CHAPTER IV

STUDIES ON ROOT AND LEAF CULTURES OF *PTERIS VITTATA* L.

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Culture of fern leaves, rhizome segments and prothalli have been extensively studied. It appears, however, that using this technique excised root cultures have not been thoroughly followed. Moreover, the roots of ferns, as in most other groups of plants, remained a relatively neglected organ. With this view, initial experiments were designed towards definition of growth conditions and media which support the active growth of roots. In fact, culture of isolated organs was quite interesting as it offered, new approach to study and understand the ability of each organ to exhibit its potential for the phenomenon of alternation of generations. Callus induction on the root-apices with proper sucrose-auxin combination was attained and the capability for differentiation of root callus was examined.

The leaf callus tissues were initiated from: (1) leaflets obtained from garden plants, (2) leaflets of sexually produced sterile plants and also (3) leaflets of apogamously produced plants. Morphogenetic potential of the leaf callus was also examined.

Experiment No. 4-1: Establishment of excised root culture

Sporelings resulting after the flooding of mature prothalli in Knudson's medium with 2% sucrose were allowed to grow for a period of 4 weeks until many roots were produced. Actively growing roots with their tips intact were excised and about one centimeter root explants were inoculated in 30 ml liquid White's medium (1954) containing 1% sucrose and supplemented with 10% CM and 300 mg/l CH.

During incubation period for 4 weeks at 26±2°C in continuous light, the roots produced several laterals (Fig.38). Clones of actively growing roots were maintained by subculturing healthy laterals every 4 weeks.

Experiment No. 4-2: Effect of Indoleacetic acid (IAA) on growth of excised roots of Pteris vittata L.

30 ml of Knudson's liquid medium containing 1 ml Nitsch's trace elements, and 1% sucrose supplemented with 0.1, 1.0 or 2.0 mg/l IAA concentrations, were inoculated with 1 cm root explants from sexually produced sterile sporophytes. After incubation for 4 weeks at 26±2°C in

Fig. 38. Growth of root explants of *Pteris vittata* in White's medium containing 10% CM, and 1% sucrose

Incubation: 4 weeks in light at 26±2°C

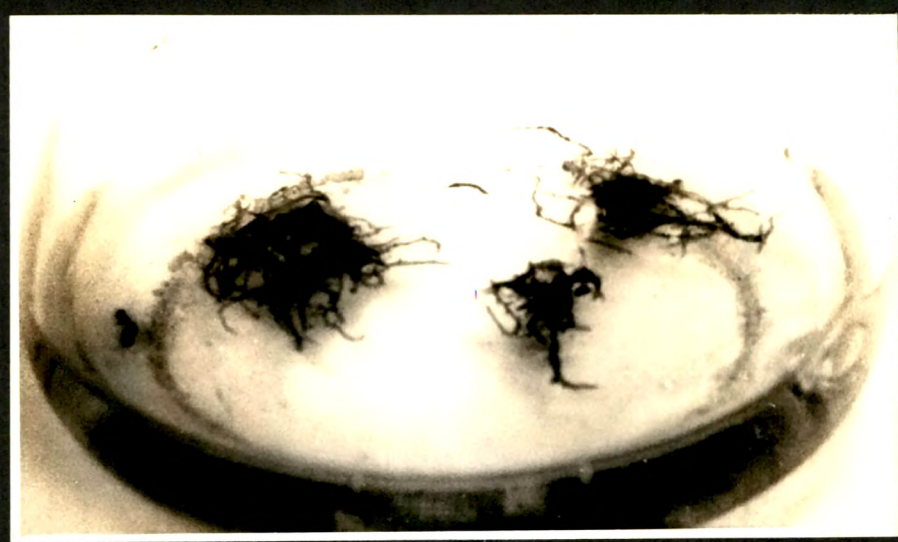

Fig.38

continuous light, it was noticed that the growth of root tips was quite slow in medium which lacked IAA. Development of numerous lateral roots from the swollen part of explants was observed, however, in medium containing 0.1 mg/l IAA. Inhibition of apical meristem and profuse development of laterals resulted in spider-like appearance (Figs. 39, 40). In media containing 1.0 mg/l and 2.0 mg/l IAA, though there was slight swelling, the explants turned necrotic on further incubation (Fig. 41).

