



# In Vitro Studies on Gametangial Ontogeny and Development of Gametophyte of Homosporous Fern- *Dryopteris chrysocoma*

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**Abstract:** In the present study spores of *Dryopteris chrysocoma* were cultured in P & T medium and 67.47% and spore germination was recorded within a week of sowing. Spore germination was found to be Vittaria Type. The viability of spores was lost in ten months, when kept in room temperature (20-25°C). Prothallial development was Aspidium Type. In vitro culture creates a new path for mass cultivation of ferns and will be very helpful for their restoration and conservation. This work advances our knowledge of the pteridophytes reproductive capacity, enabling us to better preserve, restore, grow, and protect them.

**Keywords:** *Dryopteris chrysocoma*, Gametophyte development, *in vitro*, Mass propagation

In addition to having tremendous decorative potential, pteridophytes have demonstrated their abilities as phytoremediators, bio-indicators for pollution, and bio-fertilizers. They are a type of ecological indicator and typically develop in areas where flowering plants may not thrive. India is home to 12000 living species of ferns and about 1000 species of fern allies. Fern vegetation is threatened by anthropogenic influences, natural disasters, unplanned urbanization, industrialization, agricultural extension, and soil erosion and due to these large populations of terrestrial and epiphytic ferns, as well as other angiosperms are now extinct.

*Dryopteris chrysocoma* is a member of the dryopteridaceae family, which has 1600 species and 45 genera, making it one of the largest leptosporangiate groups. This species is distinguished by a short, ascending, thick, and tufted rhizome. It flourishes in temperate parts of Europe, the British Isles, and Asia. *Dryopteris chrysocoma* is prevalent in 2,000-3,000m altitude range near Darjeeling in India. According to reports, the chemical components of *Dryopteris chrysocoma* include albaspidin, filmaron, oleoresin, flavaspidic acid, and filicic acid (Alam 2010). Root of *Dryopteris chrysocoma* has anthelmintic properties and is mainly used to eliminate tap worms. Ferns are used as medicine by several cultures, including the Reangs and Chorai and recently several ferns and fern relatives have been used for various medical treatments, biofertilizers, and as nursery plants. They can also be employed as hyper accumulators of toxic metals and as pollution indicators (Rani 2022).

The *in vitro* culture approach opens up a new avenue for fern conservation to meet the need for plant resources for commercial and restoration efforts. The morphology of the gametophyte dramatically changes as it develops, modifying

tissue architecture for newly differentiated cells. Growth of the gametophyte in ferns follows a developmental progression to gather more and more photosynthates in order to get ready for the reproductive phase. Fern gametophyte develops quickly in the culture medium, and the effect of culture medium, growth regulators, and culture conditions easily affect spore germination and gametophyte development. Gametophytes are nutritionally independent, which makes it easier to conduct experiments, make observations, and expose objects to light. The mass production of ferns will benefit greatly from *in vitro* spore culture. The production of a large population of gametophytes from spore germination in tissue culture allows us to monitor developmental patterns and investigate the role of growth regulators. The fern gametophyte is a perfect model system for the study of physiology, photobiology, and cell biology (Fernandez 2003). The tissue culture method of spore germination enables the production of spore populations free from contamination by spores of other species, infection by bacteria and fungi, and interaction with algae and mosses, all of which are common problems when developing in the natural environment.

## MATERIAL AND METHODS

**Specimen collection:** In plastic bags, mature fertile fronds of *Dryopteris chrysocoma* from Mussoorie, Uttarakhand, were collected. The fronds were kept at room temperature in brown spore packets in desiccators with silica gel.

**Spore inoculation and sterilization:** For two minutes, the spores were maintained in a solution of 2% sodium hypochlorite in water. Parker's macronutrient culture medium and Thompson's micronutrient culture medium were used as the culture medium for spore sowing.

**Scanning electron microscope studies:** Electron microscopy was used to study spore morphology. Spores were dried and adhered on the stubs using sticky tape before being scanned at various magnifications in a sputter coater (JFC-1600 Auto Coater, JEOL Japan).

