CHAPTER VII

IN VITRO STUDIES ON THELYPTERIS AUGESCENS (LINK) MUNZ ET JOHNSDON

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The importance of gametophyte morphology for better understanding of interrelationships of genera and species of ferns had been amply demonstrated as mentioned earlier. Here, an attempt was made to study spore morphology and the pattern of prothallial development in this particular species (Fig. 82) which represents an exotic variety belonging to the family Thelypteridaceae - a group of advanced Leptosporangiate ferns (Loyal, 1963).

Experiment No. 7-1: Spore-morphology and germination

Acetolysed preparation of spores mounted in glycerine jelly were used to study morphology of spore (Chapter II, Materials and Methods 8,B). Spores were oblong, brownish in colour and perinate, the perine being loosely adherent to exine and deep brown in colour. They were of bilateral type (Fig. 83).

Observations of prothallial morphology were made after sowing the spores under controlled conditions. Spores collected from the fertile fronds were sterilized

Fig. 82. Herbarium specimen of Thelypteris augescens (Link) Munz et Johnsdon

Fig. 83. Spores of Thelypteris augescens

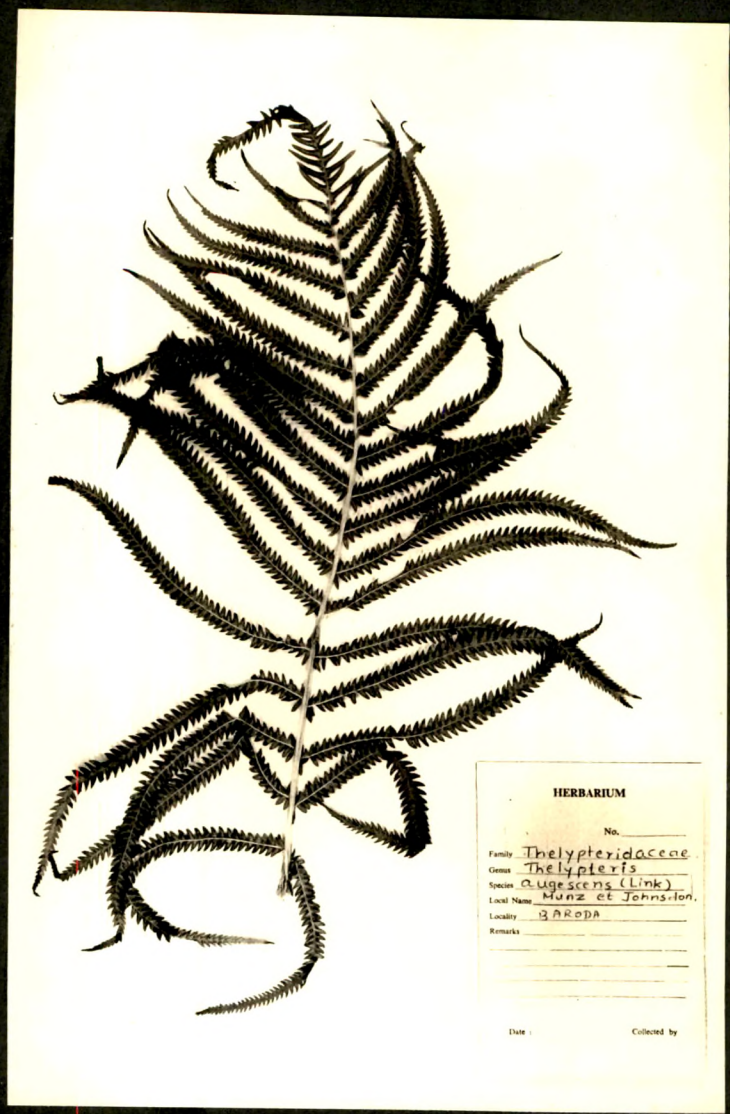
HERBARIUM
No.
Family Thelypteridaceae
Genus Thelypteris
Species augescens (Link)
Local Name Munz et Johnston.
Locality BARODA
Remarks
Date :
Collected by