Experiment No. 4-3: Effect of kinetin on induction of apospory

150 ml Erlenmeyer flasks containing 30 ml of solidified Knudson's basal medium (control), and that supplemented with different concentrations of kinetin - 0.02 mg/l, 0.2 mg/l and 2.0 mg/l - were inoculated with excised roots from clonal stock cultures. The cultures were incubated at 25±1°C in continuous light (5000 Lux) in environmental chamber for 12 weeks.

None of the media tested supported growth of the excised roots. At the end of incubation for 8 weeks in the medium supplemented with 0.02 mg/l kinetin, many root

Fig. 39. Development of lateral roots from the root explant grown in Knudson's medium containing 1% sucrose and 0.1 mg/l IAA

Incubation: 4 weeks in light at 26±2°C

Fig. 40. Development of profuse lateral roots from root explant in Knudson's medium containing 1% sucrose and 0.1 mg/l IAA

Incubation: 8 weeks in light at 26±2°C

Fig. 41. Formation of nacrosis and swellings on root explant grown in Knudson's medium containing 1% sucrose and 2.0 mg/l IAA

Incubation: 4 weeks in light at 26±2°C

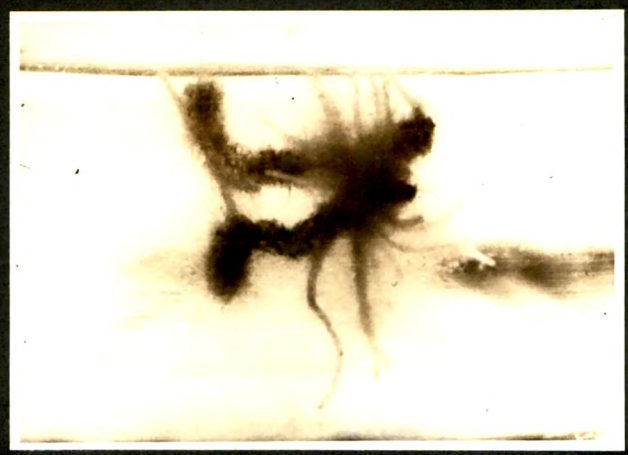

Fig.39

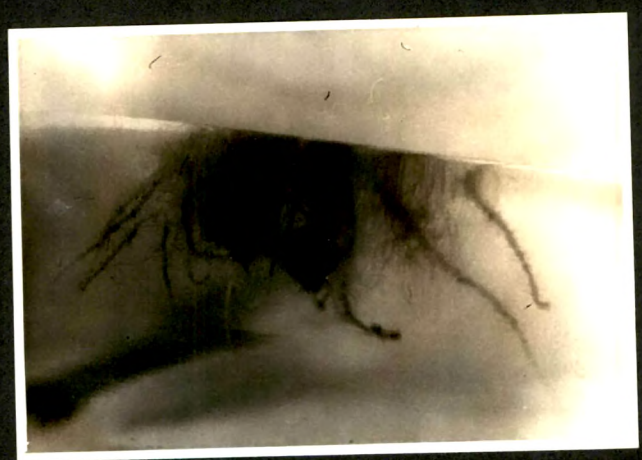

Fig.40

Fig.41

cells turned green and (aposporous) gametophytes developed from them (Fig. 42). Few aposporous gametophytes were formed on basal medium (control) also after prolonged incubation period of 16 weeks (Fig. 43).

Experiment No. 4-4: Callus initiation on excised roots

150 ml Erlenmeyer flasks containing 30 ml Knudson's medium with 2% sucrose, 10% CM and supplemented with different concentrations (0.1, 1.0 or 2.0 mg/l) of 2,4-D and solidified with 0.6% Difco-Bacto agar were inoculated with 1 cm long root explants.

