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**IN VITRO ONTOGENY OF *PYRROSIA LANCEOLATA* L. FAREWELL AN  
IMPORTANT MEDICINAL FERN**

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**ABSTRACT**

*Pyrrrosia lanceolata* L.Farewell. belongs to the family polypodiaceae. The plant is a lithophytes and its decoction is used for cold and sore throat. Tea is prepared from the fronds. Medicines prepared from this plant are used for the treatment of Soriosis and other skin diseases. The plant is used as folk remedy in the tribal community and hence seems to be of economically important and medicinally used. The spore germination as a Conservation measure is initiated under in vitro condition and its ontogeny was tried in Knops and Knudsons medium. *Vittaria* type of germination was evident and *Drynaria* type of gametophyte development was observed during the culture period. Micromorphological features were studied and tabulated. Two types of gametophyte in seen is the cultures one is having perfect cordate shaped and the other one is elongated and cordate shaped. The results facilitate the life cycle studies of this important medicinal fern.

**KEYWORDS:** *In vitro* studies, *Pyrossia lanceolata*, Spore germination and Gametophyte.

## INTRODUCTION

Pteridophytes have valuable importance to unearth the number of uncertain hypothesis of evolution. In addition, more species of the Pteridophytes have been known and validated as potential genetic resource against many diseases and source of miscellaneous articles. In India, the Pteridophytes have been found to grow in almost all climatic zones under different habitats and represented by approximately 1200 species falling under 191 genera.

Ferns reproduce via spores and have neither seeds nor flowers. Unlike mosses, Pteridophytes have xylem and phloem (making them vascular plants). They have stems, leaves, and roots like other vascular plants. Most ferns have what are called fiddleheads. The fiddleheads expand into what are called fronds, which are each delicately divided. A fertile leaf produces spores borne in sporangia. The spores germinate and produce a gametophyte with sex organs. Prothallus or gametophyte is a green, photosynthetic structure that is one cell thick, usually heart or kidney shaped, 3–10 mm long and 2–8 mm broad.

Fern propagation from spores is an elegant and challenging growing process that involves closely following and

nurturing the ancient life cycle of ferns known as alternation of generation. Spore germination is the first event in the life cycle of ferns. Germinated spores progress autotrophically through many developmental stages to form a mature gametophyte with rhizoids and gametangia (Raghavan, 1989).

Spore propagation ensures genetic diversity of native fern species and provides a high volume of fern plants for gardens and environmental restoration. Among the various biotechnological options, also reported in other agri–horticultural crops, micropropagation through tissue culture and in vitro spore germination are best applied and commercially exploited in fern species (Fay 1994). Application of this technology (in vitro spore germination) for large–scale multiplication of certain species of ferns from the Western Ghats has been demonstrated by various authors (Sara et al. 1998; Manickam et al. 2003; Johnson et al. 2005; Sara & Manickam 2005; Johnson & Manickam 2006; Sara & Manickam 2007; Johnson & Manickam 2007; Johnson et al. 2008).

The experimental plant is *Pyrossia lanceolata* (L.) Farewell. It belongs to the family Polypodiaceae. Rhizome wide and

creeping, slender, densely covered by scales. Frond is simple, linear. Lower surface of the frond is densely covered by stellate hairs, upper surface glabrous. Sori irregularly distributed mainly in the distal part of the pinna, dark brown naked; spores reniform pale brown. A decoction of the fern is used in South Africa for clods and sore throat. In Mexico, a tea prepared from the fronds is used for itch. Medicines prepared from this plant are used for the treatment of Soriosis and other skin diseases.

The plant is not explored so far for spore culture and development. Hence the plant is selected for the present study. The aim of this study is to study the germination pattern of *P.lanceolata* plant, to measure and study the growth area of the gametophytes, and also to study the micromorphology of the gametophytes of *P.lanceolata* on Knudson's and Knop's medium.

## **MATERIALS AND METHODS**

### **Collection of experimental plants**

*Pyrossia lanceolata* (L). Farwell plants were collected from the Western Ghats region of Kerala.

### **Spore collection**

The spores were collected on December by keeping the sporangia facing in a clean white paper and waiting till

dehiscence. Dark brown dust like spores were shed on the white paper and we have to collect it and keep it under 25 degree. These spores were inoculated freshly without any treatment of sterilizing reagents.

