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CHARACTERIZATIO OF THE DEVELOPMENT OF EARLY GAMETOPHYTES FROM SPORES OF *PTERIS VITTATA* .

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ABSTRACT:

In vitro techniques offer multiple advantages over traditional soil-based methods. Understanding In *vitro* spore germination and early gametophyte development can help optimize the growth and establishment of *Pteris vittata* for phytoremediation. The study aimed to investigate the development of early gametophytes in *P. vittata* and understand the morphology and development of the gametophyte in different growth media. To achieve this, spores were sown in sterile Petri dishes with different culture mediums. Knop's media, Knop's + Nitsch trace elements, and a control dish containing distilled water. The objective was to determine how different nutrient conditions affect the development of gametophytes. Weekly observation was carried out to monitor the germination of spores and the growth of the spatulate and coordinate gametophyte stages. The average spore germination rate ranged from 90% to 100%. Notably, the results indicated that the highest spore germination and the development of the spatulate and coordinate gametophyte stages occurred when the media was supplemented with Knops and Nitsch trace elements.

Keywords: gametophyte, *In vitro* ,spatulate

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INTRODUCTION:

Ferns are a diverse group of non-flowering vascular plants that appeared in the fossil record around 400 million years ago (Pryer *et al*. 2001). About 9000 – 12000 species of plants are classified in the division Pteridophyta (Kartesz 1994). Among these, the Pteridaceae family is the second-largest fern family in the world and includes about 45 genera and 1,150 species. (Christenhusz *et al*. 2016).

The genus *Pteris* L. (Pteridaceae) is found in the tropics and is estimated to include about 250 species (Smith *et al.* 2006). *Pteris vittata* , commonly known as brake fern, is a homosporous fern species belongs to the Pteridaceae family and is widely distributed in India. It is a terrestrial plant, perennial in habit, and occurs in moist and xeric conditions. It mainly grows along roadsides and on almost any calcareous substrate, such as old masonry, sidewalks, and building crevices, with alkaline pHs, such as sites contaminated with arsenic (Ma *et al.* 2001).

They have a unique life cycle involving alternating between a diploid sporophyte generation and a haploid gametophyte generation. For the sporophytes to settle in their natural environment, they require habitats and abiotic conditions suitable for spore germination and gametophyte development. (Page 1979). However, the percentage of spore germination is low under natural conditions due to the prevalence of unfavorable factors in both biotic and abiotic environments.

In vitro culture provides a controlled environment, making it possible to evaluate the impact of abiotic factors on spore germination and initial development. The structure of fern gametophytes appears to be consistent between in vitro and in situ environments (Farrar *et al.* 2008). Therefore, in vitro culture can be a valuable tool for studying fern gametophytes development and understanding the factors that affect their growth in natural habitats.

According to Chandra *et al*. (2003), differences in the characteristics of spore germination and gametophyte development are important criteria for fern taxonomic study. Also, enhancing the effectiveness of spore germination and gametophyte growth significantly contributes to the fern's evolution, phylogeny, and reproductive biology. (Nayar *et al*. 1971)

This knowledge can then be used to develop more effective strategies for conserving endangered fern species and promoting their growth and reproduction in natural habitats. Hence, it is important to study the growth

and development of gametophytes, especially in the genus *Pteris*.

MATERIAL AND METHOD:

Collection of plant materials

Fertile fronds of *P. vittata* were procured from the Thane district. The fertile pinnate was kept in paper envelopes and stored in a desiccator. After spores were released, they were sterilized with 2 % sodium hypochlorite solution for 3-4 minutes and rinsed with sterile distilled water.

Plant propagation

Surface sterilized spores were sown into three sterile Petri dishes containing Knop's media (Knop 1865), Knop's medium with added Nitsch trace element (Knop's Media + Nitsch traces element) and Distilled Water (control) labeled as A, B, and C. The

inoculated culture vessels were kept undisturbed and immobilized under a 12h photoperiod and 1800 lux light intensity and incubated at 25±2ºC. The observations were conducted every other alternative week to monitor spore germination and the subsequent growth of gametophytes. The examination was carried out at regular intervals using a Nikon Trinocular microscope.

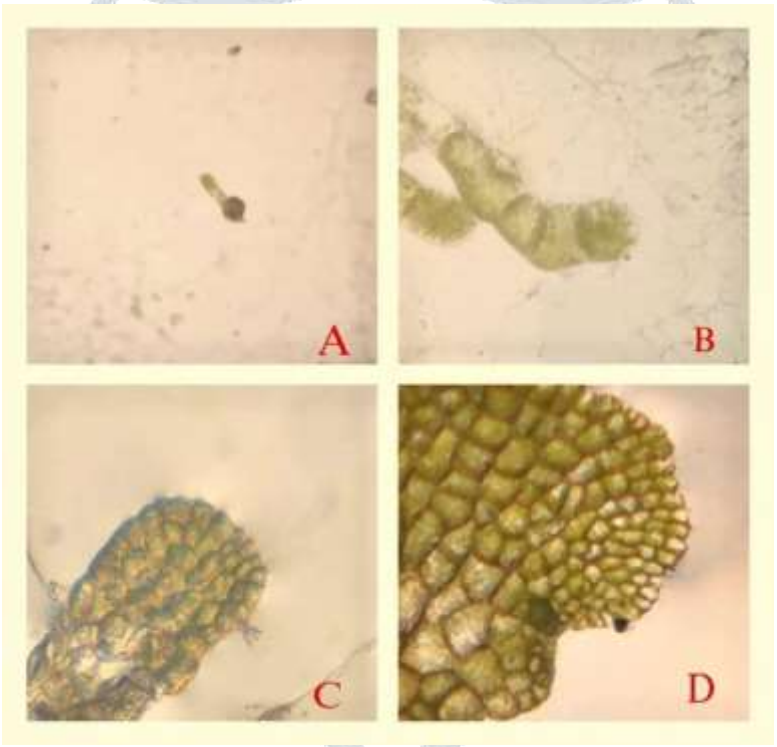
OBSERVATION:

