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# An adverse effect of meiotic abnormalities on spore fitness in medicinal fern *Glaphyropteridopsis erubescens* (Wall. ex Hook.) Ching

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**Abstract.** *Glaphyropteridopsis erubescens* plants were collected from Parvati valley, Himachal Pradesh, India. Meiotic investigations in the accessions collected from different areas of Himachal Pradesh revealed normal meiosis and good spore fertility. However, the accessions collected from Parvati valley has depicted meiotic anomalies and high spore abortion index. All populations shared the same cytological status of $2n = 72$, which is in line with previous records. Individuals depicted normal behavior at diakinesis and metaphase-I but later stages were abnormal. It includes chromatin stickiness, interbivalent connections, early and late disjunction. The formation of laggards at anaphase-I lead to series of abnormalities such as chromatin bridges, random sub-grouping of chromosomes at anaphase-II, chromatin fragmentation and irregular sporogenesis. Large numbers of micronuclei were present in triads and tetrads along with pycnotic nuclei. Empty and some abnormal sporangia with heterogenous spores were also observed.

**Keywords:** *Glaphyropteridopsis*, abnormal, meiosis, sporogenesis, pteridophyte, Parvati valley.

## INTRODUCTION

Pteridophytes are treasures of valuable secondary metabolites that helped them to withstand harsh environmental conditions. Medicinally, they are reported to have antioxidant, antimicrobial, antiviral, anti-inflammatory, antitumor and anti-HIV properties (Baskaran *et al.* 2018). Fern and fern-allies are the major medicinal resource of phenols, flavonoids, alkaloids and steroids along with some unique phytochemicals having numerous industrial applications (Shinozaki *et al.* 2008). The tribes of North West India are using crosiers, rhizomes and fronds of *Adiantum capillus-veneris*, *Actiniopteris radiata*, *Adiantum caudatum*, *Adiantum philippense*, *Adiantum venustum, Diplazium esculentum, Pteris vittata, Pteris wallichiana, Asplenium trichomanes, Asplenium nidus and Angiopteris evecta etc.* in different formulations of drugs and ointments. *Thelypteris* species have been used to

cure cold, itching, nerve pain, rheumatism, swellings, spermatorrhea, body pain, backache, cuts and wounds, bone fracture, sprain, gastric trouble, malaria fever, body pain, sprain, boils, cancer and asthma (Sureshkumar *et al.* 2018).

North West Himalayas are facing major threat to biodiversity due to habitat destruction and pollution. They are the reservoirs of medicinal ferns as they are habitat specific and needs special care if grown in nurseries and botanical gardens. Some of high altitudinal ferns are nearly impossible to grow outside their natural habitat like presently studied fern. So, evaluation of wild accessions from different parts will be helpful in finding new and stable morphotypes and chemotypes among them. The reproductive success of medicinal ferns largely depends upon spore viability. This can be influenced by meiotic behavior and sexual status of the species. Meiotic abnormalities such as chromosomal fragmentation, bridges, laggards, micronuclei and cytomixis are known to cause sterility in number of plant species. Plant chromosomes exhibit different types of aberrations caused by mutagens, radiations or temperature etc. and they acts as sensitive indicators to environmental pollutants. So, higher plant systems acts as an indicator of possible genetic damage caused by environmental mutagens (Grant 1978).

This paper deals with meiotic investigation of *Glaphyropteridopsis erubescens* (Wall. ex Hook.) Ching (=*Thelypteris erubescens* (Wall.) Ching (Thelypteridaceae), an important medicinal fern collected from disturbed areas of Parvati valley. This family comprises 20 genera and 1000 species growing in forests with ravines of low mountains, between altitudes of 800-2000m, distributed mainly in China, Taiwan, Japan, Myanmar, Pakistan, Philippines, Vietnam, Nepal and Bhutan. In India, *G. erubescens* grows well in shady habitats of Himachal Pradesh, Uttarakhand, Sikkim, Darjeeling, Arunachal Pradesh, Meghalaya and Jammu and Kashmir. Plants of *G. erubescens* are 2–3m tall, the rhizomes are stout and they are decumbent, woody and glabrous. Fronds are clustered, stipes are 1–2 m, thicker than 1 cm, ribbed, glabrous, throughout stramineous and often reddish. The pinnae are in 40–50 pairs per frond, opposite, sessile and proximal several pairs strongly oblique distally.

