Vol. 15(2), pp. 18-22, July-September 2023
DOI: 10.5897/JENE2023.0952
Article Number: 9BEFB0B71102
ISSN 2006-9847

http://www.academicjournals.org/JENE

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Journal of Ecology and The Natural Environment

Full Length Research Paper

Effect of IAA on spore germination and gametophyte development in *Ceratopteris thalictroides* (l.) brongn. from Sitamata Wild Life Sanctuary, Rajasthan

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Received 18 April, 2023; Accepted 6 June, 2023

The effect of different concentrations of Indole acetic acid (IAA) on spore germination and gametophyte development of *Ceratopteris thalictroides*, a leptosporangiate fern of the family Pteridaceae found at the marshy places near Sitamata sanctuary in Rajasthan, was observed. The highest spore germination percentage was recorded in the 2 to 6 ppm concentration range of IAA, with minimum in the 10 ppm concentration. The number of cells in the protonemal filament and percentages of 2-D growth, spatulate and cordate gametophyte were highest in the 1 ppm concentration of IAA, followed by the control. Maximum percentages of archegonia and antheridia development were also recorded in 1 ppm IAA and control treatments, with percentages gradually declining from 2 to 10 ppm treatments. However, the maximum percentage of sporophyte development was recorded in 4 and 6 ppm IAA treatments.

Key words: IAA, ppm, 2-D growth, leptosporangiate ferns, sporophytes.

INTRODUCTION

Pteridophytes, known as shade and moisture loving plant, form a sizeable component of the vegetation of Rajasthan. This group is represented by 21 genera and 39 species (Gena, 1998). Bir and Goyal (1982) were the first to provide comprehensive records of the pteridophytic flora of Mt. Abu, listing 22 species of ferns. Sitamata forest, located in Chittorgarh and Pratapgarh district in the southern part of Rajasthan, comprises 422.95 sq. km and is known to be one of the richest localities of pteridophytes in the state. Behera et al. (2022) reported that *Ceretopteris thalictroides* is consumed as a leafy vegetable and used medicinally, with an antibacterial activity against Streptococcus mutans and Shigella flexneri. However, the taxa in this area are likely facing extinction due to natural and manmade factors (Yadav, 2008). This necessitates the formulation of conservational strategies for this rapidly depleting group of plantsIn light of this present work, the effect of different concentrations of Indole-3-Acetic Acid (IAA) from 1 to 10 Parts Per Million (ppm) in an even mode after 1 ppm treatment on spore germination and gametophytic development, starting from 2-dimensional (2-D) growth, spatulate growth, cordate gametophyte and gametophyte with germinal organs of Cheilanthes thalictroide has been studied. Previous studies have investigated the effect of growth hormones on sporegermination and gametophyte

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development in ferns (Hurel-Py, 1943; Miller, 1961; Hickok and Kiriluk, 1984). An extensive review of the gametophytic generation and experimental studies has been provided by Bir (1987). Sharma and Vangani (1988) investigated the effect of Gibberellin (GA3) on spore germination, gametophyte development and sex expression in Cheilanthes farinosa Forssk. They reported that small linear gametophytes were protandrous and did not produce archegonia at any stage of development. Large bilobed gametophytes has distinct median ridge and were protogynous. According to them, there was a male to hermaphrodite sequence in prothallial development and the antheridial system was not evident. Gupta and Bhambi (1991) have investigated that cytokinins are better than other hormones for the induction and promotion of spore germination, leading to the faster rate of gametophyte and sporophyte development in *Adiantum capillus-veneris* L. Vidhya et al. (2000) tested 2, 4-D and GA_3 for their effects on spore germination and gametophyte development in three ferns viz., *Ceratopteris thallictroides* L., *Pityrogramma calomelanos* (L.) Link and *Pteris vittata* L. They observed that effect of 2, 4-D on gametophyte development in *Ceratopteris* and *Pityrogramma* were more pronounced than in *Pteris*. Higher concentration of 2, 4-D was found to be detrimental to the gametophyte development. Higher concentrations of GA_3 stimulated early germination of spores of *Pteris*; delay in *Pityrogramma* and without any significant effect in *Ceratopteris.* Low concentration of GA_3 stimulated early appearance of antheridia in *Ceratopteris* and *Pityrogramma* and reduction of number of archegonia were observed in *Ceratopteris* Purohit and Bohra (2007) described gametophyte development and impact of various plant growth regulators (GA_3, IAA and morphactin) on sex expression and sporophyte formation in *Equisetum* L., *Cheilanthes*; Sw. and *Hypodematium*; Kunze which were collected from different localities in the Aravalli hills, Rajasthan.