### **Medium preparation**

Stock solution of Kn (Knops 1865) and KC (Knudsons C, 1946) were prepared. The liquid medium was adjusted for pH 5.8 to 6.0 was directly transferred to the glass wares like conical flask and test tubes without adding agar. No carbon source was added in liquid medium. All the glass wares with medium were sterilized in the autoclave for 15 minutes at 15 lbs pressure.

### **Spore inoculation and culture conditions**

The spores were sprinkled on the surface of the various liquid medium and the culture flasks were kept undisturbed and immobilized under 12h photoperiod and 1800 Lux light intensity and incubated at 25±2°C.

### **Growth area calibration**

To study the developmental behavior and morphology of gametophyte, the prothalli were carefully removed from the culture using sterile forceps or needle. A minimum of 5 gametophytes were randomly selected, on 20<sup>th</sup>, 40<sup>th</sup> and 60<sup>th</sup> days of culture and their growth area and

morphology were observed and photographed. The magnification of pictures were denoted as bar (1cm = prescribed micro meter). Growth Area Index (GAI) was measured by calibrating its length and width of the prothalli and total growth area of the prothallus is calculated as follows by using the formula, Length ( $\mu\text{m}$ ) x Breadth ( $\mu\text{m}$ ) = Growth area ( $\text{mm}^2$ ).

## RESULTS

### Spore germination and Ontogeny (L.) Farewell. on optimal medium and prothallial development.

*P.lanceolata* possesses polypodiaceous type of sporangia. The exine of this spore is rough and the shape is more or less trilete. Size ranges from 55-63  $\mu\text{m}$ . Type, shape and colour, trilete, tetrahedral, tuberculate, spheroid, ellipsoidal and brown size. Surface is verrucate. The spores of *P. lanceolata* showed two different spores(Plate 1a) and the germination started on 15<sup>th</sup> day from the spores. The germination is of *Vittaria* type. The first rhizoids are perpendicular to the filamentous prothalli. The colour of the rhizoids is pink in colour. The prothalli filament emerged and grows up to ten celled stage (Plate 1c).

**Table-1 Showing germination and growth area of 20, and 40 days old *P. lanceolata* prothalli grown on knudson's and Knops medium**

Medium	Days	Germination percentage	Length In micrometer	Breadth In micrometer	Growth Area In micrometer
Knudsons	20	20	28.20±5.310	31.00±5.244	869.00±189.800
Knudsons	40	-	63.20±4.550	32.60±2.408	2281.20±642.843
Knops	20	10	28.00±3.162	17.20±1.643	483.60±86.907
Knops	40	-	33.60±2.074	24.20±4.025	810.40±122.649

Regarding growth area it is maximum in Knudson's medium and in 20 days time nearly 869.00 micrometer when compared to knop's with 483.60 micrometer. Length and breadth also play

important role on the formation of development of prothalli. Symmetry and micromorphology will also form an important character for the reproductive phase of an y fern species.

**Table-2. Micro morphological features of gametophytes of *P.lanceolata* grown on knudson's and knops medium on 50<sup>th</sup> day.**

Medium	Shape	Apical Notch	Rhizoids	Hairs /margins	Sex organs
Knudsons	Cordate elongated cordate	Well defined apical notch present.	Present 6-8	Undulated margins. No hairs.	No sex organs
Knops	Filamentous or Spathulate	Few pluricellular meristem is seen	Present 4-5 in numbers	Undulated margins. No hairs	sex organs

The prothallial plate of spathulate and straw shaped prothalli developed during 32-40 days of growth. The margins are undulated and pluricellular meristem appeared on 40- 50 days growth. Enormous rhizoids formed during the later days of its development. Pluricellular meristem is observed on 50 days time. Also these meristem develops by periclinal and anticlinal divisions, the apical notch which is "U" shaped. Two types of gametophyte in seen are the cultures one is having perfect cordate shaped and the other one is elongated and cordate shaped (Plate 2a and b). The measurement of both these

gametophytes varies. The development of gametophyte in *P.lanceolata* finds to be of *Drynaria* type. Rhizoids were kept on producing on the dorsal surface of the gametophyte.

