TABLE I

Developmental stage of plant	Kundson's basal medium ± % sucrose	Time in weeks after inoculation of spores	Total phenolics mg %
Gametophytes (vegetative)	0·0 sucrose	4	0·82
Gametophytes (reproductive)	0·0 sucrose	8	0·92
Gametophytes (reproductive)	+2% sucrose	8	1·20
Sporophytes (sexually produced)	+2% sucrose	12	1·52
Sporophytes (apogamously produced)	+4% sucrose	12	1·70
Sporophytes (sexually produced)	+2% sucrose	16	3·3
Sporophytes (sexually produced)	+2% sucrose	20	3·8
In vivo sporophytes (fruiting)	—	—	1·72

produced sporophyte, as compared to that in the sexually raised one could be due to higher sucrose level in the medium used. Increasing carbohydrate levels are found to enhance accumulation of polyphenols in the tissue cultures derived from the higher plants (Shah *et al.*[7]). The presence of phenolics in appreciable amounts in gametophytes and sporophytes of the fern suggests that they too have capacity to synthesize them and that the well-established pathways of phenolic metabolism in angiosperm and gymnosperm also function in ferns.

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IN VITRO STUDIES ON GAMETOPHYTES OF *PTERIS VITTATA* L.

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IN the present investigation, the influence of mineral nutrition on the induction of apogamy was investigated. Apogamy was induced in *Pteris vittata* by culturing the gametophytes on standard dose of macroelements of Knudson's medium with 4% sucrose. Decreasing the concentration of mineral salts from the culture medium prevented apogamous response. Moreover, the differentiation of the gametophytic callus was conditioned by sucrose concentration in the culture medium.

Introduction

Apogamy was experimentally induced in sexually reproducing ferns such as *Pteridium aquilinum* (Whittier[10]); *Ampelopteris prolifera* (Mehra and Sulklyan[5]) and *Acrostichum aureum* (Kshirsagar and Mehta[1]). Manton[3] suggested that the ease with which apogamy can be induced in ferns might be due to their higher chromosome number coupled with genetic imbalance brought about by hybridization. The apogamous structures produced were mostly leaves or complete sporelings. Moreover, reduction in mineral nutrition of the prothalli of *Osmunda cinnamomea* (Whittier and Steeves[11]) and *Pteridium aquilinum* (Treanor and Whittier[9]) prevented apogamous sporophyte formation.

In the present studies an attempt was made to determine the role of mineral nutrition in the induction of apogamy in *Pteris vittata* prothalli. The capacity of the gametophytic callus for differentiation was examined.

Materials and Methods

The spores of *Pteris vittata* L. were sterilized with 5% sodium hypochlorite and inoculated on Knudson's

basal medium, as modified by Steeves *et al.*[6]. The cultures were incubated at 25 ± 2° C in continuous light in a culture room.

Results

I. *Induction of apogamy*

Four week old prothalli grown on Knudson's basal medium were separately inoculated on Knudson's medium containing 1%, 2% and 4% sucrose respectively. After 4 weeks incubation, prothalli grown on 4% sucrose medium became quite thick at the apical notch and profuse hair developed over it. The leaf was the first organ to be formed from this thickened meristematic region. On further incubation for 4 weeks, after subculturing on the same medium, apogamous sporelings were developed. This apogamous embryo was derived from a group of meristematic cells just behind the sinus of the prothallus. By this time, prothalli grown on 2% sucrose showed the apogamous response in the same pattern. But the prothalli grown on 1% sucrose remained quite thin without apogamous response. In fact, a correlation was apparent between the decreasing sucrose concentrations and time taken for apogamy to set in. Thus 4% sucrose proved to be optimal concentration of sucorse for the induction of apogamy.

II. *Mineral Nutrition*

In this experiment, the effect of different levels of macroelement salts in Knudson's medium was tested. The concentrations of the macroelements tested were adjusted to 1/10, 1/2, 1 and 2 times the concentrations of macroelements present in the Knudson's medium. The ionic concentrations of the media were adjusted by proper addition of sodium or chloride ions. The level of sucrose was raised to 4% as it had been found to be optimal for apogamy from the previous experiment. Young gametophytic colonies of about equal sizes were inoculated separately on the above mentioned culture media.

