

from which the species was collected.

The ascospores of the present species are similar to those of *P. pelvetia* Sutherland², the latter, however thrive on red or brown algae. *P. triglochicola* Webster³ is another species closely related to *P. avicenniae*, however with larger ascocarps 400–500 μm in diam. and ascospores $45\text{--}65 \times 16.5\text{--}25 \mu\text{m}$.

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OBSERVATIONS ON NITROGEN NUTRITION AND SEX ORGAN FORMATION IN FERN GAMETOPHYTE

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FERN gametophytes which show very definite successive stages in their development provide an excellent material for studies of differentiation and morphogenesis. Starting from spore germination, the gametophytes pass through a filamentous 1-D stage, a spatulate 2-D stage, a cordate 3-D stage and finally form sex organs, usually antheridia first followed by archegonia. If the conditions are suitable, sporophytes are formed bringing about a culmination of the gametophytic generation. What makes the gametophyte to change over to reproductive phase from the vegetative one has so far remained an intriguing question. The present observations constitute an inquiry in this direction if nitrogen nutrition of the gametophytes has any correlation with such changes in development of the gametophytes of *Cheilanthes farinosa*, a tropical fern of local abundance.

Spores of *C. farinosa* were collected and preserved in a desiccator at 4°C. Before sowing these were surface-sterilized with 2% NaOCl for 2 min washed with sterilized water and then sown on Dyer's¹ (solid) nutrient medium containing NO_3^- as

sole source of nitrogen following the method of Raghavan². Gametophytes were grown in a culture room maintained at $22^\circ \pm 2^\circ\text{C}$ under continuous white fluorescent light of 250–300 ft-C intensity. As the gametophytes reached cordate stage, they were regularly transferred and retransferred to fresh media and as the archegonia matured the plates were flooded more than once with a thin film of sterilized water to ensure fertilization and sporophyte formation.

When spores were allowed to germinate and grown on a nitrogen-free medium the germlings grew only up to 3–4 celled filamentous stage. This indicated that the amount of stored nitrogen in the spore that could be mobilized during germination was insufficient to support growth beyond this stage, and therefore, external nitrogen source (i.e. KNO_3 and $\text{Ca}(\text{NO}_3)_2$ in Dyer's medium) should be available at a very early stage of development, if not from the time of sowing.

Gametophytes from NO_3^- containing media, at different stages of development were picked up and an *in vivo* assay of nitrate reductase (NR) was done by estimating nitrite in the incubation medium (10 mM KNO_3)³ for different replicates (table 1). Plates in replicates containing cordate prothalli, 4–5 days prior to formation of antheridia were exposed continuously to white, red and blue light of the same intensity till the formation of sporophytes. Days required for initiation of sex organs and sporophytes were recorded (table 2). A chromatographic separation of soluble aminoacids from gametophytes of different vegetative phases was done and the result shown in figure 1.

The above observations indicated that *C. farinosa* spores need external nitrogen source for normal development. The results of the *in vivo* NR assay

Table 1 NRA in different developmental stages of gametophytes

Developmental stage	NRA as $A_{543}/100 \text{ mg f. w./2 hr}$
1-D (3-celled)	0.046
2-D (initiation)	0.078
2-D (spatulate)	0.106
3-D (young)	0.144
3-D (old, vegetative)	0.124
Antheridia initiation	0.096
Archegonia initiation	0.082
Sporophyte initiation	0.072

The results presented represent the mean of 3 experiments.

Table 2 Effect of different quality of light on initiation of sex organs and sporophyte

Light quality	Antheridia days	Archegonia days	Sporophyte days
White	12	17	24
Red	11	16	24
Blue	18	23	29

The results presented represent the mean of 3 experiments.