## DISCUSSION

The important results of the present work on the experimental plant is as follows. Germination was high on knudson's medium and considered as an optical medium. *Vittaria* type of germination and *Drynaria* type of gametophyte development is evident. Micromorphology possess "U" shaped apical notch on 60 days, undulated

margins without hairs and pink colour rhizoids.

The experimental plant *P.lanceolata* is an epiphytic plant and confined to a particular ecological niche. The ontogeny of spore of *P. lanceolata* clearly expressed vittaria type germination and drynaria type of gametophyte formation. These results coincide with the classification of germination and gametophyte gives by (Nayar and kaur 1964). The experiments were carried out in liquid medium for *P. lanceolata*. Doughlas and Sheffield (1990) also confirmed the desirability of liquid instead of agar media for generating more tissues in *Pteridium aquilinum* and *Anemia phyllitidis*.

The niche specific nature may also be one of the reasons for the suppression of germination on all the light intensities on knop's medium. Germination appeared to be related to spore water content, germination rate and longevity, which differences between species with green spores and those with non green spores. Sara, (2007) explained germination studies in *A. nigripes* and *Nephrolepis*. It seems to possess low water content and high reserve or lipids and this has to be converted into chloroplast for

germination process and this made the spores to take more time (15 days).

The number of rhizoids which is an anchoring structure is specifically pink color roots and is more in number in knudsons medium whereas low in knop's medium. This observation is remarkable in this work.

The plant needs more work on studies on developmental behavior and other micromorphological activities. Micromorphology resulted two types of gametophytes and the results fall in line with the works of Sara and Manickam (2007). Formation of sex organs are evident in the cultures and hence the hermaphrodite nature is retained with the plant. Further works will reveal the full life cycle in due course of time.

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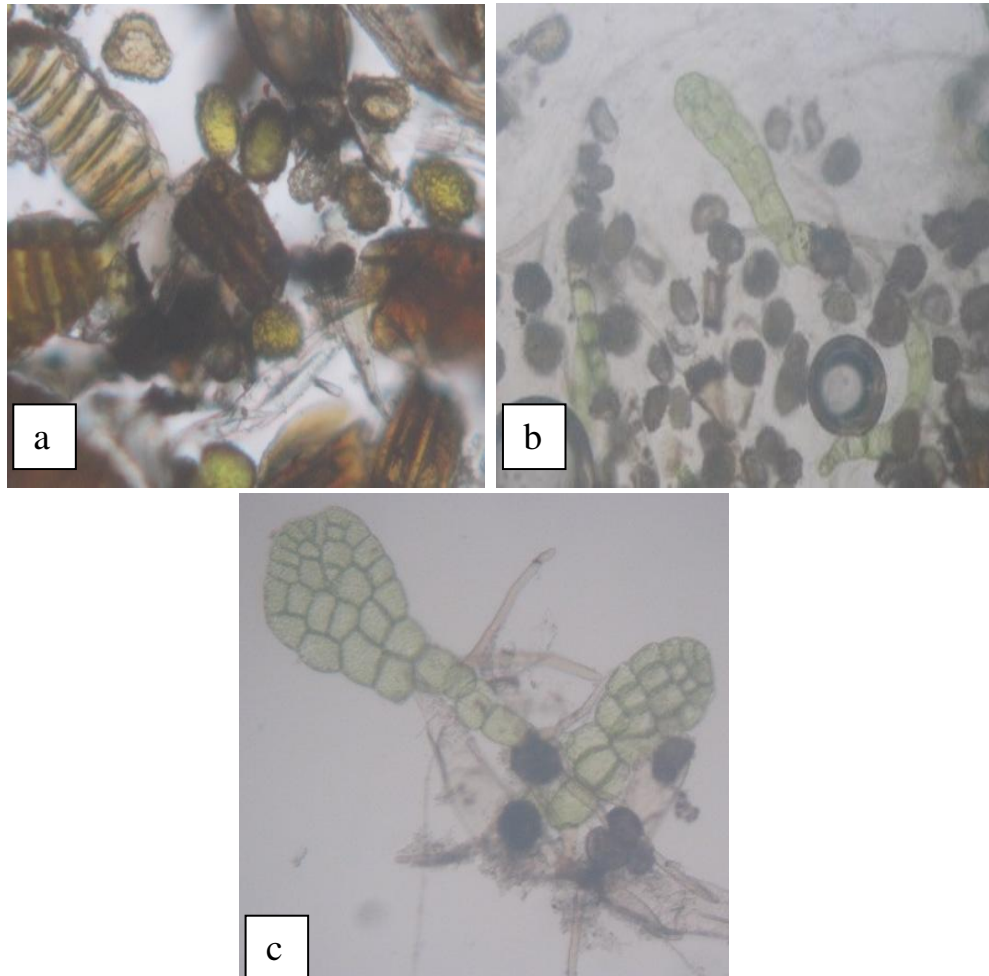
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## PLATE 1

