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IN VITRO STUDIES ON PHOTOMORPHOGENESIS OF GAMETOPHYTES OF *DRYOPTERIS DILATATA* - (HOFFM.) A.GRAY IN TWO DIFFERENT MEDIUM

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ABSTRACT

Photomorphogenesis refers to those aspects of growth and development which are subjected to relation by light shades. This photomorphogenesis processes in fern gametophytes appear to be under phytochrome control, showing the classic red/ far red reversibility. In *D.dilatata*, grown under in vitro condition in knudsons medium the growth was maximum up to the observed time limit of 60- 90 days. The growth area on knops medium was also noted. Response of various light intensities studied represents the maximum growth area of gametophytes on red light during 20 to 40 days time of illumination. Later during 60 days time Yellow light treated cultures shows maximum growth than control. In Knops medium, blue and green light treatments showed negative results and hence no effect, whereas red light and yellow light has some impact on growth area. In yellow light more growth area resulted than the control and red light. Hence it is proved that osmoticum also plays an important role in photomorphogenesis.

KEYWORDS: *In vitro* studies, *Dryopteris dilatata*, gametophyte, blue, yellow and red light

INTRODUCTION

Ferns first appear in the fossil record 360 million years ago in the Carboniferous but many of the current families and species did not appear until roughly 145 million years ago in the early Cretaceous. A fern is any one or more of a group of about 12,000 species of plants belonging to the botanical group known as Pteridophyta.

Pteridophytes are primitive plants survive on higher altitudes under the canopy of Angiosperms and in different ecological niches. These plants are least concerned for their medicinal values, studies about reproductive biology, morphogenetic studies photomorphogenesis and other branches of experimental studies.

Light has been found to govern the development stage of whole gametophyte (Turnwald *et al.* 1999). Kiss and Kiss (1998) reported that light frequency in the red region is found to promote spore germination more than in far-red region. Light not only regulates the growth mechanism in haploid gametophyte, but it also affects development of sporophyte due to involvement of a photoreceptor phytochrome (Christensen *et al.* 1998). Presence of light has been shown to increase nuclear DNA and this increase is known to have an influence on fern spore germination (Raghavan 1993).

Photomorphogenesis is the process by which plants grow and develop in response to light signals. This process is mediated by a sophisticated network of Photoreceptors among which phytochromes play a key role. Light is essential for plant growth and development, serving as an energy source for photosynthesis and as an environmental signal for photomorphogenesis (Chen et al., 2004). Plant growth and development are highly regulated by environmental light conditions. The mechanism for photomorphogenesis is not yet elucidated fully; therefore, it is critical that researchers gain further understanding of the characteristics of photoreceptors, their action mechanisms, and signal transduction pathways. *Dryopteris dilatata plant is the experimental plant and it* belongs to the family Dryopteridaceae. Plant with leaves arching and clustered in a 'shuttlecock' on a short, Leaves -Triangular-ovate, dark bluish-green Petiole - Dense, at least near base, pale brown with a broad dark central stripe even when on young crosiers. Rhizome is erect.

Various scientists have studied the moss *Physcomitrella* and fern *Adiantum* and the model plant *Arabidopsis* for analyzing photoregulatory processes. Very few works is carried out on photomorphogenesis of ferns. There is no studies currently available about the effect of light intensities on prothalli of *D.dilatata*. Hence the present study was carried out to study the effect of different wavelength of light intensity and also to optimize suitable culture medium and growth

conditions for establishment of this ferns through in vitro tissue culture methods. The study specifically was to find out the photomorphogenetic response of the young gametophytes under different light intensities like blue, green, yellow and red keeping white light as control.

MATERIALS AND METHODS

Collection of experimental plants

Dryopteris dilatata (Hoffm.) A.Gray. 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 as shown below.

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±2ºC.

Experimental set up with different light intensities

The knudson and knops medium grown gametophytes were subjected to light intensities like, blue, green, yellow and red by covering the culture vessel with cellophane paper from the day one. 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^{th}, 40^{th} and 60^{th} 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 calibration

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 (µm) x Breadth (µm) = Growth area (mm^2).