At the end of incubation for 4 weeks in culture room at 26±2°C in continuous light, there was no callus formation in the control as well as on explants grown in media containing 0.1 and 1.0 mg/l 2,4-D. Callus development - beginning at the root-tips - was, however, rapid on explants grown in the medium containing 2 mg/l 2,4-D (Fig. 44). Those explants which were once subcultured in White's medium supplemented with 0.1 mg/l NAA also produced callus when transferred to Knudson's medium containing 2 mg/l 2,4-D. However, the root-tips which had undergone several subcultures in Knudson's medium containing 0.1 mg/l IAA failed to initiate callus when transferred to Knudson's 2,4-D containing medium. Rapid and vigorous growth of the callus was maintained on

Fig. 42. Development of gametophytes (G) on roots grown on Knudson's medium containing 0.02 mg/l kinetin (no sucrose)

Incubation: 8 weeks in light (5000 Lux) in the environmental chamber at 25±1°C

Fig. 43. Development of gametophyte (G) on root explant grown on Knudson's medium (no sucrose)

Incubation: 16 weeks in light (5000 Lux) in the environmental chamber at 25±1°C

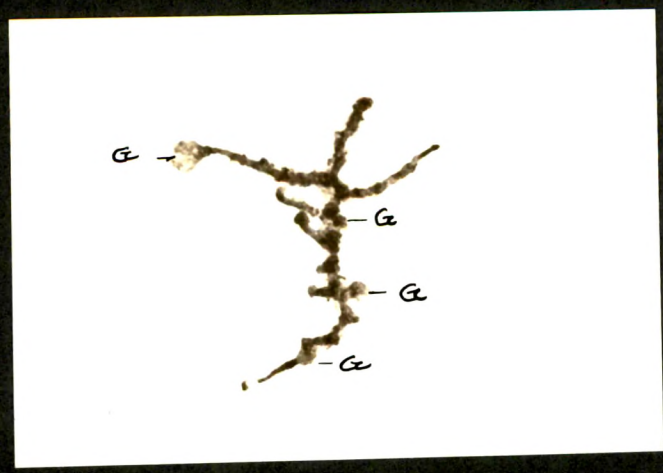

Fig.42

Fig.43

Fig. 44. Initiation on callus on root explant grown on Knudson's medium containing 10% CM, 2% sucrose and 2.0 mg/l 2,4-D

Incubation: 2 weeks in light at 26±2°C

Fig. 45. Vigorous growth of callus on Knudson's medium containing 10% CM, 2% sucrose and 2.0 mg/l 2,4-D

Incubation: 8 weeks in light at 26±2°C

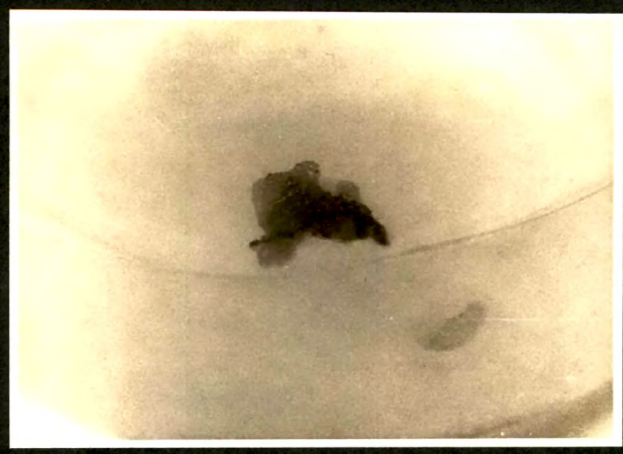

Fig.44

Fig.45

subsequent transfers to the same medium (Fig. 45). The callus was yellowish in colour and friable in texture. Though much late, callus formation occurred also on explants incubated in media containing 1 mg/l 2,4-D.

Experiment No. 4-5: Differentiation of root callus

Experiment was next set up to examine morphogenetic responses of root callus to sucrose levels in the medium. Callus pieces, about a centimeter in diameter, were transferred to Erlenmeyer flaks containing 30 ml of Knudson's medium (control) and supplemented with 1, 2, or 4% sucrose. The culture flasks were incubated at 25±1°C in continuous light (5000 Lux) and also in dark in the environmental chamber.

