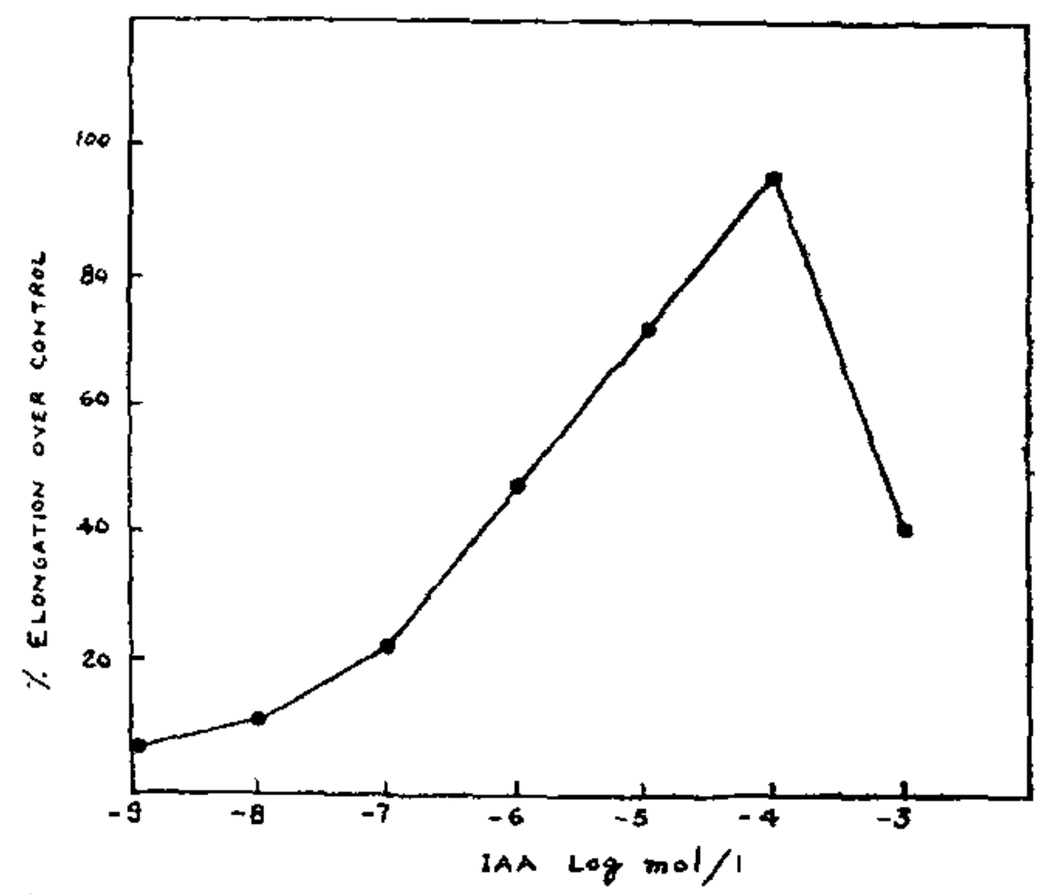

FIG. 1. Straight growth respons of rice coleoptile egments to IAA. Each point represents an average ength of 40 segmeots (4 experiments).

A linear response of rice coleoptile segments to IAA between 10^{-7} M and 10^{-4} M was observed. The total growth increment at 10^{-4} M was about 135% over original length of segments at the beginning of incubation. The control segments in sucrose solution showed only about 35% extension over their original length. Thus a net increment in growth of about 100% over control was observed at maximum.

The present results show that under the conditions tried, rice coleoptile response could well compare with that of oat coleoptile under optional conditions as reported by several workers.[2,3,4] Hence it is believed that rice plant could be an useful addition to the plants already in use for the coleoptile straight growth test for auxin.

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