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In vitro DEVELOPMENTAL STUDIES OF *SELAGINELLA CILIARIS* OF RAJASTHAN UNDER DIFFERENT LIGHT QUALITIES

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Exine bursting

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ABSTRACT

In the current study, megaspore germination and the emergence of sporelings *in vitro* under different light qualities were recorded in *Selaginella ciliaris* (Retz.) Spring. Megaspore germination in terms of exine bursting was observed after 17 days from the date of sowing in control whereas this process under red light treatment was observed two days earlier than control. Under yellow light, it was recorded after 11 days of spore sowing, which is earlier by 6 days than control while in blue light exine bursting was observed after 17 days from the date of sowing as observed in control. The emergence of sporelings was observed only in blue light treatment after 23 days of sowing. These observations indicate that yellow and red light qualities promote megaspore germination and blue light behaves as control during germination but favors the development of sporelings in this species of *Selaginella* occurring in Rajasthan.

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1 Introduction

Heterosporous genus *Selaginella* P. Beauv. with 700 species are worldwide in distribution (Pichi-Sermolli, 1977; Jermy, 1990). Amongst these 62 species were recorded from different biogeographic regions of India (Alston, 1945; Panigrahi & Dixit, 1966; Panigrahi & Dixit, 1967; Panigrahi & Dixit, 1968; Dixit, 1984; Dixit, 1992). A detailed description of sporangial and gametophytes development in two species of *Selaginella* namely *S. apus* (L.) Spring and *S. rupestris* (L.) Spring was provided by Lyon (1901). The development of the female gametophyte in *S. kraussiana* (Kunze) A. Br. was investigated by Campbell (1902). Bruchmann (1912) studied embryology in many species of *Selaginella*, including *S. kraussiana*. Slagg (1932), during the study of microgametophyte development in *S. kraussiana*, germinated microspores on the plaster of Paris blocks. Wetmore & Morel (1951) were able to maintain sterile cultures of megagametophytes of *S. pallescens* (C. Presl) Spring and *S. flabellata* (L.) Spring. Several workers have used artificial crossing techniques to determine the inheritance of pigmentation in *Selaginella* (Webster, 1979; Webster & Tanno, 1980; Tanno & Webster, 1982 a; Tanno & Webster, 1982 b). Burgeff & Filippi (1957) made inter varietal crosses between *S. martensii* Spring var. *martensii* and *S. martensii* var. *variegata*. They sowed surface-sterilized megaspores and microspores together on nutrient agar in culture tubes and after gametophytes had formed, flooded the cultures with water to achieve fertilization. After 30-40 days, sporelings appeared. Bierhorst (1964) described methods for obtaining the reproductive stages of *Selaginella* for classroom use. Webster (1967) reported the induction of sporelings under greenhouse and field conditions. Robert (1971a; 1971b; 1972) has provided a detailed account of mega-gametophyte development in *S. kraussiana*. An overview of the morphology, anatomy, and life cycle of a new model species- the lycophyte *S. apoda* (L.) Spring was provided by Schulz et al. (2010).

Rajasthan in north-west India lies between 23°3'-30°12' N and 69°30'-78°17' E is the largest state with an area of 3,42,239 sq km. Aravalli ranges, one of the oldest mountain ranges of the world, diagonally divide the state into two distinct climatic regions. The region towards the north-western side of Aravalli's is desert or semi-desert characterized by sand dunes, high wind velocity, high temperature, and thorny vegetation. The region towards the south-eastern side is a humid zone with hills of variable heights, ravines, plains, rivers, and dense forests that provides a suitable environment for the growth of pteridophytes.

Presently, four species of *Selaginella* namely *S. ciliaris* (Retz.) Spring, *S. rajasthanensis* Gena, Bhardwaja & Yadav. *S. repanda* (Desv. ex Poir.) Spring and *S. reticulata* (Hook. & Grev.) Spring are known from Rajasthan (Sharma & Bhardwaja, 1976; Gena et al.,

1979; Dulawat & Chaudhary, 2008; Yadav et al., 2011). Of these, *S. ciliaris* is highly restricted in its distribution and is confined, only to the northwestern part of Sitamata Wildlife Sanctuary of the state. Therefore, an attempt has been made to find out the developmental details of this fern ally of narrow distribution.

2 Materials and Methods

For the developmental studies spores (micro and megaspores) of *S. ciliaris* occurring in Sitamata Wildlife Sanctuary of Rajasthan were collected in August- September. Experimental studies on spore germination were conducted from November to March during the year 2013-2014.

Spores were surface sterilized with 1% sodium hypochlorite followed by repeated washing with sterilized distilled water. For spore germination and gametophyte development, sterilized spores (micro and megaspores mixed) after 48 hours of dark imbibitions were made to germinate in liquid Knop's medium (half strength) supplemented with Nitsche's trace elements using petri-dishes of 7.5cm in diameter.