This fern has noteworthy medicinal potential as leaf decoction is used to treat indigestion, dough of fronds is applied externally for rheumatism and root powder is used as an antidote in scorpion bite in Nigeria (Nwosu 2002). Powder of dried rhizome mixed with rice water can be internally administered for gonorrhea, especially for leucorrhea by the people of Deoprayag area

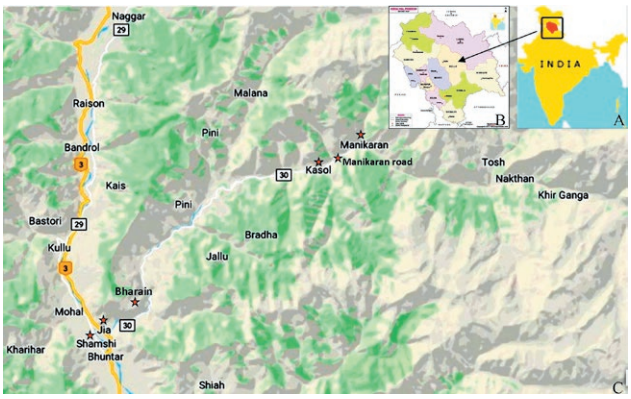


**Figure 1.** (a) Map showing the geographical location of Himachal Pradesh in India; (b) Map depicting various localities of Himachal Pradesh (Arrowed Kullu district of Parvati valley); (c) Localities visited for the collection of accessions from Parvati valley (highlighted with star).

in Garhwal Himalayas, India (Gaur and Bhatt 1994). *G. erubescens* is a natural source of kaempferol derivative kaempferol-3-O-α-L-rhamnoside involved in a variety of signaling pathways. Kaempferol has potential to significantly modulate a variety of signaling pathways that are induced in many adverse clinico pathological conditions (Kashyap *et al.* 2017). In the North East of India, fresh fronds of fern locally called "pire uneu" is used in the making of fermented cakes called marcha, amylolytic mixed starters similar to other regions of North East such as hamei of Manipur, pham, ipoh and phab of Arunachal Pradesh, humao of Assam and thiat of Meghalaya (Tamang *et al.* 2012).

During exploratory surveys to analyze diversity in medicinal plants of North West India, we had collected accessions from Parvati Valley (Figure 1). Its vernacular name is "Vaarne" and rhizomes were being used to prepare decoction for reproductive disorders by local people. Parvati valley is situated between 31°21'21"–32°25'0"N and 76°56'30"–77°52'20"E in South east of Kullu, altitude ranging between 1100–5550m above mean sea level. The valley is under extreme biotic and abiotic pressure due to rampant tourism, proliferation of concrete structures, construction of roads, dams, piles of garbage, hippie culture, drug trafficking (marijuana or hash) which had affected the biodiversity in recent years. All these activities result in degradation of forests and pose serious threat to fern inhabitants along with other plant communities (Sharma 2005).

Meiotic abnormalities were found to be consequence of extreme environmental factors in some species. Abortion in microspores of *Isoetes sinensis* was linked to anthropogenic activities causing habitat fragmentation

and decrease in numbers drastically (Heng-Chang *et al.* 2007). The present study aims to analyze the abnormal meiotic course and spore abortion index in this fern from disturbed locations within Parvati valley, Himachal Pradesh, India. The present work is the first attempt to study the cytology of this fern species from Parvati valley.