**Plate- 1. Showing different germination and development of spores of *P.lanceolata* on 20-30 days.**

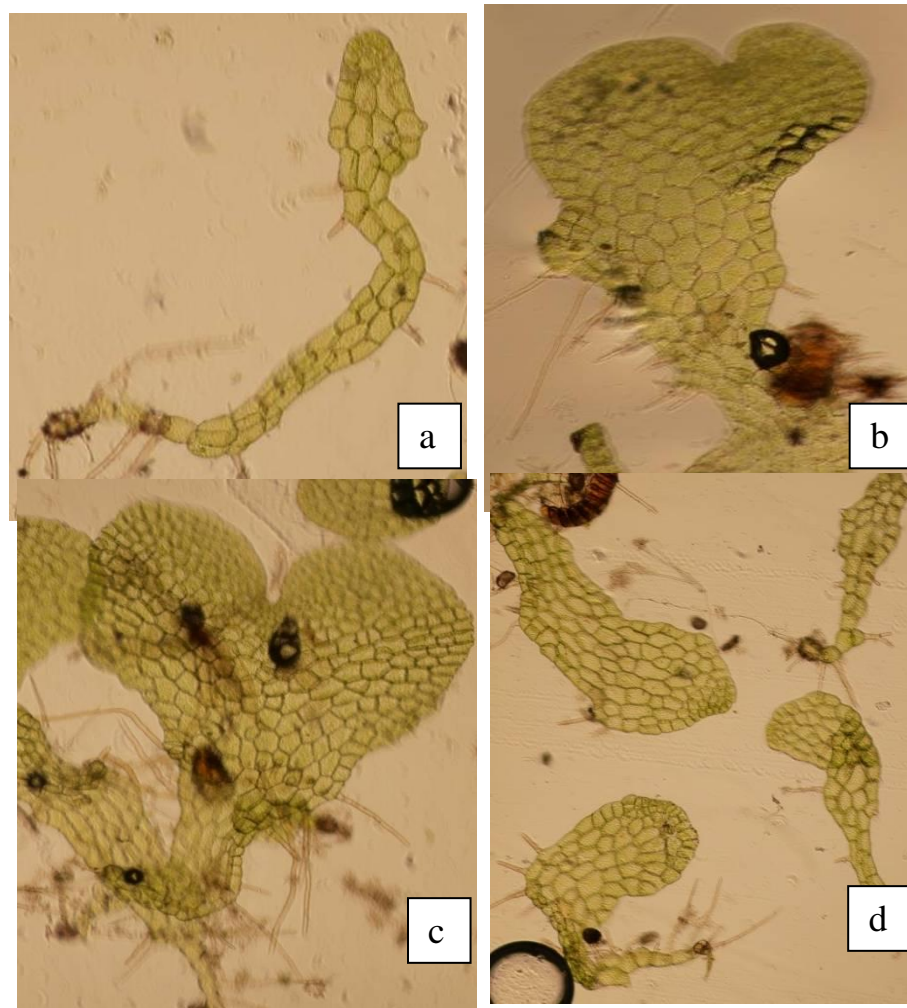


1a.Elipsoidal, brown, verrucate Spores of *P.lanceolata*. 1b.30 days old spatulate gametophyte grown on knop's medium and 1c. 30 days old gametophyte grown on knudson's medium.



## PLATE 2

**Plate-2. Showing growth pattern and development of gametophytes on 60 days time.**



2a. 40 days old elongated gametophytes of *P.lanceolata* grown on knop's medium.

2b.40 days old elongated gametophytes of *P.lanceolata* grown on knudson's medium

with apical notch. 2c.Perfect cordate shaped gametophytes grown on knudson's medium

with apical notch. 2D.Small elongated gametophytes grown on knops medium with

rhizoids and apical notch.