To provide different light qualities, white light was obtained by two fluorescent tubes fixed 60 cm above the petri-dishes and red light by covering petri-dishes with two layers of red gelatin paper. Similarly, blue and yellow lights were obtained by covering petri dishes with blue and yellow gelatin papers respectively. Controls for each were maintained under white light.

For each light treatment spores were sown in two sets of petri-dishes each containing both micro and megaspores in the culture chamber maintained at 25±2°C. A control set was invariably included in all the experiments. The data are based on counts of 100 spores from each petri dish.

3 Results

3.1 Megaspore germination under different light qualities

During the present investigation, the author found it very difficult to study the development of male gametophyte due to its very small size and endosporic in nature. Further studies are going on to find out the details of microspore germination and microgametophyte development. Megaspore germination in terms of exine bursting and emergence of sporelings were recorded under different light treatments and the data have been presented in Table 1 and figure 1-2. Dark germination has not been observed in this species, unlike the spermatophytes wherein most of the plants seed germination takes in dark.

The data in table 1 indicate that in *S. ciliaris* exine bursting in red light treatment occurs 02 days earlier than control (figure 1 N-O). It started after 17 days of sowing in control while the germination

Table 1 Effect of different light qualities on megaspore germination in *S. ciliaris* of Rajasthan (locality –Sitamata Wildlife Sanctuary).

S.No	Light qualities	Germination	Days after sowing
1	Control (White light)	+	17
2	Yellow light	+	11
3	Red light	+	15
4	Blue light	+	17

Table 2 Development of sporelings in *S. ciliaris* of Rajasthan under blue light

S. No.	Developmental stages	Days after sowing
1	Exine bursting	17
2	Emergence of rhizoids	19
3	Archegonium	23
4	Sporophyte	47
5	First pair of leaves	50
6	Second pair of leaves	63
7	Roots	63

Table 3 Percentage of sporelings of *S. ciliaris* with second and third pair of leaves and roots

S.No.	Number of days after sowing	Percentage of plants with second pair of leaf	Percentage of plants with third pair of leaf	Percentage of plants with roots
1.	63	20%	-	20%
2.	64	40%	-	50%
3.	65	50%	-	90%
4.	66	60%	-	-
5.	67	80%	-	-
6.	68	90%	10%	-
7.	69	100%	20%	-

Table 4 Morphological features of sporelings of *S. ciliaris* of Rajasthan

S. No.	Days after sowing	Height of sporelings (mm)	Breadth of sporelings (mm)	Length of leaves (mm)	Breadth of leaves (mm)
1.	58	0.36-2.10	0.15-0.30	0.30-0.63	0.15-0.60
2.	59	1.26-2.10	0.15-0.30	0.45-0.69	0.18-0.63
3.	60	1.20-2.10	0.15-0.30	0.30-0.75	0.15-0.66
4.	61	1.20-2.13	0.15-0.30	0.30-0.75	0.21-0.69
5.	62	1.20-2.13	0.16-0.30	0.30-0.75	0.21-0.75
6.	63	1.20-2.90	0.15-0.30	0.45-0.75	0.15-0.75
7.	64	1.20-2.25	0.15-0.30	0.45-0.60	0.15-0.75
8.	65	1.20-2.40	0.15-0.30	0.15-0.60	0.09-0.75
9.	66	1.20-2.40	0.15-0.30	0.36-0.60	0.15-0.75

was initiated after 15 days of sowing in red light. Thus, red light slightly promotes the germination process in this species of genus *Selaginella*. Exine bursting under yellow light was recorded after 11 days of spore sowing, which is earlier by 6 days than control (table 1, figure 1 E & F). This indicates that yellow light has promotory effect on spore germination. Interestingly the exine bursting of megaspores was observed after 17 days of sowing under blue light (table 1, 2, and figures 2 A-C) which is similar to the time taken in control (white light). Thus, blue light behaves like a control on the process of spore germination in *S. ciliaris*.

Based on these observations, it may be concluded that yellow and red light qualities promote spore germination and blue light behaves as a control in *S. ciliaris*.

3.2 Sporeling development

Sporeling development was recorded only in blue light. No sporeling formation was observed under red and yellow light. The spore germination under blue light was found to be 90%. Bulging of mega-gametophyte was observed after 23 days of the date of sowing (figure 2C). Sporelings with cotyledonary leaves (first pair) were observed after 47 days from the date of sowing (figure 2H, I, J, K, L). Initially, the cotyledons were addressed laterally, and gradually they become separate from each other (figure 2 E, F, G K, H, K). The first formed leaves were initially whitish at the time of emergence from the megaspore wall and later on these turned green in color.

The data on the percentage of sporelings with second and third pairs of leaves and roots have been presented in table 3. 20% sporelings with the second pair of leaves were observed after 63 days of spore sowing. Interestingly, the percentage of such sporelings reached 80% after 67 days of sowing. 90% sporelings were observed to have the second pair of leaves after 68 days of spore sowing along with 10% sporelings with the third pair of leaves after 68th days of sowing. Thus, within a week after the emergence of cotyledonary leaves, all the sporelings were found to bear the second pair of leaves. The percentage of sporelings with the second pair of leaves was found 100% as well as 20% with third pair were observed after 69 days of sowing. The roots were observed on 20% of plants after 63 days and on 90% of plants in 65 days of spore sowing. This indicates that the development of roots is faster than the development of leaves. After 70 days *in vitro* de-colorization of leaves started. No branching has been observed beyond the cotyledons.