RESULTS

Germination, ontogeny, growth area and photomorphogenesis on Knudson's medium (optimal medium)

The winged spinulose 35-48µm spores (Plate 1a.) of *D.dilatata* germinated on 12 days times and highest germination (95%) occurred on knudsons medium without any supplementation of carbon source at pH 5.8 under white light condition. Despite the light intensities white light promoted germination and negative growth as shown in Table 1. After 20 days of 5-6 cells of filamental growth the apical cell evidenced the glandular hairs at its tip. The gametophytes produced glandular hairs at each apical cell and were prominent. The prothalli become spathulate within 40 days, due to anticlinal and periclinal divisions. The prothalial plate showed vigorous growth and terminal cells could be seen during 60 days time. The cells of gametophytes are delicate containing more chlorophyll (Plate 2b). Since the spathulate and cordate shaped forms of gametophyte is developed in Knudsons medium, the same medium was considered as the optimal medium for growing this plant.

Table 1. Germination percentage of spores of *D.dilatata* in different medium

Germination	***Dryopteris dilatata***				
	Control	Blue	Green	Yellow	Red
Knudson's medium	(95%)	-	(81.4%)	(85.2%)	(95%)
Knop's medium	(12%)	-	-	(88.2%)	(88.4%)

Photomorphogenesis processes in fern gametophytes appear to be under phytochrome control, showing the classic red/ far red reversibility. Generally, in knudsons medium the growth of *D.dilatata* was maximum up to the observed time limit of 60- 90 days. Apart from medium other parameters under various light intensities were studied and their results were tabulated in table 2 and 3. Maximum growth of gametophytes was observed on red light intensities next to control and descending yellow follows the order.

Response of various light intensities studied represents the maximum growth area (1175.4 µm) on red light on 20th day. Next to red light, 459.8µm growth area was found on yellow light grown cultures. Green light showed very low response (128.3µm) of growth area and blue light showed negative result. On 40 days culture the same response gradually increased in all the cultures. Vigorous growth observed on red light (1952.4µm) and the growth area exceeds then control (1463µm) on 40th day. Yellow light treated cultures showed the growth area of

1017.2µm and 272.0 µm for green (Table 2.Plate 1d and e). On 60 days cultures also maximum growth area (5649.0µm) evidenced on yellow light grown cultures. Table 2 and 3 next to yellow light, red light treated cultures showed 4664 µm of growth area and these two light intensities has enhanced the growth of the gametophyte when compared with the control (4054.5µ m).

Table 2. Growth area of 20,40,60 days old *Dryopteris dilatata* gametophytes grown on Knudson's medium under various light intensities

Light intensities		Length	Breadth	Growth Area
Control	20	51.4±1.140	26.6±1.517	1366.8±1.517
	40	57.2±1.304	28.6±2.074	1463.4±95.738
	60	89.80±6.419	45.20±1.924	4054.60±270.490
Blue	20	-	-	-
	40	-	-	-
	60	-	-	-
Green	20	17.2±1.643	7.4±1.342	128.3±1.342
	40	24.2±3.194	11.2±0.837	272.0±47.666
	60	40.20±1.483	18.40±1.140	740.40±65.458
Yellow	20	29.8±1.483	15.4±2.191	459.8±78.461
	40	44.20±3.194	22.80±4.147	1017.20±252.442
	60	95.20±9.121	60.00±1.581	5643.20±487.444
Red	20	43.2±1.924	26.4±2.302	1175.4±92.173
	40	59.20±2.775	33.00±2.915	1954.40±205.899
	60	86.20±4.919	54.20±4.494	4664.00±359.065

Germination, ontogeny, growth area and photomorphogenesis on Knop's medium

The germination in *D.dilatata* sporeson knop's medium is very low (12%) in white light, whereas in yellow and red light intensities the germination is high (88.2 and 88.4%) in both the treatment (Table 1, and Plates 1b&c). Blue and green light treatment showed negative result for germination and further growth of gametophytes. Hence there is no effect of these two light intensities (blue and green) on germination as well as gametophyte formation. The growth area of 20 days old *D.dilatata* prothali in knop's medium is 310.8 µm. The growth area of red light intensity (350.8 µm) exceeds the growth of control on white light. Here also just like the knudson's medium in yellow light, the growth area is more than the control. During

40th day the yellow light irradiated prothali resulted in maximum growth area (815.2 µm) and the control (407 µm). The result shows a maximum growth area on red light also. On 60th day maximum growth area (2798 µm) was evidenced on yellow light than red (1717.8 µm) and it is greater than the control (827.4 µm) (Table-3).