MATERIALS AND METHODS

For germination experiments, spores of *C. thalictroides* found in marshy place along the road side punga pond (route to jhakham dam) of Sitamata sanctuary from Pratapgarh district were collected in the months of August-September. Spores were surface sterilized by 2% sodium hypochlorite solution and then kept on Knop's nutrient medium (Half strength) in petri-plates. For control set of experiments, one set of petri-plates containing 100 spores on nutrient medium (without growth regulators) was treated as control. Similarly, IAA treatment of vary concentration was given to another set of petri-plates containing 100 spores of selected taxa. Total seven sets of petri plates (including one set as a control) containing spores were taken for study. The culture medium used for these experiments consisted of Knop's major elements and Nitsch's trace elements (1 ppm). The composition of Knop's medium was as follows: KNO_3: 100 mg; $MgSO_4$: 100 mg; $Ca(NO_3)_2$: 400 mg; K_2HPO_4: 100 mg; Distilled water to make dilution of 1000 ml. Composition of Nitsch's medium was as follows: H_2SO_4: 0.5 ml; MnSO4: 3.0 ml; ZnSO4: 7H2O: 500.0 mg; H3BO3: 25.0 mg; CuSO4, $5H_2O$: 25.0 mg; $NaMoO_4$, $2H_2O$: 25.0 mg; $CoCl_2$: 25.0 mg; Distilled water: 1000.0 ml.

Illumination was provided by two 40 watt fluorescent tubes kept at a distance of 60 cm. Spores were allowed to germinate in a culture chamber maintained at 25 ± 2°C. For each treatment, spores were sown in two sets of petri-dishes (7.5 cm. diameter) thus total 14 petri dishes were used each pair is used for different concentration in ppm (1, 2, 4, 6, 8, and 10 ppm as well as control) of IAA. The data are based on counts of 100 spores from each petri-dish.

A control set was invariably included in all the experiments.

Treatment of IAA

For treatment of IAA on spore germination after 48 h of dark inhibition were made to germinate on liquid Knop's medium (half strength) supplemented with Nitsche's trace elements in 7.5 cm petri-dishes. White light was obtained by two fluorescent tubes fixed 60 cm above the petri-dishes. Different sets of petri dishes were supplied with different concentrations of IAA. Stock solutions of required concentration of the plant hormone were prepared to investigate the effect of IAA, and the desired concentrations for the experiment were incorporated in the culture medium and kept in petri-dishes before sprinkling spores on the surface of the medium. Stock solution of IAA was prepared by dissolving the required quantity of the chemical in 2 ml. of ethanol, the required volume of distilled water being added afterwards. Further concentrations were made by diluting this stock solution. Entire experimental work was carried out under aseptic conditions including the use of sterilized glassware in a thermostatically controlled culture chamber in the laboratory. The temperature during the course of these experiments was maintained at 25± 2°C. Microscopic observations were made on an Olympus HSA microscope. Most of the photomicrographic work was done from the temporary preparations using photomicrographic attachment on Olympus trinocular microscope.