## MATERIALS AND METHODS

The material for meiotic studies was collected from wild plants growing at various localities of Parvati valley, Himachal Pradesh, India in monsoon season (July to September). Pinnae from fronds with young sporangia were fixed in Carnoy's fixative ethanol: chloroform: glacial acetic acid (6:3:1, v/v) for 24 h at room temperature and then transferred to 70% ethanol and stored under refrigeration until use. To study meiotic course, young sporangia were squashed in 1% acetocarmine. A number of freshly prepared slides per plant were carefully examined and several spore mother cells (SMCs) were observed to determine chromosome number and frequency of meiotic abnormalities at different stages. Specimens were dried by keeping them in the folds of blotting sheets to remove excess moisture and they were supervised timely to keep them away from fungal growth or any other type of contamination. Mature spores taken onto the microscopic slide with the help of needle were treated with 1:1 glycerol and acetocarmine mixture (Marks 1954). The procedure followed for observations on number of spores per sporangium was as given by Huang *et al.* (2011). Minimum number of sporangia (8-10) was dealtwith at a time. Well stained, normal looking spores were observed for calculating average spore size and spore fertility. Distorted, deformed and poorly stained spores were taken as sterile. The spore abortion index (SAI) was calculated (Hornych and Ekrt 2017). Photomicrographs of SMCs and spores were taken from the freshly prepared slides using Magnus MLX microscope. Identification was done from fern floras (Beddome 1870; Dhir 1980 and Khullar 1994), efloras and by comparing specimens with already deposited specimens of Prof. S. S. Bir at Herbarium, Department of Botany, Punjabi University, Patiala. Finally, pressed and dried voucher specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala and their accession numbers (PUN) were obtained (Table 1).

## RESULTS AND DISCUSSION

The cytological information for Thelypteridaceae is available for 51 species and in genus Thelypteris, there are seven basic chromosome numbers, *viz*. 36, 35, 34, 32, 31, 30 and 27 (Loyal 1963). Meiotic behavior was studied in six populations of *G. erubescens* from Parvati valley, Himachal Pradesh, India. The chromosome count of the presently investigated fern is in line with previous reports of $n = 36$ from the Himalayas (Verma and Loyal 1960; Loyal 1961; Loyal 1963; Mehra and Khullar 1980; Khullar *et al.* 1983, 1988), Nepal (Roy *et al.* 1971) and South India (Irudayaraj and Manickam 1987).

Meiosis is dynamic cellular process which maintains genome stability and integrity. Any error in meiotic process can lead to variations in chromosomal structure and ploidy level. Plant species are most acceptable material for meiotic studies because of availability of more genetic resources (Cai and Xu 2007). The accessions collected from Parvati valley depicted meiotic anomalies and high spore abortion index (**Figure 2**) while large number of populations from other parts of North West India were normal. SMCs showed a high degree of abnormalities at all stages of meiosis such as stickiness, unoriented bivalents, laggards, early and late disjunction

**Table 1.** Populations with altitude, voucher specimen, percentage of abnormal SMCs in *G. erubescens*.

| Populations with altitude | PUN* | Meiotic abnormalities (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Stickiness | Unoriented bivalents | Laggards | Bridges | Early disjunction | Late disjunction | Interbivalent connections | Chromatin fragmentation |
| HP: Jia (1,125m) | 4867 | 36.31 | 7.89 | 13.15 | 11.84 | 3.94 | 5.26 | 7.89 | 19.73 |
| HP: Manikaran Road (1,423m) | 4868 | 32.30 | 7.69 | 6.15 | 10.76 | 9.23 | 4.61 | 6.15 | 9.23 |
| HP: Bharain (1,889m) | 4869 | 25.53 | 12.76 | 10.63 | 6.38 | - | - | - | - |
| HP: Shamshi (1,111m) | 4870 | 35.71 | 14.28 | 9.52 | 4.76 | 7.14 | - | 9.52 | - |
| HP: Kasol (2,619m) | 4871 | 42.37 | 6.77 | 5.08 | 8.47 | 3.38 | - | 10.16 | - |
| HP: Manikaran Sahib (1,760m) | 4872 | 46.77 | 3.22 | 6.45 | - | 4.83 | 1.61 | - | 8.06 |

PUN*= Accession number Herbarium code of Department of Botany, Punjabi University, Patiala, HP=Himachal Pradesh, India.