Table 3. **Growth area of 20, 40, 60 days old *D.dilatata* prothalli grown on knops medium under various light intensities**

Light intensities		Length	Breadth	Growth Area
Control	20	23.20±2.387	13.20±2.387	330.80±84.313
	40	27.00±1.581	15.00±1.581	406.60±63.291
	60	37.60±2.074	22.00±1.581	827.40±75.245
Blue	20	-	-	-
	40	-	-	-
	60	-	-	-
Green	20	-	-	-
	40	-	-	-
	60	-	-	-
Yellow	20	12.20±1.924	24.60±2.702	303.40±77.200
	40	24.80±2.588	32.60±2.074	815.20±125.438
	60	74.20±7.497	37.80±3.493	2798.00±324.432
Red	20	26.40±6.229	14.20±2.775	350.80±96.596
	40	31.20±1.924	22.20±2.864	695.20±120.321
	60	47.00±4.359	36.60±3.975	1717.80±229.966

Concludingly the important results of the present work on this plant were summarized as follows. Germination was effective on knudson's medium and it was considered as optimal medium. *Vittaria* type of germination and *Aspidium* type of gametophyte could be seen. Micro morphologically gametophytes possess pluricellular meristems, apical notch,unicellular glandular hairs on 60 days. Regarding the photomorphogenetic effect on *D.dilatata* In knudson's medium yellow and red light enhanced the germination and growth apart from white light. Blue light inhibit spore germination of *D.dialatata* on knudson,s and knop's medium. In knop's medium yuellow and red light enhanced the germination, but decreased in control. Growth area and micro morphology is also perfect in knudson's medium treated with yellow and red light.

DISCUSSION

Various studies on germination based on light, temperature, pH, soil, medium, hormones, and chemicals were undertaken. The microscopic observation of germination reveals that the germination of *Dryopteris dilatata* spores as started as 12 days time and it is *Vittaria* type. *Aspidium* type of gametophyte development from the results (Plates 1 and 2). This type of germination formation also has reported is *Dryopteris* s*ubtriangulari*s cultures are mixed soil by (Zhangkai mei et.al (2005). The prothalli plates were formed and mature prothalli are cordate. Profuse unicellular hairs occur at the surface and on the margins of the prothalli.

The results of growth pattern of gametophyte kundson's medium and knops medium on various wavelengths showed the effects of medium along with light factor and the physiology of these group plants. Yellow light and red light dominated during growth whereas white light dominated for germination of *D.dilatata* (Table 1 and 2 Plate 2) on knudson's medium. Phytochrome mediated photomorphogenetic responses are characterized by the complex variety of relationships between light input and physiological outputs like germination, de-etiolating and circadian rhythm.

The responses of gametophytes also shows photomorphogenetic signelling network of the incident radiation through miro morphological output. When comparing the tables --, defined apical notch and pluricellular meristem was observed only on control and red light treated cultures whereas spathulate prothalli and poor pluricellular meristem was seen in the knops grown gametophyte. Profuse unicellular glandular hairs also were the expressions of Aspidium type of development (Kaushik (1970) also has studied, green, blue, yellow, red and white light on spore germinations whereas negative growth occurs only in red, yellow and white light. This indicates a requirement photosynthetic light for spore germination and non-photosynthetic light for growth and cell division is the experimental plants. Presence of rhizoid is also an ecological adaptation on invitro cultures towards the maturity of the gametophye. Absence or inhibition of germination itself on knop's medium on blue and green light shows that medium composition and pH effects the germination and later development stage.

In knudson's medium also blue light above inhibited the germination. Raghavan 1973 has also postulated that although germination appears to be basically under phytochrome control, the presence of a blue light absorbing pigment interfere with phytochrome transformation in the spores. Favourig our results the same authors the status that is *Cheilanthes farinose* germination is inhibited by blue light, but photo reversibility of germination is also

observed in re-promoted spores irradiated successively with far red and red light.

In different germination of spores of and *D.dilatata* and gametophye development of *D.dilatata* on knudsons and knop's medium may be due to the photo receptors of the cells towards different light intensity. According to Anna (1984a and b) the spores of Wood mardia radcaus can germinate in differently either in water or in culture media containing mineral salts at temperatures(15-24 C) falling within a range behind optical for many other ferns(15-30 C). This indifference therefore due to photosensitive matures of spore on different medium.