Fig.82

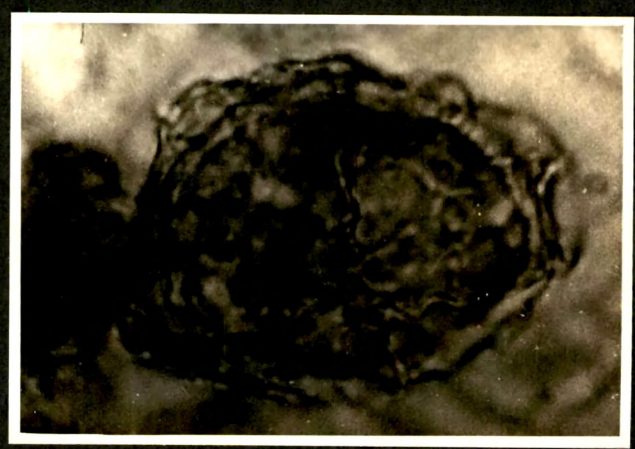

Fig.83

as described in Chapter II, Materials and Methods 3,C. Sterilised spore suspension was inoculated on agar slants of Knudson's medium (15 ml) with 1% sucrose. The pH of the medium was adjusted to 5.5 and the medium was solidified with 0.5% Difco Bacto agar, the conditions which were found to be optimal in the earlier chapter. The culture tubes were incubated at 26±2°C in continuous light.

It was observed that all the spores did not germinate; many were not viable. Germination of spore took place when spore wall cracked along the lasura (Fig. 84A) and the first protonemal cell made its appearance within three weeks. The protonemal cell entered a phase of successive transverse divisions resulting in the formation of a germ-filament consisting of 5 to 7 cells (Fig. 84B,C). The rhizoid was also produced during the same period. The apical cell of the germ filament divided by vertical division and later these cells multiplied so that a spatulate prothallial plate was formed (Fig. 84D). By six weeks an apical meristem was established (Fig. 84E). The basal cells of the germ filament produced few more rhizoids and simultaneously many unicellular glandular hairs were produced on the prothallus.

Fig. 84. Stages of prothallial development of Thelypteris augescens

(A) Spore (1500X)
(B) and (C) formation of germ filament (60X)
(D) Prothallial plate formation (150X)
(E) Prothallus with hairs developed (100X)
(F) Adult prothallus (24X)
(G) Antheridium (450X)
(H) Archegonium (450X)