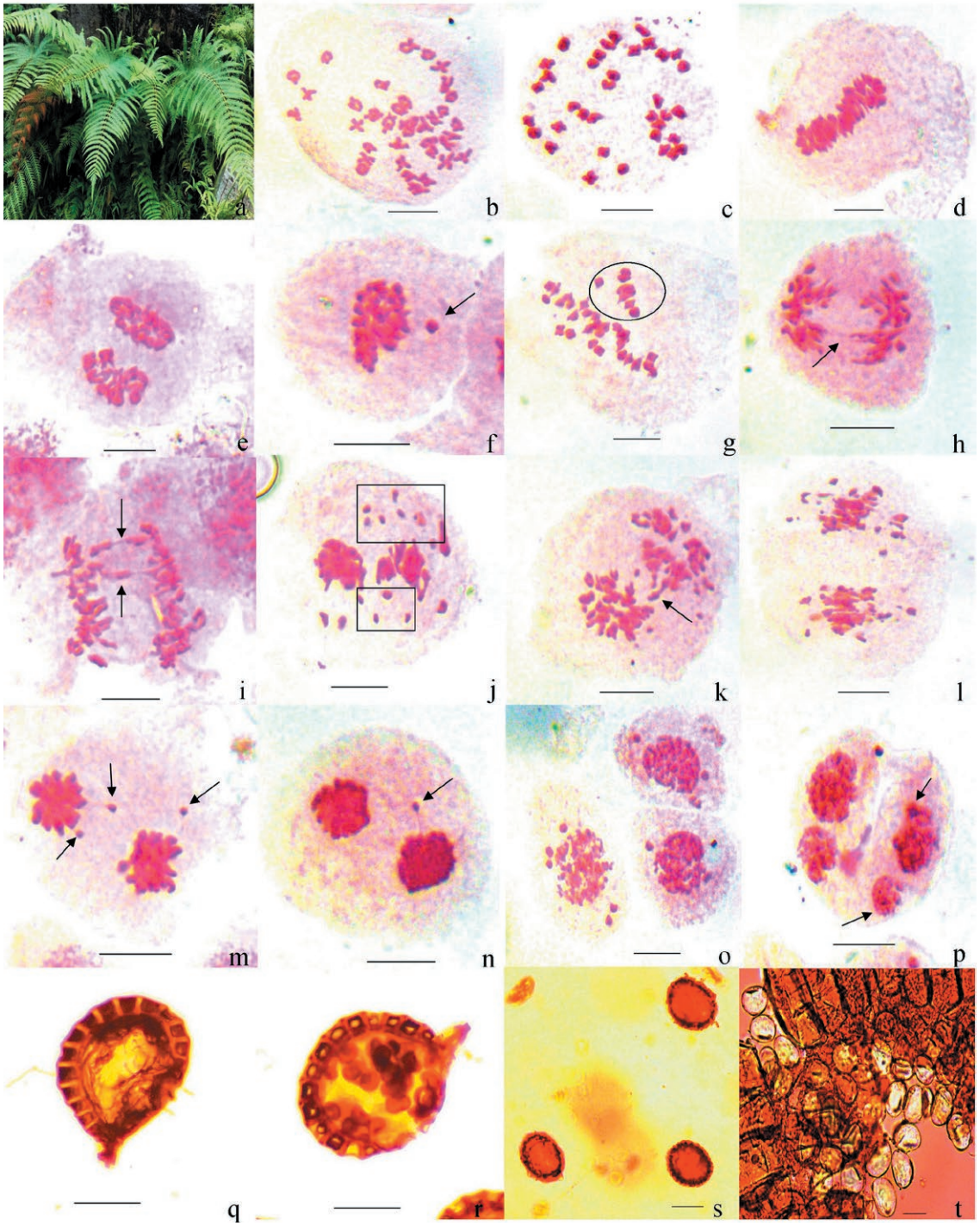


**Figure 2.** (a) *G. erubescens* growing wild; (b) SMC showing $36_{II}$ bivalents at diakinesis; (c) SMC at metaphase-I showing $36_{II}$; (d) Chromatin stickiness at metaphase-I; (e) Stickiness prevailing at anaphase-I; (f) SMC with unoriented bivalent at metaphase-I (Arrow indicating one unoriented bivalent); (g) Bivalents encircled showing early disjunction at metaphase-I; (h) Arrow pointing to single chromatin bridge at anaphase-I; (i) Arrows showing multiple bridges at anaphase-I; (j) Extreme clumping of bivalents into amorphous mass with random sub grouping of chromatin material in marked area; (k) Arrow indicating laggards at anaphase-I along with agglomerisation of chromatin; (l) SMC at telophase-I with several fragments resulting from breakage; (m) Three vagrants indicated by arrows at anaphase-I; (n) Arrow showing one vagrant at telophase-I; (o) Numerous (9) micronuclei at tetrad stage; (p) Polyad with marked pycnotic nuclei; (q) Empty sterile sporangium; (r) Abnormal sporangium with varied number of spores (instead of expected 64 spores); (s) Heterogenous spores; (t) Group of sterile spores. Scale bar = 10μm.