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PLATE- 1

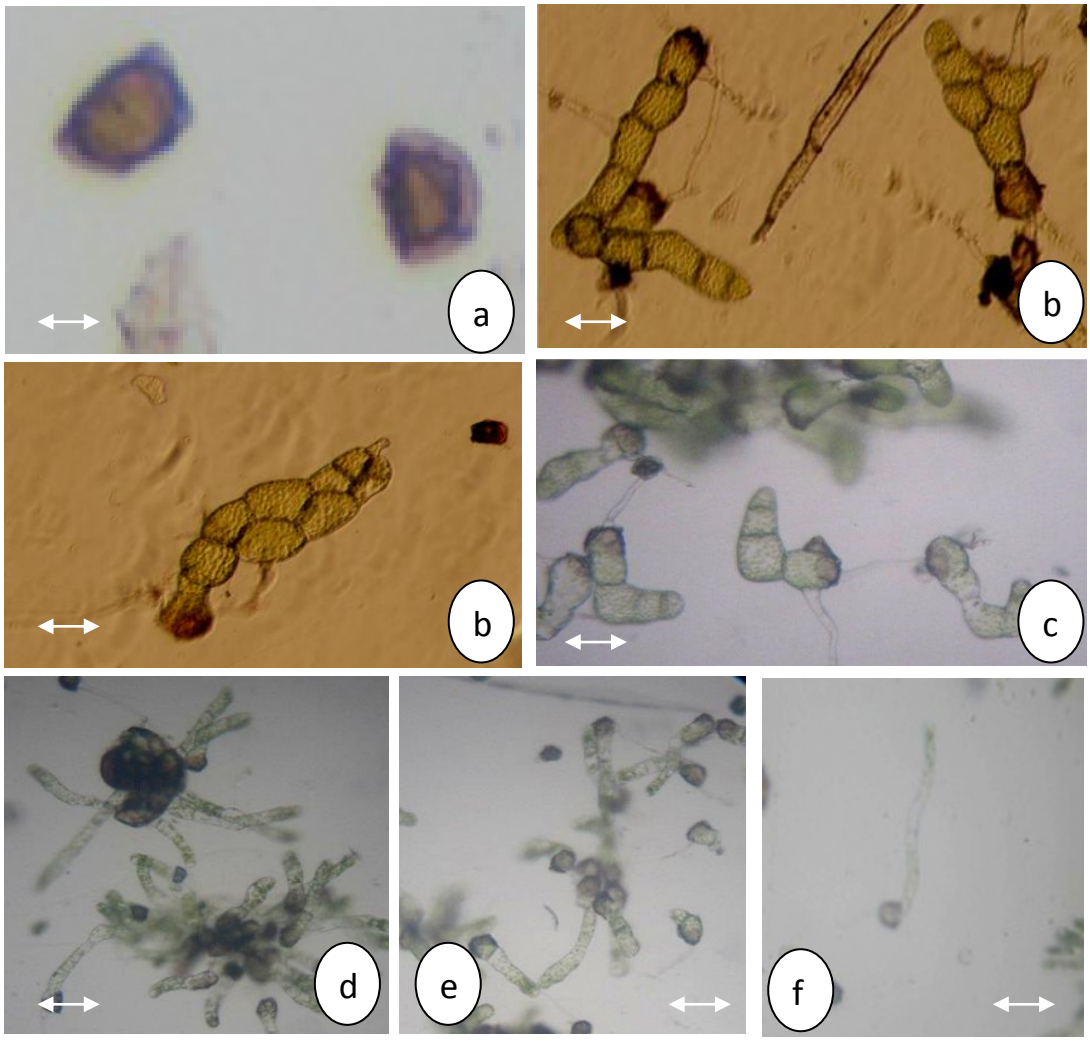

Plate 1a. Winged spinulose spores (kidney shaped) of *D.dilatata*. 1b. 3o days old prothalli of *D.dilatata* grown on kundson's medium (1 cm -10µm). 1c. 30 days old prothalli of *D.dilatata* grown on knop's medium (1 cm -10µm). 1d . Growth of protonemal filament bent from the angle of germination on knudson's medium blue light treated gametophytes. 1e Growth of straight and elongated filamentous prothalli on Kc red and yellow light treated cultures. f- Normal growth of elongated prothalli in kundson's and knop's medium.