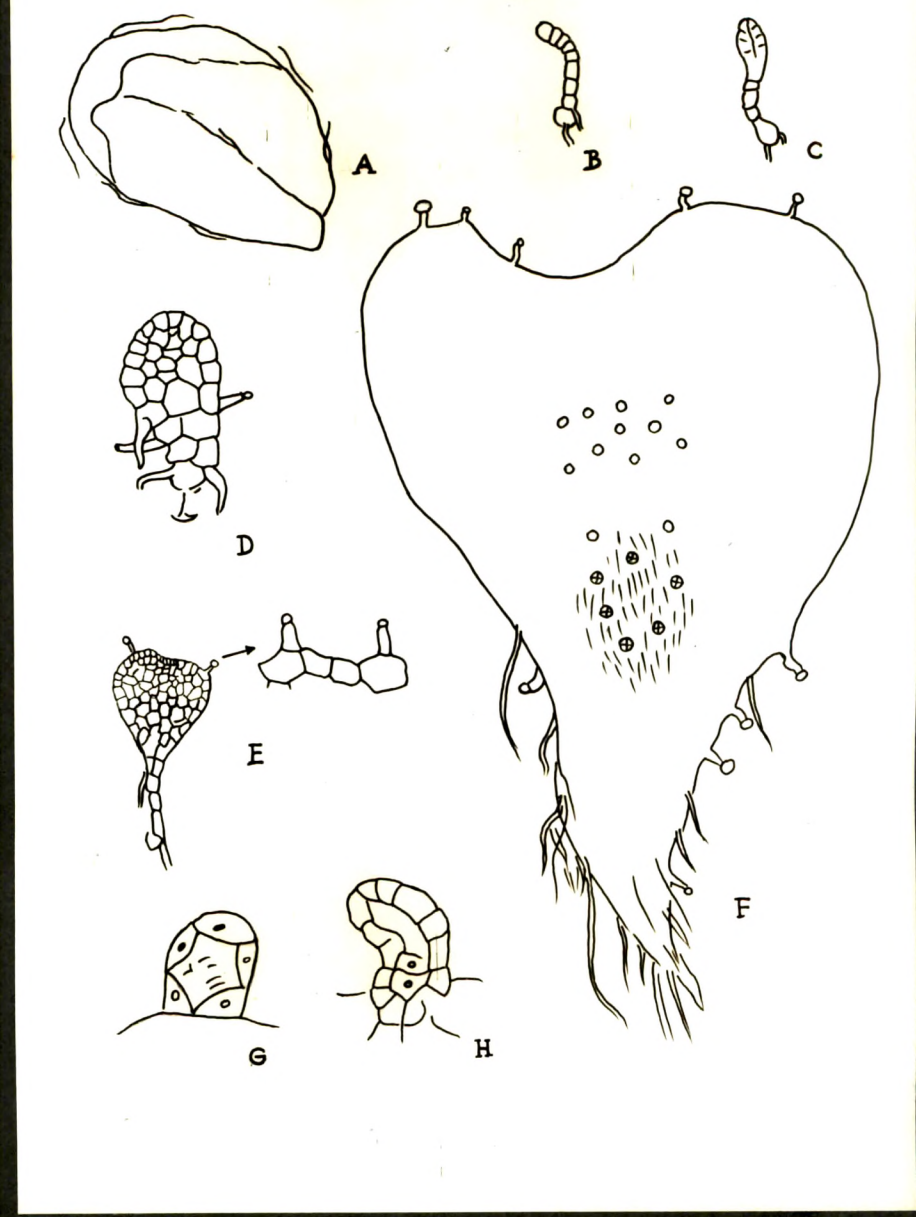

Fig.84

Experiment No. 7-2 : Prothallial development

The prothalli assumed different forms if grown in thick cultures and feeble illumination. The spatulate prothallus became notched and a cordate form was attained. Thalli of various stages of development were found growing mixed in the culture. Midrib formation was initiated 8 to 10 weeks after spore germination. Prothalli were quite large and attained maturity in 3 to 4 months (Fig. 84F).

Sex Organs

Antheridia were formed on 8 weeks old prothalli and were restricted to the ventral side. Each antheridium was small globose and of the Leptosporangiate type (Fig. 84G).

After the establishment of midrib, archegonia made their appearance (about 12 to 14 weeks from spore germination) on the thickened portion of the prothallus (Fig. 85) amongst the rhizoids. Archegonia also were of the typical Leptosporangiate type (Fig. 84H).

Fig. 85. Photomicrograph of mature prothallus showing sex organs (24X)

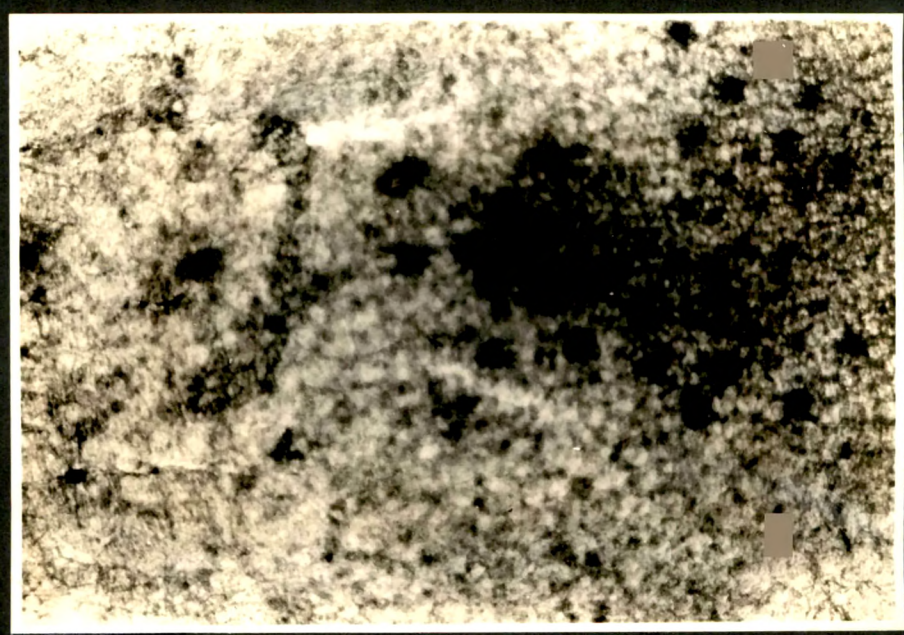

Fig.85

As the prothallus aged, the peripheral portion of the wings thickened. Old prothalli became very large and irregular in shape. They possessed copious, slender, long and tangled rhizoids on the cushion; sometimes on both the surfaces.

Experiment No. 7-3 : Development of leaf callus

Young leaftips of garden grown plants were inoculated on Knudson's agar medium containing coconut milk, sucrose and either NAA or 2,4-D. The culture flasks were incubated at 26±2°C in continuous light for 6 weeks.

Of the concentrations of 2,4-D tested 2 mg/l was found to be most suitable for callus induction on the leaf (Fig. 86). None of the NAA levels tested was found effective.

On further transfer to freshly made 2,4-D media to build up clones of tissues for more experimental work the leaf callus however, did not survive.

Fig. 86. Initiation of callus on leaflets grown on Knudson's medium containing 10% CM, 2% sucrose and 2.0 mg/l 2,4-D

Incubation: 4 weeks in light at $26 \pm 2°C$

Fig.86