RESULTS

To determine the effect of IAA, spores of *C. thalictroides* were treated with different concentrations of IAA ranging from 1 to 10 ppm. The data presented in Table 1 indicate that spores of selected taxa require different period for spore germination. The spores started to germinate after 9 days under control where only 55% of spores were able to germinate while rest undergone dormant and later disintegrate.10 ppm concentration of IAA has been recorded to induce earliest (within 7 days) with minimum spore germination (25%) as compared to other treating concentrations of IAA and control treatment (Table 1). An increase in percent spore germination was observed under 1ppm (60.00%) to 6ppm (66%) concentration as compared to control (55%) treatment. Amongst different concentrations of IAA, maximum spore germination (66.6%) was observed in 2-6 ppm. However, a decrease in percentage spore germination after 6 ppm IAA concentration has been observed. Data represented in Table 2 indicates that number of cells in protonemal filament is found to be varying in different concentrations of IAA at different time periods. 2-D gametophyte percentage was found maximum (80%) after 12 days of

Table 1. Effect of different concentrations of IAA on spore germination of *Ceratopteris thalictroides* from Sitamata wild life sanctuary, Rajasthan.

Serial number	Name of plant	*Ceratopteris thalictroides*	
	Concentration of IAA (ppm)	Days after sowing	% Spore germination
1.	Control	9	55
2.	1	8	60
3.	2	8	66
4	4	9	66
5.	6	9	66
6.	8	10	40
7.	10	7	25