## RESULTS AND DISCUSSION

### Spore Germination

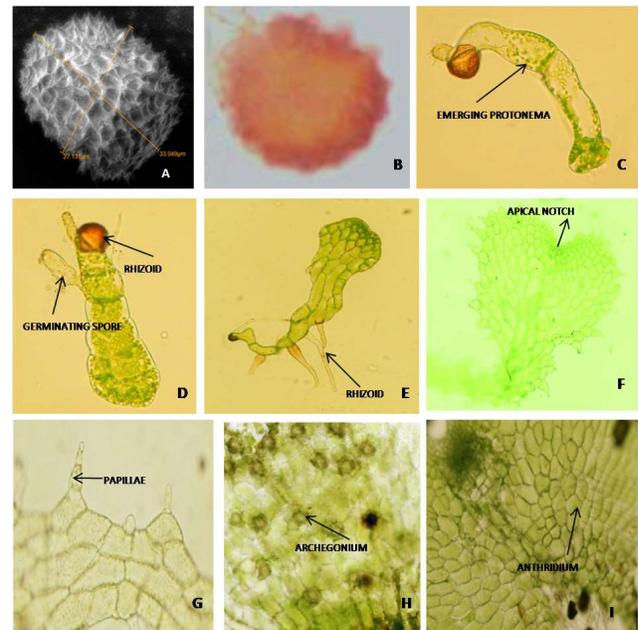
*Dryopteris chrysocoma* produced pale brown, bilateral, and perinate spores. The ornamentation on the exine and perine (or either of them) is frequently made of granulose or spinulose (Fig. 1 A, B) and measure 27.13 x 33.04  $\mu\text{m}$ . After 7–10 days of inoculation, spore germination was observed 67.47%. Spore germination was Vittaria type and the spore coat was observed to separate at the laesura area. The chlorophyllous protonemal cell and first rhizoid were followed by a series of cell divisions (Fig. 1, C). The spores have a conspicuous loose perine that has wrinkled subannular or elongated folds. Because of their limited spore viability and other physiological factors, ferns need more time to generate sporophytes than other angiosperms. The taxonomy of fern species has an impact on spore germination. A successfully fertilized spore forms a gametophyte, which then develops into a sporophyte.

### Prothallus Development

After 15 to 18 days of sowing, two-dimensional protonemal cells were visible, followed by the spatulate stage after 21 to 26 days (Fig. 1, E), and cordate gametophytes after 35 to 38 days (Fig. 1, F). The protothallial development was *Aspidium* type. The mature prothallus was huge, cordate, and thalloid, with thick midribs that were typically nearly as wide as the wings. The gametophyte had a small apical cleft in its anterior area and numerous brown-colored rhizoids and was symmetrical in nature. The adult prothallus has unicellular secretory papillate hairs all around and is heavily haired. The protonemal starting cell split into five to six cells, which generated a filamentous protonema (Fig. 1, D). Spore viability vanished completely at room temperature after 200 days. *Dryopteris chrysocoma* spores that had germinated produced filamentous prothalli that eventually transformed into cordate gametophytes. The spores produced filamentous prothalli, which eventually transformed into heart-shaped gametophytes. The gametophyte of *Dryopteris chrysocoma* is symmetrical in nature, has a shallow apical notch, and has numerous brown colored rhizoids on its posterior area. The adult prothallus of this species is cordate, thalloid, and large, with thick midribs that are typically almost as wide spread-out as the wings. Each spore in the study produced one gametophyte, which developed from a filamentous stage to a two-dimensional heart-shaped stage.

### Gametangial Development

*Dryopteris chrysocoma* has typical Leptosporangiate-type sex organs. Following maturation, the cordate gametophyte remained in a vegetative state for almost 70 days until gametangia began to develop. After 90–95 days of spore germination, the posterior area between the rhizoids on the ventral surface of the thallus began to develop antheridia (Fig. 1, I). After 100–115 days of spore germination, archegonia began to develop on the ventral surface of the thallus, right below the apical notch along the anterior portion of the midrib (Fig. 1, F). The gametophyte develops bisexuality within four months, reaching its peak after 110 days of spore seeding. Table 2 appends the specific sex ontogeny events. Antheridia in the current study developed earlier than archegonia on the same prothallus by at least 10 days to ensure cross-fertilization and genetic diversity in nature. Sporophytes in *Dryopteris chrysocoma* did not begin to form until 180 days after spore sowing. A morphological investigation of gametophytes that were unable to create sporophytes at the end of the experiment revealed that they had functioning archegonia but had depleted antheridia. A small number of functional antheridia were also found, which may have been caused by antheridiogen involvement. The absence of sporophyte production can also be explained by different combinations of gametophytic lethal (Wu 2021).



**Fig. 1.** *Dryopteris chrysocoma*: A, B: Spores, C: Emerging protonema, D: Filamentous stage, E: Spatulate stage, F: Cordate gametophyte, G: Close view of Marginal unicellular hair H: Archegonia below apical notch, I: Antheridia in between rhizoid

**Table 1.** Event of spore germination and gametophyte development of *Dryopteris chrysocoma*.

Day after sowing	Spore germination (%)	Number of protonemal cell	Number of rhizoidoidal cells	Two dimensional stage (%)	Spatulate stage (%)	Cordate stage (%)
7	67.47±1.87	1±0.03	0.6±0.58	0	0	0
14	73.96±2.32	3±1.03	1.0±0.55	0	0	0
21	80.85±1.06	7±0.6	1.1±0.4	18±0.9	0	0
28	84.35±1.43	10±0.4	1.3±0.43	36±0.75	0	0
35	96.36±0.69	19±2.08	4.2±0.77	68±0.4	8±0.63	2±0.02
42	100	59±1.38	15.1±0.8	76±0.39	36±0.25	8±0.4
49	100	105±0.6	26.0±0.56	100	78±0.19	10±0.23
56	100	156±0.41	37.7±0.35	100	100	30±0.67
63	100	169±0.32	48.2±0.69	100	100	80±0.96
70	100	192±0.61	53.4±0.41	100	100	100

