ing potential of the callus depended on the age of callus in culture. A 120-day-old callus showed maximum ability for organogenesis in terms of number of shoot-buds/callus and the organogenetic potency gradually decreased thereafter. A 240-day-old callus showed only rhizogenesis with very little shoot-forming ability (figure 2).

Young shoot-buds developed into leafy shoots after about 30 days growth in the same medium and these were transferred to MS media devoid of hormones and containing half concentration of salts. On transferring to fresh media, rooting and complete regeneration of plantlets were observed after 120 days (figure 3). Plantlets (4-5 month old) showing bulb formation were transferred to potted soil (figure 4). Regenerated plants were observed to be morphologically similar to plants growing in the field.

Cytological study of the callus tissue showed only diploid cell populations after 30 days of growth. After 90 days growth, diploid cells were 85% and polyploid cells were 15%. In a 120-day-old callus about 78% diploid cells and 22% polyploid cells were found, and in a 240-day-old callus the diploid cells were 28% and the polyploid cells were 72%. The study of root-tips and shoot-tips of regenerated plants revealed only the diploid number ($2n=30$) in plants regenerated from calli of all ages.

Thus it was noted that 2,4-D was an essential factor in inducing callus formation in the medium containing coconut milk. The omission of 2,4-D and the presence of NAA in the medium influenced the origin of shoots and roots and this resembles another monocot, *Gladiolus* sp.[6]

The callus tissue derived from explants showed heterogeneous cell populations containing both diploid and polyploid cells (figure 5) but the plants regenerating from these calli, showed only diploid cells (figure 6).

In older cultures, where the percentage of polyploid cells is high, the number of plants that can be regenerated decreases. Such a behaviour has also been reported in other species[7,8]. Thus, the karyologic state of a callus culture affects the *in vitro* regeneration of plants.

Therefore, the present study involving *in vitro* culture and cytological analysis of tissues grown *in vitro* has demonstrated the scope of utilizing this technique for rapid propagation of genetically stable plants in *S. indica*. The instability in callus offers further scope of utilization of the different genotypes.

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LEAF BUD ORGANOGENESIS *IN VITRO* IN A FERN—*AMPELOPTERIS PROLIFERA* (RETZ.) COPEL.

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Of the associated organs in fern organography, the shoot buds have attracted attention more extensively than the root and leaf buds[1]. The latter and their role in vegetative propagation have been described in several ferns[2–4] but the *in vitro* studies dealing with control mechanisms are very few[5].

Ampelopteris prolifera forms thickets by proliferous buds borne on fronds of two distinct growth patterns viz determinate and indeterminate (= runner fronds). The bud organogenesis *in vivo*[6,7] is depicted in figure 1. Our present study is aimed at assessing the organogenetic potentials of buds in response to interacting effects of sucrose (S), a combination of indole-3-acetic acid (IAA) and kinetin (KN) and the age of buds.

Bud-bearing rachis segments were cultured on modified Knudson's 'C' basal medium (= BM) under 12 hr duration in white light (1000 lux). Three replicates of each of the following culture media were tested. (i) On sucrose-free BM, the buds were activated 4 days after inoculation (ii) On 4% sucrose-containing BM, the response was delayed by about 48 hr. The delayed response was followed by differentiation of fronds with diminutive pinnae and markedly reduced extension growth of petioles.