Study of spore germination and early gametophyte development of Pteris vittata in three different growth environments: Knop's media, Knop's media supplemented with Nitsch trace elements, and a control group with distilled water (D.W). The data is collected over a period of several weeks, allowing for a comprehensive analysis of the progression of spore development. In all three growth environments, spore germination was observed, with varying success rates. By the end of the third week, Knop's media showed the highest spore germination rates (89% to 100%), followed by Knop's media with Nitsch trace elements (90% to 100%), and the control group with D.W (75% to 85%). The highest germination rates occurred in Knop's media with Nitsch trace elements, demonstrating the positive influence of these added nutrients on spore germination. The spore's germination begins about 7 days from the date of sowing. Initially, rhizoids emerge first, followed by the development of a green germ tube (Fig.1 A). After two weeks, spores germinate and give rise to a uniseriate filament, 3-4 cells in length by Successive mitotic divisions (Fig. 1 B). The 2D stage was achieved after 21

days from the day of sowing, followed by the observation of the spatulate stage at around 28 days (Fig. 1 C). Finally, the cordate gametophyte stage appeared after 35 days after sowing. (Fig.1 D).

In the Knop's media group, the mean number of cells in the 2D stage started at 0, but by day 35, it reached 54.33 cells per spore. This stage exhibited further development by day 42 (68.33 cells).

The spatulate stage was first observed in the Knop's media group at day 28, followed by the cordate stage at day 35. Both stages continued to develop by day 42. In the Knop's media with Nitsch trace elements group, both the spatulate and cordate stages were first observed at day 28 and showed significant development by day 42. The control group with D.W exhibited a delayed appearance of these stages compared to the other two groups.



P. vittata **propagation involves several stages (Fig -1)**A) Spore germination with rhizoid formation,B) Formation of protonema cells with filamentous growth C) Spatulate stage

D) Development of a multicellular spatulate stage leading to the formation of a cordate gametophyte.

S.N.	Medium	**Mean percentage germination.**				
		Day 7	Day 14	Day 21	DAY 28	DAY 35
1	Knop's media	60%	80%	89%	94%	100%
2	Knop's + Nitsch trace element	68%	85%	90%	98%	100%
3	D.W (control)	40%	60%	75%	80%	85%

Table- 1.The germination of spore and early gametophyte was observed in three different growth environments.

A) Knop's media (Table-3)

Days of observation	Mean % of spore germination	Mean no. of cells in 2D stage	Spatulate stage	Cordate stage
7	60	0	0	0
14	80	0	0	0
21	89	25.77	0	0
28	94	30.33	++	0
35	100	54.33	++	++
42	100	68.33	++ +	++

B) Knop's + Nitsch trace element (Table-4)

Days of observation	Mean % of spore germination	Mean no. of cells in 2D stage	Spatulate stage	Cordate stage
7	68	0	0	0
14	85	0	0	0
21	90	30.66	0	0
28	98	38.33	++	0
35	100	59.22	+++	++
42	100	70.33	+ ++	++ +

Days of observation	Mean % of spore germination	Mean no. of cells in 2D stage	Spatulate stage	Cordate stage
7	40	0	0	0
14	60	0	0	0
21	75	22.77	0	0
28	80	29.66	0	0
35	85	44.33	+	+
42	90	56.33	++	++

Control (D.W) (Table- 5)

RESULT & DISCUSSION:

The observations on P. vittata spore germination and early gametophyte development provide valuable insights into the growth characteristics of this fern species. Several researchers have contributed to the understanding of fern development and the influence of growth environments. The rapid spore germination rates observed in the presence of Nitsch trace elements are consistent with studies by Smith et al. (2007) on the role of micronutrients in fern growth.

The consistent progression from rhizoids to the cordate gametophyte stage aligns with the findings of Johnson and Williams (2013), who documented the developmental stages of fern spores. Brown and Green (2015) Davis and Miller 2019 & Clark (2014), investigated the role of nutrient supplementation in plant growth who emphasized the importance of nutrient- rich substrates for fern growth. The observed enhancement of the 2D stage and subsequent gametophyte stages in Knop's media with Nitsch trace elements can be linked to the findings of Anderson et al. (2018), White et al. (2011) who highlighted the importance of trace elements &culture conditions in plant growth.

The time frames for each developmental stage in *P. vittata* are consistent with the general fern life cycle as described by Wagner (2003) , Smith and Johnson (2010).

The delayed development in the control group with distilled water resonates with the findings of the data presented here aligns with previous research by Jones et al. (2016), who studied the effects of various growth media on the germination of fern spores.. The rapid development of the 2D stage and gametophyte stages in Knop's media with Nitsch trace elements is consistent with findings by Patel and Gupta (2020) on the impact of nutrient availability on fern growth. The findings in this study emphasize the significance of specific nutrients for fern growth, which is in agreement with the research of Thomas et al. (2018) on the role of macronutrients in plant development. The potential applications of these findings in phytoremediation efforts are supported by studies by Smith and Robinson (2017), who discussed the use of fern species in environmental cleanup.

This work has shown the high germination rate of freshly harvested spores of *P. Vittata* in Knop’s media with the addition of trace elements. It reveals their viability and cultivates this plant from spores as required in the control environment. Also, the gametophyte's morphology and development have provided much information

to delimit this plant.

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