**Table 2.** Sporogenesis of populations with spore abortion index and spore size.

| Sporogenesis | Populations* | | | | | |
|---|---|---|---|---|---|---|
| | Jia | Manikaran Road | Bharain | Shamshi | Kasol | Manikaran Sahib |
| Number of sporads observed | 98 | 103 | 84 | 92 | 106 | 107 |
| Normal tetrad number (%) | 45(45.91) | 61(59.22) | 52(61.90) | 51(55.43) | 60(56.60) | 65(60.75) |
| Tetrad with micronuclei (%) | 40(40.81) | 31(30.09) | 15(17.86) | 21(22.83) | 29(27.36) | 20(18.69) |
| Tetrad with micronuclei+pycnotic nuclei (%) | 8(8.16) | 6(5.82) | 10(11.90) | 16(17.39) | 12(11.32) | 10(9.35) |
| Triad with micronuclei (%) | 5(5.10) | 5(4.85) | 7(8.33) | 4(4.25) | 5(4.71) | 12(11.21) |
| Spore abortion index | 55.47±2.64 | 45.17±1.93 | 38.30±2.39 | 49.80±3.06 | 43.63±1.31 | 37.55±0.52 |
| Spore size (μm) | 42.42×33.42<br>39.51×29.57 | 41.85×32.38<br>39.74×29.01 | 42.72×33.83<br>38.76×29.02 | 42.56×32.26<br>38.71×28.97 | 42.35×31.64<br>39.86×29.65 | 42.23×32.09<br>38.67×29.82 |

Populations* = All the populations were collected from different localities of Parvati valley.

of chromosomes, interbivalent connections, chromatin bridges and chromatin fragmentation which leads to abnormal sporogenesis (Table 1, 2).

SMC with $n = 36$ bivalents were recorded at diakinesis (Figure 2b) and metaphase-I (Figure 2c). Chromatin stickiness prevailed at almost every stage, more prevalent at metaphase-I (Figure 2d) and anaphase-I (Figure 2e) in every population ranging from mild to severe (chromosomes appeared to be an amorphous mass with no identity) affecting 25–47% of SMCs. Chromatin stickiness can lead to serious consequences of spore sterility as these abnormalities were found to be interlinked to each other. This abnormality becomes evident at metaphase-I stage when chromosomes form intense chromatin mass but if it continues to anaphase-I obviously it will pose difficulty in bivalent separation, forming chromatin bridges. The maximum percentage of stickiness was present in population collected from Manikaran Sahib (HP) (46.77%). It has certainly affected pollen viability in many other species as they are unbalanced through irregular chromosome segregation and fragmentation (Golubovskaya 1989 and Rao *et al.* 1990). The bivalents which failed to orient themselves on the equatorial plane due to spindle abnormalities were present as unoriented bivalents (Figure 2f). The maximum percentage of unoriented bivalents was found to be present in population collected from Shamshi (HP) *i.e.* 14.28% and minimum in case of Manikaran Sahib population (3.22%). This random orientation of chromosomes and their consequent sub-grouping (Figure 2j) results due to irregular spindle activity while a single and transient spindle during mitosis and meiosis ensures the proper chromosome segregation (Caetano-Pereira and Pagliarini 2001). Interbivalents connections involving 3–4 bivalents was another phenomenon observed in four populations in which maximum percentage was in Kasol (HP) population (10.16%). These interbivalent connections were meant to keep the bivalents together before spindle formation in order to guarantee orientation of chromosomes in division plane but fusion of heterochromatic regions will result in formation of chromatic knots (Viinikka and Nokkala 1981). These diffused connections between the bivalents are associated with origin of polyploid species (Malgwi *et al.* 1997).