Callus grown in medium containing 4% sucrose developed hairs after 3 weeks (Fig. 46). On further incubation, the callus turned brown to black and produced roots (Fig. 47). On 2% sucrose the callus took much longer (8 weeks) to turn dark and develop hairs. Roots were produced still later (after 12 weeks). On still lower sucrose level (1% sucrose) change in colour of callus and hair formation were observed after 12 to 14 weeks. On the other hand, callus grown in light on medium without sucrose (control) showed formation of a few gametophytes

Fig. 46. Hair formation on callus grown on Knudson's medium containing 4% sucrose (no auxin)

Incubation: 4 weeks in light in the environmental chamber (5000 Lux) at 25±1°C

Fig. 47. Development of root (R) on callus grown on Knudson's medium containing 4% sucrose (no auxin)

Incubation: 8 weeks in light in the environmental chamber (5000 Lux) at 25±1°C

Fig.46

Fig.47

along its margin after 10 weeks. The control grown in dark did not show any growth nor morphogenetic response.

Experiment No. 4-6: Initiation of callus on leaves of Pteris vittata L.

In this experiment the leaflets derived from (1) garden plant, (2) from sexually produced sporophyte, (3) from apogamously produced sporophytes, and (4) leaves regenerated on rhizome segments were used as inocula. Leaflets cut from sterile plants were transferred to culture media solidified with agar (0.8%); while the leaflets obtained from garden plants were sterilized as mentioned in Chapter II, Materials and Methods, 3,B before inoculation.

The sucrose and 2,4-D concentrations and their combinations incorporated in White's medium containing 10% CM and 300 mg/l CH are shown in the chart below:

		Sucrose (%)		
		0.0	0.5	1.0
2,4-D (mg/l)	0.0			
	0.1			
	1.0			

After incubation for 8 weeks in culture room it was observed that in media containing 0.5% and 1% sucrose with 1.0 mg/l 2,4-D, callus was formed on leaflets from (1) sexually produced plants, (2) apogamously produced plants (Fig. 48), and (3) from leaves regenerated on rhizome segments; but not from leaves of garden plant. Induction of callus was more pronounced in case of younger leaflets than in older ones. The leaflets from garden plants showed, however, initiation of callus when the medium containing higher level of sucrose and 2,4-D (2% sucrose and 2 mg/l 2,4-D) was tried (Fig. 49). Compared to other leaflets, callus formation was most rapid on leaflets obtained from apogamously produced sporophytes. Here too, like rhizome segments, no callus initiation occurred in absence of sugar and auxin. Low concentrations of sugar and auxin, alone and in combination, were also equally ineffective.

Experiment No. 4-7 : Differentiation of Leaf Callus

Callus obtained from leaflets of garden grown Pteris vittata L. plant was used for morphogenetic studies. Callus pieces, about 1.0 cm in diameter, were inoculated in culture vessels containing 60 ml media. The media tested were A: White's basal medium (i.e. no sucrose, no CM, no

Fig. 48. Initiation of callus on apogamously produced leaves grown on White's medium containing 10% CM, 1% sucrose and 1.0 mg/l 2,4-D

Incubation: 4 weeks in light at 26±2°C

Fig. 49. Initiation of callus on leaflets of garden grown plant grown on White's medium containing 10% CM, 2% sucrose and 2.0 mg/l 2,4-D