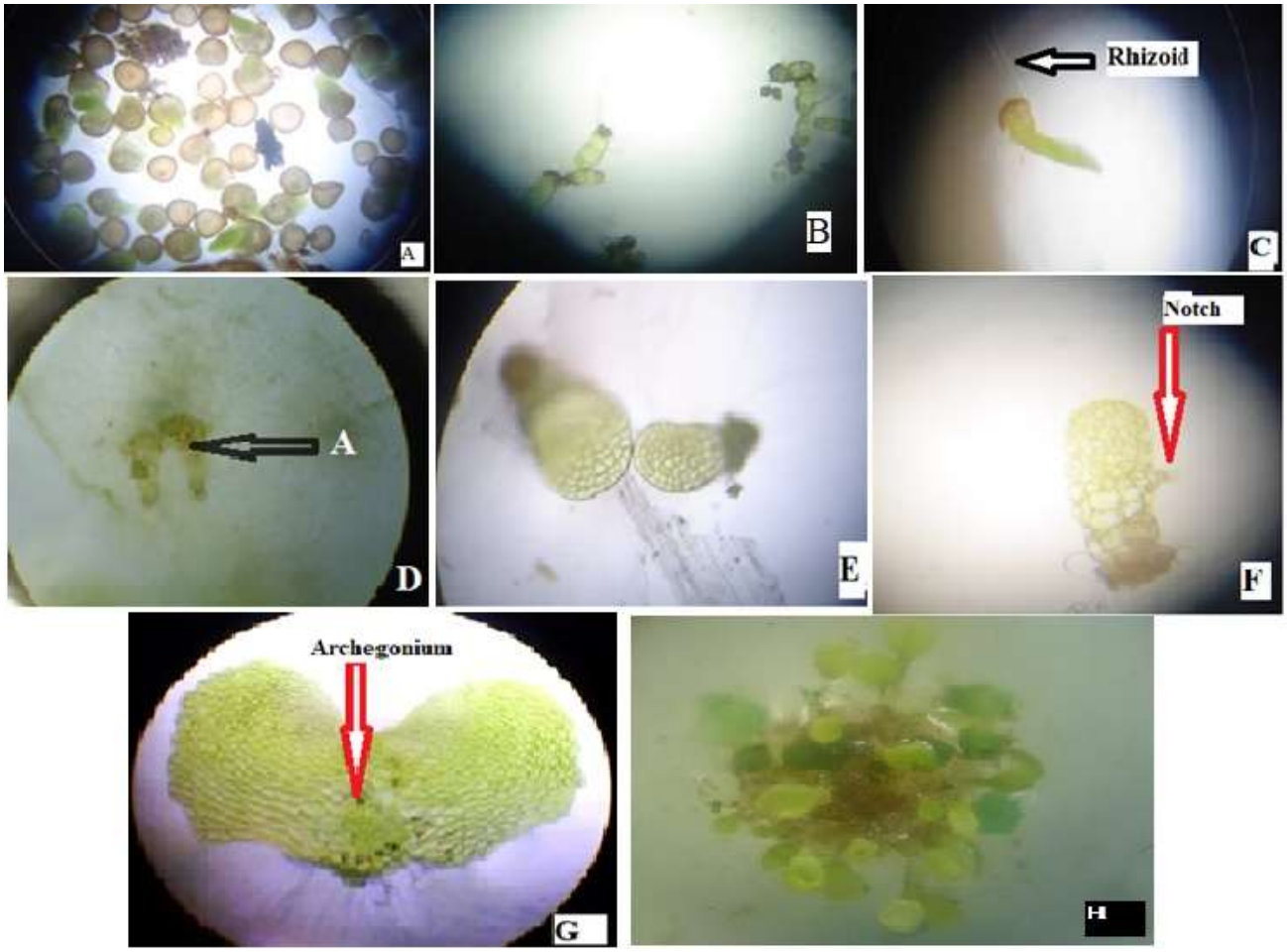

Figure 1. Spore germination and different stages of gametophyte development in *Ceretopteris thalictroides*, Sitamata forest (A-H). A. Initiation of Spore germination in 4 ppm concentration of IAA (after 9 days of sowing); B-C. Protonema formation with rhizoids in 8 and 2 ppm of IAA respectively; D. Spatulate gametophyte with "Antheridia (A)" in control (after 14 days of sowing); E. Spatulate gametophyte in 6 ppm of IAA (after 17 days of sowing); F. Cordate gametophyte showing notch in 6 ppm IAA (after 21 days of sowing); G. Mature prothallus showing Archegonia in 2 ppm IAA (after 32 days of sowing); H. Clusters of Sporophyte in 8 ppm concentration of IAA (after 38 days of sowing).

sowing in 1ppm of IAA while minimum (23%) after 19 days of sowing in 10 ppm and percentage growth of others were lies in between these two. Similarly, the highest percentage growth of spatulate gametophyte

Table 2. Effect of different concentrations of IAA on gametophyte development in *Ceratopteris thalictroides.*

Serial number	IAA Conc. (ppm)	Protonemal filament		Initiation of 2-D growth		Spatulate gametophyte		Cordate gametophytes		Development of sex organs				% of gametophyte with sporophyte	
		Days after sowing	No. of cells sowing	Days after sowing	% of 2-D gametophyte	Days after sowing	% of spatulate gametophyte	Days after sowing	% of cordate gametophyte	Days after sowing	Gametophyte with Antheridia%	Days after sowing	Gametophyte with Archegonia %	Days after sowing	Gametophyte with sporophyte %
1	Control	10	3	12	40	14	64	18	63	27	66	35	45	43	72
2	1 ppm	10	7	12	80	13	67	17	71	27	52	30	61	35	70
3	2 ppm	10	7	11	45	13	56	18	54	26	35	32	42	36	65
4	4 ppm	11	3	12	44	15	60	19	50	26	43	32	43	38	80
5	6 ppm	10	5	12	64	17	52	21	53	25	42	31	28	36	80
6	8 ppm	12	6	13	36	18	36	20	20	23	37	28	24	38	50
7	10 ppm	9	6	19	21	18	23	17	16	53	28	46	15	60	42