Early disjunction at metaphase-I (Figure 2g) and late disjunction at anaphase-I was found to be the possible reason for the presence of laggards and chromatin bridges at later stages. It has been found that late disjunction was present in three populations only ranging from 1–5%, and early disjunction *i.e.* within range of 3–9%. We came across two types of non-synchronization disjunction of bivalents early and late which arises due to different rates of terminalisation of various chromosomes of complement (Darlington 1937), changed homology of chromosomes (Koul 1971) or absence of coordination between chromosome and spindle (Sharma 1976). Early disjunction of bivalents does not affect the normal distribution of chromosomes at anaphase I but late disjunction which is more pronounced in hybrids and diploids with meiotic abnormalities will lead to bridges and laggard formation and consequently pollen malformation (Wang *et al.* 2004). This abnormal phenomenon is quite significant as it can lead to formation of gametes with numerical variations in chromosome number (Singhal *et al.* 2010). Laggards at anaphase-I (Figure 2k) are noticeable phenomenon as compared to other abnormalities involving 5–13% of SMCs, with maximum percentage in case of Jia (HP) population (13.15%). Sometimes these laggards were found to be stretched in between two poles forming single (Figure 2h) or multiple chromatin bridges (Figure 2i). Bhattacharjee (1953) observed bridge like con-

figuration which might be originated due to interlocking of chromosomes starting from prophase, persists till metaphase and during its separation at anaphase stringing out of cytoplasmic strands between them, resulting in one or two false bridge like configurations. These bridge fragment configuration is considered to be the result of crossing over in heterozygous paracentric inversions (Saylor and Smith 1966). This may lead to unequal distribution of chromosomes resulting in abortion and heterogenous pollen grains. Chromatin fragmentation resulting from breakage of chromosomes was observed at anaphase-I and telophase-I (Figure 2l) and some vagrants were observed at anaphase-I (Figure 2m) and telophase-I (Figure 2n). The possible causes of presence of laggards at anaphase were asynapsis, desynapsis, failure of chiasma formation and premature disjunction of bivalents (Gupta and Priyadarshan 1982) and abnormal spindle formation (Tarar and Dhyansagar 1980). These laggards when permanently failed to reach poles often constituted micronuclei during microsporogenesis (Jiang *et al.* 2011). One such example is the case of diploid species of *Clematis flammula* L. where meiotic abnormalities and cytomixis had resulted in pollen malformation (Kumar *et al.* 2008). Extra chromosomes were reported in case of *Ophioglossum* but in present case chromatin fragmentation were found to be present at anaphase-I and telophase-I stages, so we cannot confirm them as extra chromosomes (Goswami and Khandelwal 1980).

The incidence of meiotic aberrations (*e.g.* irregular disjunction resulting from univalent and multivalent formation) and other processes may lead to the production of non-viable spores (Ramsey and Schemske 2002). The effect of abnormal meiotic behavior on spore fertility seems to be independent of ploidy status of plant and it varies with each species. The observations regarding the effect of meiotic abnormalities on reduced pollen fertility in flowering plants have been made by Daniela *et al.* 2005; Guan *et al.* 2012; Jaryal *et al.* 2015; Kumari and Saggoo 2017; Mandal and Nandi 2017; Ramanpreet and Gupta, 2019 and Andrada 2019. Sexual species of ferns usually produced normal fertile spores whereas hybrids, apomicts, triploids etc. produce unbalanced predominantly aborted spores (Hornych and Ekrt 2017). It has been seen that if the population is seriously affected by the abnormalities, its spore abortion index will be also high. The population collected from Jia (HP) is highly abnormal with more than 75% of SMCs affected resulting in high spore abortion index (55%). Due to presence of large number of micronuclei at tetrad stage (Figure 2o), triad stage and polyad with pycnotic nuclei (Figure 2p) sporogenesis was severely affected. Empty (Figure 2q), abnormal sporangia (Figure 2r), group of sterile spores (Figure 2t) were present along with heterogenous spores (Figure 2s) leading to low spore fertility.

More investigations in row on meiotic abnormalities in different fern species will be helpful in drawing conclusions about their origin whether these are genetic, physiological or environmental. The general paradigm that can be drawn from the present observations is the fact that large percentage of meiotic abnormalities will lead to abnormal sporogenesis in this species. So, we can conclude that spore sterility may not pose problem in diploid populations unless they are seriously affected by meiotic abnormalities. These studies will be significant in studying the effect of meiotic abnormalities on reproductive behavior and calculating spore abortion index in fern species. Further, cytotaxonomic studies can solve the problems regarding scientifically correct identification of medicinal plants which is crucial for their appropriate use in pharmaceutical industry. Nowadays, conservation of medicinal plants is top most priority by conservation biologists and government agencies. Thus, evaluation of meiotic course of these plants from unexplored areas is also significant in conservation because one can opt for stable plants with high spore fertility to ensure propagation of elite germplasm.

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