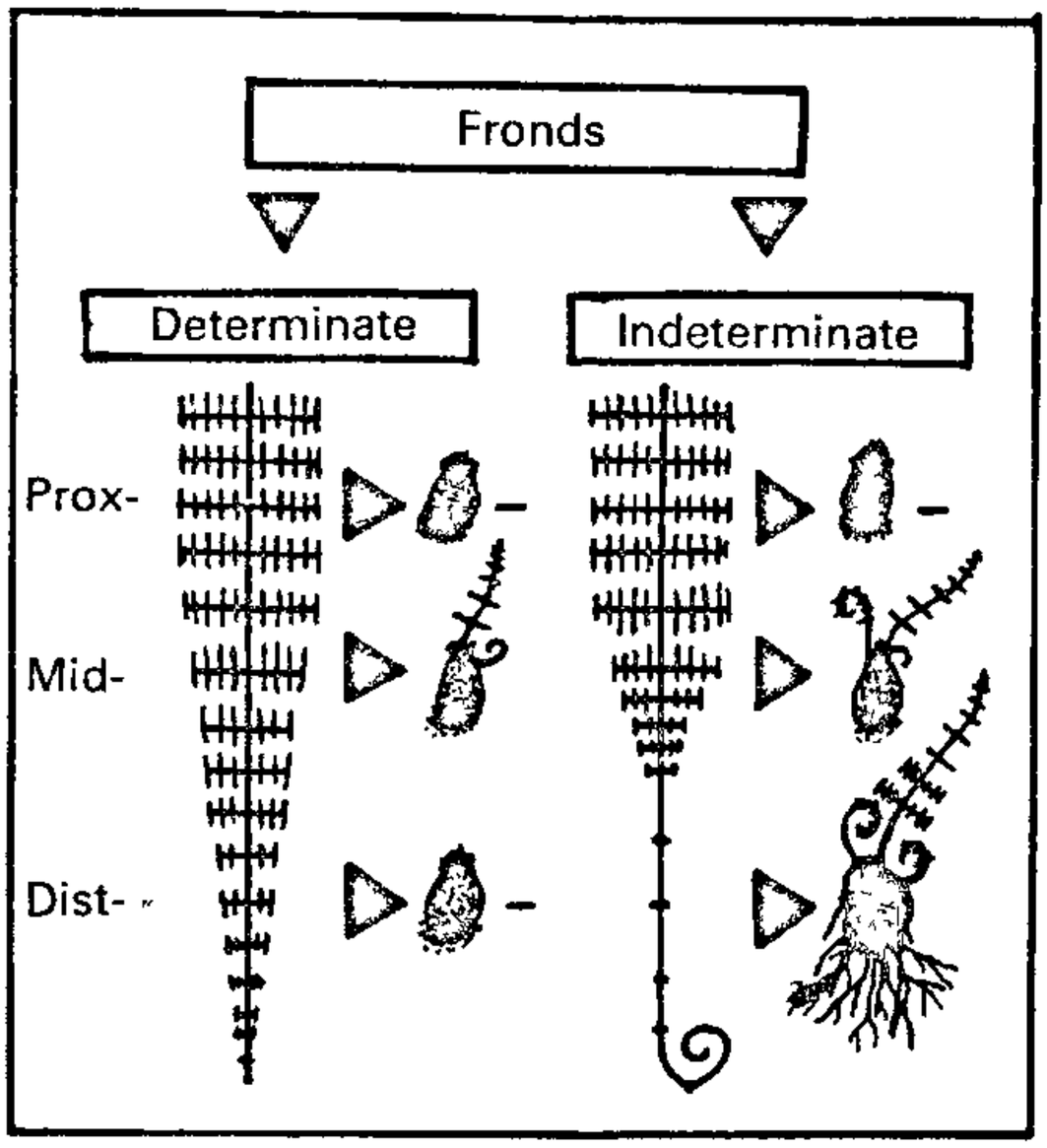

Figure 1. Schematic representation of bud organogenesis in proximal, middle and distal part of rachis *in vivo*. (–) denotes arrested buds.

The leaf-trace was unitary (i.e. a composite of 2 xylem masses) on (a) medium in contrast to 2-stranded, normal trace at a comparable level on (b) medium. Also, the ground parenchyma cells in the latter case were spherical with ample starch accumulation. (c) Buds regenerated on BM + 2% S + 1.5% IAA + 0.2% KN differentiated fronds only and the formation of pinnae on one side of the rachis remained suppressed; while the shoot remained inhibited, roots were differentiated in large numbers in 50-day-old cultures. (d) Buds turned green on BM + 2% S + 1.5% IAA + 1% KN but further regeneration was inhibited as seen in 50-day-old cultures. Perhaps the higher kinetin:auxin ratio, compared to other media, proved inhibitory. (e) On cultures containing BM + 2% S + 2% IAA + 0.2% KN, the buds differentiated fronds as well as roots comparable to those *in vivo*. (f) In 35-day-old explants on BM + 2% S + 2% IAA + 1% KN, the shoot formation was noted in addition to fronds comparable to those of the preceding culture. It appears that the aforesaid auxin:kinetin ratio was optimal for the differentiation of plantlet.

If relatively younger buds were cultured on (e) and (f) media cylindrical appendages were differentiated during the period of experiment.

In conclusion it can be stated that the normal bud organogenesis is a consequence of subtle interactions of carbohydrate metabolism and appropriate ratios of growth regulators. *In vivo* such interactions emanate from the frond, and like the shoot apex, one can regard the frond apex of this fern as capable of morphogenetic control on the subtended buds (cf. figure 1). Secondly, in sharp contrast to calluses which are prone to chromosomal as well as cytoplasmic upsets, the buds in different spatial position on the rachis, can be used as genotypically stable systems (barring those rare cases of spontaneous mutations) for performing more critical experiments to uncover the integrational aspect of fern organ categories.

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POLYEMBRYONY IN *MELOTHRIA MADERASPATANA* (L.) COGN.

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THE embryo in Angiosperms, at times, is produced from other constituents of the embryo sac, besides egg, with or without fertilization. The literature on the subject of polyembryony in Angiosperms has been reviewed by Lakshmanan and Ambegaokar[1]. During a study of the embryology of *Melothria maderaspatana* (Cucurbitaceae) an interesting case