After 8 weeks incubation in light, apogamous shoots were produced from the prothalli. The gametophytes with their apogamous shoots were weighed and the number of observable shoots noted down. Out of the four treatments tested, the medium with standard concentration of macroelements with 4% sucrose showed mximum number of apogamous shoots and highest fresh weight (Fig. 1). At the lower doses of macroelements there was no apogamous shoot formation. Doubling the level of macroelement salts also did not invoke the apogamous response nor any increase in fresh weight. Clearly, 4% sucrose with standard dose of macroelement was found to be optimal concentration for induction of apogamy in this species.

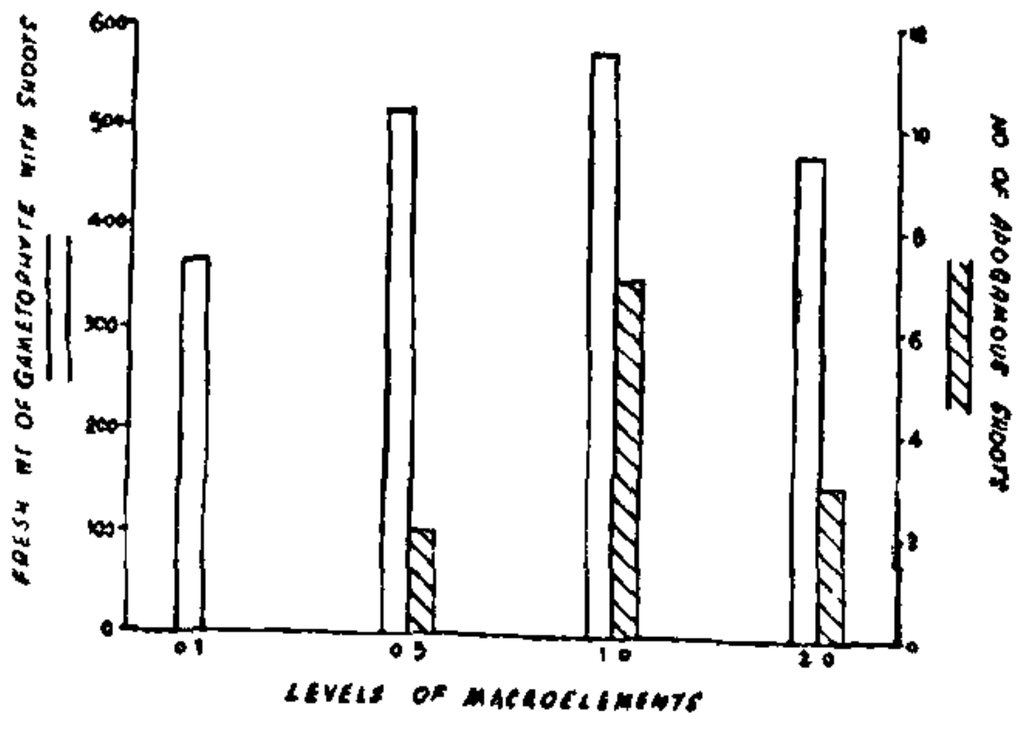

FIG. 1. Effect of mineral nutrients on apogamy on gametophytes of *Pteris vittata* L. The concentrations of macroelements adjusted to 1/10th, 1/2, 1 and 2 times the concentrations as present in Knudson's basal medium containing 4% sucrose. Equal sized gametophyte colony inoculated. Incubation : 8 weeks in continuous light at 25 ± 2° C.

III. *Initiation of callus from gametophytes*

Aseptically grown 6 week old prothalli were inoculated on Knudson's medium containing 2% sucrose, 2 mg/l 2, 4-D and 10% coconut milk. This medium was found to be suitable for callus initiation from gametophytes (Padhya and Mehta[4]). Within 4 weeks callus initiation occurred from the apical meristematic region of the prothallus (Fig. 2). The callus when subcultured on the medium of the same composition, grew profusely into a green friable mass.

