S U M M A R Y

SUMMARY

The thesis incorporates results of investigation aimed at:-

(i) the initiation of callus from the prothallus and different parts of the sporophyte,

(ii) to gain information concerning the nutrition and growth in continuous culture of the sporophytic and gametophytic callus tissues, and

(iii) to have some insight into the physiology of regeneration by regulating the type of differentiation of the callus cultures derived from sporophytic and gametophytic life forms of the fern life cycle.

The thesis is divided into eight chapters. Chapter I gives historical background and introduces the various problems tackled. It further emphasizes the advantages of tissue-culture research method as an experimental tool in morphogenetic studies such as are undertaken in the present investigation. The methods employed are described in Chapter II. The work is mainly done with three fern species which belong to different families:-

(i) Pteris vittata L.
(Family: Pteridaceae)

(ii) Adiantum trapeziforme L.
(Family: Adiantaceae) and

(iii) Thelypteris augescens (Link)
Munz et Johnsdon
(Family: Thelypteridaceae).

Chapter III embodies in vitro studies on the initiation, growth and differentiation of rhizome tissues of Pteris vittata L. White's medium containing 2% sucrose and supplemented with 2.0 mg/l 2,4-D and 10% coconut milk was found most suitable for callus development on rhizome explants. Callus induction on Knudson's medium which lacked vitamins demonstrated simple nutrient requirements of sporophytic tissues in culture.

The rhizome segments as well as rhizome callus regenerated into sporophytes or gametophytes depending on the presence or absence of sucrose in the medium. In both the cases, the regenerative outgrowths ranged from cylindrical structures to complete sporophytes with increasing sucrose level in the medium. Root formation occurred when high sucrose (4%) medium was supplemented with low (0.2 mg/l) 2,4-D.

Aposporous gametophytes were observed on rhizome segments and rhizome callus after very prolonged period of incubation in light. By this time the parent tissues had ceased growth and almost senesced.

Callus subjected to sucrose-NAA combinations produced leaves or roots depending upon the NAA concentration. In presence of low (0.1 mg/l) conc. of NAA leaves were first formed and roots much later; while only roots were differentiated in presence of high (1.0 mg/l) NAA level.

Callus on transfer to agitated liquid medium produced a suspension of fine cells and small groups of cells. When plated on semi solid agar media, the less than 300 μ in diameter suspension produced colonies, which on transfer to auxin free medium showed organisation of shoot-apices.

Studies on root and leaf cultures of Pteris vittata L. are described in Chapter IV. White's liquid medium containing coconut milk supported rapid growth of excised roots in culture. Incorporation of IAA enhanced lateral formation. Presence of minute traces of kinetin in the medium promoted the development of aposporous gametophytes on the root segments.

Callus was induced on the excised roots when the medium contained 2,4-D. The root callus also possessed the capacity to form (sporophytic) roots of (gametophytic) prothalli in presence and absence of sucrose respectively.

Leaflets of garden plants also produced callus; but their sucrose and auxin requirements were much higher than those of sterile, apogamously sporophytes. In fact, the callus developed from the latter grew more vigorously. Like the rhizome and root callus, the leaf callus also exhibited the potentiality for differentiation. In presence of sucrose, sporophytic structures developed, while in absence of sucrose, gametophytes were regenerated.

Chapter V includes studies on the gametophyte and callus derived from prothalli of Pteris vittata L. only well developed, cordate gametophytes responded to sucrose-2,4-D treatment to produce callus. The callus on transfer to media, devoid of auxin, but containing sucrose at increasing levels, developed apogamous sporophytes. The number of sporophytes induced and the time taken for their appearance directly varied with sucrose level. On low sucrose medium, roots were formed first, while on sucrose-free basal medium prothalli were formed. With lapse of

time as sucrose became depleted from the medium, prothalli were produced in other treatments as well.

Cell suspensions obtained by gentle maceration of the prothalli when aseptically spreaded on sloppy agar regenerated into entire prothalli. The capacity for such regeneration was more pronounced in suspension of prothalli compared to that exhibited by gametophytic callus.

Examination of inorganic nutrition of gametophytes and development of apogamous sporophytes revealed that nutrition which supported vigorous growth of the prothalli was most conducive to appogamous shoot formation.

Attempts to induce fruiting of the in vitro produced sporophytic plants by supplying increasing level of sucrose or by incorporating yeast extract in the medium or by 'starving' cultures for over a year were unsuccessful. When such sterile plants raised in culture, either sexually or apogamously, were screened for antibacterial substances, the results were negative.

Studies on the gametophyte and gametophytic callus of Adiantum trapeziforme are dealt with in Chapter VI. After examining spore and its germination, development of the prothallus is followed. Profuse regeneration of

gametophytes from young prothalli led to development of rosette-shaped prothallial colonies. Apogamous shoots were produced in high sucrose medium when the prothalli were transferred to a range of sucrose containing media.

Free prothallial cells obtained by gentle maceration by hand displayed the potentiality to regenerate into gametophytes.

The callus induced from adult prothalli proved promising for morphogenetic studies. As in preceding cases, it differentiated into sporophytic or gametophytic forms depending upon the presence or absence of sucrose in the medium.

Chapter VII incorporates examination of spore germination and development of prothallus in Thelypteris augescens (Link) Munz et Johnsdon. Callus was induced on leaflets of garden grown plants in presence of sucrose, coconut milk and 2,4-D.

All the foregoing results obtained are discussed in light of available information in Chapter VIII. The significance of apospory and apogamy and the phenomenon of alternation of generations are elaborated in view of experimental evidences gathered. The cultural manipulations

made to regulate the regeneration of sporophytic or gametophytic forms clearly suggested that the strict barrier between the sporophyte and gametophyte generations could be made to break by a simple substance like sugar. Besides, of course, providing respiratory substrate, the exact role of sugars in trigerring the differentiation of different life forms still remains unknown.