(67%) and cordate gametophyte (71%) are observed in 1 ppm of IAA treatment after 13 and 17 days of sowing respectively (Figure 1, D and E). Sex organs were developed after a month of spore inoculation. However, the further growth of these gametophytic portions underwent decline from 6 to 10 ppm (Table 2). The gametophytes were found protandrous under control and hormonal treatment. Antheridial formation took place in spatulate gametophyte (Figure 1D). Sex organs begin to develop after 20 days of sowing. Antheridial formation takes place earlier then archegonia and it is found that treatment of IAA usually has not promontory effect for the development of antheridia in compare to control (66%) except 1 ppm (55%). However, 1 ppm of IAA causes earlier promoter effect for the development of archegonia (61%) in compare to control (45%) and other concentrations of IAA. Similarly, Yadav and Uniyal (2021) studied in vitro spore germination and gametophyte development of *Drynaria mollis* Beddome, and *Pteris aspericaulis* wall and noticed early development of antheridia after 90 days of sowing and later archegonial formation began after 100 days of sowing in both the treated species. Delayed formation of sex organs and the least number of antheridia and archegonia are recorded in 10 ppm. Sporophytes were observed after 45 days in control while in treated plates they appeared earlier between 35-38 days of sowing. Maximum percentage of sporophytes (80%) was calculated in 4 ppm and 6 ppm hormonal treatment (Figure 1). The least percentage observed in 10 ppm after 2 months of sowing.

DISCUSSION

The effect of growth regulators on spore germination under different concentration of IAA indicate that the spores of *C. thalictroides* start to germinate earlier in different concentrations of treated hormone except 8 ppm where it causes little delayed to germinate. In control the germination was recorded on 9th days of sowing. Raghavan (1971b) reported that the longer period of exposure of white light for the induction of spore germination may be due to longer hydration period. Under 10 ppm of IAA treatment the spores of *C. thalictroides* germinate earlier while percent spore germination decreases gradually from the concentration of 1to 8 ppm. Thus, the spore germination is not much affected under PGRs in the selected fern taxa of Sitamata forest as reported by Sharma et al. (1996) in some common ferns of Rajasthan. Roshni and Hegde (2020) suggested that the spore germination of *Pityrogramma calomelanos* L. needed knops medium for in vitro culture and follow vittaria-type of spore germination pattern. Prothallus development is of Ceretopteris-type. In general, germination and protonemal development take one to two weeks under optimum conditions and mature gametophytes are formed after 6-8 weeks (Table 2). Similar observations were made by

Praptosuwiryo (2017) who reported that the germination of spores in *Platycerium wande* Racib takes place between 7-14 days on natural media. However, in *C. thalictroides*, fertilization can take place only in two weeks after germination (Stein, 1971). The whole life cycle can be completed in vitro in 3-4 months in *C. thalictroides* and *C. pteridiodes* (Loyal and Chopra, 1977). The gametophytic development of *Christella hispidula* (Deene) Holttum has been described and illustrated by Bejoy et al. (1994).

Sex organs were developed after a month of spore inoculation. The gametophytes were found protandrous under control and hormonal treatment. Maximum percentage of gametophytes with sporophytes in *C. thalictroides* (Table 1) was observed in 4 and 6 ppm concentration of IAA in comparison to the control. A decrease in percentage development of sporophytes in selected taxa is observed under higher concentration of IAA. 1ppm concentration of IAA has been found effective in antheridial development however; Ohishi et al. (2021) suggested that GA_4 stimulates both protonemal elongation and antheridium formation in *Lygodium japonicum* Thunb. On the other hand, IAA promoted protonema elongation but causes reduced antheridium formation. Overall it may be concluded that formation of spatulate gametophytes and antheridial development in this taxa are not favoured by hormone, especially at higher concentration of IAA, while archegonial development is enhanced by the minimum concentration of treated hormone as compare to control in the selected fern taxa.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

ACKNOWLEDGMENT

The author is grateful to Dr. B.L. Yadav for his valuable guidance and cooperation during the research and to M.L.V. Govt. College, Bhilwara (Raj.) for the laboratory facility provided.

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