The morphological features such as height and diameter of sporelings, length, and breadth of leaves of sporelings developed under blue light exposures have been presented in table 4. A perusal of data presented in table 4 indicates that the height of

Fig -1
WHITE LIGHT

YELLOW LIGHT

RED LIGHT

Figure 1 (A-T) In vitro Developmental studies of *Selaginella ciliaris* under different light qualities – Effect of white (control), yellow and red light; A-D Effect of white light (control) Exine bursting in megaspores; E-L Effect of Yellow light Exine bursting in megaspores and emergence of papilla; M-T Effect of Red light Exine bursting in megaspores and emergence of papilla

Fig.-2
BLUE LIGHT

Figure 2 (A-T) *In vitro* Developmental studies of *Selaginella ciliaris* of Rajasthan under different light qualities - Effect of blue light; A-C Exine bursting in Megaspore, D - Megaspore with papilla, E-F Emergence of sporeling; G Emergence of root, H-J Sporeling with cotyledons (1st pair of leaves), K-L Cotyledons and subtending hypocotyle, O-P Young sporophyte with green cotyledons, hypocotyle and roots. M-Q Emergence of second pair of leaves, expanded cotyledons, R-T Emergence of third pair of leaves

sporelings varies from 0.36 mm to the maximum 2.4 mm in between 58-67 days after the date of sowing. The diameter in this period ranges from 0.15 to 0.30 mm. Leaves of sporelings were ovate-rotund, obtuse, green 0.15 mm to 0.75 mm in length, and 0.09 mm to 0.75 mm in breadth. The vascular tissue was also visible in the sporelings.

The data on the development of lateral leaves, median leaves, and roots have been presented in table 5. These data indicate that the development of median leaves (2nd pair of leaves) take more time as only 25% of sporelings with median leaves were observed in between 61-65 days of sowing and 50% in between 66-70 days after sowing. The leaf area of lateral leaves was generally higher which is essential for efficient trapping of light in shady habitats. The quick development of roots was observed as there were 75 % sporelings with root in between 56-60 days and 95% in between 61-65 days.

4 Discussion and Conclusions

The gametophytic generation of *Selaginella* has received far less attention than the sporophyte. The small size of the endosporic micro and megagametophytes makes them more difficult to study and gives the impression that there is little variation in gametophyte morphology throughout the genus (Webster, 1992). Several workers have tried to get male and female gametophytes as well as the sporelings of different species of *Selaginella*. Slagg (1932) in a study of microgametophytic development in *Selaginella kraussiana*, germinated microspores on the plaster of Paris blocks. A similar method for germination of both micro and megaspores was described by Bold (1967). Methods for obtaining the reproductive stages of *Selaginella* species have also been described by Bierhorst (1964).

Webster (1967) has described the induction of sporelings under greenhouse and field conditions. The micro and megagametophytes have also been obtained in *Selaginella kraussiana* through micro and megaspore germination on filter paper keeping wet with water by Webster (1979). Wetmore & Morel (1951) germinated megaspores of *S. pallescens* and *S. flabellata* on nutrient agar under sterile conditions. Observations of the present study too, support these findings as during present investigations spore germination and sporelings were obtained on Knop's liquid culture medium.

The data on megaspore germination under different light qualities indicate that yellow and red light qualities promote spore germination and blue light behaves like control in *S. ciliaris*. Borodin (1868), Schulz (1902), and Hartt (1925) have reported that the process of spore germination is enhanced in red light and delayed in blue light. Similar results have also been reported by Tripathi (2002), Kothari (2004), Meena (2008), and Hussain (2015) in homosporous ferns. Blue light-induced inhibition suggested that phytochrome is not involved in the inhibition.

Another pigment violaxanthin, a carotenoid of wide occurrence in plants was suggested for the inhibition process. By irradiation, with blue light, the pigment is possibly converted to an inhibitory system by giving rise to an inhibitory substance. The detailed sporeling development has been observed only in *S. ciliaris* under blue light quality. Sporelings with the first pair of leaves were obtained after 47 days from the date of megaspore sowing. The development of the second pair of leaves (median leaves) and roots have been observed up to the 70 days from the date of sowing.

A switch over to 2D phase of gametophytes is possible only in light-grown gametophytes and involves a change in the division of cells from transverse to longitudinal plane. This reorientation of mitotic spindle requires short wavelength light below 500 nm (blue light) in many pteridophytes (Mohr, 1956a; Mohr, 1956b; Davis, 1968). Raghawan (1973) has reported the biplanar growth in protonema of some pteridophytes under blue light. Present investigations also, support these observations as biplanar growth and sporeling development have been observed under blue light.

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Conflict Of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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