IV. *Differentiation of callus*

The callus was grown on sucrose-auxin free medium for a week, to minimise any carry over effects. Pieces of callus tissues were inoculated on Knudson's medium containing 0, 0·5, 1, 2 and 4% sucrose respectively. After 4 weeks incubation in continuous light, callus on 4% sucrose showed formation of many apogamous shoots (Fig. 3). Callus grown on 2% sucrose showed few shoots produced while callus grown on 1% sucrose showed very few shoots with roots formed (Fig. 4). The number of apogamous shoots produced were more on higher (4%) sucrose medium than on lower sucrose containing media (2% and 1%). On still further incubation for 4 weeks, callus grown on 4% sucrose produced roots and complete plantlet formation occurred. Similarly callus from 2% sucrose showed root formation. Callus from 1% sucrose showed more of root formation. Callus on 0·5% sucrose showed development of intermediate forms between sporophyte and gametophytes (Fig. 5). These intermediate structures were flat, thick, cushion-like with multicellular scales at the margin. Cells of

FIGS. 2–5. Fig. 2. Gametophytes of *Pteris vittata* grown on Knudson's medium containing 2% sucrose 2 mg/l 2, 4–D and 10% coconut milk, showing callus initiation. Incubation : 4 weeks in light at 25 ± 2° C. Fig. 3. Formation of many well developed apogamous shoots from callus grown on Knudsons' medium with 4% sucrose. Incubation: 4 weeks in light at 25 ± 2° C Fig. 4. Formation of few apogamous shoots and then roots from callus grown on Knudson's medium containing 1% sucrose. Incubation : 4 weeks in light at 25 ± 2° C. Fig. 5. Intermediate structures developed from callus grown on Knudson's medium containing 0·5 % sucrose. Incubation : 4 weeks in light at 25 ± 2° C.

these structures were found to be rich in starch when squashed and stained with iodine. Regeneration of gametophytes occurred from callus grown on sucrose-free medium after 8 weeks incubation.

Discussion

In the present studies with *Pteris vittata* gametophytes, vigorous growth occurred in the presence of standard dose of macroelements as present in the Knudson's medium along with 4% sucrose. Similar results were reported by Schwabe[5] who showed that mineral deficiencies reduced the growth of the prothalli and apogamy was not promoted by elimination or decrease of these macroelements from the culture medium. In *Pteris vittata* the apogamous structures formed were leaves which ultimately produced sporelings while Sulklyan and Mehra[7] reported in *Nephrolepis cordifolia* only apogamous roots formation. They thought this might be due to high level of endogenous native auxin (IAA) present in this species.

Gametophytic callus differentiated into sporophytic structures in the presence of sucrose. This might be due to the complex sporophytic forms required more of energy source for their formation. Thorpe[8] reported that for shoot formation and its further development from tobacco callus a continuous supply of carbohydrates was essential. In ferns such as *Pteris vittata* autotrophic gametophytic forms can be produced from the gametophytic callus in the absence of sucrose.

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CHROMOSOME NUMBER OF THE TREE FERN—*CYATHEA GIGANTEA*

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Cyathea gigantea (Wall. ex Hook.) Holtt. (= *Alsophila gigantea* Wall. ex Hook, *Alsophila glabra* sensu Bedd.) is one of the very few tree ferns, indigenous to South India. It is usually found on banks of streams in the mountains (1000–1500 m).

Sporangial materials for the present study were collected from plants growing in the reserve forest at Bonnacade in Trivandrum District. The sporangia were fixed in acetic alcohol (1 : 3) and smeared in 1% acetocarmine. The spore mother cells showed 69 bivalents at first metaphse of meiosis (Fig. 1). This is the first report of chromosome number in the species from South India. Plants of this species from Ceylon are also known to have $n = 69$ bivalents in spore mother cells[1].

Chromosome numbers in 10 species of *Cyathea* are so far known. A cytogeographic analysis of the data shows that the same gametic chromosome number $n = 69$ occurs in specimens of both *C. lutebrosa*[2–4] and *C. gigantea*[1] from different geographic regions. The remaining eight species also show $n = 69$ bivalents[5–9]. The absence of any variation in chromosome number