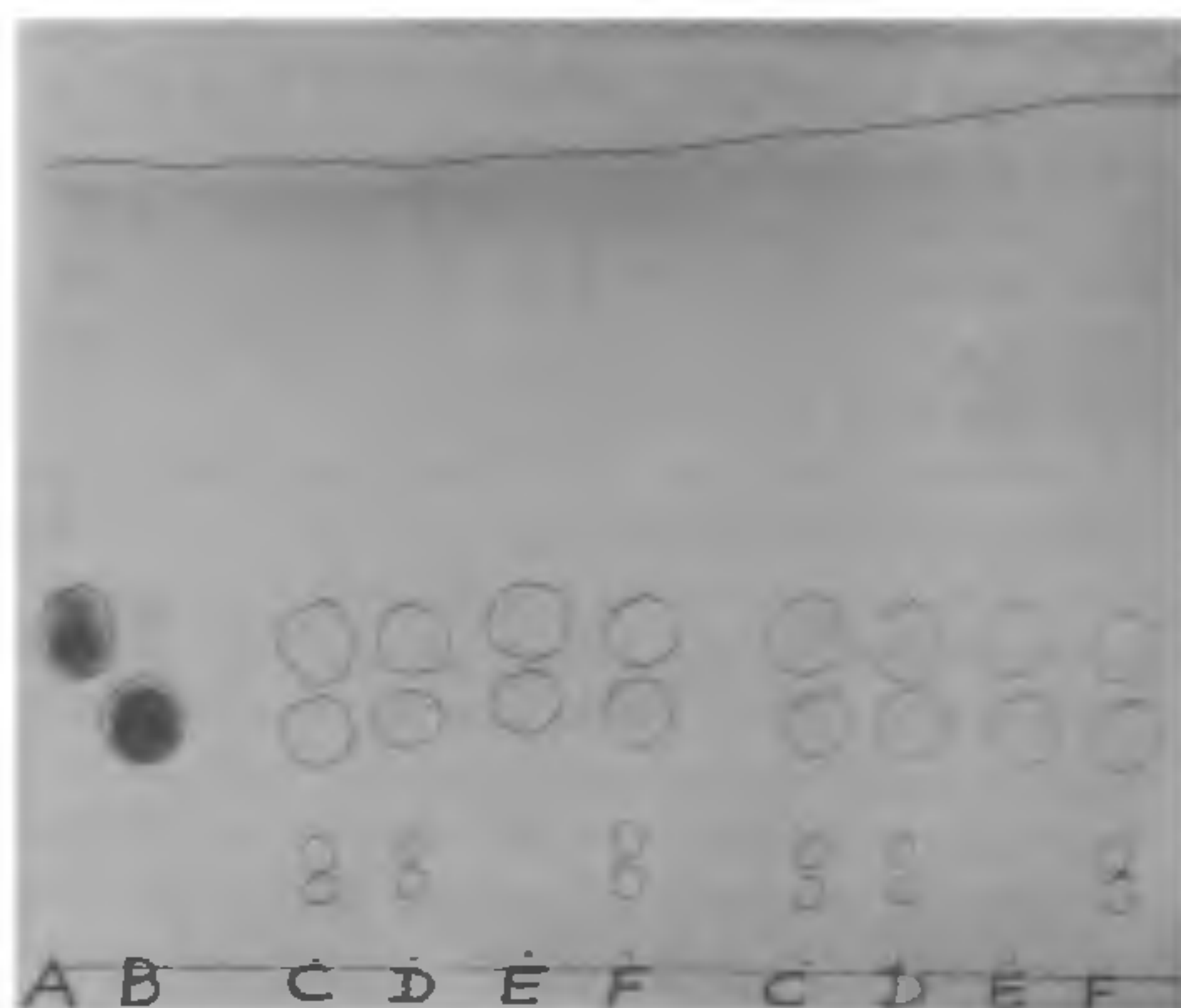


Figure 1. TLC of soluble amino acids A. Glutamic acid (reference spot); B. Glutamine (reference spot); C. 1-D, stage; D. 2-D, stage; E. -N medium, (1-D stage); F. 3-D, stage.

showed a gradual increase in nitrate reductase activity (NRA) from germination to 3-D stage formation whereafter it started decreasing as the cordate prothallus matured and sex organs appeared, minimum activity being reached at the initiation of sporophyte. Since NR is the key enzyme in nitrogen metabolism when NO_3^- is the sole source of nitrogen, the above observations suggest that a decreased nitrogen metabolism perhaps triggers the reproductive phase initiation in *C. farinosa* gametophytes under culture condition. There are earlier reports that nitrogen deficiency in the substratum causes the liverwort, *Riccia* to form sex organs⁴ and also that induction of flowering in a short day species of *Lemna* sp. by suppression of nitrogen metabolism⁵. The experiment with different quality of light also suggested a probable role of NR in switching over to reproductive phase. While red light had no visible effect, blue light definitely

delayed the process by about a week, without affecting the periods required for initiation of either archegonia or sporophytes, initiation of both of which are almost rhythmic once the antheridia are formed. This shows that blue light delayed the change over to reproductive phase. *In vitro*, blue light has been found to enhance NRA⁶ so also *in vivo* in ferns (unpublished data from this laboratory). However, at this stage of our experimentation it is not possible to suggest unequivocally the role played by blue light though our results could be taken as indirect evidence supporting the contention that reduced NRA is a probable prerequisite for antheridia initiation in *C. farinosa*.

The TLC of soluble amino acids showed constant presence of glutamic acid and glutamine in all the three stages, 1-D, 2-D and 3-D suggesting the presence of GS/GOGAT pathway of assimilation of ammonia, the ultimate product of NO_3^- assimilation.

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VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI IN ROOTS AND SCALE-LIKE LEAVES OF *ACORUS CALAMUS* LINN. AND *COLACASIA ESCULENTA* LINN.

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VESICULAR-ARBUSCULAR mycorrhiza (VAM) occur on almost all tropical crop plants and are known to enhance the plant growth by augmenting the