Incubation: 4 weeks in light at 26±2°C

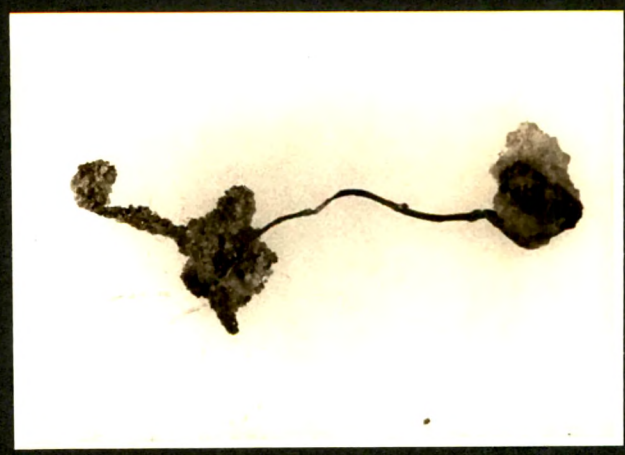

Fig.48

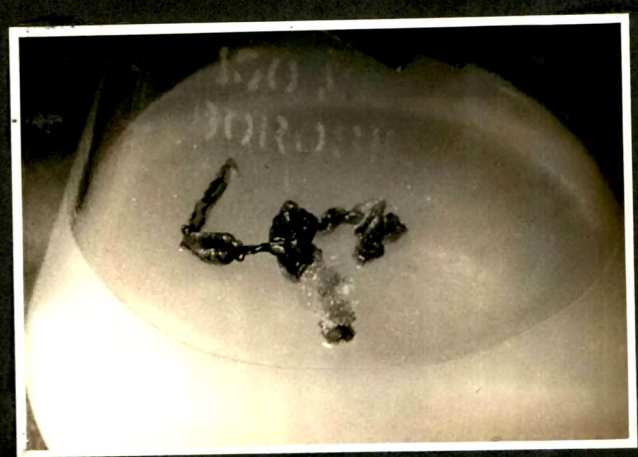

Fig.49

auxin), and B: White's medium with 2% sucrose and 10% CM (but no auxin).

After incubation for 4 weeks, leaves developed on the callus grown on medium containing 2% sucrose (Medium B). On further incubation roots were also produced from it (Fig. 50). On the other hand, gametophytes regenerated on the callus mass near the points of contact with the basal medium (Medium A) after prolonged incubation (Fig. 51).

DISCUSSION

It was observed that White's liquid medium with coconut milk supported good growth of excised root (Expt. 4-1). In Knudson's medium supplemented with low concentrations of IAA, the growth of main axis ceased but profuse laterals were produced (Expt. 4-2). Incorporation of very low concentrations of kinetin promoted the aposporous development of gametophytes from the excised root segments. Peripheral root cells became green and more gametophytes were produced within short time on kinetin medium than on the control (Expt. 4-3).

Fig. 50. Development of leaves and roots on callus developed on leaflets of garden plant, grown on White's medium containing 10% CM and 2% sucrose (no auxin)

Incubation: 4 weeks in light at 26±2°C

Fig. 51. Formation of gametophytes (G) on callus developed on leaflets of garden plant, grown on White's medium (no CM, no sucrose, and no auxin)

Incubation: 8 weeks in light at 26±2°C

Fig.50

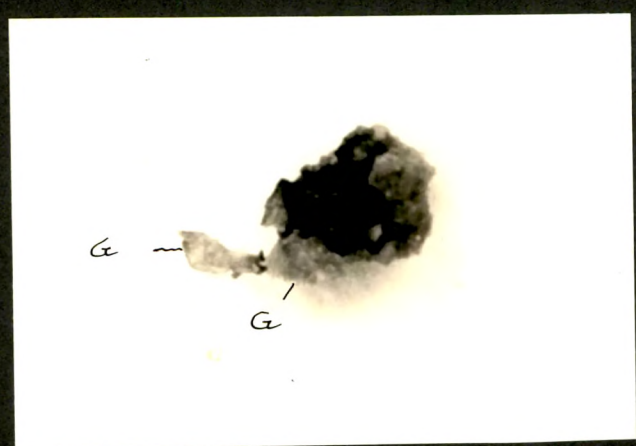

Fig.51

Callus formation was induced from the excised roots in presence of 2,4-D in the medium (Expt. 4-4). The root callus showed the potentiality for differentiation either into sporophytic organs such as roots in presence of sucrose or autotrophic gametophytes in complete absence of sucrose (Expt. 4-5). It appeared that carbohydrate starvation was prerequisite for switching on the autotrophic gametophyte form in the fern life-cycle. The property of production of aposporous gametophytes from fern roots was unique, because the roots of higher plants when grown in medium deprived of carbohydrate energy source are known to form only wound callus which soon senesces. This showed that the organism's genetic information for the less nutritionally demanding gametophyte stage might be easily called forth, in adverse conditions and permits a special kind of regeneration capacity, apparently not found in higher plants. Roots and root callus showing the capacity for the formation of aposporous gametophytes was quite striking in that just like the aerial organs, these organs too possess this potentiality.

Leaflets of garden plant showed requirement of higher sucrose and auxin concentrations for callus initiation (Expt. 4-6). The ability of apogamously

produced leaflets was more pronounced; they formed callus in relatively short period of time as compared with other leaflets. The callus developed from the leaflets showed potentiality for differentiation. In presence of sucrose sporophytic structures developed, while in absence of sucrose, gametophytes were regenerated (Expt. 4-7).