Parentheses showing Mean± Standard Deviation

**Table 2.** Chronological changes in sex ratio in a composite culture of *Dryopteris chrysocoma*.

Day after sowing	Number of neuter	Number of male	Number of female	Number of bisexual
80	20±0.3	0	0	0
90	14±0.69	1±0.63	0	0
100	11±0.21	4±0.89	1±0.36	0
110	6±0.69	5±0.23	1±0.21	1±0.42
120	7±0.84	10±0.48	2±0.64	1±0.13
130	1±0.66	11±0.32	3±0.98	2±0.28
140	0	11±0.14	5±0.78	4±0.14
150	2±0.87	3±0.19	6±0.16	3±0.38
160	3±0.1	4±0.73	10±0.35	6±0.43

According to studies (Bharati 2013, Parihar 2010) low germination rates, fungal and algal contamination, sporophyte development, and growth inhibition brought on by invading species can all be overcome through in vitro culture. Rare species like *Angiopterisboivinii* (Seychelles) and *Cibotiumschiedei* (Central America) are observed to demonstrate good growth through in vitro culture (Goswami 2016). By adjusting nutrients like nitrogen and carbon in the medium, one can accomplish the early creation of the gametangia. The gametophyte needs almost three months in the regular nutritional medium to create gametangia (Suneetha 2022). The maturity of spores at the time of collecting may have an impact on different growth patterns. The variable responses of various fern species may also be explained by the spore viability at the moment of collection.

The gametophyte not only permits sexual reproduction but also affects migration, recruitment, habitat choice, and adaption (Li 2022). Little research has been done on important aspects of gametophyte biology, such as the selection of habitats by gametophytes based on their morphological and physiological diversity, the timing and method of development and maturation, the breeding system

and habitats that produce new recruits for the sporophytic population. The haploid, straightforward structure of the fern gametophyte is primarily made up of a single layer of cells. Although it lacks sophisticated tissues and organs, it has a tremendous deal of potential to provide important information and understanding about how plants develop. It is an excellent experimental system since it is simple to cultivate and may be handled in a destructive-free manner. For the study of morphogenesis in response to environmental stimuli, the gametophyte of ferns makes an excellent model system (Goller 2007). Additionally, these discoveries offer knowledge to aid in the management, cultivation, and protection of the species (Hanyuan 2003).

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#### REFERENCES

Alam SM, Qureshi M and Jahan N 2010. Antimicrobial screening of some medicinal plants of Pakistan. *Pakistan Journal of Botany* 42: 4281-4284.

- Bharati SK, Manabendra DC and Behari MP 2013. In vitro propagation in pteridophytes: A review. *International Journal of Research in Ayurveda and Pharmacy* **4**(2): 297-303.
- Fernández H and Revilla MA 2003. In vitro culture of ornamental ferns. *Plant Cell, Tissue and Organ Culture* **73**(1): 1-13.
- Goller K and Rybczynski JJ 2007. Gametophyte and sporophyte of tree ferns in vitro culture. *Acta Societatis Botanicorum Poloniae* **76**(3): 193-199.
- Goswami HK, Sen K and Mukhopadhyay R 2016. Pteridophytes: evolutionary boon as medicinal plants. *Plant Genetic Resources* **14**(4): 328-355.
- Hanyuan Z and Bingyang D 2003. Studies on the gametophytes development in ferns. *Zhiwu Yanjiu* **23**(2): 154-158.
- Li W and Ma H 2002. Gametophyte development. *Current Biology* **12**(21): 718-721.
- Parihar P, Parihar L and Bohra A 2010. In vitro antibacterial activity of fronds (leaves) of some important pteridophytes. *Journal of Microbiology and Antimicrobials* **2**(2): 19-22.
- Rani A, Uzair M, Ali S, Qamar M, Ahmad N, Abbas MW and Esatbeyoglu T 2022. *Dryopteris juxtapostia* Root and Shoot: Determination of Phytochemicals; Antioxidant, Anti-Inflammatory, and Hepatoprotective Effects; and Toxicity Assessment. *Antioxidants* **11**(9): 1670-1687.
- Suneetha C and Hegde S 2022. Micropropagation of Pteridophytes. In Ferns: *Biotechnology, Propagation, Medicinal Uses and Environmental Regulation*. Singapore: Springer Nature Singapore. 201-242
- Wu X, Yan A, Mcadam SA, Banks JA, Zhang S and Zhou Y 2021. Timing of meristem initiation and maintenance determines the morphology of fern gametophytes. *Journal of Experimental Botany* **72**(20): 6990-7001.

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