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Research Article

EXPERIMENTAL STUDIES ON GAMETOPHYTIC GENERATION OF *PTERIS* SPECIES FROM MT. KAROL (DIST. SOLAN) HIMACHAL PRADESH

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A R T I C L E I N F O

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A B S T R A C T

A fern gametophyte is a small, photosynthetic organism which has the capability to grow normally in a culture. It bears both the sex organs indicating that self- fertilization is probably the rule. But this would lead to a great deal of homozygosity and hence little morphological variation. Actually ferns exhibit a lot of morphological and cytological variation. The objective of this study was to investigatethe reason for these variations. In the present work the gametophytic development of the three species of *Pteris* have been described, *P. pseudoquadriaurita, P. stenophylla and P. vittata* subsp. *vittata.* The ripe spores of these species were raised on half Knop's nutrient medium and their growth was studied.

INTRODUCTION

Fern gametophyte is the simplest photosynthetic organism capable of normal development in a culture. That there is a distinct potentiality to produce homothallic prothalli along with the formation of two types of gametangia simultaneously in homosporous ferns has been very well documented in the literature. In the absence of any barrier to self-fertilization, it is to be expected that the occurrence of intra-gametophytic selfing in the systems with hermaphrodite prothalli will yield completely homozygous sporophytes. But the ferns exhibit a lot of morphological and cytological variations which is obviously due to cross fertilization. A great deal of work has been done in this field by Klekowski and Lloyd(1968), Lovis(1977), Walker(1979), Cousens(1979), Verma(2013) etc. The gametophyte generation requires to be understood to realise its various adaptations which tend to control or influence their mating systems. In the present work the reproductive biology of three *Pteris* species of homosporous ferns from Mt. Karol have been investigated and all of them have been found to possess features that tend to favour or ensure intergametophytic mating to various degrees.

Mt. Karol or Karol tibba is located on Kalka- Shimla highway in Solan district, Himachal Pradesh, 2kms before Kandaghat and 12 kms beyond Solan towards Shimla. Mt.Karol is the highest peak of this area with a maximum altitude of around 2000mts..

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The forest here is quite rich in ferns due to water being plenty. It shows a great deal of diversity and variation. Ferns grow here on the forest floor, on forest slopes, banks of waterways, in open or in shaded places.

Pteris L.belongs to the family Pteridaceae. About 280 species of *Pteris* are distributed in tropical and subtropical regions of the world. Species of *Pteris* can grow in both warmer and cold temperatures. The *Pteris* species can easily be distinguished because of their sori characters. The sori are linear located in the margins of the leaves. A false indusium protects each sorus.

MATERIALS AND METHODS

Fertile fronds of the three *Pteris* species were collected from Karol area in the months of September and October. These fertile leaves were put in paper envelopes and were kept in dry conditions till the spores were released from the sporangia. Although the gametophytes raised on soil would be ideal for mating system analysis, the periodic examination of such prothalli is rather difficult since these cannot be placed back in the soil. So the technique of sowing fern spores by floating them on the surface of liquid medium was carried out. The cultures of gametophytes were raised on simple inorganic half Knop's nutrient solution without supplementing with sugar and/or growth hormones. The cultures were kept under conditions of diurnal variation of room temperature and day length.

The spores were sown in November in sterilized petri dishes containing 20 ml of ½ Knop's solution. A small quantity of spores were spread evenly on a clean paper. The paper was

then inverted over the culture dish and gently tapped. An almost uniform distribution of spores was obtained on the surface of the nutrient solution. The cultures were replenished with fresh nutrient solution whenever required. Random samples of 30-50 gametophytes per culture dish were scored. All the studied prothalli were placed back in the parent culture.

Observations

The spores germinated within 4-12 days of sowing. Prothallial initial appears first followed by a filamentous initial. It was noticed that there are some fast growing gametophytes and some are slow growing. All the prothalli were studied periodically at 10 days intervals. The slow growing gametophytes usually remain only male throughout their life due to certain chemicals that are leached out from the fast growing gametophytes.

Pteris pseudoquadriaurita Khullar
It is a very common fern of the forest growing between 1300-2000 m.

The spores are trilete, roughly triangular, measuring about 35-45 X 50-56 µm. These are dark brown to yellowish brown in colour and without the perine. A single prominent band of thickening runs along the equator of the spore.

The spores germinate within 6-8 days of sowing. An apical cell is cut off within 15 days of sowing and as the thallus grows it becomes asymmetrical with one wing smaller than the other. After a few days the thallus becomes cordate but in a few cases it retains its asymmetry for long. The antheridia appear on the spathulate and ameristematic prothalli in 9-13 weeks, both at the margin as well as on the wings. Archegonia appear on the same prothallus after 23-24 weeks of germination. (Fig. 1).

Fig 1 Pteris Pseudoquadriaurita

***Pteris stenophylla* Wall**

Found commonly between altitude of 1000-1500 m in moist and open places.
The spores are tetrahedral, measuring about 35-45 X 50-56 µm. with a granulose to rugulose exospore and a collar like equatorial ridge. Perine is absent. The spores germinate within 8-9 days of sowing by cutting off a prothallial initial. The antheridia appear as spathulate and cordate gametophytes within 18-19 days of sowing. The antheridia are present on the margins and on the lower half of the cushion. The archegonia appear within 21-22 weeks of germination.(Fig.2).