PLATE -2

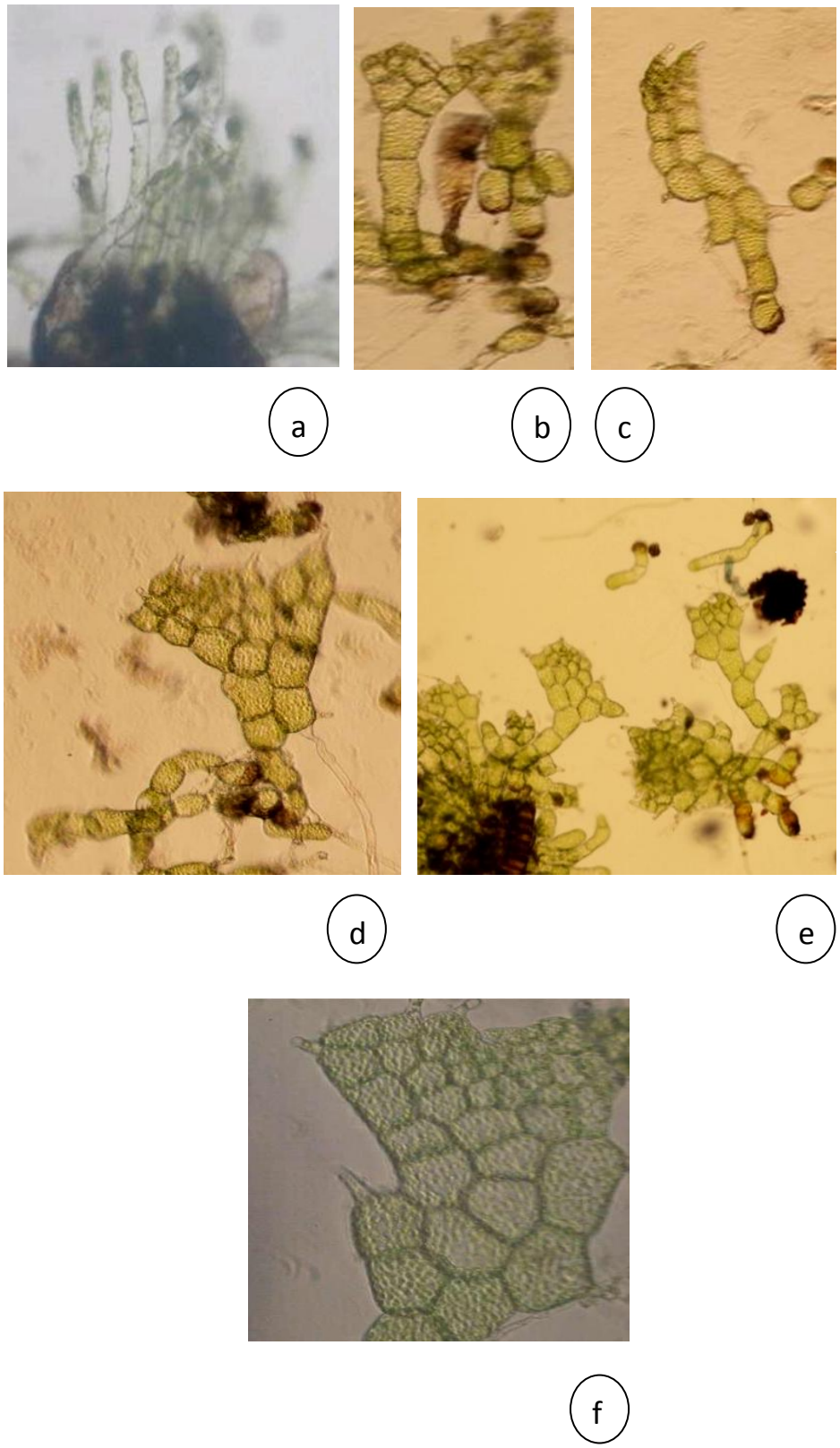

Plate 2- Showing various growth pattern of *D.dilatata* on different light intensities on 40 days. 2a-. Growth of short filamentous prothalli on knudson's medium. 2b- Growth of short filamentous prothalli on knudsons medium. 2c Growth of strap shaped prothalli on knops medium. 2d.45 days old prothalli grown on red light on Knudson's medium. 2e. .45 days old prothalli grown on green light on Knop's medium 3f-.Close up view of spathulate prothalli showing unicellular hairs grown on knudson's