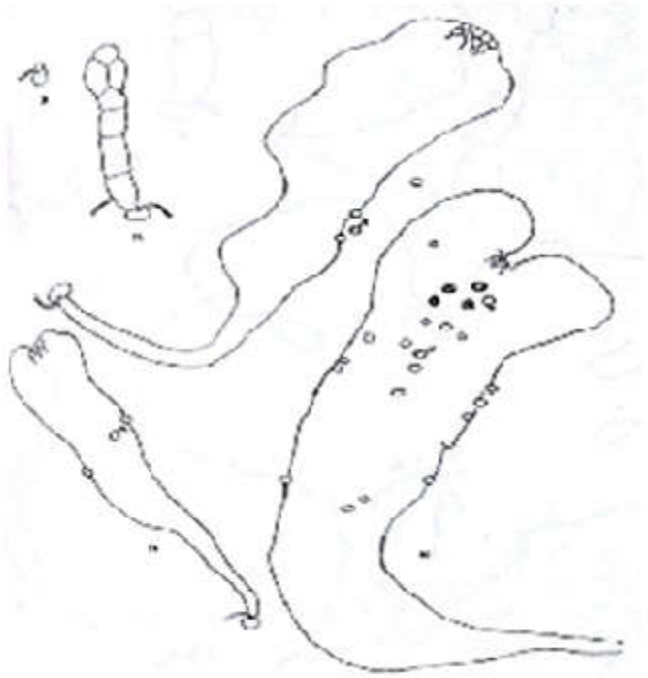

Fig 2 Pteris Stenophlla

Pteris vittata L. subsp. *vittata*

Very common along the streamlets or river banks, on open roadsides or on walls up to 2000m.

The spores are tetrahedral 35-45 X 50- 66 µm in size with a granulose to rugulose exospore. Perine is absent. A thick band is present along the equator of the spore.

On germination the spores first cut off a prothallial initial. Soon the gametophyte assumes a spathulate outline which later becomes cordate. The adult prothallus is naked with a distinct midrib and broad wings that become uplifted so that the gametophyte has a funnel shaped outline. The prothallus is quick growing and reaches maturity in 13-20 weeks. Rhizoids are restricted to the lower surface of the midrib, are thin walled, nearly hyaline, non- chlorophyllous with a slightly dilated base. Antheridia are produced at the early stages of their development. Some of the antheridia bearing young gametophytes exhibit arrested growth, remaining small and non-meristematic. (Fig.3).

Fig 3 Pteris vittata subsp vittata

Table Showing Percentage of Germination

S.No	Name of the species	Germination(No. of days from the date of Sowing)				Initial sex	% of males	% of bisexual gametophytes
		5%	10%	50%	%			
1.	*Pteris quadriaurita*	6	10	20	96	Male	68%	87%
2.	*Pteris stenophylla*	8	13	23	89	Male	65%	81%
3.	*Pteris vittata* subsp.*vittata*	7	9	14	95	Male	70%	90%

RESULTS

The starting point of spore germination was the emergence of rhizoids/ green prothallial cell from the ruptured triradiate mark of spores. Usually the germination begins about 6-8 days of sowing and 20 - 30% and sometimes up to 50% of the spores germinate earlier than the bulk of the spores. Maximum germination of up to 96% was recorded in the three species of *Pteris.* In the above three species studies antheridia were the first to appear. Bisexual prothalli originated from initially male prothalli.

DISCUSSION

The primary protonemal cells give rise to 4-9 celled filaments which become abundant during the third week from sowing. These develop further into spathulate and cordate gametophytes of different types. The fully grown meristematic prothalli, often referred to as" heart- shaped"or cordate are initially asymmetrically bilobed which later on become more or less symmetrically bilobed, dorsiventrally flattened and consist of thickened midrib and unistratose lateral wings with elongated cells.The developing population of gametophytes contain different forms of prothalli in variable frequencies when scored at intervals. The differentiation of archegonia occurs with the development of meristem as well as the central cushion, whereas the antheridial production is independent of the development of this 3-dimensional, central cushion on the meristematic prothalli and these may even be borne on spathulate and filamentous prothalli.

The filamentous and spathulate prothalli bear only antheridia whereas the meristematic prothalli can bear both antheridia and archegonia. Nayar and Kaur(1971). The production and persistence of antheridiogen in the cultures suggest some antheridiogen activity which needs to be explored further. Kurumatani *et al*. (2001). It is now realized that not all homosporous ferns can undergo intragametophytic selfing habitually which can further promote successful colonization in some homosporous ferns. Suter *et al*.,(2000).

The ability to reproduce by intergametophytic selfing facilitates rapid spread. Some adaptations that tend to favour intergametophytic mating are (i) different timing in the appearance of sex organs in the same gametophyte. (ii) mutual effect of gametophytes i.e. when a fast growing gametophyte reaches a spathulate stage certain chemicals called as antheridiogens are leached out which inhibit the growth of other gametophytes so that they grow slowly.and produce sex organs a little later than the fast growing gametophytes.

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